



Article

4-Hydroxypiperidines and Their Flexible 3-(Amino)propyloxy Analogues as Non-Imidazole Histamine H₃ Receptor Antagonist: Further Structure–Activity Relationship Exploration and In Vitro and In Vivo Pharmacological Evaluation

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Abstract: Presynaptic histamine H₃ receptors (H₃R) act as auto- or heteroreceptors controlling, respectively, the release of histamine and of other neurotransmitters in the central nervous system (CNS). The extracellular levels of several neurotransmitters are enhanced by H₃R antagonists, and there is a great interest for potent, brain-penetrating H₃ receptor antagonists/inverse agonists to compensate for the neurotransmitter deficits present in various neurological disorders. We have shown that 1-[(benzylfuran-2-yl)methyl]piperidinyl-4-oxyl- and benzyl- derivatives of *N*-propylpentan-1-amines exhibit high in vitro potencies toward the guinea pig H₃ receptor (jejunum), with pA₂ = 8.47 and 7.79, respectively (the reference compound used was thioperamide with pA₂ = 8.67). Furthermore, following the replacement of 4-hydroxypiperidine with a 3-(methylamino)propyloxy chain, the pA₂ value for the first group decreased, whereas it increased for the second group. Here, we present data on the impact of elongating the aliphatic chain between the nitrogen of 4-hydroxypiperidine or 3-(methylamino)propan-1-ol and the lipophilic residue. Additionally, the most active compound in this series of non-imidazole H₃ receptor antagonists/inverse agonists, i.e., **ADS-003**, was evaluated for its affinity to the recombinant rat and human histamine H₃ receptors transiently expressed in HEK-293T cells. It was shown that **ADS-003**, given parenterally for 5 days, reduced the food intake of rats, as well as changed histamine and noradrenaline concentrations in the rats' brain in a manner and degree similar to the reference H₃ antagonist Ciproxifan.

Keywords: histamine H₃ receptor non-imidazole antagonists; *N*-methyl-5-[[1-(ω -substitutedalkyl)piperidin-4-yl]oxy]-*N*-propylpentan-1-amines; 5-{3-[ω -substitutedalkyl](methyl)aminopropoxy}-*N*-methyl-*N*-propylpentan-1-amines

1. Introduction

The histamine H₃ receptors were first identified in 1983 in the rat brain by J.-Ch. Schwartz and coworkers [1]. The presence of H₃ receptors was confirmed in the human brain a few years later [2]. The cDNA encoding the human H₃ receptor was successfully cloned and functionally expressed by Lovenberg et al. [3]. Histamine H₃ receptors predominantly have a presynaptic localization in histaminergic or other neurons and they modulate, through a negative feedback mechanism, the biosynthesis and release of histamine as autoreceptors [4] or the release of various other neurotransmitters, including norepinephrine [5], dopamine [6], serotonin [7], gamma-aminobutyric acid (GABA) [8], glutamate [9], and acetylcholine [10], acting as heteroreceptors. Consequently, it has been observed that administration of H₃-antagonists to the CNS enhances neurotransmission and improves cognition and attention in relevant animal models of CNS diseases [11]. Therefore, H₃-antagonists/inverse agonists have been proposed for the treatment of cognitive disorders, such as attention-deficit hyperactivity disorder (ADHD) [12] and Alzheimer's disease [13] as well as of memory and learning deficits [10]. They may also be useful in epilepsy [14], schizophrenia [15], and obesity [16].

The discovery of thioperamide [17], the prototype of the H₃ antagonist, led to the development of a wide range of imidazole-containing ligands [18]. Although many of them have found utility as pharmacological tools, the presence of an imidazole ring greatly limited brain penetration [19] and also introduced the potential for cytochrome P450 interactions [20]. For these reasons, efforts have been directed toward the design and synthesis of non-imidazole H₃ antagonists with a good binding affinity, CNS penetration ability, and reduced/no potential for cytochrome P₄₅₀ inhibition. A number of such antagonists with high selectivity and specificity have since been reported [21].

The marine natural product aplysamine-1 patented by the Harbor Branch Oceanographic Institution as a weak histamine H₃ receptor antagonist [22], possesses the characteristic 3-aminopropan-1-ol functionality in its structure [23]. This moiety has successfully been used by several laboratories for the development of non-imidazole histamine H₃ receptor antagonists and resulted in a number of highly potent and selective compounds, for example, JNJ-5207852 [24] and Pitolisant BF2.649 (Wakix) [25], the potent and selective H₃R antagonist, which was approved by the European Medicine Agency (EMA) in March 2016 for the treatment of the orphan disease narcolepsy with and without cataplexy (Figure 1). Later on, the successful replacement of the highly flexible 3-aminopropoxy link with the 4-phenoxy piperidine moiety JNJ-7737782 [26] (Figure 1) or the partially rigid 2-aminoethylbenzofuran substructure ABT-239 [27] (Figure 1) was demonstrated.

Previously, our laboratory has described several non-imidazole piperazine- [28,29] and 4-hydroxypiperidine-based histamine H₃ antagonists with a moderate to pronounced affinity for the receptor [30,31]. The structure-activity relationship (SAR) of 4-hydroxypiperidines series showed that the most potent compounds, under in vitro screening conditions, were the benzofuranyl piperidinyloxy **1a** (ADS-003) (pA₂ = 8.47; Figure 1) [31] (for reference, thioperamide pA₂ = 8.67) and benzyl **1d** (pA₂ = 7.79; Figure 1) derivatives [30]. None of the compounds showed any activity following binding to the histamine H₁ receptor. Two analogues of the compounds **1a** and **1d**, i.e., **2a** and **2d** (Figure 1), in which the 4-hydroxypiperidine ring was replaced by a flexible 3-(methylamino)propoxy chain, were synthesized and pharmacologically evaluated in vitro. In the case of derivatives carrying a (benzofuran-2-yl)methyl substituent (**1a**), a drastic reduction in potency was observed (**2a**, pA₂ = 6.23; Figure 1) [31]. For compounds bearing a benzyl substituent, an inverse relationship was observed—5-[3-[benzyl(methyl)amino]propoxy]-*N*-methyl-*N*-propylpentane-1-amine **2d** (pA₂ = 8.06; Scheme 2) expressed higher potency than its 4-hydroxypiperidine analogue **2a** (pA₂ = 7.79; Scheme 1) [31].

In continuation of our search for new highly active and selective non-imidazole histamine H₃ receptor antagonists, the first step of this study aimed to clarify the significant difference between the potencies of the 4-hydroxypiperidine derivatives and their 3-(methylamino)propoxy analogues, bearing ω-(benzofuran-2-yl)alkyl and ω-phenylalkyl moieties, respectively. We synthesized and in vitro pharmacologically [32] evaluated a series of derivatives in which the aliphatic chain

between the nitrogen of the 4-hydroxypiperidine or the 3-(methylamino)propan-1-ol and the lipophilic residue was elongated by two to three methylene groups (compounds **1b,c,e,f**, and **2b,c,e,f**; Figure 1, respectively). This was to check whether the earlier observed tendency would still be kept, or if it was an exceptional case only for short methyl chain. We also wanted to study how the elongation of the alkyl chain would affect the potency for the H₃ receptor. Additionally, the affinity of **ADS-003**—the most active compound that we have so far synthesized in this series—was estimated for the recombinant rH₃R and hH₃R (respectively), transiently expressed in HEK-293T cells. This derivative has also been proven to cross the blood–brain barrier; given parenterally for 5 days, **ADS-003** reduced the food intake by rats as well as changed the cerebral histamine and noradrenaline concentrations in these animals in a manner and degree similar to the reference H₃ antagonist—Ciproxifan.

