4-(3-Aminoazetidin-1-yl)pyrimidin-2-amines as High-Affinity Non-imidazole Histamine H₃ Receptor Agonists with in Vivo Central Nervous System Activity

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Supporting Information

ABSTRACT: Despite the high diversity of histamine H₃ receptor (H₃R) antagonist/inverse agonist structures, partial or full H₃R agonists have typically been imidazole derivatives. An in-house screening campaign intriguingly afforded the non-imidazole 4-(3-azetidin-1-yl)pyrimidin-2-amine 11b as a partial H₃R agonist. Here, the design, synthesis, and structure−activity relationships of 11b analogues are described. This series yields several non-imidazole full agonists with potencies varying with the alkyl substitution pattern on the basic amine following the in vitro evaluation of H₃R agonism using a cyclic adenosine monophosphate response element-luciferase reporter gene assay. The key compound VUF16839 (14d) combines nanomolar on-target activity (pKi = 8.5, pEC50 = 9.5) with weak activity on cytochrome P450 enzymes and good metabolic stability. The proposed H₃R binding mode of 14d indicates key interactions similar to those attained by histamine. In vivo evaluation of 14d in a social recognition test in mice revealed an amnesic effect at 5 mg/kg intraperitoneally. The excellent in vitro and in vivo pharmacological profiles and the non-imidazole structure of 14d make it a promising tool compound in H₃R research.

INTRODUCTION

The four histamine receptors (H₁R, H₂R, H₃R, H₄R) belong to class A of the G protein-coupled receptor (GPCR) family.¹ The histamine H₃ receptor (H₃R) was discovered in 1983 by Arrang et al.² and regulates the release of several neurotransmitters such as histamine (1), acetylcholine, serotonin, noradrenaline, and dopamine, as both auto- and hetero-receptor.¹ Due to its expression in the cortex, striatum, and hippocampus, H₃R regulates several physiological processes such as sleep−wake regulation, cognition, and food intake.¹,³

During the early years of discovery of H₃R ligands, the natural ligand histamine served as an initial structure for drug design, leading to a plethora of imidazole-containing ligands. However, imidazole-containing ligands are associated with drug−drug interactions due to the propensity for cytochrome P450 (CYP) inhibition and having poor brain penetration.⁴,⁵ Therefore, research toward therapeutically relevant H₃R antagonists focused on druglike non-imidazole structures.⁶,⁷

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and these efforts have led to numerous clinical candidates for different indications connected to central nervous system diseases, for example, Alzheimer’s disease, attention deficit hyperactivity disorders, sleep disorders, schizophrenia, obesity, epilepsy, and neuropathic pain and narcolepsy.8,9 For the latter condition, the H3R ligand pitolisant (Wakix) was approved by the European Medicines Agency in 201610 and, most recently, by the Food and Drug Administration in 2019.11

In sharp contrast, developing non-imidazole H3R agonists has not been very successful. The best agonists contain an imidazole ring12−23 and, compared to the endogenous ligand histamine (1), these derivatives show similar [e.g., imbutamine (2)] or significantly higher affinity (pKi) and/or functional activity (pEC50) on H3R [e.g., imetit (3), methimepip (4), and (1S,2S)-2-(2-aminoethyl)-1-(1H-imidazol-4-yl)cyclopropane (5)] (Figure 1A). Imidazole-containing agonists have shown some potential application in different therapeutic areas, such as mechanical nociception,23 obesity, and diabetes mellitus (diet-induced obesity mice test)24 and stress (rodent-intruder mice test).21 Some data also support the hypothesis of cardioprotective effect of H3R receptor activation.25,26 However, it is fair to say that imidazole-containing agonists may suffer from the same imidazole-related drawbacks that were associated with the first generation of imidazole-containing H3R antagonists (vide supra). Future studies on the pharmacological and therapeutic roles of H3R agonists can therefore be helped by having non-imidazole H3R agonists available.

A very limited number of non-imidazole agonists have been published to date (Figure 1B). VUF8430 (6) was designed as an H3R agonist based on the H3R agonist dimaprit and shows micromolar affinity and full H3R agonism as well.27 The histamine analogues amthamine (7a) and amselamine (7b) were identified as H3R agonists, but both show weak H3R agonist activity in an electrically stimulated guinea-pig jejunum model.28,29 Three pentacyclic spiroindolinone derivatives were isolated from Penicillium waksmanii, of which PF1270A (8) shows the best affinity for H3R and moderate functional H3R activity in a guanosine 5′-O-[γ-thio]triphosphate (GTPγS) accumulation assay.30 ZEL-H16 (9) has been reported to have nanomolar binding affinity to the H3R, partial H3R agonism in forskolin-stimulated cAMP accumulation and ERK1/2 signaling assays, and full H3R agonism in a guinea-pig ileum contraction assay.31 Finally, a compound set with 94 examples in four compound families with either a β-lactam or a pyrrolidinone central core without basic amino moiety was published recently and surprisingly included compounds with nanomolar functional H3R agonist activities (e.g., compound 10).32 The fungal isolates and the multicomponent reaction product 10 are large and complex molecules, which are difficult to align with known H3R pharmacophores or H3R binding modes for agonists and antagonists. We therefore started a search for novel high-affinity non-imidazole H3R full agonists with simpler structures to generate fundamental knowledge on ligand recognition and signaling of the H3R.

**RESULTS**

**Design.** During an in-house compound screen aimed at identifying agonist activities in a set of ligands using a H3R-driven reporter gene assay in HEK293T cells, dianimopyr-
Figure 2. (A) Initial functional data of compounds 11b and 11i compared to histamine, as obtained by ligand-induced activation of hH3R expressed on HEK293T cells measured by the CRE-luc reporter gene assay. Shown is a representative graph of at least three experiments performed in triplicate. Data are mean ± standard deviation (S.D.). (B) Structures of 11b and 11i, the closest relevant structure (14a) resulting from a subsequent extensive patent search and the compound set designed for the current study.

