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Original article

Diamine-based human histamine H_3 receptor antagonists: (4-Aminobutyn-1-yl)benzylamines

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ABSTRACT

A series of (4-aminobutyn-1-yl)benzylamines were prepared and the SAR around three key areas: (1) the amine attached to the butynyl linker (R^3R^4N–); (2) the benzylamine moiety (R^1R^2N–); and (3) the point of attachment of the benzylamine group (R^1R^2N– in the *ortho*, *meta*, or *para* positions) was examined. One compound, 4-[3-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-morpholine (**9s**) was chosen for further profiling and found to be a selective histamine H_3 antagonist with desirable drug-like properties. Ex vivo receptor occupancy studies established that **9s** does occupy H_3 binding sites in the brain of rats after oral administration. Subcutaneous doses of **9s** (10 mg/kg) given during the natural sleep phase demonstrated robust wake-promoting effects.

1. Introduction

Since its initial discovery, the histamine H_3 receptor [1] has attracted the attention of numerous research groups exploring the receptors' pharmacology thus beginning the search for potent and selective ligands. The successful cloning of the human H_3 receptor [2], and the design of selective ligands lacking the imidazole ring found in the endogenous ligand histamine itself were significant milestones in the H_3 field [3]. Increasingly, medicinal chemistry efforts are now focused on the refinement of these non-imidazole based ligands thereby improving the physical–chemical properties of the compounds [4]. This refinement is in part to address the next key hurdle for histamine H_3 ligands, human clinical trials, which will ultimately determine their potential therapeutic value for the treatment of human diseases.

The roles of histamine as a neurotransmitter and the H_3 receptor in the central nervous system (CNS) are now becoming quite clear. The synthesis and release of histamine are regulated in the CNS by H_3 pre-synaptic autoreceptors through a negative-feedback mechanism [5]. Thus, H_3 inverse agonists or antagonists would increase the amount of histamine available in the synapse. In its capacity as a heteroreceptor [6], the H_3 receptor also mediates the release of several other neurotransmitters [7] including dopamine, noradrenaline, GABA, serotonin, and acetylcholine [8]. These effects on neurotransmission have led to numerous suggestions for therapeutic applications for H_3 ligands including Parkinson's and Alzheimer's diseases, schizophrenia, obesity, cognition, learning and sleep disorders [9]. Other clinical uses have been suggested due to the presence of H_3 receptors outside the CNS, for instance in the heart, where they are involved in the regulation of noradrenaline release. Thus, H_3 agonists might be potentially useful in the treatment of ischemic heart conditions [10]. Indeed, compared with the marketed successes of compounds targeting histamine H_1 and H_2 receptors, the potential of histamine H_3 receptor inverse agonists, antagonists or agonists still remains to be determined [11].

We previously reported that several members of a series of simple diamines **1** possessed high potency at the human histamine H_3 receptor (Chart 1) [12]. Structure–activity trends of these relatively simple diamines **1** suggested a minimal H_3 receptor pharmacophore could be deduced consisting of a lipophilic core flanked by two alkylamine groups. This conclusion is further reinforced by the high potency of the marine natural product Aplysamine **4** [13] and other more rigid diamines recently appearing in the literature (e.g. **2** [14] and **3** [15]). In an effort to further improve our diamine H_3 pharmacophore model, and potentially enhancing the drug-like properties of the molecules, we considered adding a conformationally restricted side-chain to replace the propyloxy group linking

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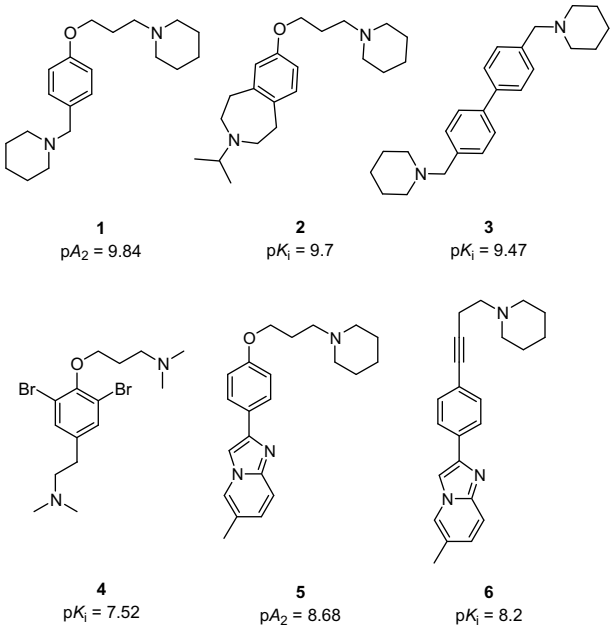


Chart 1. Representative non-imidazole antagonists with activities at the cloned recombinant human histamine H_3 receptor.

the NR^3R^4 amine and the aryl ring found in **8**. This resulted in a series of aryloxypiperidines [16] (**7**) that utilized a hydroxy piperidine attached to the core aryl ring thereby reducing the number of rotatable bonds found in the structures [17]. Continued refinement to our ligand design has led us to consider the use of a butyne element as another conformationally restricted side-chain replacement for the propyloxy moiety to give structures of type **9** providing a more rigid core (Fig. 1).

The use of alkynes as structural elements within non-imidazole H_3 receptor ligands has received relatively little attention. We previously reported nearly identical human H_3 receptor affinities of imidazopyridines **5** and **6** [18], suggesting that the structural changes in the manner we have envisioned may not adversely affect the binding affinity. Subsequently, Stark et al. reported that phenylalkyne **16** (Table 1) was a weak H_3 receptor antagonist, although some analogues showed improved potency [19]. Thus, we set out to examine the structure–activity relationships (SAR) of a series of diamines that utilize a 4-amino butynyl element as a potential 3-amino propyloxy replacement.

2. Chemistry

The assembly of the compounds was designed to be modular, flexible and adaptable for a parallel format whenever possible in order to quickly elucidate the SAR. Two major disconnections were

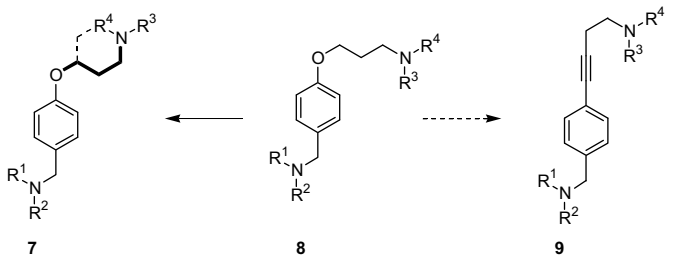


Fig. 1. Design of (4-aminobutyn-1-yl)benzylamines.

utilized in the preparation of (4-aminobutyn-1-yl)benzylamines **9** and are shown in Fig. 2. Therefore, in order to explore the influence of the amine attached to the butyne (R^3R^4N–), commercially available 3-butynyl *p*-toluenesulfonate was treated with various amines giving the corresponding 4-aminobutynes **10**. The 3-butynyl amines were then coupled to the appropriate appended bromobenzyl amine fragments under Sonogashira conditions with $PdCl_2(PPh_3)_2$ and CuI to provide compounds **9a**–**g** (Scheme 1).

The remaining compounds were synthesized by coupling 3-butynyl *p*-toluenesulfonate to either *o*-, *m*- or *p*-bromobenzaldehyde with modified Sonogashira conditions developed by Fu et al. [20] utilizing $PdCl_2(PhCN)_2$, CuI, and *t*-Bu_3P at ambient temperature. The corresponding tosylates were then treated with piperidine in DMF in the presence of Na_2CO_3 thus providing benzaldehydes **15**. The benzaldehydes **15** were then treated with a diverse set of amines and sodium triacetoxyborohydride [21] to install the benzylamine moieties to provide additional target compounds **9h**–**w** (Scheme 2).

3. Pharmacological results and discussion

The human histamine H_3 receptor binding affinities for a series of (4-aminobutyn-1-yl)benzylamines and related compounds were determined. The functional activities were also obtained for selected analogues. Compounds were selected for their ability to elucidate structure–activity relationships around three key structural areas of the molecules as mentioned above: (1) the butynylamine group (R^3R^4N–); (2) the benzylamine group (R^1R^2N–); and (3) the point of attachment of the benzylamine group (R^1R^2N– in the *ortho*, *meta*, or *para* positions). In addition to determining these structural modifications on potency and SAR trends, ultimately the physical–chemical properties and any positive effects on the pharmacokinetic profile were examined.