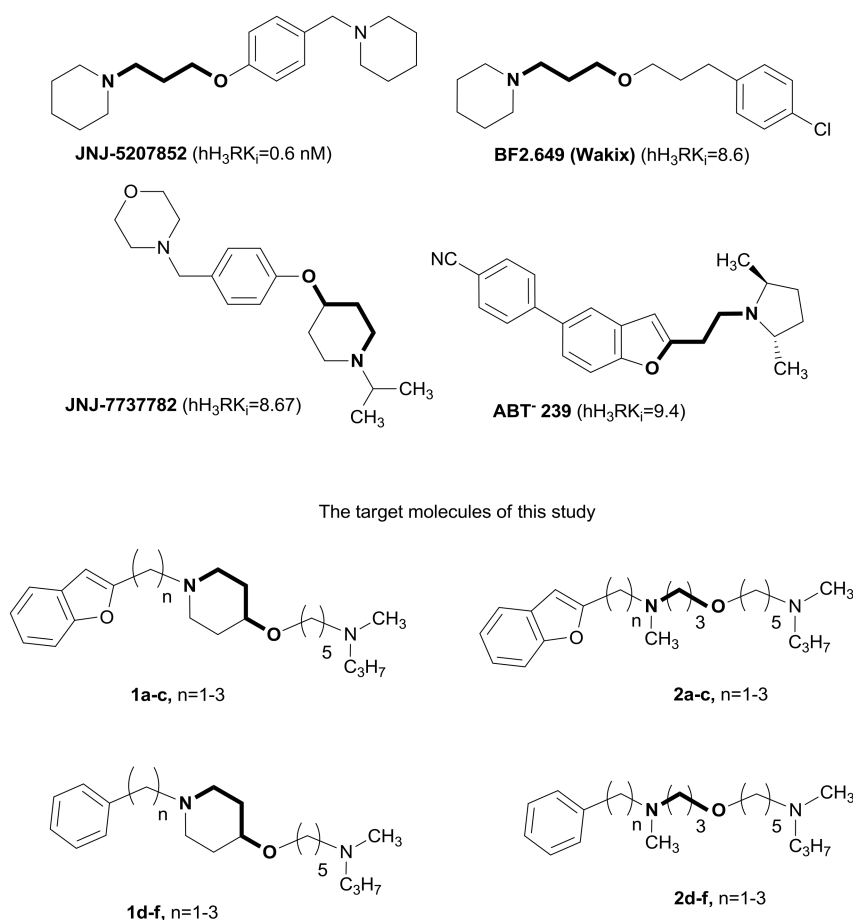
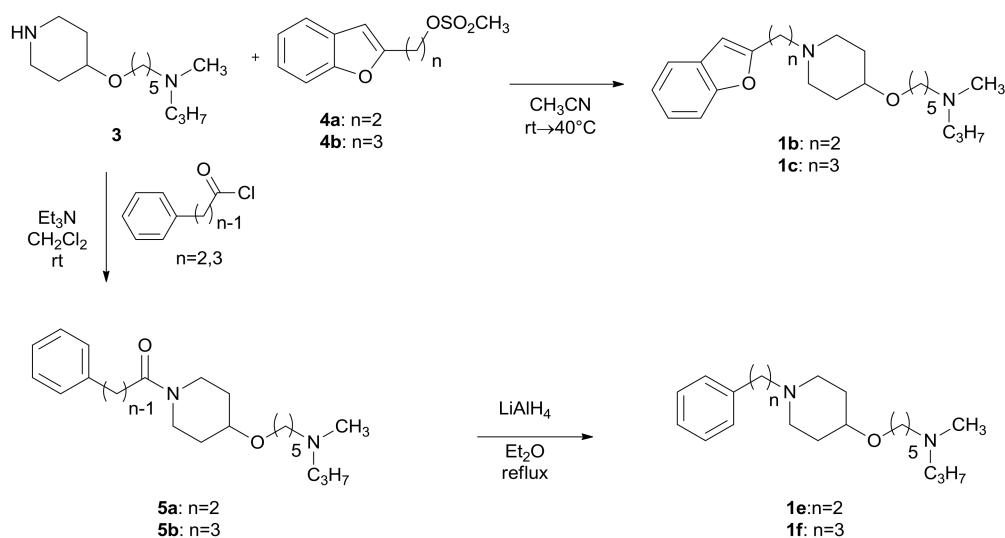
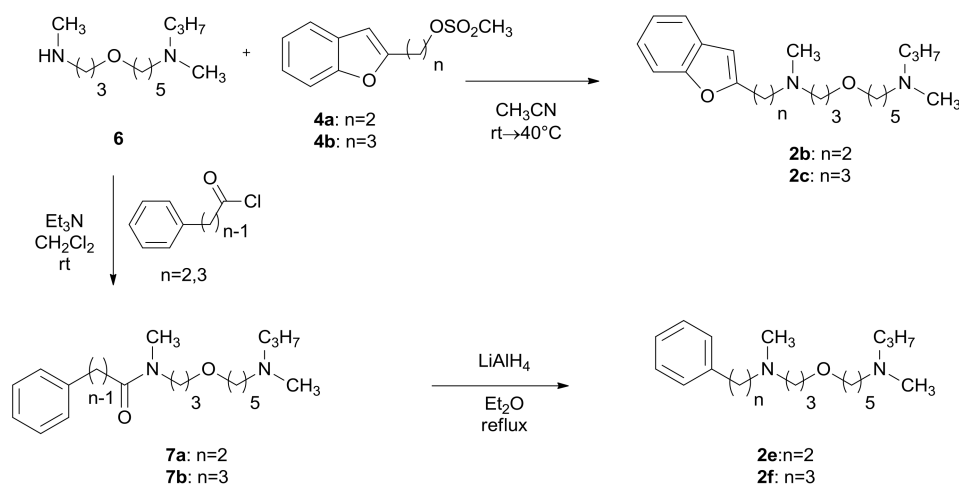


Figure 1. Representative non-imidazole H₃-histamine receptor antagonists containing the characteristic 3-aminopropan-1-ol functionality, its rigid analogues, and the target molecules of this study.

2. Results and Discussion

2.1. Chemistry

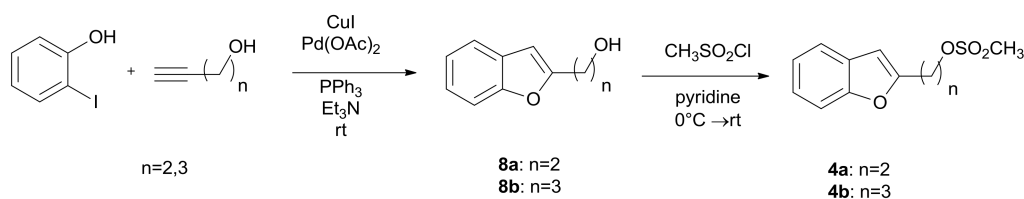
The general synthetic procedures used in this study are illustrated in Schemes 1 and 2. The key intermediates for all novel synthesized 4-hydroxypiperidines (**1b,c,e,f**; Scheme 1) and 3-(methylamino)propan-1-ols (**2b,c,e,f**; Scheme 2) were *N*-methyl-5-[(piperidin-4-yl)oxy]-*N*-propylpentan-1-amine (**3**) and *N*-methyl-5-[3-(methylamino)propoxy]-*N*-propylpentane-1-amine (**6**), respectively. Both mentioned intermediates were prepared by hydrogenation of their corresponding benzyl derivatives with a catalytic amount of palladium in charcoal in ethanol [31].

Scheme 1. Synthetic routes to compounds **1b,c,e,f**.Scheme 2. Synthetic routes to compounds **2b,c,e,f**.

The ω -(benzofuran-2-yl)alkyl derivatives of 4-hydroxypiperidine (**1b,c**; Scheme 1) and 3-(methyl)aminopropan-1-ol (**2b,c**; Scheme 2) were obtained from compounds **3** or **6** by alkylation with the corresponding ω -(benzofuran-2-yl)alkyl methanesulfonate in acetonitrile followed by purification by column chromatography.

The ω -phenylalkyl derivatives of 4-hydroxypiperidine (**1e,f**; Scheme 1) and 3-(methyl)aminopropan-1-ol (**2e,f**; Scheme 2) were obtained from compound **3** and **6** by a two-step synthesis: (1) acylation with the corresponding ω -phenylalkyl acid chloride in dry dichloromethane in the presence of triethylamine (TEA) to amides **5a,b** (Scheme 1) and **7a,b** (Scheme 2), (2) reduction of amides with LiAlH_4 in dry ethyl ether to compounds **1e,f** (Scheme 1) and compounds **2e,f** (Scheme 2), respectively. Each step was followed by purification by column chromatography.

The synthesis of the required intermediates **4a,b** (Scheme 3) was carried out by first treating the 2-iodophenol with the appropriate ω -alkyn-1-ol in the presence of palladium acetate, copper(I) iodide, and triphenylphosphine in dry triethylamine [33], followed by mesylation of the hydroxyl group by methanesulfonyl chloride in pyridine to yield the corresponding methanesulfonate with a 2 and 3 methylene linker. The detailed synthetic procedure and analytical data for compounds **8a,b** and **4a,b** are shown in Supplementary Material A (Section 1).



Scheme 3. Synthesis of ω -(1-benzofuran-2-yl)alkyl methanesulfonates **4a,b**.

2.2. Pharmacology

2.2.1. In Vitro Pharmacological Studies

H₃ Antagonistic Activity for Compounds **1a–f**, and **2a–f**

All newly synthesized compounds were converted into their dihydrogenoxolates salts and in vitro evaluated as H₃ receptor antagonists against H₃ agonist-induced inhibition of the electrically evoked contraction of the guinea pig jejunum [32].

The potencies of compounds **1b,c**, **1e,f**, **2b,c**, and **2e,f**, are reported in Table 1, as well as the previously described data for compounds **1a**, [31] **1d** [32], **2a** [32], and **2d** [32]. Derivatives **1b,c**, **1e,f**, **2b,c**, and **2e,f**, showed moderate to weak antagonist activity at H₃-receptor.

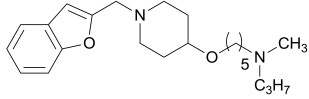
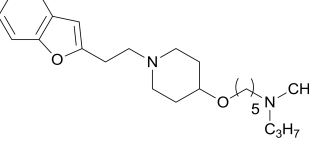
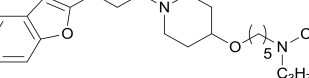
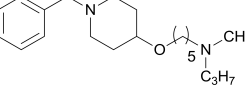
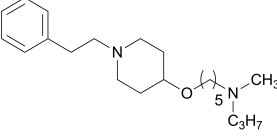
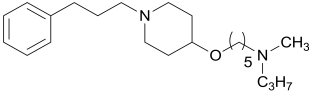
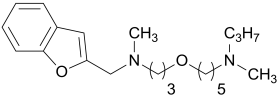
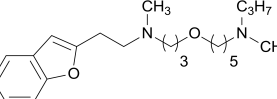
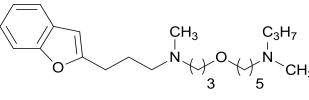
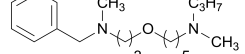
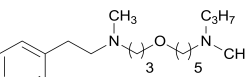
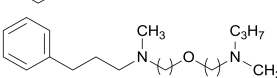
With the aim of clarifying the significant difference between the change in the potency of derivatives **1a/2a** ($pA_2 = 8.27/6.23$) carrying a 2-methylbenzofuranyl substituent versus their benzyl-analogues **1d/2d** ($pA_2 = 7.79/8.06$), we compared the homologues pairs of compounds **1b/2b** versus **1e/2e**, and **1c/2c** versus **1f/2f** (Table 1). It was found that **1b** ($pA_2 = 7.26$) had a higher activity than its 3-(methylamino)propyloxy analogue **2b** ($pA_2 = 6.32$), as also observed for derivatives **1a/2a**. In contrast, in the homologues pair **1e/2e**, the 3-(methylamine)propan-1-ol derivative **2e** ($pA_2 = 6.72$) with the phenylethyl moiety showed antagonistic activity at the same level as its 4-hydroxy piperidine analogue **1e** ($pA_2 = 6.67$). In contrast to the above results, further elongation of the alkyl chain to three methylene groups led to a decrease of potency for both pairs **1c/2c** versus **1f/2f**. Derivative **1c** ($pA_2 = 7.11$), again, showed a higher potency than its 3-(methylamino)propyloxy analogue **2c** ($pA_2 = 6.37$), but the phenylpropyl derivative of 4-hydroxypiperidine **1f** ($pA_2 = 7.51$) had a higher potency than its analogue **2f** ($pA_2 = 6.79$), which is in opposition to the previously obtained results for derivatives **1d/2d**.