Scheme 1. Syntheses Routes for Final Compounds

Reagents and conditions: (i) POCl3, 110 °C, 3 h, 26–52%; (ii) N,N-diisopropylethylamine (DIPEA), dioxane or N-methyl-2-pyrrolidone (NMP), microwave (μW), 120–150 °C, 0.5–2 h, 27–50% (a, b) or 35–71% (c, d, f, two steps from benzhydryl deprotection); (iii) HCl, dichloromethane (DCM), MeOH, room temperature (rt) to 50 °C, 3 h to overnight, 10% to quant.; (iv) aldehyde/ketone, AcOH, NaHB(OAc)3, DCM, MeOH, rt, 3 h to overnight, 16–44%; or 1,4-diiodobutane, K2CO3, MeCN, reflux, 16 h, 9%; (v) N,N-dimethyldiethylaminodihydrochloride, DIPEA, dioxane, μW, 150 °C, 30 min, 65%; (vi) di-tert-butyl dicarbonate, triethylamine (TEA), tetrahydrofuran (THF), rt, overnight, 63%; (vii) NaH, R2I, THF, 0 °C to rt, overnight, 28–58%; (viii) H2, Pd/C, MeOH, EtOH, rt to 60 °C, 1 h to overnight, not purified and used crude.
imidine 11b emerged as a H₃R partial agonist hit (α = 0.7), while its close derivative 11i showed only weak agonist activity (α = 0.4) (Figure 2A). Interestingly, a set of four diaminopyrimidine compounds has been tested before by others on H₃R en route to imbutamine (2) analogues, but the majority of these diaminopyrimidines were rather inactive and, where applicable, all were shown to be antagonists/inverse agonists. Intrigued by the agonist activity of the diaminopyrimidine 11b and recognizing its core as a thoroughly explored heterocycle in the H₃R area, we decided to perform an in-depth patent search on this scaffold in an effort to capture the full array of industrial contributions. This resulted in the identification of 14a (Figure 2B) as the closest derivative with data associated with H₃R (Figure 2B). Remarkably, 14a was claimed as a H₃R agonist by Abbott, although its actual synthesis was not included and only the potential synthetic route was described. In the same patent, 25 related examples have been prepared, and partial agonism at the human H₃R is reported.

Based on 11b and 14a, we designed a focused series of compounds to explore the H₃R affinity and activity in the chemical space between 14a and 11b (Figure 2B). The design strategy targets four series with iPr, Et, Me, or H as the R₁ group at position 6 (11–14). These R₁ groups were combined with different substituents on the basic amino groups (R² and R³). Beyond the evident H (a) and Me (b) substituents, dimethyl derivatives (i) were synthesized due to the potential functional switch that appears to reside in the cases of 11b and 11i. Based on the initial results of the designed compound set (vide infra), the R₁ = H series was extended with elongated (d and f), branched (e, g, h), and disubstituted (j, k) amino derivatives. This second design iteration was also inspired by previous work from our labs on the imidazole-containing agonist imbutamine (2), which harbors a functional switch on the basic amine. Beyond 14a, some exact compounds from this designed set are known but none in a context of H₃R. That is, 11a, 11b, and 12b have been claimed as H₃R ligands, while 12i, 13i, 14i, 14j, and 14k were offered in chemical catalogs (April 2019) without any synthesis description and analytical or pharmacological data.

**Chemistry.** The designed compound set shown in Figure 2B was synthesized as outlined in Scheme 1. The key step of the synthetic route was a nucleophilic aromatic substitution on the appropriate aromatic cores with aminoazetidine moieties (step ii). The 4-chloro-2-aminopyrimidines were commercially available (19 and 20) or were synthesized (17 and 18) from the appropriate pyrimidin-4(3H)-one derivatives (15 and 16) with POCl₃. The R² = H or Me derivatives of the Boc-protected 3-aminoazetidines (28a and 28b) were commercially available, while the R² = Et, nPr, and nBu derivatives were synthesized. These intermediates were built from benzhydryl-protected 3-aminoazetidine (25) with Boc protection of the

### Table 1. Pharmacological Evaluation of Designed Compound Set

| compound | R¹ | R² | R³ | pKᵢ⁺ | pEC₅₀ | α |
|----------|----|----|----|------|-------|---|
| histamine | | | | 7.9 ± 0.2 | 8.6 ± 0.0 | 1.0 ± 0.0 |
| 11a | iPr | H | H | 6.7 ± 0.0 | 7.1 ± 0.1 | 0.7 ± 0.0 |
| 11b | iPr | Me | H | 7.0 ± 0.0 | 7.9 ± 0.4 | 0.7 ± 0.1 |
| 11c | iPr | Et | H | 7.0 ± 0.1 | 6.8 ± 0.3 | 0.3 ± 0.1 |
| 11i | iPr | Me | Me | 6.9 ± 0.0 | 7.0 ± 0.2 | 0.4 ± 0.0 |
| 12a | Et | H | H | 7.1 ± 0.0 | 7.5 ± 0.0 | 0.9 ± 0.0 |
| 12b | Et | Me | Me | 7.3 ± 0.1 | 7.6 ± 0.0 | 0.8 ± 0.0 |
| 12c | Et | Et | H | 6.9 ± 0.1 | 7.5 ± 0.1 | 0.9 ± 0.0 |
| 12i | Et | Me | Me | 6.6 ± 0.2 | 7.2 ± 0.1 | 0.8 ± 0.0 |
| 13a | Me | H | H | 7.3 ± 0.1 | 7.8 ± 0.0 | 0.9 ± 0.0 |
| 13b | Me | Me | H | 7.5 ± 0.1 | 8.0 ± 0.0 | 0.8 ± 0.1 |
| 13c | Me | Et | H | 7.1 ± 0.1 | 7.9 ± 0.0 | 1.0 ± 0.0 |
| 13i | Me | Me | Me | 6.5 ± 0.2 | 7.4 ± 0.0 | 0.9 ± 0.1 |
| 14a | H | H | H | 7.8 ± 0.1 | 8.3 ± 0.0 | 1.1 ± 0.0 |
| 14b | H | Me | Me | 8.2 ± 0.1 | 8.9 ± 0.1 | 1.1 ± 0.1 |
| 14c | H | Et | Me | 8.0 ± 0.1 | 9.2 ± 0.0 | 1.0 ± 0.1 |
| 14d | H | nPr | H | 8.5 ± 0.1 | 9.5 ± 0.1 | 1.2 ± 0.1 |
| 14e | H | iPr | H | 7.4 ± 0.1 | 8.5 ± 0.1 | 1.2 ± 0.1 |
| 14f | H | nBu | H | 7.8 ± 0.1 | 9.1 ± 0.3 | 1.2 ± 0.0 |
| 14g | H | rac-iBu | H | 7.9 ± 0.2 | 8.7 ± 0.1 | 1.1 ± 0.0 |
| 14h | H | iBu | H | 7.4 ± 0.2 | 8.3 ± 0.0 | 1.1 ± 0.0 |
| 14i | H | Me | Me | 7.3 ± 0.1 | 8.4 ± 0.1 | 1.0 ± 0.1 |
| 14j | H | Et | Et | 7.2 ± 0.1 | 8.1 ± 0.1 | 1.2 ± 0.1 |
| 14k | H | -(CH₂)₄ | | 7.4 ± 0.1 | 8.1 ± 0.0 | 1.3 ± 0.2 |