Initially holding the benzylamine group (R^1R^2N–) constant as piperidinyl, compounds bearing cyclic and lipophilic –NR^3R^4 groups showed the highest binding affinity. Thus, binding affinity increased slightly over the series of diethylamino, pyrrolidinyl and piperidinyl (**9a**–**c**). Similarly, replacing a carbon atom in the piperidine ring of the –NR^3R^4 group with a heteroatom led to a

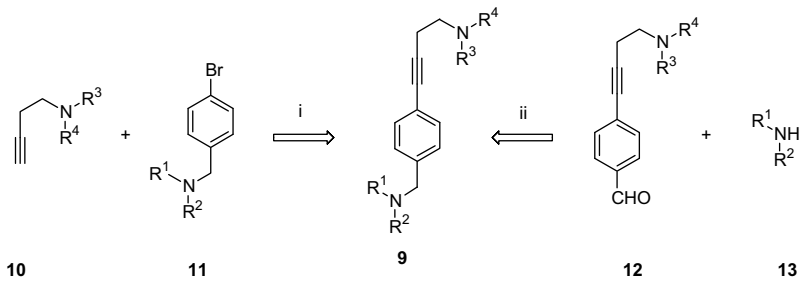
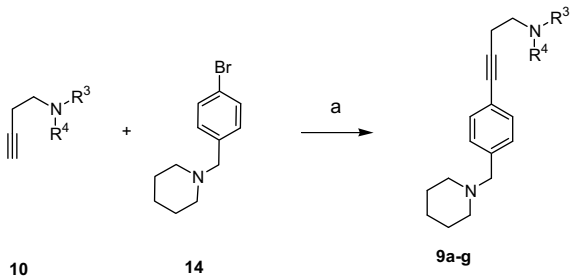


Fig. 2. Retrosynthesis of (4-aminobutyn-1-yl)benzylamines **9**.

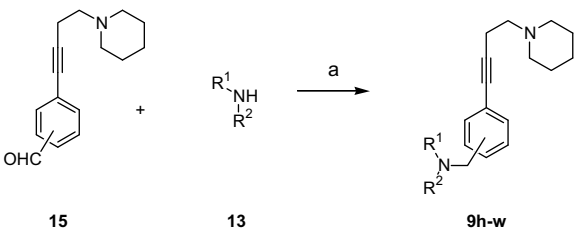


Scheme 1. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, CuI, PPh_3, Et_2NH, DMF, 125 °C.

10-fold decrease in binding affinity across the series piperidino- (**9c**), thiomorpholino- (**9d**), morpholino- (**9e**) and methylpiperazinyl (**9f**). This drop in binding affinity correlates well with the lipophilic nature (*c*log *P*) of the $-NR^3R^4$ fragments as well as the corresponding pK_a's of the amines.

To estimate the ligand binding contribution of the benzylamine group ($-CH_2NR^1R^2$) itself, the binding affinities of bispiperidine **9c** and monopiperidine **16** were compared. This analysis revealed diamine **9c** bound with higher affinity to the human histamine H_3 receptor by over two orders of magnitude. Additional modifications of the benzylamine group ($-NR^1R^2$) in the series of 4-(1-piperidinly)-butynes revealed a high degree of functional group tolerance. Most compounds displayed high H_3 receptor affinity, particularly piperidine and pyrrolidine **9c** and **9j**, *N*-methylpiperazine **9l**, and carboxamide **9p**. Functional groups tolerated include secondary amines (**9l** and **9m**), hydroxyl groups (**9q**) and primary amides (**9p**). The marked increase in potency combined with tolerance for a wide variety of functionality in this region ($-NR^1R^2$) represents a key area allowing for the selection of optimized physical properties (log *P*, amine pK_a, TPSA, etc.)

A series of compounds differing by virtue of the position of the benzylamine substituent ($-CH_2NR^1R^2$) on the aromatic ring were also examined. Positional isomers of bispiperidine **9c** exhibited decreasing binding affinity in the order *para* (**9c**), *meta* (**9r**), then *ortho* (**9w**), although the *meta* and *para* isomers were nearly equipotent. Additional *meta* substituted phenylalkynes were prepared to further explore the binding affinities of *meta* substituted phenylalkynes. Thus, both the *meta* and *para* carboxamides (**9p** and **9u**) bound with nearly equal affinity and the methylpiperazinyl analogues (**9t** vs **9l**) slightly favored the *para* orientation. The pyrrolidine **9v**, piperidine **9r** and morpholine **9s** *meta* analogues all had equivalent binding affinity with a preference over the *para* isomer. In addition, a 10-fold loss in activity was observed in both the *meta* and *para* analogues when the butynylamine group (R^3R^4N-) was changed from piperidine to morpholine (**9s** and **9e**). Overall, both the *para* and *meta* benzylamine isomers of the 4-(1-piperidinly)-butynes series were determined to be highly potent ligands at the human H_3 receptor. The SAR of the butyne linker on potency at the human H_3 receptor was comparable to both the propyloxy chain linking the NR^3R^4 group and the central aryl ring as well as the 4-aryloxypiperidines suggesting that the different scaffolds may allow the two basic amine moieties to obtain similar orientations. Structure–activity trends of diamines **7**, **8** and **9** further reinforce the proposed minimal H_3 receptor pharmacophore model consisting of a lipophilic core flanked by two alkylamine groups.



Scheme 2. Reagents and conditions: (a) $NaBH(OAc)_3$, $(R_1)R_2NH$, CH_2Cl_2.

Selected compounds were evaluated in a whole cell-based model of receptor activation. All were found to be neutral antagonists with good correlation between the observed binding affinities and functional activities. The compounds were triaged for possible further profiling by receptor occupancy experiments in rodents (sc or po). This useful and powerful tool provides plasma and brain exposures and receptor occupancy over time as related to dose. Given our experience with related structures, the importance in shorter acting compounds to compliment **1** was desired for additional first-in-class CNS clinical candidates. As reported earlier in a review of our H_3 program, **9s** (JNJ 10181457) is cleared from the brain in rats within 24 h following a 10 mg/kg dose [22]. Full dose–response ex vivo autoradiography of **9s** was performed using [^{3}H]-R-α-methylhistamine [23] as the radioligand. Dose-dependency of the H_3 receptor occupancy 1 h after oral administration of **9s** (0.1, 0.3, 1, 3, 10, 30 mg/kg) is presented in Fig. 3. Maximal ex vivo occupancy of the H_3 receptor was observed at 30 mg/kg at 1 h post-dose with an ED_{50} for occupancy being ~6.5 mg/kg.

Compound **9s** was selected for more detailed evaluation. The potency of **9s** was found to be slightly less at the rat H_3 receptor vs the human (pK_i 8.15 rH_3 vs 8.93 hH_3). The selectivity of **9s** over the H_1 and H_4 receptors is very high with pK_i's of <5. Overall selectivity against a panel of 50 neurotransmitter receptors, ion channels and other drug targets indicated that **9s** binds specifically to the H_3 receptor (IC_{50}'s of >1 μM at all targets). In addition, **9s** had minimal effects on human hERG channel with an activity of 13.7% and 8.3% at 10 and 3 μM respectively and displayed high non-efflux limited absorption potential (Caco-2). Moreover, **9s** has minimal drug–drug interaction (DDI) potential having no effect on Cpy450's 1A2, 2C9, 2C19, 2D6 and 3A4 at levels >40 μM. Plasma protein binding was also determined and **9s** displayed 45% binding to human plasma proteins. The oral bioavailability (%F) of **9s** was determined to be 11% in the rat. The potency of **9s** at the rat receptor was considered to be sufficient to explore animal models of efficacy (pA_2 $rH_3 = 8.33 \pm 0.08$) (Table 2).

Histaminergic neurons, which originate in the tuberomammillary nuclei and project throughout the brain, play an important role in arousal mechanisms, and histamine H_3 receptors

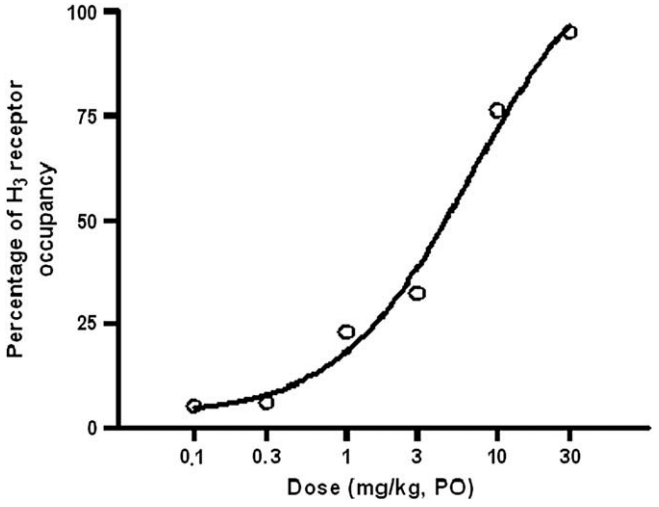
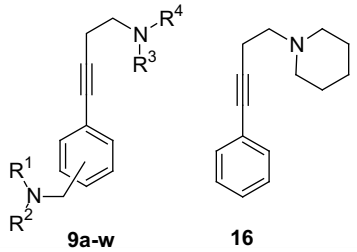


Fig. 3. Ex vivo occupancy of H_3 receptor by **9s** in rat striatum (coronal section): dose-dependency after oral administration (1 h post-dose). Results are represented as mean value of $n = 2$.