The differences were observed within the **1a–c** and **1d–f** series. While the previously reported 4-hydroxypiperidine derivative bearing a 2-benzofuranylmethyl moiety **1a** showed a high potency, the newly synthesized compounds **1b,c**, where the aliphatic chain between the piperidine nitrogen and the benzofuranyl residue is elongated from 2 to 3 methylene groups, resulted in a decrease of potency ($pA_2 = 7.26$ and 7.11 , respectively). A similar effect was observed when the 2-benzofuranylmethyl substituent was replaced by a ω -phenylalkyl one (compounds **1d–f**). In this series, derivative **1d** ($pA_2 = 7.79$) showed a high potency, but an increase in the alkyl chain length to 2 methylene groups resulted in a decrease of antagonist activity for compound **1e** ($pA_2 = 6.67$), and the activity increased again on further lengthening to 3 methylene groups (**1f**; $pA_2 = 7.51$).

In the series of derivatives **2a–c**, bearing a ω -(benzofuran-2-yl)alkyl substituent—analogue of compounds **1a–c**—only a weak activity (pA_2 ranging from 6.23 to 6.37), independent of the alkyl chain length, was observed. Elongation of the alkyl chain from 2 to 3 methylene groups in the analogue series of compounds **1e,f** i.e., **2e,f** resulted in a drastic reduction of potency ($pA_2 = 6.72$ and 6.79 , respectively) in comparison to the parent compound **2d** ($pA_2 = 8.06$).

Representative graphs of the antagonism by ADS-003 (**1a**) and thioperamide of the inhibitory effect of *R*-(-)- α -methylhistamine (*R*- α -MH) on the electrically induced contraction of guinea pig ileum strips are shown in Supplementary Material B (Section 2.2.1; Figure S2).

Table 1. H₃ antagonistic potency of 4-hydroxypiperidine derivatives (**1a–f**) and their 3-(methyl-amino)propoxyanalogues (**2a–f**) as tested in the in vitro test system in the guinea pig jejunum.

Cpd.	Code Cpd.	Structure	pA ₂ (sem) ^a	N (caviae)
1a [31]	ADS-003		8.27 (0.05) (8.47) *	12 (4)
1b	ADS-013		7.26 (0.25)	12 (4)
1c	ADS-014		7.11 (0.12)	12 (4)
1d [30]	ADS-009		7.79 (0.06)	12 (4)
1e	ADS-015		6.67 (0.12)	9 (4)
1f	ADS-016		7.51 (0.03)	8 (4)
2a [31]	ADS-012		6.23 (0.12)	12 (4)
2b	ADS-017		6.32 (0.52)	5 (4)
2c	ADS-018		6.37 (0.01)	6 (4)
2d [31]	ADS-011		8.06 (0.05)	12 (4)
2e	ADS-019		6.72 (0.06)	6 (4)
2f	ADS-020		6.79 (0.01)	6 (4)
	Thioperamide		8.44 (0.26)	36 (12)

^a sem—standard error of the mean; N—number of different animal preparations; (caviae)—number of animals; * pA₂ value obtained previously [31]; for Thioperamide *, pA₂ H₃ = 8.65, (sem; 0.07); N (caviae)—36 (12).

H₁ Antagonistic Activity for Compounds **1b** and **1f**

The compounds **1b** and **1f**, possessing the highest potency for the H₃ receptors, were also tested for H₁ antagonistic effects in vitro, using standard methods [34]. Derivatives **1b** and **1f** did not show any antagonistic activity for the H₁-receptor (pA₂ < 4; for pyrilamine pA₂ = 9.37).

2.2.2. Histamine H₃ Receptor Affinity

The affinity, based on the SARs obtained for both series (compounds: **1a–f** and **2a–f**), of the most active compound **1a** (**ADS-003**) was evaluated by measuring the displacement curve of [³H]-N^α-methylhistamine from the rat (rH₃R) and human histamine H₃ receptor (hH₃R) in HEK-293T cell membranes, as described by Bonger [35].

Saturation of Rat and Human H₃ Receptors

The saturation of rat and human H₃ receptors were carried out as described previously [36].

A representative graph of saturation of rat and human H₃R can be found in Supplementary Material B (Section 2.2.2; Figure S3).

The analysis of the [³H]-N^α-MH saturation binding yielded, at rH₃R, a K_D value of 2.72 ± 0.34 nM and a B_{max} value of 2715 ± 445 fmol/mg protein and, at hH₃R, a K_D value of 0.9 ± 0.08 nM and a B_{max} value of 632 ± 52 fmol/mg protein.

Competition Binding of H₃ Receptor Ligands

The affinity of **ADS-003** (**1a**), histamine, and thioperamide was determined—the reference compounds were evaluated by measuring the displacement curves of [³H]-N^α-methylhistamine binding to rat and human histamine H₃ receptor expressed in HEK-293T membranes. Derivative **ADS-003** (**1a**) possessed a high nanomolar affinity for the rat H₃R, with a pK_i value of 7.9 ± 0.1, similar to thioperamide (pK_i value 7.9 ± 0.1) and slightly higher than histamine (pK_i = 7.3 ± 0.1). A significantly lower affinity was observed for **ADS-003** (**1a**) for the human H₃R, with pK_i 6.6 ± 0.1, in comparison with the pK_i of thioperamide (7.2 ± 0.1) and the pK_i of histamine (7.7 ± 0.1). Representative graphs of competition binding of H₃R ligands to rat and human H₃ receptor are shown in Supplementary Material B (Section 2.2.2; Figures S4 and S5, respectively).

2.2.3. Verification of In Vivo Activity for Compound **ADS-003** (**1a**)

Finally, compound **ADS-003** (**1a**) was subjected to an in vivo evaluation of its impact on brain neurotransmitter systems. This assessment concerned:

- The effects of the compound on the feeding behavior of rats after its repeated peripheral administration. Given that the compound enters the CNS and blocks the H₃R, it should release histamine. Histamine, in turn, acting via H₁R, would induce loss of appetite, i.e., the food intake of rats would decrease,
- The influence on the cerebral amine neurotransmitter concentrations, as well as the activity of the monoamine oxidases A and B and histamine N-methyltransferase. The latter was accomplished by postmortem analyses of the brain tissues of the treated rats.

To evaluate the central effects of peripheral administration of **ADS-003** (**1a**) to rats, the feeding behavior of rats was monitored after drug administration. The neuronal histaminergic system is known to be one of the regulatory systems in food intake. Studies in various animal models have convincingly shown that histamine H₁R and H₃R receptors play an important role in this respect. Activation of both histamine receptors is a critical part of the diurnal rhythm of the food consumption regulatory mechanism, as well as in energy intake and expenditure [37–41]. In a comprehensive study done in rats, it was demonstrated that centrally infused histamine or H₁ receptor agonists invariably decreased food intake, as did the strategies leading either to an enhanced release of hypothalamic histamine by the blocking the H₃ receptor or to an increase of available histamine by inhibiting its degradation. The opposite, i.e., hyperphagia, was seen for H₁ antagonists [38]. Compatible with the earlier reported experimental data, it was assumed that, if an H₃ receptor antagonist subcutaneously injected into rats crossed the blood–brain barrier (BBB), it would affect the animal food consumption [37]. As presented in Figure 2, the treatment of rats with either of the two H₃R antagonists **ADS-003** (3 mg/kg s.c.) and

Ciproxifan (3 mg/kg s.c.) over a period of 5 days evoked a statistically significant reduction in the amount of consumed food by rats compared to the pre-treatment period.

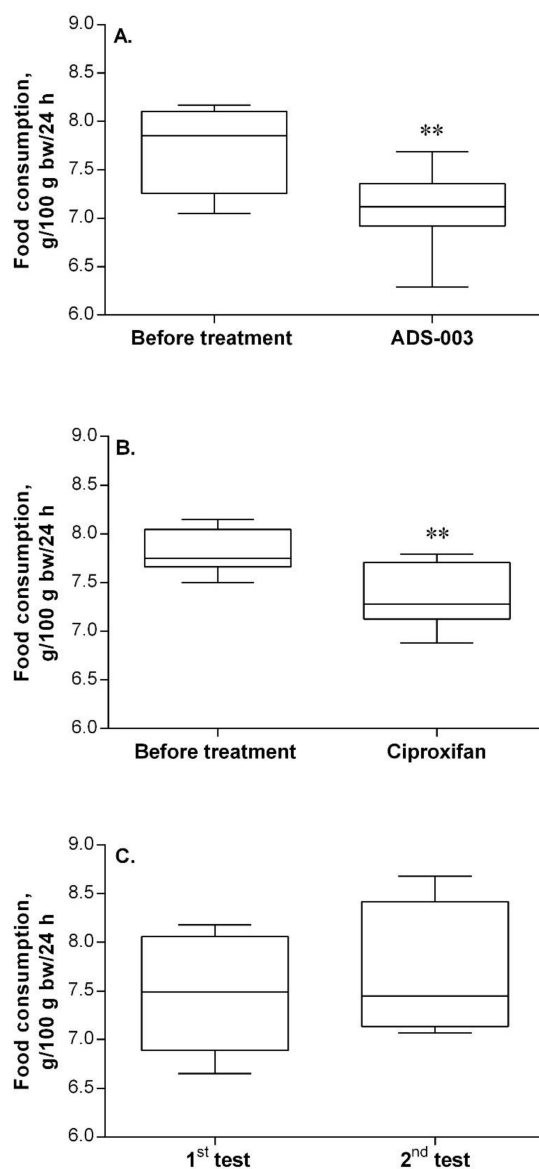


Figure 2. The effect of ADS-003 (s.c. 3 mg/kg/daily for 5 days), the newly synthesized histamine H₃ receptor antagonist (A), and of the reference compound Ciproxifan (s.c. 3 mg/kg/daily for 5 days; B) on food intake. Consumption by untreated rats (C). The median (the line in the middle of the box) and the range of values (whiskers) are given for eight rats. Paired *t*-test: ** $p < 0.01$ versus before treatment.