*Measured as fumarate salt. Measured as dihydrochloride salt. Affinity values (pKᵢ) were determined by [³H]NAMH displacement assay on hH₃R expressed on HEK293T cell homogenates. Potency (pEC₅₀) and intrinsic activity (α) were determined by ligand-induced activation of hH₃R expressed on HEK293T cells as measured by a CRE-luciferase reporter gene assay. Data are mean ± standard error of the mean (S.E.M.) of at least three experiments performed in triplicate.
primary amino group to give 26, followed by alkylation with the corresponding iodoalkyl reagents resulting in the orthogonally protected intermediates (27c, 27d, 27f) and the removal of the benzhydryl group with hydrogenation. The resulting mixtures of unprotected azetidine intermediate and diphenylmethane were used directly for the ensuing nucleophilic substitution with 17–20. The key nucleophilic aromatic substitution of the appropriate Boc-protected intermediates was performed in a microwave at 120–150 °C to give 21–24. This was followed by the deprotection under acidic condition to afford the majority of monosubstituted (R² = H or linear alkyl, R³ = H) products 11–14. Although the Boc protection was necessary at the precursor stage to avoid overalkylation in the case of linear alkyl derivates, overalkylation was not a problem in the case of the branched-alkyl derivates as a result of steric hindrance. Therefore, the branched-alkyl derivatives 14a, g, and h as well as the dialkylated derivatives 12i, 13i, 14i, and 14j were synthesized from 14a with a reductive amination. Last, 11i was synthesized directly from 17 and N,N-dimethylazetidin-3-amine with a nucleophilic substitution, while the pyrrolidine ring of 14k was obtained from 1,4-diododobutane and 14a.

Pharmacological Evaluation. The synthesized compound set was tested for its activity at the human H₁,R transiently expressed in HEK293T cells. Binding affinity (Kᵢ) was evaluated using a [³H]NAMH displacement assay, while potency (EC₅₀) and intrinsic activity (α) were determined as the H₁,R-mediated inhibition of forskolin-induced CRE-driven luciferase reporter gene activity with histamine as the control (Table 1 and Figure 3). During the first iteration, the isopropyl group of 11b was gradually decreased in size to give 11–14, which were all combined with small-size R²/R³ amino substituents (a, b, c, i). The affinities of the unsubstituted derivatives (14) stand out especially within the monomethylated (b) and monoethylated series (c), with both 14b and 14c exceeding the affinity of histamine. Although monomethylated (b) derivatives generally show the highest affinities in each R¹ subseries 11–14, the most notable variation was observed in the case of the monoethylated series c, with affinities of Me/Et/iPr derivatives 11c, 12c, and 13c being considerably reduced compared with that of 14c (Figure 3A). A more indicative trend was observed in the case of the functional affinities of this c series. Compound 14c has a higher potency than 14b and produces the same intrinsic activity (α) as histamine but with higher potency (EC₅₀), while 12c and 13c show >1 log unit weaker EC₅₀ albeit with maintained full agonism (α > 1.0). Interestingly, isopropyl substitution on the R¹ position (11c) turns this full agonism to partial agonism (α = 0.3) (Figure 3B). A similar trend was observed in the other three series, with the pyrimidine derivatives bearing R¹ = H (14a–c and 14i) reaching or exceeding the affinity (Kᵢ) and potency (EC₅₀) of histamine, while any alkyl substituent at position 6 (R³) on the pyrimidine leads to inferior results. The intrinsic activity (α) indicates full or almost full agonism (α ≥ 0.8) in the methyl (12a, 12b, 12i) and ethyl (13a, 13b, 13i) series, while it drops to partial agonism in the isopropyl series (11a, 11b, 11i) (Table 1).

Due to the better results of the pyrimidines lacking an R¹ substituent (14), in a subsequent iteration the amine NH₃ substituent was replaced with longer linear groups (14d, 14f), branched groups (14e, 14g, 14h), or dialkylamino (14j, 14k) moieties. Representative curves illustrate the structure–activity relationship (SAR) (Figure 3C) and SFR (Figure 3D) of this series. The nPr derivative 14d shows the highest affinity (pKᵢ = 8.5) from all linear monoalkyl substituents. Although both shorter and longer R² moieties resulted in lower binding affinities (e.g., 14c: pKᵢ = 8.0 or 14f: pKᵢ = 7.8), all derivatives remained in the same affinity range (Figure 3C). The branched-alkyl moieties as well as the dialkylated derivatives display loss of affinity (compare, e.g., 14d vs 14e, or 14b vs 14j).
The potencies (EC\textsubscript{50}) show almost the same trends as observed for the affinities (Figure 3D). Highly noteworthy, the potency of 14d (pEC\textsubscript{50} = 9.5) is almost a log unit higher than that of histamine (pEC\textsubscript{50} = 8.6), while chain shortening (e.g., 14b, 14c), chain lengthening (14f), chain branching (e.g., 14e), or dialkylation (e.g., 14i) results in lower potencies. In contrast to the observed differences in affinity and potency, the intrinsic activities indicate that all derivatives of 14 remain full agonists (\(\alpha \geq 1.0\)) (Table 1). A combination of highest affinity (pK\textsubscript{i} = 8.5), highest potency (pEC\textsubscript{50} = 9.5), and full agonism resides in 14d. The potential aggregation of GPCR ligands might cause non-specific effects on the receptor activity, but nephelometry revealed no microprecipitation of 14d up to 100 mM concentration (Figure S1) and underscores the high aqueous (aq) solubility of 14d (soluble up to at least 100 mM in 50 mM Tris—HCl, pH 7.4). All this led to identification of 14d as a key compound (VUF16839) in this study.