Table 1
In vitro human histamine H_3 receptor binding affinities.



9a-w **16**

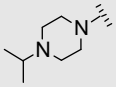
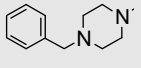
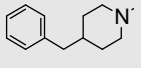
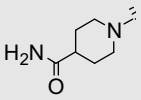
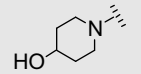
Compds (**9**)	NR^1R^2	NR^3R^4	Isomer (*o*, *m*, *p*)	$pK_i \pm SEM$[a, b]
a			*p*	8.87 ± 0.18
b			*p*	9.17 ± 0.02
c			*p*	9.29 ± 0.19
d			*p*	8.42 ± 0.23
e			*p*	8.30 ± 0.05
f			*p*	8.28 ± 0.05
g			*m*	7.84 ± 0.23
h			*p*	9.04 ± 0.01
i			*p*	8.96 ± 0.01
j			*p*	9.21 ± 0.06
k			*p*	8.76 ± 0.03
l			*p*	9.01 ± 0.10
m			*p*	9.06 ± 0.03

Table 1 (*continued*)

Compds (**9**)	NR^1R^2	NR^3R^4	Isomer (*o*, *m*, *p*)	$pK_i \pm SEM$[a, b]
n			*p*	8.72 ± 0.04
o			*p*	8.46 ± 0.22
p			*p*	9.12 ± 0.02
q			*p*	9.05 ± 0.03
r			*m*	9.11 ± 0.06
s			*m*	8.93 ± 0.16
t			*m*	9.14 ± 0.09
u			*m*	9.26 ± 0.09
v			*m*	7.09 ± 0.09
w			*o*	7.08 ± 0.09
16	– Thioperamide	–	–	7.25 ± 0.06 7.50 ± 0.10

[a] Displacement of *N*-[^{3}H]-methylhistamine from human H_3 receptors expressed in SK-N-MC cells.
[b] Values reported as the mean of at least three determinations.

Table 2
In vitro human histamine H_3 receptor functional activities of selected compounds.

Compds	$pA_2 \pm SEM$[a, b]
9c	9.83 ± 0.06
9k	8.49 ± 0.05
9r	10.09 ± 0.07
9s	9.31 ± 0.08
9s	8.33 ± 0.08[c]

[a] pA_2 values are derived from Schild regression analysis of the compound-induced rightward shifts in dose–response curves of histamine-induced inhibition of forskolin-stimulated cAMP accumulation in SK-N-MC cells overexpressing the human histamine H_3 receptor.
[b] Values reported as the mean of at least three determinations.
[c] Functional activity at the rat H_3 receptor.

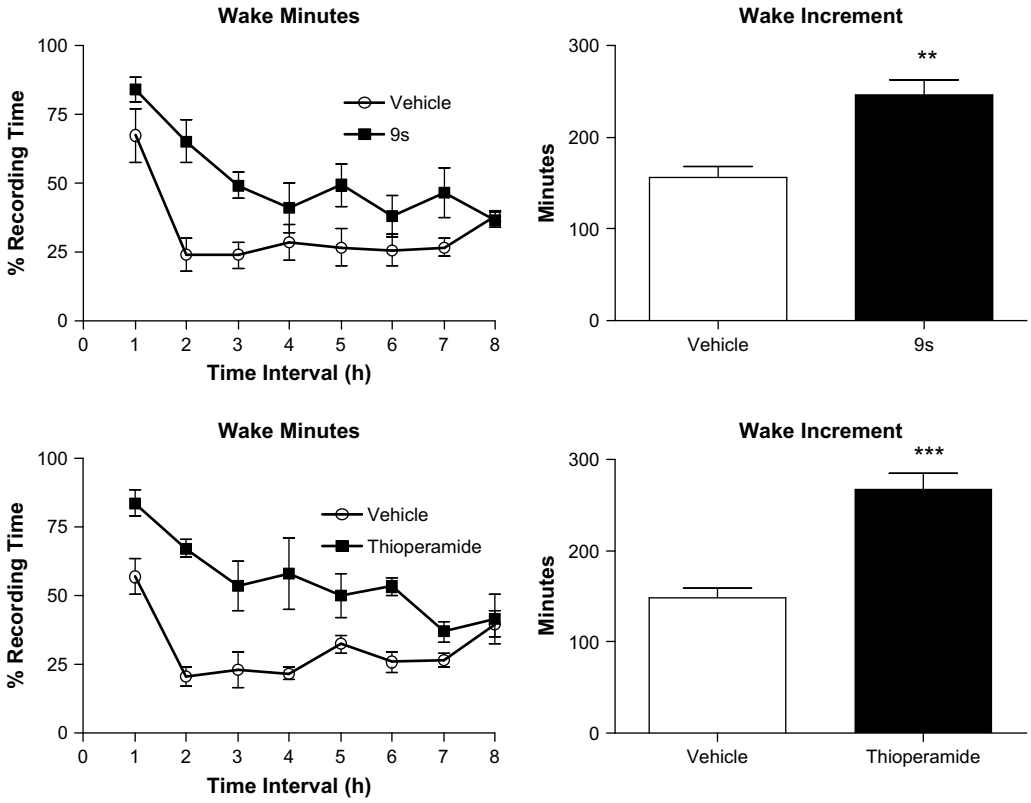


Fig. 4. Wake-promoting effects of **9s** and thioperamide in rats. Male adult Sprague-Dawley rats chronically implanted with EEG/EMG electrodes received the compound (10 mg/kg s.c.) or vehicle (saline) 2 h after the onset of the light phase. Polysomnographic traces were recorded for 8 h following the treatment and were visually scored by 10 s epoch as wake, non-rapid eye movement (NREM) or rapid eye movement (REM) sleep. The increased time spent awake was analyzed for each rat per 1 h intervals and expressed as percentage of recording time. In addition, the increment in wake was averaged over the 8-h recording period and expressed in minutes. Data are reported as mean ± SEM ($n = 5$ animals per compound), and statistical significance was determined through comparison of vehicle vs compound treatment by paired t-test ($^{**}p < 0.01$, $^{***}p < 0.001$).

are involved in this process by an inhibitory control of histamine release and synthesis. Indeed, the pharmacological blockade of presynaptic H_3 receptors promotes wakefulness in different animal models [22]. The development of specific H_3 receptor antagonists has therefore become a very attractive strategy for the treatment of daytime sleepiness associated with various conditions (narcolepsy, obstructive sleep apnea and shift work sleep disorders). Fig. 4 illustrates the wake-promoting effects of **9s** in comparison with thioperamide in rats after subcutaneous administration. Both compounds significantly increased the time spent awake with a similar duration of 7 h after injection. Concomitantly, the duration of non-rapid eye movement (NREM) sleep was decreased whereas REM sleep was not altered (data not shown).

4. Conclusion

A series of (4-aminobutyn-1-yl)benzylamines were prepared and the SAR around three key areas: (1) the amine attached to the butynyl linker (R^3R^4N–); (2) the benzylamine moiety (R^1R^2N–); and (3) the point of attachment of the benzylamine group (R^1R^2N– in the *ortho*, *meta*, or *para* positions) was examined. One compound, 4-[3-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-morpholine (**9s**) was selected for further profiling and found to be a selective histamine H_3 antagonist that promotes wakefulness in rats as compared to thioperamide. The compound distinguishes itself from previous compounds mainly by its pharmacokinetic profile such that it has a shorter plasma half-life (1.5 h) after single oral dose administration in rats. In addition, although **9s** readily penetrates the blood–brain barrier, it does not persist in the brain to an appreciable extent beyond 24 h, with brain concentrations that roughly parallel plasma concentrations.