In *in vivo* studies, **Ciproxifan** was used as a reference instead of thioperamide, because the latter demonstrated lower bioavailability due to restricted brain penetration [42].

There was no difference in the efficacy of **ADS-003 (1a)** and **Ciproxifan** (the reference compound). These results suggest that **ADS-003** crosses the BBB, and its potency at H₃R is similar to that of **Ciproxifan**.

Post-Mortem Biochemical Analysis of the Brain Tissues of **ADS-003 (1a)**-Treated Rats

Postmortem biochemical analysis of the brain tissues of **ADS-003**-treated rats quantified the brain concentration of histamine, serotonin, dopamine, noradrenaline, and the activities of monoamine oxidase (MAO)-A, MAO-B, and HNMT. As shown in Figure 3, the histamine concentration in the

hypothalamus, where histaminergic cell bodies are located, showed a tendency to increase—which could be explained by the stimulation of the amine synthesis following its release by H₃R blockade with **1a** (**ADS-003**) or **Ciproxifan** to replenish vesicular stores. Yet, one-way ANOVA and Tukey's multiple comparisons test showed no statistically significant differences. Similarly, no changes were found in the histamine levels in the cerebral cortex of the treated rats (Figure 3).

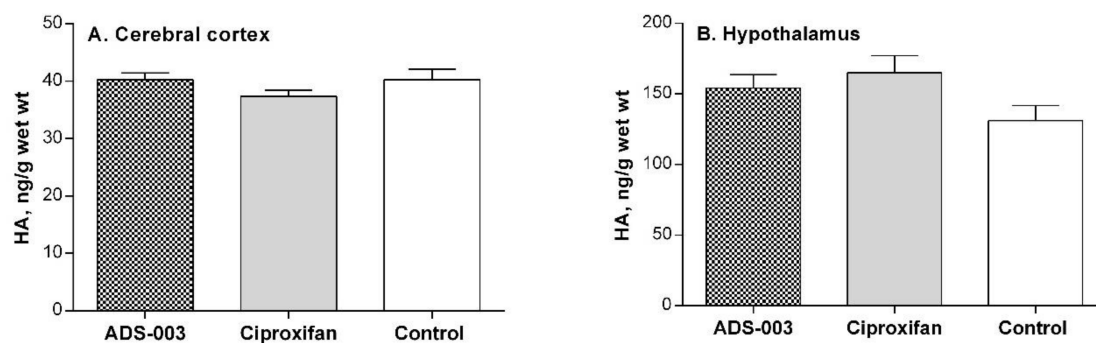


Figure 3. Cerebral histamine concentration in rats subchronically treated with Ciproxifan and the newly synthesized **ADS-003** histamine H₃ receptor antagonist. The median (the line in the middle of the box) and the range of values (whiskers) are given for eight rats. Paired *t*-test: $p < 0.05$ versus “before treatment” for eight rats. Paired *t*-test, $p < 0.05$, $p < 0.01$ versus “before treatment”.

On the other hand, both H₃R antagonists caused a significant increase in noradrenaline levels in the cerebral cortex (Figure 4).

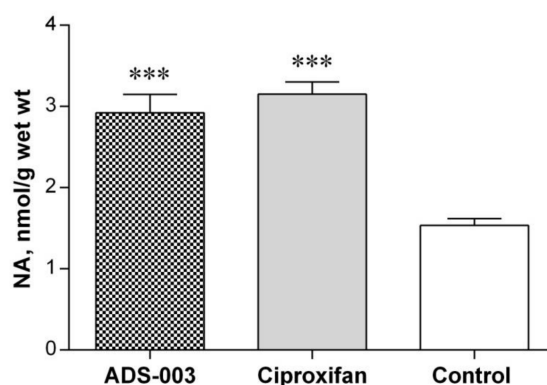


Figure 4. The concentration of noradrenaline (NA) in the cerebral cortex of rats subchronically treated with the newly synthesized **ADS-003** histamine H₃ receptor antagonist or with Ciproxifan. The values are means \pm SEM for four–nine rats. One-way ANOVA and Tukey's multiple: *** $p < 0.05$, three symbols: $p < 0.001$.

There were no changes in serotonin and dopamine concentration.

The increase in tissue NA is compatible with previous data reporting an inhibitory control exerted by H₃ histamine receptors on NA neuronal function in the cortex [43,44]. The fact that both histamine H₃ receptor antagonists, Ciproxifan and **ADS-003**, enhanced the tissue levels of NA in a similar manner strengthens this idea.

Using sensitive isotopic assays, neither changes in monoamine oxidase A and B nor in histamine *N*-methyltransferase in the brain tissues of rats were observed (Table 2).

Table 2. The effect of subchronic administration of ADS-003 (s.c. 3 mg/kg/daily for 5 days) or Ciproxifan (s.c. 3 mg/kg/daily for 5 days) on cerebral MAOs and HNMT activities.

Group	MAO-A	MAO-B	HMNT	
	pmol/min/mg Protein		pmol/min/mg Protein	
	CTX	CTX	CTX	HTH
ADS-003	1536 ± 50	1065 ± 48	44.41 ± 1.06	41.09 ± 0.80
Ciproxifan	1654 ± 31	1049 ± 56	46.90 ± 3.59	51.76 ± 5.67
Control	1506 ± 29	1054 ± 19	45.97 ± 1.28	39.81 ± 1.94

The values are given as means ± SEM for four–eight rats. The drugs were administered subcutaneously (s.c.) at a dose of 3 mg/kg of body mass for 5 consecutive days. MAO—monoamine oxidase, HNMT—histamine N-methyltransferase, CTX—cerebral cortex, HTH—hypothalamus. one-way ANOVA and Tukey's multiple comparisons test showed no statistically significant differences.

3. Materials and Methods

3.1. Chemistry

3.1.1. General Methods

All melting points (mp) were measured in open capillaries in an electrothermal apparatus and are uncorrected. The ¹H NMR spectra were recorded in CDCl₃ as a solvent in a 600 MHz spectrometer, a Bruker Avance III spectrometer at ambient temperature. The chemical shifts are reported in ppm on scale downfield from tetramethylsilane (TMS) as internal standard, and the signal patterns are indicated as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad; * exchangeable by D₂O; a number of protons, and J approximate coupling constant in Hertz. The ¹³C NMR spectra were recorded in a 600 MHz spectrometer, a Bruker Avance III (150 MHz). Elemental analyses (C, H, N) for all compounds were measured in Perkin Elmer Series II CHNS/O Analyzer 2400 and agreed with the theoretical values within ±0.4%. TLC data were obtained with Merck silica gel 60F₂₅₄ aluminum sheets. For flash column chromatography using silica gel, 60 Å, 50 μm (J. T. Baker B. V.), the same solvent system as for TLC, was used. All obtained final free bases were treated with methanolic oxalic acid, and the dihydrogenoxolates were precipitated with dry diethyl ether and crystallized twice from ethanol. All dihydrogenoxolates were obtained as white crystalline solids.

Chemicals

The reagents 2-iodophenol, palladium acetate, copper(I) iodide, triphenylphosphine, 3-butyne-1-ol, 4-pentyn-1-ol, methanesulfonyl chloride, lithium aluminum hydride, and all solvents were purchased from Sigma-Aldrich (Saint Louis, MO, USA) and Alfa Aesar (Haverhill, MA, USA) and were used without any purification.

3.1.2. General Procedure for the Preparation of Compounds **1b,c** and **2b,c**

To a solution of the corresponding amines **3** or **6** (1.26 mmol) in acetonitrile (5 mL), the appropriate methanesulfonate **4a** or **4b** (1.05 mmol) was added. The reaction mixture was stirred at 40 °C for 24 h. After completion of the reaction, water was added. The mixture was extracted with dichloromethane (3 × 20 mL), and the organic layer was dried over MgSO₄ and filtered. The solvent was evaporated, and the residue was purified by silica gel-flash column chromatography to yield a sticky oil.