**Computational Studies on 14d.** A combination of molecular docking and molecular dynamics (MD) simulations was used to evaluate the potential binding mode of the key compound 14d and to compare it to the binding mode of the endogenous ligand histamine. A homology model of H\(_3\)R based on the available crystal structure of H\(_1\)R was used (see the Experimental section). This model was validated by its ability to retrospectively discriminate between known H\(_3\)R fragmentlike ligands and true inactives.\(^{40}\) Histamine was docked in the receptor model using PLANTS 1.1 (Figure 4A). The best-scored docking pose showing interactions with both D114\(_{3.32}\) and E206\(_{5.461}\) (residues known to be involved in H\(_3\)R ligand binding\(^{40-45}\)) was selected. During 100 ns of molecular dynamics (MD) simulations, histamine is able to maintain stable interactions with residues D114\(_{3.32}\) and E206\(_{5.461}\) as well as with Y374\(_{6.51}\) (Figure 4B,E). Using similar procedures, compound 14d was also docked into the same homology model using PLANTS 1.1 (Figure 4A), and the best-scored docking results show similar interactions of 14d with D114\(_{3.32}\) and E206\(_{5.461}\). The basic amine of 14d forms an ionic interaction with the negatively charged side chain of D114\(_{3.32}\), and the amino group in the pyrimidine ring makes a hydrogen bond with E206\(_{5.461}\). Different docking poses maintain these key interactions but show a different positioning of the linear nPr moiety at the R\(_2\) position: toward the extracellular surface of the receptor or toward the intracellular side (Figure S2A,B, respectively). MD simulations of the two alternative models were performed (Movies 1 and 2, Supporting Information). The model in which the nPr group of 14d is directed toward the intracellular half of the receptor was not stable along 100 ns of MD simulations (Figure S2D, Movie 2, Supporting Information), while the model where the nPr group of 14d is pointing toward the extracellular vestibule remained stable throughout the entire simulation time (Figure S2C and Movie 1, Supporting Information). This binding mode is shown in Figure 4D, and the interactions that remained stable during the simulations are depicted in Figure 4C and the interaction fingerprints (IFPs) in Figure 4E. It can be concluded that the non-imidazole H\(_3\)R ligand 14d exerts its unusual agonist H\(_3\)R activity by showing a similar pharmacophore as the endogenous H\(_3\)R ligand. That is, it may achieve its

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**Figure 4.** Predicted binding mode of 14d. (A) Overview of the H\(_3\)R homology model based on the H\(_1\)R crystal structure (PDB ID: 3RZE).\(^{46}\) The experimentally validated binding mode of histamine (magenta) is shown in more detail in (B), and the predicted binding mode of 14d (green) is schematically represented in (C) and shown in more detail in (D). Interaction fingerprint representations of histamine and compound 14d are shown in (E), where a one represents the presence of an interaction according to the color coding: blue for apolar, olive green for face-to-face aromatic, green for edge-to-face aromatic, red for protein hydrogen bond donor, purple for ligand hydrogen bond donor, gray for ligand-negative charge, and orange for protein-negative charge.
agonist activity by forming similar interactions with the same residues as histamine.

**Pharmacological and Pharmacokinetic Characterization of 14d.** Functional characterization of key compound 14d in a direct G protein activation assay, that is, the $[^{35}S]$-GTPγS accumulation assay on hH3R expressing cell homogenates (Figure 5A), results in potent but partial agonism ($pEC_{50} = 9.0 \pm 0.1$) compared with histamine ($pEC_{50} = 8.5 \pm 0.1$). However, 14d was tested for its H3R/H4R selectivity. The pyrimidine shows only marginal selectivity with respect to the binding $hH3R$ or $hH4R$ ($hH3R$ $pK_i = 8.5 \pm 0.1$ vs $hH4R$ $pK_i = 8.1 \pm 0.0$), but encouragingly a 10-fold selectivity in potency is observed in favor of the $hH4R$ ($hH4R$ $pEC_{50} = 9.5 \pm 0.1$ vs $hH3R$ $pEC_{50} = 8.5 \pm 0.2$) with full agonism on $hH4R$ ($\alpha = 1.1 \pm 0.1$) in a CRE-luciferase reporter gene assay. Moreover, 14d does not activate the $H3R$ and $H4R$ up to 10 $\muM$ (Figure S3).

Equally encouraging, the binding affinity of 14d is increased for $mH3R$ ($pK_i = 9.0 \pm 0.1$) compared with $hH3R$, while for $mH4R$, the $pK_i$ value is decreased to 7.8 $\pm 0.0$, thus yielding a substantial $H3R/H4R$ binding selectivity for mouse receptors. Compound 14d was also functionally evaluated as an agonist on the $mH3R$ and $mH4R$ using the CRE-luciferase reporter gene assay. In these experiments, 14d displays a 10-fold selectivity in potency ($mH3R$ $pEC_{50} = 10.0 \pm 0.1$ vs $mH4R$ $pEC_{50} = 9.0 \pm 0.1$), while it acts as a full agonist on both murine receptors ($mH3R$ $\alpha = 1.2 \pm 0.1$ and $mH4R$ $\alpha = 1.1 \pm 0.1$) (Figure 5B,C).

The metabolic stability of 14d was determined in vitro by incubation with rodent liver microsomes (Table 2). The pharmacokinetic properties for mouse ($t_{1/2} = 130.8$ min; $Cl_{int} = 20.7$ mL/(min*kg)) indicate more than 2 times slower elimination compared with the reference control verapamil. For rats, this difference between 14d and verapamil is even more pronounced.

The imidazole ring is known to generally be able to interact with CYP enzymes via coordination of the imidazole with the prosthetic heme iron, which can cause unwanted drug–drug interaction. Since the 2-aminoypyridine core contains a pattern of adjacent nitrogen atoms, we measured its propensity for CYP inhibition. Compound 14d shows only weak activity on three key CYP enzymes (Figure S4) with IC50 values for binding to CYP3A4, CYP2C9, and CYP2D6 all being larger than 25 $\muM$.