5. Experimental protocols

5.1. Chemistry

Reagents were purchased from commercial suppliers and were used without purification. Anhydrous solvents were obtained from a Glass Contour Solvent Dispensing System. Reactions were performed at room temperature (20–23 °C) under an atmosphere of N_2 unless otherwise noted. Chromatography was performed using prepacked ISCO RediSep™ silica cartridges utilizing gradient elution. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 or 500 MHz spectrometer. Chemical shifts are reported in parts per million downfield from an internal standard (Me_4Si). All spectra were taken in $CDCl_3$ unless otherwise noted. Mass spectra were obtained on an Agilent series 1100 MSD using electrospray ionization (ESI) in either positive or negative modes as indicated. Thin-layer chromatography was performed using Merck silica gel 60 F_{254} 2.5 cm × 7.5 cm, 250 μm or 5.0 cm × 10.0 cm, 250 μm pre-coated silica gel plates. Preparative thin-layer chromatography was performed using EM Science silica gel 60 F_{254} 20 cm × 20 cm 0.5 mm pre-coated plates with a 20 cm × 4 cm concentrating zone. Melting points are uncorrected and were obtained on a MelTemp apparatus. Analytical reverse phase HPLC was performed on a Hewlett Packard Series 1100 instrument with an Agilent ZORBAX® Bonus RP, column utilizing an acetonitrile/water (0.05%TFA) gradient. Combustion analyses were performed by Desert Analytics or NuMega Resonance Labs.

5.1.1. Method A. General procedure for the preparation of 3-butynyl amines

*5.1.1.1. 4-But-3-ynyl-morpholine (**10a**).* To a solution of 3-butynyl *p*-toluenesulfonate (4.8 g, 22 mmol) in DMF (25 mL) were added sodium carbonate (2.6 g, 24 mmol) and morpholine (2.1 mL, 24 mmol) and the reaction mixture was heated to 80 °C. After 16 h, the reaction was quenched with water and ether was added. The organic portion was washed with brine, dried over Na_2SO_4 and concentrated to provide 1.6 g (51%) of 4-but-3-ynyl-morpholine. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.69 (t, $J = 4.7$ Hz, 4H), 2.56 (dd, $J = 7.8$, 7.1 Hz, 2H), 2.46 (br t, $J = 4.4$ Hz, 4H), 2.36 (dt, $J = 2.6$, 7.5 Hz, 2H), 1.97 (t, $J = 2.6$ Hz, 1H); MS *m*/*z* 140.2 (M + H).

*5.1.1.2. 1-But-3-ynyl-piperidine (**10b**).* Prepared according to method A (48%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.56 (dd, $J = 8.1$, 7.4 Hz, 2H), 2.44–2.34 (m, 6H), 1.96 (t, $J = 2.6$ Hz, 1H), 1.61–1.54 (m, 4H), 1.46–1.38 (m, 2H); MS *m*/*z* 138.2 (M + H).

*5.1.1.3. 4-But-3-ynyl-thiomorpholine (**10c**).* Prepared according to method A (74%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.79–2.73 (m, 4H), 2.68–2.65 (m, 4H), 2.63 (t, $J = 7.7$ Hz, 2H), 2.35 (dt, $J = 2.2$, 7.7 Hz, 2H), 2.00 (t, $J = 2.6$ Hz, 1H); MS *m*/*z* 156.2 (M + H).

*5.1.1.4. 1-But-3-ynyl-4-methyl-piperazine (**10d**).* Prepared according to method A (12%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.62 (t, $J = 7.4$ Hz, 3H), 2.59–2.43 (m, 6H), 2.39 (dt, $J = 2.6$, 8.1 Hz, 3H), 2.30 (s, 3H), 1.99 (t, $J = 2.7$ Hz, 1H); MS *m*/*z* 153.2 (M + H).

*5.1.1.5. But-3-ynyl-diethyl-amine (**10e**).* Prepared according to method A (14%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.70 (t, $J = 7.4$ Hz, 2H), 2.54 (q, $J = 7.2$ Hz, 4H), 2.31 (dt, $J = 2.7$, 8.0 Hz, 2H), 1.96 (t, $J = 2.6$ Hz, 1H), 1.04 (t, $J = 7.1$ Hz, 6H); MS *m*/*z* 126.2 (M + H).

*5.1.1.6. 1-But-3-ynyl-pyrrolidine (**10f**).* Prepared according to method A (28%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.65 (t, $J = 7.6$ Hz, 2H), 2.55–2.48 (m, 4H), 2.39 (dt, $J = 2.7$, 7.4 Hz, 2H), 1.97 (t, $J = 2.6$ Hz, 1H), 1.81–1.74 (m, 4H); MS *m*/*z* 124.2 (M + H).

5.1.1.7. Toluene-4-sulfonic acid 4-(4-formyl-phenyl)-but-3-ynyl ester. To a solution of 4-bromobenzaldehyde (2.80 g, 15.1 mmol) in dioxane (15 mL) were added 3-butynyl *p*-toluenesulfonate (3.1 mL, 17.1 mmol), iPr_2NH (3.0 mL) and the flask was flushed with nitrogen for ca. 10 min. The solid reagents, $PdCl_2(PhCN)_2$ (141.5 mg, 0.37 mmol), CuI (45.6 mg, 0.24 mmol), and P(*t*-Bu)$_3\cdot HBF_4$ (211.1 mg, 0.73 mmol) were then added together in one portion. The mixture was stirred at rt for 16 h. A solid ppt. was observed after initial mild exotherm at ca. 5 min. The dark brown mixture was then diluted with CH_2Cl_2 (400 mL) and washed with water. The organic phase was dried over sodium sulfate and evaporated. Chromatography of the residue (0–15% EtOAc/Hexanes) provided 3.65 g (73%) of the desired tosylate. ^{1}H NMR (500 MHz) δ 10.0 (s, 1H), 7.84–7.79 (m, 4H), 7.48 (d, $J = 8.2$ Hz, 2H), 7.32 (d, $J = 8.2$ Hz, 2H), 4.20 (t, $J = 7.1$ Hz, 2H), 2.82 (t, $J = 7.1$ Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (125 MHz) δ 191.3, 144.9, 135.4, 132.9, 132.2, 129.8, 129.4, 129.2, 127.9, 88.3, 81.9, 67.3, 21.6, 20.5; MS *m*/*z* 329.2 (M + H).

*5.1.1.8. 4-(4-Piperidin-1-yl-but-1-ynyl)-benzaldehyde (**14**).* To a solution of toluene-4-sulfonic acid 4-(4-formyl-phenyl)-but-3-ynyl ester (3.62 g, 11.0 mmol) in DMF (5 mL) were added piperidine (2.2 mL, 22 mmol) and Na_2CO_3 (2.41 g, 23 mmol). The mixture was placed into a preheated oil bath (80 °C) and heated for ca. 2 h. The mixture was then diluted with water (400 mL) and extracted with Et_2O (3 × 100 mL). The combined organic phases were dried over sodium sulfate and evaporated. Chromatography of the residue (0–5% 2 M methanolic ammonia/CH_2Cl_2) provided 1.35 g (51%) of the desired aldehyde. ^{1}H NMR (500 MHz) δ 9.98 (s, 1H), 7.81–7.78 (m, 2H), 7.54–7.51 (m, 2H), 2.69–2.61 (m, 4H), 2.51–2.43 (m, 4H), 1.63–1.57 (m, 4H), 1.48–1.42 (m, 2H); ^{13}C NMR (125 MHz) δ 191.4, 135.0, 132.0, 130.3, 129.4, 93.4, 80.7, 57.7, 54.2, 25.9, 24.2, 17.8; MS *m*/*z* 242.2 (M + H).

5.1.1.9. Toluene-4-sulfonic acid 4-(3-formyl-phenyl)-but-3-ynyl ester. To a solution of 3-bromobenzaldehyde (2.80 g, 15.1 mmol) in dioxane (15 mL) were added 3-butynyl *p*-toluenesulfonate (3.1 mL, 17.1 mmol), iPr_2NH (3.0 mL) and the flask was flushed with nitrogen for ca. 10 min. The solid reagents, $PdCl_2(PhCN)_2$ (140.3 mg, 0.37 mmol), CuI (43.8 mg, 0.23 mmol), and P(*t*-Bu)$_3\cdot HBF_4$ (210.0 mg, 0.72 mmol) were then added together in one portion. The mixture was stirred at rt for 16 h. A solid ppt. was observed after initial mild exotherm at ca. 5 min. The dark brown mixture was then diluted with CH_2Cl_2 (400 mL) and washed with water. The organic phase was dried over sodium sulfate and evaporated. Chromatography of the residue (0–15% EtOAc/Hexanes) provided 3.37 g (68%) of the desired tosylate. ^{1}H NMR (500 MHz) δ 9.98 (s, 1H), 7.85–7.79 (m, 4H), 4.60–7.57 (m, 1H), 7.46 (t, $J = 7.6$ Hz, 1H), 7.33 (d, $J = 7.6$ Hz, 2H), 4.20 (t, $J = 7.1$ Hz, 2H), 2.81 (t, $J = 7.1$ Hz, 2H), 2.42 (s, 3H); ^{13}C NMR (125 MHz) δ 191.4, 144.9, 137.1, 136.3, 132.8, 129.8, 128.9, 127.9, 124.1, 85.7, 81.3, 67.4, 21.6, 20.3; MS *m*/*z* 329.2 (M + H).