5-{{1-[2-(1-Benzofuran-2-yl)ethyl]piperidin-4-yl}oxy}-N-methyl-N-propylpentan-1-amine (**1b**): (119 mg, 29.0%): R_f = 0.49 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.90 (t, J = 7.4 Hz, 3H, NCH₂CH₂CH₃), 1.34–1.38 (m, 2H, H-3), 1.49–1.65 (m, 10H, H-2, H-4, NCH₂CH₂CH₃, 2xCH₂^{PiP}), 1.87–1.92 (m, 2H, CH₂^{PiP}), 2.28 (s, 3H, CH₃), 2.36 (t, J = 7.8 Hz, 2H, H-5), 2.41 (t, J = 7.7 Hz, 2H, NCH₂CH₂CH₃), 2.76 (t, J = 7.4 Hz, 2H, C₈H₅OCH₂CH₂N), 2.81–2.83 (m, 2H, CH₂^{PiP}), 2.97 (t, J = 7.4 Hz, 2H, C₈H₅OCH₂CH₂N), 3.27–3.31 (m, 1H, CH^{PiP}), 3.42 (t, J = 6.5 Hz, 2H, H-1), 6.42 (s,

1H, CH^{furan}), 7.15–7.21 (m, 2H, C₆H₄), 7.39 (d, *J* = 7.6 Hz, 1H, C₆H₄), 7.46 ppm (d, *J* = 7.2 Hz, 1H, C₆H₄); ¹³C NMR (150 MHz, CDCl₃): δ = 12.04 (NCH₂CH₂CH₃), 20.08 (NCH₂CH₂CH₃), 24.41 (C-3), 26.73 (C₈H₅OCH₂CH₂N), 29.90 (CH₂^{PiP}), 30.19 (C-4), 31.08 (C-2), 42.05 (CH₃), 51.28 (CH₂^{PiP}), 56.59 (C₈H₅OCH₂CH₂N), 57.59 (C-5), 59.61 (NCH₂CH₂CH₃), 67.93 (C-1), 75.06 (CH^{PiP}), 102.66 (C^{furan}), 110.94, 120.47, 122.66, 123.43, 129.12, 154.85 (C₆H₄), 157.83 ppm (C^{furan}).

Anal. calcd for dihydrogenoxolate (C₂₄H₃₈N₂O₂·2C₂H₂O₄): C 59.35, H 7.47, N 4.94; found: C 59.08, H 7.83, N 5.07; mp_{dihydrogenoxolate} = 133.2–134.9 °C.

5-{{1-[3-(1-Benzofuran-2-yl)propyl]piperidin-4-yl}oxy}-*N*-methyl-*N*-propylpentan-1-amine (**1c**): (226 mg, 54.0%): R_f = 0.38 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.88 (t, *J* = 7.4 Hz, 3H, NCH₂CH₂CH₃), 1.31–1.36 (m, 2H, H-3), 1.45–1.51 (m, 4H, NCH₂CH₂CH₃, CH₂^{PiP}), 1.55–1.62 (m, 6H, H-2, H-4, CH₂^{PiP}), 1.87–1.89 (m, 2H, CH₂^{PiP}), 1.91–1.94 (m, 2H, C₈H₅OCH₂CH₂CH₂N), 2.20 (s, 3H, CH₃), 2.28 (t, *J* = 7.7 Hz, 2H, H-5), 2.32 (t, *J* = 7.8 Hz, 2H, NCH₂CH₂CH₃), 2.39 (t, *J* = 7.6 Hz, 2H, C₈H₅OCH₂CH₂CH₂N), 2.74–2.80 (m, 4H, CH₂^{PiP}, C₈H₅OCH₂CH₂CH₂N), 3.24–3.27 (m, 1H, CH^{PiP}), 3.42 (t, *J* = 6.6 Hz, 2H, H-1), 6.37 (s, 1H, CH^{furan}), 7.14–7.20 (m, 2H, C₆H₄), 7.38 (d, *J* = 7.8 Hz, 1H, C₆H₄), 7.46 ppm (d, *J* = 7.2 Hz, 1H, C₆H₄); ¹³C NMR (150 MHz, CDCl₃): δ = 12.15 (NCH₂CH₂CH₃), 20.60 (NCH₂CH₂CH₃), 24.46 (C₈H₅OCH₂CH₂CH₂N), 25.49 (C-3), 26.64 (C₈H₅OCH₂CH₂CH₂N), 27.32 (C-4), 30.29 (CH₂^{PiP}), 31.65 (C-2), 42.49 (CH₃), 51.56 (CH₂^{PiP}), 57.95 (C₈H₅OCH₂CH₂CH₂N and C-5), 60.06 (NCH₂CH₂CH₃), 67.98 (C-1), 74.06 (CH^{PiP}), 102.66 (C^{furan}), 110.94, 120.47, 122.66, 123.43, 129.12, 154.85 (C₆H₄), 157.83 ppm (C^{furan}).

Anal. calcd for dihydrogenoxolate (C₂₅H₄₀N₂O₂·2C₂H₂O₄·0.5 H₂O): C 59.07, H 7.69, N 4.75; found: C 59.05, H 7.75, N 4.80; mp_{dihydrogenoxolate} = 157–159 °C.

5-{3-[(2-Benzofuran-2-yl)ethyl](methyl)amino}propoxy}-*N*-methyl-*N*-propylpentan-1-amine (**2b**): (109 mg, 28.0%): R_f = 0.51 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.88 (t, *J* = 7.4 Hz, 3H, NCH₂CH₂CH₃), 1.31–1.36 (m, 2H, H-6), 1.43–1.50 (m, 4H, H-7, NCH₂CH₂CH₃), 1.54–1.59 (m, 2H, H-5), 1.72–1.77 (m, 2H, H-2), 2.19 (s, 3H, CH₃), 2.27 (t, *J* = 7.6 Hz, 2H, NCH₂CH₂CH₃), 2.29–2.32 (m, 5H, H-8, CH₃), 2.49 (t, *J* = 7.2 Hz, 2H, H-1), 2.76–2.79 (m, 2H, C₈H₅OCH₂CH₂N), 2.91–2.95 (m, 2H, C₈H₅OCH₂CH₂N), 3.36 (t, *J* = 6.7 Hz, 2H, H-4), 3.42 (t, *J* = 6.5 Hz, 2H, H-3), 6.41 (s, 1H, CH^{furan}), 7.13–7.20 (m, 2H, C₆H₄), 7.38–7.40 (m, 1H, C₆H₄), 7.45–7.47 ppm (m, 1H, C₆H₄); ¹³C NMR (150 MHz, CDCl₃): δ = 12.15 (NCH₂CH₂CH₃), 20.65 (C-7), 24.44 (C-6), 26.92 (NCH₂CH₂CH₃), 27.37 (C-2), 27.92 (C₈H₅OCH₂CH₂N), 29.94 (C-5), 42.33, 42.49 (2 × CH₃), 54.58 (C-1), 55.83 (C₈H₅OCH₂CH₂N), 58.00 (NCH₂CH₂CH₃), 60.10 (C-8), 69.23 (C-4), 71.13 (C-3), 102.64 (C^{furan}), 110.91, 120.43, 122.62, 123.36, 129.17, 154.87 (C₆H₄), 158.00 ppm (C^{furan}).

Anal. calcd for dihydrogenoxolate (C₂₃H₃₈N₂O₂·2C₂H₂O₄·0.5 H₂O): C 57.54, H 7.69, N 4.97; found: C 57.37, H 7.95, N 5.02; mp_{dihydrogenoxolate} = 144.5–145.7 °C.

5-{3-[(3-Benzofuran-2-yl)propyl](methyl)amino}propoxy}-*N*-methyl-*N*-propylpentan-1-amine (**2c**): (134 mg, 34.0%): R_f = 0.45; (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.88 (t, *J* = 7.4 Hz, 3H, NCH₂CH₂CH₃), 1.30–1.35 (m, 2H, H-6), 1.44–1.50 (m, 4H, H-7, NCH₂CH₂CH₃), 1.55–1.59 (m, 2H, H-5), 1.71–1.76 (m, 2H, H-2), 1.88–1.93 (m, 2H, C₈H₅OCH₂CH₂CH₂N), 2.19 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 2.28 (t, *J* = 7.6 Hz, 2H, NCH₂CH₂CH₃), 2.31 (t, *J* = 7.6 Hz, 2H, H-8), 2.41–2.43 (m, 4H, H-3, C₈H₅OCH₂CH₂CH₂N), 2.78 (t, *J* = 7.4 Hz, 2H, C₈H₅OCH₂CH₂CH₂N), 3.38 (t, *J* = 6.7 Hz, 2H, H-4), 3.41–3.44 (m, 2H, H-1), 6.37 (s, 1H, CH^{furan}), 7.14–7.20 (m, 2H, C₆H₄), 7.39 (d, *J* = 8.1 Hz, 1H, C₆H₄), 7.46 ppm (d, *J* = 8.1 Hz, 1H, C₆H₄); ¹³C NMR (150 MHz, CDCl₃): δ = 12.09 (C-11), 20.39 (C-7), 24.39 (C-6), 25.67 (C-10), 26.50 (C-2), 27.83 (C₈H₅OCH₂CH₂CH₂N), 29.90 (C₈H₅OCH₂CH₂CH₂N and C-5), 42.30, 42.40 (2 × CH₃), 54.80 (C₈H₅OCH₂CH₂CH₂N), 57.19 (C-1), 57.83 (C-9), 59.89 (C-8), 69.33 (C-3), 71.08 (C-4), 102.16 (C^{furan}), 110.92, 120.39, 112.60, 123.30, 129.20, 154.89 (C₆H₄), 159.51 ppm (C^{furan}).

Anal. calcd for dihydrogenoxolate (C₂₄H₄₀N₂O₂·2C₂H₂O₄·0.5 H₂O): C 58.22, H 7.85, N 4.85; found: C 58.61, H 8.30, N 4.97; mp_{dihydrogenoxolate} = 106.0–108.0 °C.

3.1.3. General Procedure for the Preparation of Compounds **5a,b** and **7a,b**

The corresponding amines **3** or **6** (2.6 mmol) and Et₃N (3.12 mmol) were dissolved in dichloromethane and cooled to 0°C. After flushing with argon, 2-phenylacetyl chloride (3.12 mmol) or 3-phenylpropanoyl chloride (3.12 mmol) was slowly added to the mixture, and the reaction was stirred at room temperature for 12 h. After completion of the reaction, an aqueous solution of K₂CO₃ was added. The reaction mixture was extracted with dichloromethane (3 × 20 mL), and the organic layer was dried over MgSO₄ and filtered. The solvent was evaporated, and the residue was purified by silica gel-flash column chromatography to yield a sticky oil.