**Effect of 14d on Social Recognition in Mice.** Given the notion that CNS penetration of $H3R$ agonists, including 14d, is not evident, we evaluated the in vivo CNS effects of 14d in a standard paradigm for $H3R$ action. It is well known that histamine, acting in different brain sites, is an important regulator of memory consolidation and retrieval in various learning paradigms, including the social recognition test. We used this behavioral paradigm to investigate the $H3R$-related CNS activity of compound 14d in vivo. The social recognition memory investigates the ability to remember the identity of a conspecific, which is crucial to the building of social relationships and survival. Twenty-four hours after animals’ habituation to the apparatus, the subject mouse was placed in an open-field arena with an empty cage and another one containing a juvenile mouse. Mice tend to spend more time in the proximity of the cage containing the juvenile mouse than the empty one, offering an indication of sociability. One hour later, the experimental mouse was placed again in the same arena, but this time one cage contained the familiar mouse and the second one a novel juvenile mouse. The exploration times of the familiar and the novel mouse were recorded separately. Compound 14d at a dose of 5 mg/kg or vehicle was given intraperitoneally (i.p.) 30 min before the training session (Figure 6A). Compound 14d did not affect animals’ sociability as revealed by the longer time that they spent exploring the cage containing the social stimulus compared with the empty cage (Figure S5). In this respect, mice treated with compound 14d behaved like controls. During the test session, control mice recognized the familiar juvenile, since they spent more time exploring the novel one. Conversely, mice treated with 14d did not discriminate between the novel and the familiar mouse (Figure 6B). This result clearly suggests a social memory impairment, further confirmed by the negative discrimination index (DI) calculated for the group of animals receiving injections of 14d (Figure 6C).

| Compound | $t_{1/2}$ (min) | $Cl_{int}$ (mL/(min*kg)) |
|----------|----------------|--------------------------|
| 14d      | 239.0          | 7.9                      |
|          | 130.8          | 20.7                     |
| verapamil| 50.9           | 37.3                     |
|          | 57.3           | 47.3                     |
reported as agonists at H4R.27–32 Indeed, replacements of the imidazole ring while maintaining agonism have so far yielded little success. To illustrate, several imbutamine (2) analogues in which the imidazole moiety was replaced with an aminopropimidine, aminopryidine or aminotriazole ring studied were not effective as H3R agonists.34 Comparing the activities of the aminopropimidine analog of 2 with 14d, the effective activation of H3R by 14d suggests a very important role of its azetidine side chain. Molecular docking combined with MD studies affords a predicted binding mode (Figure 4) in which 14d interacts with the same key amino acids (D11432, E20634, Y37431) as histamine (1) (Figure 4E), suggesting that the 2-aminopyrimidine moiety mimics the imidazole ring. The computational studies also suggest that the azetidine side chain makes an ionic interaction with the same amino acid (D11432) as the amine group in the ethylamine side chain of histamine. Clearly, both 14d and histamine are able to interact with the same key amino acids of H3R, despite the fact that the binding modes of 14d and histamine do not substantially overlap.

The alkyl substitution of the basic amino moiety of 2 was studied previously in our group,35 and subtle differences in alkyl substituents on the basic amine strongly influence the functional activity of such imbutamine analogs. Based on this SAR, we hypothesize that the basic amine of the 2-aminopyrimidine compound series 11–14 might be a functional “hot spot” as well. In the case of the R1 = iPr series (11), this appears to partially pan out as the chain elongation and dialkylation show moderate drops in intrinsic activity (α) (compare 11b vs 11c and 11i in Table 1). However, such a trend was not observed within the other series 12–14 (Table 1 and Figure 3D).

The analysis of additional properties for key compound 14d underscores its suitability for in vivo characterization in mice. That is, mouse potency data (mH3R: pEC50 = 10.0, mH4R: pEC50 = 9.0) and in vitro elimination parameters in mouse [(t1/2 = 130.8 min; Clint = 20.7 mL/min/kg)] all bode well. The inhibition of CYP enzymes is a general issue of imidazole-containing ligands.30 Although the diaminopyrimidine core might conceivably also be prone to CYP inhibition, 14d only weakly (IC50 > 25 μM) interacts with selected key CYP enzymes (CYP3A4, CYP2D6, CYP2C9).

Poor brain penetration is a known problem of some imidazole-containing ligands,37 limiting their potential administration routes in CNS-related experiments. As indicated by the effects in the in vivo social memory test, the 2-aminopyrimidine 14d (5 mg/kg i.p.) is clearly penetrating the CNS. Histamine is a known modulator of different types of memory.52,53 H3R antagonists, such as thioperamide, improve short-term memory, while H3R agonists such as immepip (4) cause amnesia in the social recognition test.51 The results shown here confirm and expand these observations, since a memory impairment was observed also for systemic treatment with compound 14d in the same paradigm (Figure 6). It should be noted that H3R activation affects the circadian rhythm by increasing slow wave sleep and dose-dependently attenuates ciproxifan-induced waking effects.55 Also, H3R agonists reduce stress-induced behavior in preclinical models.54 These observations suggest that H3R activation may affect exploratory activity, which could negatively impact arousal and cognition. We did not specifically measure 14d-induced alterations of the sleep–wake cycle; however, 14d-associated social memory impairment does not seem to be related to

Figure 6. Compound 14d impairs social recognition in mice. (A) Schematic drawings showing the sequence of procedures and treatment administrations. (B) Results are calculated as means of individual percentage of time spent exploring familiar (white columns) and novel (black columns) social stimuli. **p < 0.01 vs the respective familiar subject [two-way analysis of variance (ANOVA) and Bonferroni’s MCT]. (C) Discrimination index calculated according to the formula tN = IFlN + Ft. **p < 0.01 vs vehicle (unpaired t-test). Shown are means ± S.E.M. of 10–11 animals per experimental group.