*5.1.1.10. 3-(4-Piperidin-1-yl-but-1-ynyl)-benzaldehyde (**15a**).* To a solution of toluene-4-sulfonic acid 4-(3-formyl-phenyl)-but-3-ynyl ester (3.35 g, 10.2 mmol) in DMF (5 mL) were added piperidine (2.2 mL, 22 mmol) and Na_2CO_3 (2.36 g, 22 mmol). The mixture then placed into a preheated oil bath (80 °C) and heated for ca. 2 h. The mixture was then diluted with water (400 mL) and extracted with Et_2O (3 × 100 mL). The combined organic phases were dried over magnesium sulfate and evaporated. Chromatography of the residue (0–5% 2 M methanolic ammonia/CH_2Cl_2) provided 1.64 g (67%) of the desired aldehyde. ^{1}H NMR (500 MHz) δ 9.98 (s, 1H), 7.88 (s, 1H), 7.78 (d, $J = 7.7$ Hz, 1H), 7.63 (d, $J = 7.7$ Hz, 1H), 7.45 (t, $J = 7.7$ Hz, 1H), 2.69–2.59 (m, 4H), 2.51–2.43 (m, 4H), 1.63–1.57 (m, 4H), 1.48–1.42 (m, 2H); ^{13}C NMR (125 MHz) δ 191.6, 137.1, 136.3, 132.9, 128.9, 128.3, 125.0, 90.5, 79.9, 57.8, 54.2, 25.9, 24.2, 17.6; MS *m*/*z* 242.2 (M + H).

5.1.1.11. Toluene-4-sulfonic acid 4-(2-formyl-phenyl)-but-3-ynyl ester. To a solution of 2-bromobenzaldehyde (0.55 g, 2.9 mmol) in dioxane (3 mL) were added 3-butynyl *p*-toluenesulfonate (0.6 mL, 3.4 mmol), iPr_2NH (0.6 mL) and the flask was flushed with nitrogen for ca. 10 min. The solid reagents, $PdCl_2(PhCN)_2$ (37.1 mg, 0.09 mmol), CuI (13.6 mg, 0.07 mmol), and P(*t*-Bu)$_3\cdot HBF_4$ (57.1 mg, 0.19 mmol) were then added together in one portion. The mixture was stirred at rt for 16 h. A solid ppt. was observed after initial mild exotherm at ca. 5 min. The dark brown mixture was then diluted with CH_2Cl_2 (400 mL) and washed with water. The organic phase was dried over sodium sulfate and evaporated. Chromatography of the residue (0–15% EtOAc/Hexanes) provided 0.52 g (54%) of the desired tosylate. ^{1}H NMR (500 MHz) δ 10.36 (s, 1H), 7.88 (d, $J = 6.6$ Hz, 1H), 7.82 (d, $J = 8.2$ Hz, 2H), 7.55–7.51 (m, 1H), 7.48–7.40 (m, 2H), 7.31 (d, $J = 7.7$ Hz, 2H), 4.23 (t, $J = 6.6$ Hz, 2H), 2.87 (t, $J = 6.6$ Hz, 2H), 2.40 (s, 3H); ^{13}C NMR (125 MHz) δ 191.4, 145.0, 136.0, 133.6, 133.4, 132.8, 129.9, 128.5, 127.9, 127.1, 126.5, 91.4, 78.4, 67.3, 21.6, 20.6; MS *m*/*z* 329.2 (M + H).

*5.1.1.12. 2-(4-Piperidin-1-yl-but-1-ynyl)-benzaldehyde (**15b**).* To a solution of toluene-4-sulfonic acid 4-(2-formyl-phenyl)-but-3-ynyl ester (0.39 g, 1.2 mmol) in DMF (2 mL) were added piperidine (0.5 mL, 5 mmol) and Na_2CO_3 (0.25 g, 2.4 mmol). The mixture then placed into a preheated oil bath (80 °C) and heated for ca. 12 h. The mixture was then diluted with water (50 mL) and extracted with

Et_2O (3 × 10 mL). The combined organic phases were dried over magnesium sulfate and evaporated. Chromatography of the residue (0–5% 2 M methanolic ammonia/CH_2Cl_2) provided 0.13 g (45%) of the desired aldehyde. ^1H NMR (500 MHz) δ 10.54 (s, 1H), 7.88 (d, $J = 7.7$ Hz, 1H), 7.53–7.47 (m, 2H), 7.40–7.36 (m, 1H), 2.67 (s, 4H), 2.51–2.42 (m, 4H), 1.64–1.57 (m, 4H), 1.47–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 192.2, 136.0, 133.6, 133.1, 127.9, 127.6, 126.9, 96.3, 57.6, 54.2, 25.9, 24.2, 17.9; MS m/z 242.2 (M + H).

5.1.2. Method B. General procedure for the Sonogashira coupling

*5.1.2.1. Diethyl-[4-(4-piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-amine (**9a**).* But-3-ynyl-diethyl-amine (300 mg, 2.4 mmol) was combined in a sealed tube with 1-(4-bromo-benzyl)-piperidine (300 mg, 1.2 mmol), triphenylphosphine (56 mg, 0.21 mmol), copper (I) iodide (11 mg, 0.06 mmol), dicholordiphenylphosphorylpalladium (41 mg, 0.06 mmol) in a mixture of DMF (0.5 mL) and diethylamine (2.5 mL). The reaction mixture was heated to 125 °C for 1 h. After cooling to room temperature, ether was added and all solids were filtered off. The filtrate was concentrated and purified by RP-HPLC to provide 221 mg (63%) of diethyl-[4-(4-piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-amine. The dihydrochloride salt was prepared by addition of 2 mL of 1 M HCl in ether to the above compound in methanol (7 mL). After 10 min a white precipitate was observed. The solid was filtered off and rinsed with ether to provide 179 mg (65%) of the dihydrochloride salt of diethyl-[4-(4-piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-amine ^1H NMR (CD_3OD, 500 MHz) δ 7.55 (d, $J = 8.2$ Hz, 2H), 7.45 (d, $J = 8.8$ Hz, 2H), 4.19 (s, 2H), 3.44–3.37 (m, 2H), 3.36–3.31 (m, 3H), 3.30–3.22 (m, 4H), 3.03 (t, $J = 7.2$ Hz, 2H), 2.87–2.73 (m, 2H), 2.07–1.93 (m, 2H), 1.92–1.77 (m, 3H), 1.41 (t, $J = 7.3$ Hz, 6H); ^{13}C NMR (CD_3OD, 125 MHz) δ 132.6, 131.8, 128.9, 124.9, 85.9, 82.7, 60.5, 53.1, 50.2, 47.7, 23.0, 22.0, 15.7, 8.8; MS m/z 299.3 (M + H); Anal. ($C_{20}H_{32}Cl_2N_2$) C, H, N.

*5.1.2.2. 1-[4-(4-Pyrrolidin-1-yl-but-1-ynyl)-benzyl]-piperidine (**9b**).* Prepared according to method B (24%). ^1H NMR (CD_3OD, 500 MHz) δ 7.52 (d, $J = 8.3$ Hz, 2H), 7.43 (d, $J = 8.3$ Hz, 2H), 4.18 (s, 2H), 3.96–3.73 (m, 1H), 3.49–3.32 (m, 5H), 3.18–2.95 (m, 4H), 2.79–2.63 (m, 2H), 2.27–1.99 (m, 6H), 1.93–1.78 (m, 3H), 1.51–1.31 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 132.6, 131.6, 128.5, 124.9, 86.0, 82.7, 60.4, 54.3, 53.6, 53.0, 23.4, 22.8, 22.0, 17.2; MS m/z 297.3 (M + H); Anal. ($C_{20}H_{30}Cl_2N_2$) C, H, N.

*5.1.2.3. 4-[4-(4-Piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-piperidine (**9c**).* Prepared according to method B (42%). ^1H NMR (CD_3OD, 500 MHz) δ 7.53 (d, $J = 8.2$ Hz, 2H), 7.41 (d, $J = 8.1$ Hz, 2H), 4.15 (s, 2H), 3.60 (d, $J = 11.7$ Hz, 2H), 3.43–3.35 (m, 2H), 3.25 (t, $J = 7.36$ Hz, 2H), 3.07 (t, $J = 7.1$ Hz, 2H), 2.88–2.79 (m, 2H), 2.73–2.64 (m, 2H), 2.18–1.98 (m, 4H), 1.93–1.77 (m, 6H), 1.53–1.28 (m, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 132.5, 131.6, 128.5, 124.8, 86.2, 82.6, 60.4, 55.5, 53.6, 53.0, 22.8, 22.7, 22.0, 21.9, 15.6; MS m/z 311.3 (M + H); Anal. ($C_{21}H_{32}Cl_2N_2$) C, H, N.