1-{4-[[5-[Methyl(propyl)amino]pentyl]oxy]piperidin-1-yl}-2-phenylethanone (5a): (818 mg, 87.0%); R_f = 0.42 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.89 (t, J = 7.3 Hz, 2H, NCH₂CH₂CH₃), 1.30–1.35 (m, 3H, H-3, CH^{PIP}), 1.46–1.49 (m, 4H, NCH₂CH₂CH₃, H-4), 1.53–1.57 (m, 4H, H-2, CH₂^{PIP}), 1.74–1.80 (m, 1H, CH^{PIP}), 2.21 (s, 3H, CH₃), 2.28 (t, J = 7.6 Hz, 2H, H-5), 2.32 (t, J = 7.6 Hz, 2H, NCH₂CH₂CH₃), 3.17–3.21 (m, 1H, CH^{PIP}), 3.31–3.43 (m, 4H, H-1, CH^{PIP}, CH^{PIP}), 3.60–3.65 (m, 1H, CH^{PIP}), 3.73 (s, 2H, C₆H₅CH₂C(O)), 3.88–3.93 (m, 1H, CH^{PIP}), 7.21–7.24 (m, 3H, C₆H₅), 7.29–7.32 ppm (m, 2H, C₆H₅); ¹³C NMR (150 MHz, CDCl₃): δ = 12.09 (NCH₂CH₂CH₃), 20.40 (NCH₂CH₂CH₃), 24.40 (C-3), 30.17 (C-4), 30.84 (C-2), 31.65 (CH₂^{PIP}), 39.32 (CH₂^{PIP}), 41.39 (C₆H₅CH₂C(O)), 43.65 (CH₃), 57.83 (C-5), 59.92 (NCH₂CH₂CH₃), 68.25 (C-1), 73.98 (CH^{PIP}), 126.93, 128.75, 128.92, 135.52 (C₆H₅), 169.50 ppm (C=O).

1-{4-[[5-[Methyl(propyl)amino]pentyl]oxy]piperidin-1-yl}-3-phenylpropan-1-one (5b): (815 mg, 84.0%); R_f = 0.45 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.88 (t, J = 7.4 Hz, 3H, NCH₂CH₂CH₃), 1.33–1.37 (m, 2H, H-3), 1.45–1.53 (m, 6H, H-4, NCH₂CH₂CH₃, CH₂^{PIP}), 1.54–1.60 (m, 2H, H-2), 1.69–1.72 (m, 1H, CH^{PIP}), 1.76–1.80 (m, 1H, CH^{PIP}), 2.21 (s, 3H, CH₃), 2.27–2.34 (m, 4H, H-5, NCH₂CH₂CH₃), 2.62 (t, J = 8.2 Hz, 2H, C₆H₅CH₂CH₂C(O)), 2.96 (t, J = 7.7 Hz, 2H, C₆H₅CH₂CH₂C(O)), 3.15–3.18 (s, 1H, CH^{PIP}), 3.30–3.34 (s, 1H, CH^{PIP}), 3.40–3.48 (m, 3H, H-1, CH^{PIP}), 3.57–3.61 (m, 1H, CH^{PIP}), 3.88–3.92 (m, 1H, CH^{PIP}), 7.18–7.22 (m, 3H, C₆H₅), 7.26–7.29 ppm (m, 2H, C₆H₅); ¹³C NMR (150 MHz, CDCl₃): δ = 12.15 (NCH₂CH₂CH₃), 20.61 (NCH₂CH₂CH₃), 24.46 (C-3), 30.25 (C-2 and C-4), 30.91 (CH₂^{PIP}), 31.88 (C₆H₅CH₂CH₂C(O)), 35.33 (C₆H₅CH₂CH₂C(O)), 29.19 (CH₂^{PIP}), 43.03 (CH₃), 57.98 (C-5), 60.09 (NCH₂CH₂CH₃), 68.28 (C-1), 74.03 (CH^{PIP}), 126.36, 128.65, 128.72 (C₆H₅), 141.66 (C₆H₅), 170.72 ppm (C=O).

N-Methyl-N-{3-[[5-[methyl(propyl)amino]pentyl]oxy]propyl}-2-phenylacetamide (7a): (382 mg, 42.0%); R_f = 0.48 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.86 (t, J = 7.4 Hz, 3H, NCH₂CH₂CH₃), 1.29–1.38 (m, 2H, H-6), 1.47–1.52 (m, 4H, H-5, NCH₂CH₂CH₃), 1.55–1.62 (m, 2H, H-7), 1.72–1.76 (m, 1H, H-2), 1.77–1.82 (m, 1H, H-2), 2.22 (s, 1.5H, CH₃), 2.23 (s, 1.5H, CH₃), 2.29–2.37 (m, 4H, H-8, NCH₂CH₂CH₃), 2.92 (s, 1.5H, CH₃), 2.97 (s, 1.5H, CH₃), 3.34–3.46 (m, 6H, H-1, H-3, H-4), 3.69 (s, 1H, C₆H₅CH₂C(O)), 3.74 (s, 1H, C₆H₅CH₂C(O)), 7.20–7.32 ppm (m, 5H, C₆H₅); ¹³C NMR (150 MHz, CDCl₃): δ = 11.74 and 11.78 (NCH₂CH₂CH₃), 19.39 and 19.58 (NCH₂CH₂CH₃), 24.07 and 24.13 (C-6), 25.90 and 26.25 (C-7), 27.67 and 28.56 (C-2), 29.49 and 29.63 (C-5), 33.35 and 36.18 (CH₃), 40.48 and 41.37 (CH₃), 41.43 and 41.61 (C₆H₅CH₂C(O)), 45.59 and 47.21 (C-1), 57.09 and 57.23 (C-8), 59.02 and 59.23 (NCH₂CH₂CH₃), 67.17 and 68.35 (C-3), 70.73 and 70.93 (C-4), 126.71, 126.74, 128.63, 128.67, 128.82, 128.89, 129.01, 129.29, 135.19, 135.63 (C₆H₅), 170.91 and 171.20 ppm (C=O).

N-Methyl-N-{3-[[5-[methyl(propyl)amino]pentyl]oxy]propyl}-3-phenylpropanamide (7b): (705 mg, 75.0%); R_f = 0.70 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.88 (t, J = 7.3 Hz, 3H, NCH₂CH₂CH₃), 1.26–1.36 (m, 2H, H-6), 1.42–1.59 (m, 6H, H-5, H-7, NCH₂CH₂CH₃), 1.72–1.80 (m, 2H, H-2), 2.19 (s, 3H, CH₃), 2.25–2.33 (m, 4H, H-8, NCH₂CH₂CH₃), 2.59 (t, J = 7.7 Hz, 1H, C₆H₅CH₂CH₂C(O)), 2.65 (t, J = 7.7 Hz, 1H, C₆H₅CH₂CH₂C(O)), 2.91 (s, 3H, CH₃), 2.97 (q, J = 6.7 Hz, 2H, C₆H₅CH₂CH₂C(O)), 3.32–3.36 (m, 3H, H-1, H-3), 3.37–3.41 (m, 2H, H-4), 3.43 (t, J = 6.9 Hz, 1H, H-3), 7.17–7.28 ppm (m, 5H, C₆H₅); ¹³C NMR (150 MHz, CDCl₃): δ = 12.14 (NCH₂CH₂CH₃), 20.61 (NCH₂CH₂CH₃), 24.37 and 24.42 (C-6), 27.34 (C-7), 27.98 and 28.82 (C-2), 29.87 and 29.92 (C-5), 31.56

and 31.83 (C₆H₅CH₂CH₂C(O)), 34.94 (CH₃), 35.70 and 35.89 (C₆H₅CH₂CH₂C(O)), 42.47 (CH₃), 45.69 and 46.88 (C-1), 57.94 and 57.96 (C-8), 60.07 and 60.09 (NCH₂CH₂CH₃), 67.26 and 68.59 (C-3), 71.17 and 71.29 (C-4), 126.25, 126.28, 128.62, 128.65, 141.76, 141.80 (C₆H₅), 172.19 and 172.50 ppm (C=O).

3.1.4. General Procedure for the Preparation of Compounds **1e,f** and **2e,f**

To a vigorous stirred solution of the corresponding amides **5a,b** or **7a,b** (1.0 mmol) in 250 mL of anhydrous diethyl ether, LiAlH₄ (2.5 mmol) was added portionwise. The mixture was stirred at reflux for 2 h, cooled to room temperature, and quenched by a dropwise addition of water (0.5 mL). The suspension was stirred for 30 min and filtered. The filter cake was washed with diethyl ether. The solvent was evaporated, and the residue was purified by silica gel-flash column chromatography (eluent: CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%) to yield a sticky oil.