■ DISCUSSION AND CONCLUSIONS

We present 2-aminopyrimidine derivatives with an alkylated 3-amino-azetidine moiety showing low nanomolar affinities for the H4R. Based on the non-imidazole partial agonist 11b identified as an in-house hit, a 23-membered compound set was synthesized and tested on H3R. The reduction of the substituent size at position 6 of the pyrimidine ring (R1) improved both affinity (pKᵢ) and potency (pEC50) on H3R. Each member of the extended R1 = H series (14) shows full agonism in a CRE-luciferase reporter gene assay, with three weakly (IC50 > 25 μM) interacts with selected key CYP enzymes (CYP3A4, CYP2D6, CYP2C9).

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sedative effects, as there were no statistically significant differences between the control group and 14d-treated mice in exploration time of the cage in the presence or absence of the social stimulus (during training: vehicle = 144.0 ± 60.0 s; 14d = 136.2 ± 44.1 s; during test: vehicle = 150.5 ± 42.4 s; 14d = 148.3 ± 69.4 s). Moreover, based on the mH3R/mH3R selectivity profile of 14d (vide supra), available literature on H3R expression, H3R agonist actions in the CNS, and the behavioral profile of H3R-deficient mice excluding a relevant role of this receptor on the histaminergic modulation of memory processing, it is highly unlikely that the in vivo amnesic effects of 14d are confounded by its H3R activity.55,56 Indeed, the amnesic effects of 14d are consistent with the memory impairments observed following treatment with different H3R agonists [imetit (3) and (R)-α-methylhistamine] in the object recognition and passive avoidance tests in rats.57 To conclude, in this study, 2-aminoypyrimidine derivatives with an alkylated 3-amino-azetidine side chain are presented as highly potent, non-imidazole agonists for the H3R. The key n-propyl derivative (14d, VUF16839) shows attractive in vitro pharmacological properties on human H3R (pKᵢ = 8.5, pEC50 = 9.5, α ≥ 1.0) in a CRE-luciferase reporter gene assay with a 10-fold lower potency at H2R and mouse H3R (pKᵢ = 9.0, pEC50 = 10.0, with >10-fold lower potency at mH1R). It exerts reasonable metabolic stability in rodent liver microsomes and weak activity on CYP enzymes. Moreover, 14d causes amnesic effects in social recognition tests in mice at 5 mg/kg, which is in line with the reported memory loss after administration of other H3R agonists.51,57 The observed in vivo H3R effects also indicate appreciable brain penetration of 14d. Compound 14d can serve as a useful tool compound for fundamental studies concerning H3R, given its excellent affinity and potency, H3R agonism, and effective brain penetration.

### EXPERIMENTAL SECTION

**Pharmacology and ADME. Materials.** [3H]NAMH (specific activity: 79.7 Ci/mmol) and [3H]histamine (specific activity: 17.5 Ci/mmol) were purchased from PerkinElmer (Groningen, the Netherlands). Human embryonic kidney 293T cells (HEK293T cells) were obtained from ATCC. Kotorazol, quinidine, sulfaphenazole, and verapamil were obtained from Sigma-Aldrich (St. Louis, MO). For radioligand displacement assays, HEK293T cells were transfected using the polyethylenimine (PEI) method.58 Cells were transfected with 2500 ng of complementary DNA (cDNA) encoding the hH3R (genbank: AF140538), mH3R (genbank: NM_133849.3), hH4R (genbank: NM_153087.2) and 2500 ng of empty plasmid pcDEF3. The DNA/PEI mixture (ratio 1:4) was incubated for 20 min at 22 °C before addition to the cells. **Preparation of Cell Homogenates.** Cell homogenates expressing the hH3R were harvested 48 h after transfection as reported previously.59

**Radioligand Displacement Assays.** [3H]NAMH and [3H]histamine displacement assays were performed in a binding buffer (50 mM Tris–HCl pH 7.4, 25 °C) by incubation of 2 nM [3H]NAMH or 10 nM [3H]histamine, increasing concentrations of unlabeled ligand and cell homogenates expressing the hH3R/mH3R or hH3R, respectively. For mH3R displacement, similar studies were performed but with 30 nM [3H]histamine. The assay mixture was incubated for 2 h at 25 °C before rapid filtration over a 0.5% PElcoated GF/C filter with a PerkinElmer filtermate harvester. The filter plate was dried, and 300 min after 25 μL of Microsint O was added, filter-bound radioactivity was measured with a Microbeta scintillation counter (PerkinElmer).

[125I]GTPγS Accumulation Assay. [35S]GTPγS accumulation experiments on hH3R were performed as described previously.60

**Reporter Gene Assay.** HEK293T cells were transfected in suspension with cDNA encoding hH3R, mH3R, hH4R, mH4R, H1R (1000 ng) or H2R (2500 ng), CRE-luciferase (2500 ng) or NFAT-luciferase (2000 ng) for H3R, and empty pcDEF3 plasmid, and 50,000 cells per well were plated on a poly-l-lysine-coated white 96-well plate and grown for an additional 24 h. Cells were stimulated with increasing ligand concentrations for H3R and H2R in the presence of 1 μM forskolin at 37 °C and 5% CO₂. After 6 h, the medium was aspirated and 25 μL of luciferase assay reagent [0.83 mM adenosine 5’-triphosphate, 0.83 mM MgCl₂, 18.7 mM MgCl₂, 0.78 μM Na₃PO₄, 38.9 mM Tris–HCl (pH 7.8), 0.39% glycerol, 0.03% Triton-X 100, and 2.6 μM dithiothreitol] was added to each well. After 30 min of incubation at 37 °C, luminescence was measured with a Mithras plate reader (Berthold, Germany).

**Data Analysis.** Data were analyzed using GraphPad prism 7.02 (GraphPad Software Inc., San Diego). Shown data are mean ± S.E.M. of three individual experiments performed in triplicate unless stated otherwise. Competition binding curves were fitted to a one-site binding model. Obtained IC₅₀ values were converted into pIC₅₀ values using the Cheng–Prusoff equation.61 Dose–response curves were fitted using nonlinear regression.