*5.1.2.4. 4-[4-(4-Piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-thiomorpholine (**9d**).* Prepared according to method B (36%). ^1H NMR (CD_3OD, 500 MHz) δ 7.54 (d, $J = 8.1$ Hz, 2H), 7.48 (d, $J = 8.1$ Hz, 2H), 4.24 (s, 2H), 3.88 (br d, $J = 11.9$ Hz, 2H), 3.44–3.33 (m, 6H), 3.30–3.24 (m, 2H), 3.09 (dd, $J = 7.3$, 7.2 Hz, 2H), 2.95–2.85 (m, 2H), 2.84–2.76 (m, 2H), 1.97–1.81 (m, 5H), 1.56–1.40 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 132.1, 131.3, 128.7, 124.6, 85.6, 82.4, 60.1, 55.8, 54.1, 52.7, 24.6, 22.7, 21.5, 14.9; MS m/z 329.3 (M + H); Anal. ($C_{20}H_{30}Cl_2N_2S$) C, H, N.

*5.1.2.5. 4-[4-(4-Piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-morpholine (**9e**).* Prepared according to method B (68%). ^1H NMR (CD_3OD, 500 MHz) δ 7.32 (d, $J = 8.1$ Hz, 2H), 7.23 (d, $J = 8.0$ Hz, 2H), 4.20–4.06 (m, 4H), 4.02–3.92 (m, 2H), 3.54 (d, $J = 12$ Hz, 2H), 3.37 (d, $J = 11.8$ Hz, 2H), 3.20–3.06 (m, 7H), 3.06–2.97 (m, 2H), 2.63 (t, $J = 12.6$ Hz, 2H), 2.17–2.01 (m, 2H), 1.88–1.75 (m, 3H), 1.40–1.25 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 132.4, 131.6, 128.5, 124.6, 85.9, 82.7, 63.7, 60.3, 55.8, 52.9, 22.7, 22.0, 15.3; MS m/z 313.3 (M + H); Anal. ($C_{20}H_{30}Cl_2N_2O$) C, H, N.

*5.1.2.6. 1-Methyl-4-[4-(4-piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-piperazine (**9f**).* Prepared according to method B (63%). ^1H NMR (CD_3OD, 500 MHz) δ 7.53 (d, $J = 8.3$ Hz, 2H), 7.47 (d, $J = 8.3$ Hz, 2H) 4.17–4.03 (m, 10H), 3.88–3.75 (m, 6H), 3.75–3.63 (m, 2H), 3.47 (dd, $J = 7.2$, 7.0 Hz, 2H), 3.43–3.36 (m, 2H), 3.08 (dd, $J = 7.2$, 7.0, 2H), 2.95 (s, 3H), 2.74 (dt, $J = 2.0$, 12.3 Hz, 2H), 2.09–1.94 (m, 2H), 1.91–1.79 (m, 3H), 1.46–1.33 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 132.4, 131.4, 128.5, 124.5, 85.2, 82.8, 60.1, 55.1, 52.7, 49.9, 48.4, 22.6, 21.7, 15.4; MS m/z 326.3 (M + H); Anal. ($C_{21}H_{34}Cl_3N_3$) C, H, N.

*5.1.2.7. 4-[4-(3-Piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-morpholine (**9g**).* Prepared according to method B (51%). ^1H NMR (CD_3OD, 500 MHz) δ 7.78 (s, 1H), 7.53 (d, $J = 7.3$ Hz, 1H), 7.48 (d, $J = 7.8$ Hz, 1H), 7.40 (t, $J = 7.7$ Hz, 1H), 4.22 (s, 2H), 4.12–3.97 (m, 4H), 3.59 (d, $J = 12.3$ Hz, 2H), 3.46–3.36 (m, 4H), 3.28–3.18 (m, 2H), 3.08 (t, $J = 6.8$ Hz, 2H), 2.96–2.85 (m, 2H), 2.01–1.81 (m, 5H), 1.55–1.40 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 134.5, 132.9, 131.2, 129.2, 129.1, 123.8, 123.8, 85.0, 85.5, 63.6, 60.0, 55.4, 52.8, 52.1, 22.7, 21.6, 15.0; MS m/z 313.3 (M + H); Anal. ($C_{20}H_{30}Cl_2N_2O$) C, H, N.

5.1.3. Method C. General procedure for reductive amination

A solution of the benzaldehyde (1 equiv) and amine (1.2 equiv) in CH_2Cl_2 or THF (0.1 M) was treated with $NaBH(OAc)_3$ (1.6 equiv). After 16 h, the resulting mixture was treated with 10% sodium hydroxide (5 mL), and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over sodium sulfate and evaporated. Chromatography of the residue (0–5% 2 M methanolic ammonia/CH_2Cl_2) provided the desired product. Benzylamines were then dissolved in Et_2O and treated with HCl (2.5 equiv) and the resulting suspension stirred for 30 min. Solvent removed and solids dried under high vacuum.

*5.1.3.1. Dimethyl-[4-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-amine (**9h**).* Prepared according to method C (75%). ^1H NMR (500 MHz) δ 7.35–7.32 (m, 2H), 7.23–7.20 (m, 2H), 3.38 (s, 2H), 2.68–2.63 (m, 2H), 2.61–2.57 (m, 2H), 2.49–2.44 (m, 4H), 2.21 (s, 6H), 1.62–1.57 (m, 4H), 1.47–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 138.5, 131.4, 128.9, 122.4, 88.3, 81.1, 64.0, 58.0, 54.2, 45.3, 25.9, 24.2, 17.5; MS m/z 271.3 (M + H); Anal. ($C_{18}H_{26}N_2$) C, H, N.

*5.1.3.2. Diethyl-[4-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-amine (**9i**).* Prepared according to method C (74%). ^1H NMR (400 MHz) δ 7.33 (d, $J = 8.2$ Hz, 2H), 7.24 (d, $J = 8.2$ Hz, 2H), 3.53 (s, 2H), 2.68–2.57 (m, 4H), 2.52–2.45 (m, 8H), 1.63–1.57 (m, 4H), 1.47–1.41 (m, 2H), 1.02 (t, $J = 7.1$ Hz, 6H); ^{13}C NMR (125 MHz) δ 139.7, 131.3, 128.6, 122.0, 88.1, 81.2, 58.1, 57.3, 54.2, 46.7, 25.9, 24.3, 17.6, 11.7; MS m/z 299.3 (M + H); Anal. ($C_{20}H_{30}N_2$) C, H, N.

*5.1.3.3. 1-[4-(4-Pyrrolidin-1-ylmethyl-phenyl)-but-3-ynyl]-piperidine (**9j**).* Prepared according to method C (96%). ^1H NMR (400 MHz) δ 7.34 (d, $J = 8.0$ Hz, 2H), 7.24 (d, $J = 8.0$ Hz, 2H), 3.58 (s, 2H), 2.68–2.57 (m, 4H), 2.50–2.45 (m, 8H), 1.79–1.76 (m, 4H), 1.63–1.57 (m, 4H), 1.47–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 139.0, 131.3, 128.7, 122.2, 88.2, 81.1, 60.4, 58.1, 54.2, 54.1, 25.9, 24.2, 23.4, 17.6; MS m/z 297.3 (M + H); Anal. ($C_{20}H_{28}N_2$) C, H, N.

*5.1.3.4. 4-[4-(4-Piperidin-1-yl-but-1-ynyl)-benzyl]-morpholine (**9k**).* Prepared according to method C (88%). ^1H NMR (400 MHz) δ 7.34 (d, $J = 8.2$ Hz, 2H), 7.24 (d, $J = 8.2$ Hz, 2H), 3.70 (t, $J = 4.6$ Hz, 4H), 3.47

(s, 2H), 2.68–2.57 (m, 4H), 2.50–2.41 (m, 8H), 1.63–1.57 (m, 4H), 1.48–1.42 (m, 2H); ^{13}C NMR (125 MHz) δ 137.4, 131.4, 128.9, 122.6, 88.4, 81.0, 66.9, 63.1, 58.0, 54.2, 53.5, 25.9, 24.2, 17.5; MS *m*/*z* 313.3 (M + H); Anal. ($C_{20}H_{28}N_2O$) C, H, N.