N-Methyl-5-[(1-phenethylpiperidin-4-yl)oxy]-*N*-propylpentan-1-amine (**1e**): (252 mg, 72.0%): R_f = 0.32 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%) = 0.32; ¹H NMR (600 MHz, CDCl₃): δ = 0.89 (t, J = 7.3 Hz, 3H, NCH₂CH₂CH₃), 1.32–1.37 (m, 2H, H-3), 1.45–1.51 (m, 4H, H-4, NCH₂CH₂CH₃), 1.56–1.65 (m, 4H, H-2, CH₂^{PiP}), 1.87–1.92 (m, 2H, CH₂^{PiP}), 2.18–2.22 (m, 5H, CH₃, CH₂^{PiP}), 2.28 (t, J = 7.4 Hz, 2H, NCH₂CH₂CH₃), 2.32 (t, J = 7.3 Hz, 2H, H-5), 2.57 (t, J = 8.6 Hz, 2H, C₆H₅CH₂CH₂), 2.78–2.84 (m, 4H, C₆H₅CH₂CH₂, CH₂^{PiP}), 3.26–3.31 (m, 1H, CH^{PiP}), 3.43 (t, J = 6.6 Hz, 2H, H-1), 7.17–7.19 (m, 3H, C₆H₅), 7.25–7.28 ppm (m, 2H, C₆H₅); ¹³C NMR (150 MHz, CDCl₃): δ = 12.17 (NCH₂CH₂CH₃), 20.65 (NCH₂CH₂CH₃), 24.49 (C-3), 27.37 (C-4), 30.32 (C-2), 31.67 (CH₂^{PiP}), 34.16 (C₆H₅CH₂CH₂), 42.54 (CH₃), 51.53 (CH₂^{PiP}), 58.03 (C-5), 60.12 (C₆H₅CH₂CH₂), 60.77 (NCH₂CH₂CH₃), 68.02 (C-1), 75.01 (CH^{PiP}), 126.19, 128.57, 128.92, 140.80 ppm (C₆H₅).

Anal. calcd for dihydrogenoxolate (C₂₂H₃₈N₂O 2C₂H₂O₄): C 59.30, H 8.04, N 5.32; found: C 58.86, H 8.44, N 5.38; mp_{dihydrogenoxolate} = 131.0–133.0 °C.

N-Methyl-5-[[1-(3-phenylpropyl)piperidin-4-yl]oxy]-*N*-propylpentan-1-amine (**1f**): (342 mg, 95.0%): R_f = 0.45 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1% CH₂Cl₂/MeOH/NH_{3(aq)}); ¹H NMR (600 MHz, CDCl₃): δ = 0.88 (t, J = 7.3 Hz, 2H, NCH₂CH₂CH₃), 1.31–1.36 (m, 2H, H-3), 1.44–1.50 (m, 4H, H-4, NCH₂CH₂CH₃), 1.55–1.61 (m, 4H, H-2, CH₂^{PiP}), 1.81 (t, J = 7.6 Hz, 2H, C₆H₅CH₂CH₂CH₂), 1.85–1.88 (m, 2H, CH₂^{PiP}), 2.07–2.11 (m, 2H, CH₂^{PiP}), 2.20 (s, 3H, CH₃), 2.27 (t, J = 7.6 Hz, 2H, H-5), 2.30–2.35 (m, 4H, NCH₂CH₂CH₃, C₆H₅CH₂CH₂CH₂), 2.62 (t, J = 7.6 Hz, 2H, C₆H₅CH₂CH₂CH₂), 2.72–2.76 (m, 2H, CH₂^{PiP}), 3.23–3.28 (m, 1H, CH^{PiP}), 3.41 (t, J = 6.6 Hz, 2H, H-1), 7.15–7.18 (m, 3H, C₆H₅), 7.25–7.28 ppm (m, 2H, C₆H₅); ¹³C NMR (150 MHz, CDCl₃): δ = 12.16 (NCH₂CH₂CH₃), 20.66 (NCH₂CH₂CH₃), 24.47 (C-3), 27.37 (C-4), 29.11 (C₆H₅CH₂CH₂CH₂), 30.31 (C-2), 31.68 (CH₂^{PiP}), 34.02 (C₆H₅CH₂CH₂CH₂), 42.53 (CH₃), 51.57 (CH₂^{PiP}), 58.03 (C₆H₅CH₂CH₂CH₂), 58.21 (NCH₂CH₂CH₃), 60.12 (C-5), 67.98 (C-1), 75.28 (CH^{PiP}), 125.91, 128.48, 128.61, 142.49 ppm (C₆H₅).

Anal. calcd for dihydrogenoxolate (C₂₃H₄₀N₂O 2C₂H₂O₄ 0.5 H₂O): C 59.00, H 8.25, N 5.10; found: C 58.90, H 8.42, N 5.20; mp_{dihydrogenoxolate} = 134.5–137.8 °C.

N-Methyl-5-[3-[methyl(phenethyl)amino]propoxy]-*N*-propylpentan-1-amine (**2e**): (264 mg, 80.0%): R_f = 0.48 (CH₂Cl₂/MeOH/NH_{3(aq)} 8:1:1%); ¹H NMR (600 MHz, CDCl₃): δ = 0.88 (t, J = 7.4 Hz, 3H, NCH₂CH₂CH₃), 1.31–1.36 (m, 2H, H-6), 1.45–1.49 (m, 4H, H-7, NCH₂CH₂CH₃), 1.55–1.59 (m, 2H, H-5), 1.72–1.77 (m, 2H, H-2), 2.19 (s, 3H, CH₃), 2.58–2.32 (m, 7H, H-8, NCH₂CH₂CH₃, CH₃), 2.48 (t, J = 7.3 Hz, 2H, H-1), 2.59–2.62 (m, 2H, C₆H₅CH₂CH₂), 2.75–2.78 (m, 2H, C₆H₅CH₂CH₂), 3.39 (t, J = 6.7 Hz, 2H, H-4), 3.42 (t, J = 6.5 Hz, 2H, H-3), 7.18–7.28 ppm (m, 5H, C₆H₅); ¹³C NMR (150 MHz, CDCl₃): δ = 12.18 (NCH₂CH₂CH₃), 20.67 (NCH₂CH₂CH₃), 24.46 (C-6), 27.40 (C-2), 2.89 (C-7), 29.96 (C-5), 34.08 (C₆H₅CH₂CH₂), 42.47 and 42.53 (2 × CH₃), 54.66 (C-1), 58.03 (C₆H₅CH₂CH₂), 59.86 (C-8), 60.13 (NCH₂CH₂CH₃), 69.36 (C-3), 71.14 (C-4), 126.14, 128.55, 128.92, 140.88 ppm (C₆H₅).

Anal. calcd for dihydrogenoxolate (C₂₁H₃₈N₂O 2C₂H₂O₄ 0.5 H₂O): C 57.34, H 8.28, N 5.35; found: C 57.32, H 8.53, N 5.40; mp_{dihydrogenoxolate} = 110.0–112.0 °C.

N-Methyl-5-(3-(methyl(3-phenylpropyl)amino)propoxy)-*N*-propylpentan-1-amine (**2f**): (175 mg, 50.0%): $R_f = 0.63$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_3(\text{aq})$ 8:1:1%) = 0.63. ^1H NMR (600 MHz, CDCl_3): $\delta = 0.88$ (t, $J = 7.4$ Hz, 3H, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 1.30–1.36 (m, 2H, H-6), 1.43–1.49 (m, 4H, H-7, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 1.55–1.60 (m, 2H, H-5), 1.69–1.72 (m, 2H, H-2), 1.76–1.80 (m, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2$), 2.19 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.26 (t, $J = 7.6$ Hz, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_3$), 2.30 (t, $J = 7.5$ Hz, 2H, H-8), 2.36 (t, $J = 7.3$ Hz, 2H, H-1), 2.39 (t, $J = 7.1$ Hz, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2$), 2.62 (t, $J = 7.7$ Hz, 2H, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2$), 3.39 (t, $J = 6.7$ Hz, 2H, H-3), 3.43 (t, $J = 6.5$ Hz, 2H, H-4), 7.15–7.18 (m, 2H, C_6H_5), 7.25–7.27 ppm (m, 3H, C_6H_5); ^{13}C NMR (150 MHz, CDCl_3): $\delta = 12.17$ ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 20.71 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 24.44 (C-6), 27.44 (C-7), 27.88 (C-2), 29.31 ($\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2$), 29.96 (C-5), 33.94 ($\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2$), 42.44 and 42.55 ($2 \times \text{CH}_3$), 54.80 ($\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2$), 57.53 (C-1), 58.05 (C-8), 60.16 ($\text{NCH}_2\text{CH}_2\text{CH}_3$), 69.44 (C-4), 71.14 (C-3), 125.89, 128.49, 128.61, 142.64 ppm (C_6H_5).

Anal. calcd for dihydrogenoxolate ($\text{C}_{22}\text{H}_{40}\text{N}_2\text{O} \cdot 2\text{C}_2\text{H}_2\text{O}_4$): C 59.07, H 8.39, N 5.30; found: C 58.84, H 8.80, N 5.39; $\text{mp}_{\text{dihydrogenoxolate}} = 104.5\text{--}107.0$ °C.

^1H and ^{13}C NMR spectral data of final compounds **1b,c,e,f** and **2b,c,e,f** can be found in Supplementary Material A (Section 3).

3.2. In Vitro Pharmacology

3.2.1. H_3 Antagonistic Activity for Compounds **1a–f** and **2a–f**

In the first step, all the obtained compounds were tested for their H_3 antagonistic effects in vitro, following standard methods, using the electrically contracting guinea pig jejunum [32].

Male guinea pigs weighing 300–400 g were sacrificed, and a portion of the small intestine, 20–50 cm proximal to the ileocaecal valve (jejunum), was removed and placed in Krebs buffer (composition (mM) NaCl 118; KCl 5.6; MgSO_4 1.18; CaCl_2 2.5; NaH_2PO_4 1.28; NaHCO_3 25; glucose 5.5; indomethacin (1×10^{-6} mol/L)). Whole jejunum segments (2 cm) were prepared and mounted between two platinum electrodes (4 mm apart) in 20 mL Krebs buffer, continuously gassed with 95% O_2 :5% CO_2 , and maintained at 37 °C. The contractions were recorded isotonicly under 1.0 g tension with a Hugo Sachs Hebel–Messvorsatz (TI-2)/HF-modem (Hugo Sachs Elektronik, Hugstetten, Germany) connected to a pen recorder. The equilibration lasted for one hour with washings every 10 min. The muscle segments were then stimulated at a maximum between 15 and 20 Volts, continuously at a frequency of 0.1 Hz for a duration of 0.5 msec, with rectangular wave electrical pulses, delivered by a Grass Stimulator S-88 (Grass Instruments Co., Quincy, MA, USA). After 30 min of stimulation and 5 minutes before adding (*R*)- α -methylhistamine, pyrilamine (1×10^{-5} mol/L concentration in organ bath) was added, and then cumulative concentration–response curves (half-log increments) of (*R*)- α -methylhistamine, an H_3 -agonist, were recorded until no further change in the responses was found. Five minutes before adding the tested compounds, the pyrilamine (1×10^{-5} mol/L concentration in an organ bath) was added. The antagonists were preincubated for 20 min during the stimulation, before the preparation were challenged with (*R*)- α -methylhistamine. Antagonist potency was determined by the construction of a Schild plot [34], using three different concentrations of the antagonist. The potency of an antagonist is expressed by its pA_2 value. The pA_2 values were compared with those indicating the potency of thioperamide.