**Metabolic Stability.** The pharmacokinetic parameters of 14d and the reference drug verapamil were estimated by using rat liver microsomes (RLMs) or mouse liver microsomes (MLMs) obtained from Sigma-Aldrich (St. Louis, MO). The tested compounds (50 μM) were incubated in the presence of microsomes (1 mg/mL) for 5, 15, 30, and 45 min in 10 mM Tris–HCl buffer (pH = 7.4) at 37 °C. Cold methanol with an internal standard (IS) was added to terminate each reaction. Next, the reaction mixtures were centrifuged at 14 500 rpm. The disappearance of the tested compounds in time was calculated by the UPLC/MS Waters ACQUITY TQD system with a TQ Detector (Waters, Milford). The course of the reaction was followed by using the analyte/IS peak height ratio values. For the determination of the t₁/₂ value, the slope of linear regression from log concentration remaining versus time relationships (−k) was used according to Obach66 (eq 1)

\[
t_{1/2} = \frac{-0.683}{k}
\]

Conversion of t₁/₂ to intrinsic clearance Clᵢn in [units of mL/(min·kg)] was done by using eq 2

\[
Clᵢn = \frac{0.693 \times mL \text{ incubation} \times mg \text{ microsomes}}{t_{1/2} \times mg \text{ liver}} \times \frac{g \text{ liver}}{kg \text{ b.w.}}
\]

where 45 mg of microsomal protein per gram of liver tissue (g liver) and 87 g of liver per kilogram of body weight (kg b.w.) were applied to calculate Clᵢn in mice, whereas 61 mg of microsomal protein per g liver and 45 g of liver per kg b.w. were applied to calculate Clᵢn in rats, according to Huang et al.62 and Smith et al.63

**Effect on CYP.** Luminescent CYP3A4, CYP2D6, and CYP2C9 P450-Glo assays and protocols were obtained from Promega (Madison, WI). Compound 14d was tested in triplicate at the final concentrations in range from 0.01 to 25 μM. The luminescent signal was measured by using a microplate reader EnSpire PerkinElmer (Waltham, MA).

**Social Recognition Test.** Male C57BL/6 mice (8–9 weeks old) behavior was assessed in a test apparatus comprising an open-field pleiexglass arena (45 × 25 cm² and 20 cm high) placed in a sound-attenuated room. The assay paradigm comprises three sessions. In the first session, mice were placed in the arena containing two empty pencil-wire cups placed on opposing sides and left free to explore for 10 min. Twenty-four hours after this session, a juvenile mouse (stimulus, 4–5 weeks old), which had no prior contact with the
subject mice, was placed under one of the wire cups while the other cup remained empty. The subject mouse was then placed in the arena and was left free to explore for 10 min. During the third session, performed 1 h later, the same stimulus animal was again placed under the wire cup and a novel unfamiliar juvenile mouse was placed under the opposing cup. Subject mice were then placed again in the arena and tested for discrimination between novel and familiar mice in a 10 min session. Each mouse was subjected to the procedure separately, and care was taken to remove any olfactory/taste cues by cleaning carefully the arena and wire cups between trials. The positions of the social stimuli (empty × social; familiar × novel) were counter-balanced across subjects and trials to prevent bias from place preference. Stimulus mice were habituated to remain under the wire cups several days before behavioral testing. Vehicle or 14d (5 mg/kg) was injected systemically (i.p.) 30 min before the second session. The animal's behavior during all sessions was videotaped, and the time spent actively exploring the stimuli was analyzed by experienced observers unaware of the experimental groups. Exploration was defined as direct snout-to-cup contact, and the time spent climbing on the cups was not considered. Data are expressed as a percentage of time spent exploring each cup (social × nonsocial during the second session or familiar × novel during the third session), and statistical significance was determined by the two-way ANOVA followed by Bonferroni's test. We also determined a sociability index, calculated according to the formula \( \text{time exploring social cup (TS)} + \text{total exploration time (TS + TNS)} \), and a discrimination index (DI), which was calculated according to the formula \( \text{time exploring the novel mouse (TN) + total exploration time (TNS + TN)} \), both analyzed using unpaired t-tests.

**Computational Studies.** Residue Numbering. Residue numbering is displayed throughout the manuscript as absolute sequence numbers and with generic numbering from GPCRdb also in superscript, in which the first number denotes the helix, 1–8, and the second number denotes the residue position relative to the most-conserved residue, defined as number 50, in a gapped sequence alignment.

**Homology Modeling.** A three-dimensional model of the H2R was constructed on Modeller v9.15.56 based on the crystal structure of H2R (PDB ID: 3RZE).28 The sequence of H2R was obtained from UniProt66 and aligned to the crystal structure sequence based on the structure-based alignment of GPCRdb. An optimal structure was selected based on its ability to retrospectively discriminate between known H2R fragmentlike ligands and true inactives as described elsewhere.68 Docking. A conformational library of all of the compounds was obtained with Corina v3.49 and protonated in ChemAxon Calculator.66 The most energetically favorable conformations were docked using PLANTS v1.69 Hundred docking poses were generated per conformation and postprocessed with interaction fingerprints (IPFs) inferred from OpenEye's OChem 1.3 library.67,68 IPFs are bit vectors that are switched off (0) or on (1) depending on the occurrence of predefined intermolecular interactions [apolar, face-to-face, and face-to-edge aromatic interactions, hydrogen bonds (acceptor or donor), and ionic interactions (cationic or anionic)]

**Molecular Dynamics Simulations and Analysis.** Ligands were parametrized using the AM1-BCC charges in Antechamber.72 The selected models were energy minimized to optimize protein–ligand interactions and used to run membrane-embedded MD simulations in GROMACS.73 Each system was simulated for 100 ns after an equilibration of 5 ns, with the parameters and conditions described elsewhere.7 Potential energy, root-mean-square deviation, root-mean-square fluctuation, and dihedrals of the simulations were analyzed with GROMACS tools, and residue interactions were analyzed with IPFs.