*5.1.3.5. 1-Methyl-4-[4-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-piperazine (**9l**).* Prepared according to method C (84%). ^{1}H NMR (400 MHz) δ 7.33 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 3.47 (s, 2H), 2.68–2.57 (m, 4H), 2.47 (m, 12H), 2.28 (s, 3H), 1.62–1.57 (m, 4H), 1.47–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 137.9, 131.3, 128.9, 122.4, 88.3, 81.1, 62.7, 58.1, 55.1, 54.2, 53.1, 46.0, 25.9, 24.2, 17.6; MS *m*/*z* 326.4 (M + H); Anal. ($C_{21}H_{31}N_3$) C, H, N.

*5.1.3.6. 1-Isopropyl-4-[4-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-piperazine (**9m**).* Prepared according to method C (97%). ^{1}H NMR (400 MHz) δ 7.33 (d, *J* = 8.2 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 3.48 (s, 2H), 2.68–2.40 (m, 17H), 1.63–1.57 (m, 4H), 1.48–1.41 (m, 2H), 1.04 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (125 MHz) δ 137.8, 131.3, 129.0, 122.4, 88.3, 81.1, 62.8, 58.1, 54.4, 54.2, 53.4, 48.6, 25.9, 24.2, 18.6, 17.6; MS *m*/*z* 354.4 (M + H); Anal. ($C_{23}H_{35}N_3$) C, H, N.

*5.1.3.7. 1-Benzyl-4-[4-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-piperazine (**9n**).* Prepared according to method C (38%). ^{1}H NMR (400 MHz) δ 7.38–7.21 (m, 9H), 3.51 (s, 2H), 3.48 (s, 2H), 2.68–2.56 (m, 4H), 2.46 (m, 10H), 1.62–1.56 (m, 6H), 1.74–1.42 (m, 2H); ^{13}C NMR (125 MHz) δ 138.1, 137.8, 131.3, 129.1, 128.9, 128.1, 126.9, 122.4, 88.3, 81.1, 63.0, 62.7, 58.0, 54.2, 53.1, 53.0, 25.9, 24.2, 17.6; MS *m*/*z* 402.4 (M + H); Anal. ($C_{27}H_{35}N_3$) C, H, N.

*5.1.3.8. 4-{4-[4-(4-Benzyl-piperidin-1-ylmethyl)-phenyl]-but-3-ynyl}-piperidine (**9o**).* Prepared according to method C (19%). ^{1}H NMR (400 MHz) δ 7.32 (d, *J* = 8.0 Hz, 2H), 7.28–7.15 (m, 5H), 7.12 (d, *J* = 7.1 Hz, 2H), 3.43 (s, 2H), 2.83–2.80 (m, 2H), 2.68–2.56 (m, 4H), 2.52 (d, 7.0 Hz, 2H), 2.46 (m, 4H), 1.87 (t, *J* = 9.9 Hz, 2H), 1.62–1.57 (m, 6H), 1.53–1.41 (m, 3H), 1.34–1.24 (m, 2H); ^{13}C NMR (125 MHz) δ 140.7, 131.3, 129.1, 128.9, 128.1, 125.7, 63.1, 58.1, 54.2, 53.8, 43.2, 37.8, 32.1, 28.5, 25.9, 24.2, 17.6; MS 401.4 *m*/*z* (M + H); Anal. ($C_{28}H_{36}N_2$) C, H, N.

*5.1.3.9. 1-[4-(4-Piperidin-1-yl-but-1-ynyl)-benzyl]-piperidine-4-carboxylic acid amide (**9p**).* Prepared according to method C (49%). ^{1}H NMR (400 MHz) δ 7.33 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 3.94 (s, 2H), 3.49 (s, 2H), 2.67–2.57 (m, 4H), 2.51–2.45 (m, 8H), 1.77–1.71 (m, 5H), 1.63–1.57 (m, 4H), 1.47–1.42 (m, 2H); ^{13}C NMR (125 MHz) δ 177.3, 138.1, 131.4, 128.8, 122.4, 88.4, 81.1, 62.8, 58.1, 54.2, 53.0, 42.7, 28.9, 25.9, 24.3, 17.6; MS *m*/*z* 354.3 (M + H); Anal. ($C_{22}H_{31}N_3O$) C, H, N.

*5.1.3.10. 1-[4-(4-Piperidin-1-yl-but-1-ynyl)-benzyl]-piperidin-4-ol (**9q**).* Prepared according to method C (84%). ^{1}H NMR (400 MHz) δ 7.34 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 3.72–3.65 (m, 1H), 3.47 (s, 2H), 2.75–2.57 (m, 6H); 2.47 (m, 4H), 2.13 (t, *J* = 9.6 Hz, 2H), 1.90–1.84 (m, 2H), 1.63–1.53 (m, 5H), 1.47–1.41 (m, 3H); ^{13}C NMR (125 MHz) δ 138.2, 131.3, 128.8, 122.4, 88.3, 81.1, 68.0, 62.6, 58.1, 54.2, 50.9, 34.5, 25.9, 24.2, 17.5; MS *m*/*z* 327.3 (M + H); Anal. ($C_{21}H_{30}N_2O$) C, H, N.

*5.1.3.11. 4-[4-(3-Piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-piperidine (**9r**).* Prepared according to method C (92%). ^{1}H NMR (400 MHz) δ 7.35 (br s, 1H), 7.28–7.21 (m, 3H), 3.42 (s, 2H), 2.67–2.57 (m, 4H), 2.50–2.43 (m, 4H), 2.39–2.31 (m, 4H), 1.63–1.53 (m, 8H), 1.48–1.38 (m, 3H); ^{13}C NMR (125 MHz) δ 138.7, 132.2, 130.0, 128.5, 127.9, 123.5, 88.3, 81.3, 63.5, 58.1, 54.4, 54.2, 26.0, 25.9, 24.3, 24.2, 17.6; MS *m*/*z* (M + H); Anal. ($C_{21}H_{30}N_2$) C, H, N.

*5.1.3.12. 4-[3-(4-Piperidin-1-yl-but-1-ynyl)-benzyl]-morpholine (**9s**).* Prepared according to method C (90%). ^{1}H NMR (400 MHz) δ 7.36 (br s, 1H), 7.30–7.22 (m, 3H), 3.70 (t, *J* = 4.6 Hz, 4H), 3.45 (s, 2H), 2.68–2.57 (m, 4H), 2.51–2.40 (m, 8H), 1.64–1.57 (m, 4H), 1.48–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 137.9, 132.2, 130.3, 128.5, 128.1, 123.7, 88.5, 81.1, 66.9, 63.0, 58.0, 54.2, 53.5, 25.9, 24.2, 17.5; MS *m*/*z* 313.3 (M + H); Anal. ($C_{20}H_{28}N_2O$) C, H, N.

*5.1.3.13. 1-Methyl-4-[3-(4-piperidin-1-yl-but-1-ynyl)-benzyl]-piperazine (**9t**).* Prepared according to method C (95%). ^{1}H NMR (500 MHz) δ 7.36 (s, 1H), 7.29–7.20 (m, 3H), 3.45 (s, 2H), 2.68–2.57 (m, 4H), 2.56–2.30 (m, 11H), 2.28 (s, 3H), 1.87 (br s, 1H), 1.63–1.57 (m, 4H), 1.48–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 138.3, 132.2, 130.2, 128.5, 128.0, 123.6, 88.4, 81.2, 62.6, 58.1, 55.1, 54.2, 53.0, 46.0, 25.9, 24.2, 17.6; MS *m*/*z* 326.4 (M + H); Anal. ($C_{21}H_{31}N_3$) C, H, N.

*5.1.3.14. 1-[3-(4-Piperidin-1-yl-but-1-ynyl)-benzyl]-piperidine-4-carboxylic acid amide (**9u**).* Prepared according to method C (84%). ^{1}H NMR (500 MHz) δ 7.35 (s, 1H), 7.29–7.21 (m, 3H), 5.45 (br s, 1H), 5.36 (br s, 1H), 3.44 (s, 2H) 2.93–2.88 (m, 2H), 2.68–2.57 (m, 4H), 2.51–2.42 (m, 4H), 2.18–2.11 (m, 1H), 2.02–1.96 (m, 2H), 1.89–1.82 (m, 2H), 1.80–1.68 (m, 3H), 1.63–1.57 (m, 4H), 1.48–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 177.2, 138.5, 132.0, 130.1, 128.3, 128.0, 123.6, 88.5, 81.2, 62.7, 58.1, 54.2, 53.0, 42.7, 28.9, 25.9, 24.2, 17.6; MS *m*/*z* 354.3 (M + H); Anal. ($C_{22}H_{31}N_3O$) C, H, N.