3.2.2. H_1 Antagonistic Activity for Compounds **1b** and **1f**

In addition, the compounds **1b** and **1f** with the highest potency at the H_3 receptors were also tested for H_1 antagonistic effects in vitro, using standard methods [34]. The potencies of the aforementioned ligands were determined for the guinea pig ileum histamine H_3 receptors as described previously [36], using histamine as a competing agonist.

The pA_2 values were calculated according to Arunlakshana and Schild [34]. The pA_2 values were compared with those of pyrilamine.

Chemicals

Thioperamide maleate, (*R*)- α -methylhistamine dihydrochloride, indomethacin, pyrilamine maleate, and histamine dihydrochloride were purchased from Sigma Aldrich (Saint Louis, MO, USA).

3.2.3. Antagonist Binding to Rat rH₃R and Human hH₃R

Cell Culture and Transfection

Human Embryonic Kidney cells (HEK293T) were cultured in DMEM supplemented with 10% Fetal Bovine Serum, 100 IU·mL⁻¹ penicillin, and 100 μ g·mL⁻¹ streptomycin at 37 °C and 5% CO₂. The day prior to transfection, 2 million cells were seeded in 10 cm dishes. Approximately 4 million cells were transfected by the polyethyleneimine (PEI) method with 5 μ g of cDNA in a ratio of 1:4 (DNA/PEI). Briefly, 0.5 μ g of pcDNA3-rH₃R or pcDNA3.1-hH₃R and 4.5 μ g of empty plasmid (pcDNA3.1) were mixed with 20 μ g of 25 kDa linear PEI in 500 μ L of 150 mM NaCl and incubated for 30 min at 22 °C. Meanwhile, the medium in the 10 cm dishes was replaced with fresh culture medium, and the transfection mix was subsequently added dropwise to the cells, which were incubated for 48 h at 37 °C and 5% CO₂.

Crude Membrane Extracts

Forty-eight hours after transfection, the cells were washed with ice-cold phosphate buffered saline (PBS) and scrapped, and the homogenate was centrifuged for 10 min at $\sim 2000 \times g$, 4 °C. The supernatant was aspirated, and the cell pellets were resuspended in 1 ml ice-cold PBS and centrifuged again under the same conditions, aspirating the supernatant. The membranes were stored at -20 °C until further use.

[³H]-N α -Methylhistamine Binding

The affinities of the derivative **ADS-003** were determined for rat and human histamine H₃ receptors (445 isoforms) as described previously [36], using [³H]-N α MH as a competing radioligand.

Chemicals

Dubelcco's Modified Eagles Medium (DMEM), Phosphate Buffered Saline (PBS), Trizma Base, polyethyleneimine solution (50%, PEI) were purchased from Sigma Aldrich (Saint Louis, MO, USA). Fetal Bovine Serum (FBS, Bodinco BV, Alkmaar, The Netherlands), Penicillin/Streptomycin (streptomycin 10,000 IU·mL⁻¹; penicillin 10,000 μ g·mL⁻¹, Thermo Fischer Scientific, (p/a Perbio Science BVBA, Etten-Leur, The Netherlands) , linear 25 kDa polyethyleneimine (PEI, Polysciences, Warrington, PA, USA), [³H]-N- α -methylhistamine (specific activity 79.7 Ci/mmol, Perkin Elmer, (Waltham, MA, USA), thioperamide (Abcam, Cambridge, UK) histamine (TCI, Nihonbashi-honcho, Chuo-ku, Tokyo, Japan).

3.3. Verification of In Vivo Activity of Compound **ADS-003** (**1a**)

All animal experimental procedures were in accordance with EU directives and local ethical regulations. Male Wistar rats (260–300 g) were used. The animals were maintained under standard laboratory conditions (liquid and food available ad libitum, 12 h light–dark cycle). For feeding behavior examination, the rats were placed individually in metabolic cages (TecniplastGazzada, Buguggiate, Italy) and kept there throughout the entire test. Pharmacotherapy was preceded by a control period of 4 days aimed to determine the basal feed and water consumptions as well as urine excretion. The rats ($n = 8$ per group) were randomly given the H₃R antagonists **ADS-003** or Ciproxifan, the latter serving as a reference compound [40]. Control rats were treated with an equivalent volume of physiological saline. The compounds, dissolved in distilled water with DMSO (7%, *v/v*), were administered subcutaneously at a dose of 3 mg/kg of body mass for 5 consecutive days, always

during the morning hours, following the record of feeding parameters. The dose of **ADS-003** had been determined on the basis of a thorough characterization *in vitro*. The recorded volumes of consumed food are expressed in g or ml per 100 g of body weight or in ml per 24 h, respectively. The final results are given as means, with SEM calculated for each 24 h period, computed from a four-day (before treatment) or a five-day (treatment) monitoring

3.3.1. Post-Mortem Biochemical Analyses

Following the behavioral study, the rats were sacrificed, and their brains were collected. From each brain, the cerebral cortex and hypothalamus were quickly dissected according to the Glowinski and Iversen method [37]. The samples were immediately frozen in liquid nitrogen and kept at $-70\text{ }^{\circ}\text{C}$ until assayed.

The tissue concentration of histamine (HA) was measured by radioisotopic assay according to Taylor and Snyder [45], while catecholamine (dopamine, DA; noradrenaline, NA) and serotonin (5-HT) concentrations were measured by radioimmunoassays using RIA kits (DIAsourceImmunoAssays S.A., Nivelles, Belgium). The amine concentrations were expressed as ng/g wet weight (HA) or nmol/g wet weight (DA, NA, 5-HT).

Monoamine oxidase A and B (EC 1.4.3.4; MAO A and B) activities were estimated in cerebral homogenates with radioassays, using serotonin (fine conc. $200\text{ }\mu\text{M}$) and β -phenylethylamine (fine conc. $20\text{ }\mu\text{M}$), as well as specific inhibitors—clorgyline and deprenyl ($0.3\text{ }\mu\text{M}$ each), respectively [46]. Histamine *N*-methyltransferase (EC 2.1.1.8; HMNT) activity was determined radioenzymatically, as described by Taylor and Snyder [45], by measurements of radioactive N^T -methylhistamine formed in a transmethylation reaction catalyzed by the enzyme, as previously described; *S*-Adenosyl-L-(methyl- ^{14}C)-Methionine was used as a donor of the methyl group.

The enzyme activities are expressed as pmol/min/mg protein. Protein concentration was analyzed according to Lowry's method [47].

Chemicals

Adenosyl-L-methionine, S [methyl- ^{14}C] (specific activity $50\text{ }\mu\text{Ci}$) and β -phenylethylamine hydrochloride [ethyl- ^{14}C] (specific activity $250\text{ }\mu\text{Ci}$) were purchased from ARC.

4. Conclusions

The highest *in vitro* potency as an H_3 receptor antagonist for both series was observed for the (benzylfuran-2-yl)methyl derivative of 4-hydroxypiperidine **1a** (**ADS-003**) and the benzyl derivative of 3-(methylamino)propan-1-ol **2d**. Compared to **1a** and **2d**, the *in vitro* potency was found to decrease with increasing chain length (**1a–f**; **2d–f**), with the exception of the series **2a–c**, where only weak potency was observed, independent of the alkyl chain length.

In competition radioligand binding studies to the rat histamine H_3 receptor, compound **ADS-003** ($\text{pK}_i = 7.9 \pm 0.1$) showed nanomolar affinity at the same level as the reference compound thioperamide ($\text{pK}_i = 7.9 \pm 0.1$) and a slightly higher affinity than histamine ($\text{pK}_i = 7.3 \pm 0.1$). A significantly lower affinity was observed for **ADS-003** for the human H_3R , with $\text{pK}_i 6.6 \pm 0.1$, in comparison with the pK_i of thioperamide (7.2 ± 0.1) and the pK_{ii} of histamine (7.7 ± 0.1).

ADS-003, given parenterally for 5 days, reduced the food intake of rats as well as changed rats' brain amine concentrations in a manner and degree similar to those observed for the reference H_3 antagonist **Ciproxifan**. These results indicate that the compound crosses the blood–brain barrier and acts also as an H_3R antagonist *in vivo* in the rat brain.

Supplementary Materials: Supplementary materials can be found at <http://www.mdpi.com/1422-0067/19/4/1243/s1>.

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Author Contributions: Krzysztof Walczyński was responsible for the supervision and development of the whole project. Beata Olszewska performed the chemical syntheses of the newly synthesized compounds and performed preliminary pharmacological studies in vitro, both at H₃ and H₁ receptor. Anna Stasiak performed the extended pharmacological studies in vivo, elaborated and described the results. Daniel McNaught Flores performed the hH₃ and rH₃ binding affinity test, elaborated and described the results. Agnieszka Fogel coordinated the advanced pharmacological studies in vivo and interpreted the obtained results. Rob Leurs coordinated the hH₃ and rH₃ binding affinity test and interpreted the obtained results.

Conflicts of Interest: The authors have declared no conflict of interest.

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