*5.1.3.15. 1-[4-(3-Pyrrolidin-1-ylmethyl-phenyl)-but-3-ynyl]-piperidine (**9v**).* Prepared according to method C (90%). ^{1}H NMR (400 MHz) δ 7.37 (s, 1H), 7.28–7.22 (m, 3H), 3.56 (s, 2H), 2.68–2.57 (m, 4H), 2.51–2.42 (m, 8H), 1.82–1.74 (m, 4H), 1.63–1.57 (m, 4H) 1.48–1.41 (m, 2H); ^{13}C NMR (125 MHz) δ 139.5, 131.9, 130.0, 128.2, 128.0, 123.6, 88.3, 81.2, 60.3, 58.1, 54.2, 54.1, 25.9, 24.2, 23.4, 17.5; MS *m*/*z* 297.3 (M + H); Anal. ($C_{20}H_{28}N_2$) C, H, N.

*5.1.3.16. 4-[4-(2-Piperidin-1-ylmethyl-phenyl)-but-3-ynyl]-piperidine (**9w**).* Prepared according to method C (85%). ^{1}H NMR (400 MHz) δ 7.43 (d, *J* = 7.1 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 7.26–7.22 (m, 1H), 7.16–7.12 (m, 1H), 3.63 (s, 2H), 2.70–2.61 (m, 4H), 2.53–2.37 (m, 8H), 1.64–1.55 (m, 8H), 1.49–1.39 (m, 4H); ^{13}C NMR (125 MHz) δ 140.3, 131.9, 129.0, 127.5, 126.3, 123.6, 92.8, 79.7, 61.0, 58.1, 54.5, 54.2, 26.0, 25.9, 24.3, 24.2, 17.8; MS *m*/*z* (M + H); Anal. ($C_{21}H_{30}N_2$) C, H, N.

5.2. Biological evaluation

5.2.1. In vitro pharmacology

5.2.1.1. Human and rat histamine H_3 binding assays. Binding of compounds to the cloned human H_3 receptor, stably expressed in SK-N-MC cells, was performed as described earlier [24]. Briefly, cell pellets from SK-N-MC cells expressing either the rat or human H_3 receptor were homogenized in 50 mM Tris–HCl/5 mM EDTA and recentrifuged at 30,000 *g* for 30 min. Pellets were rehomogenized in 50 mM Tris/5 mM EDTA (pH 7.4). Membranes were incubated with 0.8 nM [^{3}H]-N^α-methylhistamine plus/minus test compounds for 60 min at 25 °C and harvested by rapid filtration over GF/C glass fiber filters (pretreated with 0.3% polyethylenimine) followed by four washes with ice-cold buffer. Nonspecific binding was defined in the presence of 10 μM histamine. IC_{50} values were determined by a single site curve-fitting program (GraphPad, San Diego, CA) and converted to K_i values based on a [^{3}H]-N^α-methylhistamine K_d of 800 pM and a ligand concentration of 800 pM [25].

5.2.1.2. Additional in vitro data. The compound was screened at a concentration of 1 μM against a panel of 50 drug targets (receptors, ion channels, transporters) by CEREP (15318 NE 95th Street, Redmond WA; www.cerep.com). Human plasma protein binding

and Caco-2 permeability were determined by Absorbtion Systems, Exton, PA.

5.2.1.3. Human histamine H_3 functional assay. Sublines of SK-N-MC cells were created that expressed a reporter construct and the human H_3 receptor. The reporter gene was β-galactosidase under the control of multiple cyclic AMP responsive elements. In 96-well plates, histamine was added directly to the cell media followed 5 min later by an addition of forskolin (5 μM final concentration). After a 6-h incubation at 37 °C, the media were aspirated and the cells washed with 200 μL of phosphate-buffered saline followed by a second aspiration. Antagonists were added 10 min prior to the addition of histamine. Cells were lysed with 25 μL 0.1× assay buffer (10 mM Na-phosphate, pH 8, 0.2 mM $MgSO_4$, 0.01 mM $MnCl_2$) and incubated at room temperature for 10 min. Cells were then incubated for 10 min with 100 μL of 1× assay buffer containing 0.5% Triton and 40 mM β-mercaptoethanol. Color was developed using 25 mL of 1 mg/mL substrate solution (cholorphenolred β-D-galactopyranoside; Roche Molecular Biochemicals, Indianapolis, IN). Color was quantitated on a microplate reader at absorbance 570 nm. The pA_2 values were calculated by Schild regression analysis of the EC_{50} values [26].

Appendix

Table A1
Combustion analysis data for all biologically-assayed compounds.

Compd.	Formula	Anal. Calcd.	Anal. Found
9a	$C_{20}H_{32}Cl_2N_2$	C, 64.68; H, 8.68; N, 7.54	C, 64.69; H, 8.43; N, 7.37
9b	$C_{20}H_{30}Cl_2N_2 \times 0.2\ H_2O$	C, 64.41; H, 8.22; N, 7.51	C, 64.11; H, 7.83; N, 7.54
9c	$C_{21}H_{32}Cl_2N_2 \times 0.01\ H_2O$	C, 65.48; H, 8.43; N, 7.27	C, 65.17; H, 8.04; N, 7.11
9d	$C_{20}H_{30}Cl_2N_2S \times 0.2\ H_2O$	C, 59.31; H, 7.57; N, 6.92	C, 59.05; H, 7.19; N, 7.08
9e	$C_{20}H_{30}Cl_2N_2O \times 0.05\ H_2O$	C, 62.19; H, 7.85; N, 7.25	C, 61.70; H, 7.30; N, 7.19
9f	$C_{20}H_{34}Cl_3N_3$	C, 58.00; H, 7.88; N, 9.66	C, 57.72; H, 7.79; N, 9.51
9g	$C_{20}H_{30}Cl_2N_2O \times 0.15\ H_2O$	C, 62.33; H, 7.85; N, 7.27	C, 61.66; H, 7.59; N, 7.18
9h	$C_{18}H_{28}Cl_2N_2$	C, 62.97; H, 8.22; N, 8.16	C, 62.83; H, 7.99; N, 8.00
9i	$C_{20}H_{32}Cl_2N_2$	C, 64.68; H, 8.68; N, 7.54	C, 64.97; H, 8.28; N, 7.46
9j	$C_{20}H_{30}Cl_2N_2$	C, 65.03; H, 8.19; N, 7.58	C, 64.78; H, 7.82; N, 7.36
9k	$C_{20}H_{30}Cl_2N_2O$	C, 62.33; H, 7.85; N, 7.27	C, 62.48; H, 7.71; N, 7.05
9l	$C_{21}H_{34}Cl_3N_3 \times 0.5\ H_2O$	C, 56.82; H, 7.95; N, 9.47	C, 56.88; H, 7.91; N, 9.40
9m	$C_{23}H_{38}Cl_3N_3 \times 0.8\ H_2O$	C, 57.87; H, 8.36; N, 8.80	C, 58.08; H, 8.10; N, 8.59
9n	$C_{27}H_{35}N_3 \times 0.05\ CH_2Cl_2$	C, 80.06; H, 8.72; N, 10.35	C, 80.24; H, 8.72; N, 10.19
9o	$C_{28}H_{36}N_2 \times 0.05\ CH_2Cl_2$	C, 83.22; H, 8.99; N, 6.92	C, 83.30; H, 9.05; N, 6.86
9p	$C_{22}H_{31}N_3O$	C, 74.75; H, 8.84; N, 11.89	C, 74.89; H, 8.93; N, 11.59
9q	$C_{21}H_{32}Cl_2N_2O \times 0.8\ H_2O$	C, 60.95; H, 8.18; N, 6.77	C, 61.04; H, 8.17; N, 6.61
9r	$C_{21}H_{32}Cl_2N_2$	C, 65.79; H, 8.41; N, 7.31	C, 65.44; H, 8.28; N, 6.97
9s	$C_{20}H_{30}Cl_2N_2O$	C, 62.33; H, 7.85; N, 7.27	C, 62.11; H, 7.90; N, 7.10
9t	$C_{21}H_{34}Cl_3N_3$	C, 58.00; H, 7.88; N, 9.66	C, 58.02; H, 7.78; N, 9.28
9u	$C_{22}H_{31}N_3O$	C, 74.75; H, 8.84; N, 11.89	C, 74.59; H, 8.76; N, 11.80
9v	$C_{20}H_{30}Cl_2N_2$	C, 65.03; H, 8.19; N, 7.58	C, 65.02; H, 7.95; N, 7.47
9w	$C_{21}H_{32}Cl_2N_2 \times 1.65\ H_2O$	C, 61.05; H, 8.61; N, 6.78	C, 61.12; H, 8.29; N, 6.54

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