**Nephelometry.** In transparent flat-bottom 96-well plates, 14d was placed at different concentrations in triplicate (10−2, 10−1.5, 10−2, 10−2.5, 10−3, 10−3.5, 10−4, and 10−4.5 M) in Tris–HCl binding buffer (50 mM Tris–HCl, pH 7.4) at least 1 h before the measurement. A Kaolin dispersion was used as a positive control75 in each plate at different concentrations (10−2.5, 10−3, 10−3.5, 10−4, 10−4.5, and 10−5.5 M) under the same conditions as with compound 14d. Nephelometry measurements were performed with a NEPHELO star Plus (BMG Labtech, Germany) with the following settings: one cycle, measurement start time 0.1 s, measurement interval time 0.1 s, laser intensity 80%, beam focus 2.0 mm, orbital shaking mode at 200 rpm with an additional shaking time of 10 s before each cycle. Results were analyzed using Matlab R2014A (8.3.0.532) software, plotting all available data points and plotting mean and standard deviation values in a line chart compared to the Kaolin control. The linear fit (R2) of the Kaolin control was above 0.985 in all cases.
mg, 22%). mp: 157.0–157.6 °C. 1H NMR (600 MHz, CD3OD) δ 5.58 (s, 1H), 4.25 (t, J = 8.1 Hz, 2H), 3.95–3.85 (m, 1H), 3.72 (dd, J = 9.0, 5.2 Hz, 2H), 2.64 (hept, J = 7.0 Hz, 1H), 1.21 (d, J = 6.9 Hz, 6H). 13C NMR (151 MHz, CD3OD) δ 175.4, 165.7, 164.0, 89.8, 59.9, 44.1, 36.6, 22.0. HPLC-MS (basic mode): tR = 2.9 min, purity: > 99%, [M + H]+: 208.1577, found 208.1564.

4-Isopropyl-6-(3-(methylamino)azetidin-1-yl)pyrimidin-2-amine (12b). To a solution of carbamate 22b (450 mg, 1.46 mmol) in MeOH (20 mL) was added aq HCl (37%, 0.36 mL, 4.35 mmol). The reaction mixture was stirred at rt overnight. The solvents were removed under reduced pressure. The residue was dissolved in hot EtOH (5 mL), and after addition of EtOAc (15 mL), a precipitate formed. The formed solid was collected by filtration, washed, and dried in vacuo. Dissolving in H2O (5 mL) and freeze-drying gave the title compound as a white fluffy solid (150 mg, 50%). mp: 208.8–203.3 °C. 1H NMR (600 MHz, CD3OD) δ 8.59 (s, 1H), 4.51–4.39 (m, 2H), 4.11 (dd, J = 10.1, 3.5 Hz, 2H), 3.96 (br, 1H), 2.58 (q, J = 7.9 Hz, 2H), 2.55 (s, 1H), 1.27 (t, J = 7.6 Hz, 3H). 13C NMR (151 MHz, CD3OD) δ 164.1, 160.4, 157.6, 92.6, 55.9, 50.4, 33.3, 27.2, 12.2. HPLC-MS (basic mode): tR = 2.6 min, purity: > 99%, [M + H]+: 208. HR-MS [M + H]+ calc for C9H16N5O+: 208.1557, found 208.1556.

4-Aminoazetidin-1-yl-6-ethylpyrimidin-2-amine (12a). To a solution of carbamate 22a (250 mg, 0.85 mmol) in MeOH (20 mL) was added aq HCl (37%, 0.21 mL, 2.54 mmol). The reaction mixture was stirred at rt overnight. The solvents were removed under reduced pressure. The residue was dissolved in DCM/MeOH (10:1, 40 mL). The pH was adjusted to above 10 with NH3 solution (7 N) in MeOH. The suspension was filtered. The solvents were removed under reduced pressure. The crude product was dissolved in hot MeOH (10 mL), and after addition of EtOAc (15 mL), a precipitate formed. The formed solid was collected by filtration, washed, and dried in vacuo. Dissolving in H2O (5 mL) and freeze-drying gave the title compound as a white fluffy solid (95 mg, 58%). mp: 224.7–225.9 °C. 1H NMR (600 MHz, CD3OD) δ 5.83 (s, 1H), 4.51–4.37 (m, 2H), 4.16–4.02 (m, 3H), 2.50 (q, J = 7.6 Hz, 2H), 1.18 (t, J = 7.6 Hz, 3H). 13C NMR (151 MHz, CD3OD) δ 164.1, 160.2, 157.4, 92.7, 56.5, 42.6, 27.1, 12.2. HPLC-MS (basic mode): tR = 2.3 min, purity: > 99%, [M + H]+: 194. HR-MS [M + H]+ calc for C10H18N5O+: 194.1400, found 194.1401.

The title compound was obtained as a white solid (25 mg, 45%). mp: 226.2 °C. MeOH (4:1, 12 mL). The suspension was stirred at rt overnight. The solvents were removed under reduced pressure. The residue was dissolved in MeOH (4 mL) and freeze-drying gave the title compound as a white solid (150 mg, 50%). mp: 222.8–223.7 °C. 1H NMR (600 MHz, CD3OD) δ 5.97 (s, 1H), 4.63 (br, 1H), 4.57 (br, 1H), 4.46 (br, 1H), 4.37 (br, 1H), 4.35–4.27 (m, 1H), 3.13 (q, J = 7.2 Hz, 2H), 2.87 (t, J = 6.9 Hz, 1H), 1.38 (t, J = 7.2 Hz, 3H), 1.32 (d, J = 6.9 Hz, 6H). 13C NMR (151 MHz, CD3OD) δ 164.2, 163.6, 157.0, 91.5, 54.8, 54.4, 48.2, 42.4, 33.3, 20.9, 11.7. HPLC-MS (basic mode): tR = 2.3 min, purity: > 99%, [M + H]+: 236. HR-MS [M + H]+ calc for C10H18N5O+: 236.1870, found 236.1868.

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min, purity: >99%; [M + H]+: 222. HR-MS [M + H]+ calc for C7H12N2O2: 222.1713, found 222.1705.

4-(3-Aminooazidin-1-yl)-6-methylpyrimidin-2-amine (13a). To a solution of carbamate 23a (723 mg, 2.59 mmol) in MeOH (25 mL) was added aq HCl (37%, 3.26 mL, 39.4 mmol). The mixture reaction was stirred at rt overnight. The solvents were removed under reduced pressure. The residue was dissolved in DCM/MeOH (15:1, 40 mL). The pH was adjusted to above 10 with NH4 solution (7 N) in MeOH. The suspension was filtered. The solvents were removed under reduced pressure. The crude product was recrystallized from EtOH. The crystals were collected by filtration, washed, and dried in vacuo. Dissolving in H2O (5 mL) and freeze-drying gave the title compound as a white fluffy solid (189 mg, 43%). 1H NMR (600 MHz, CD3OD) δ 8.89 (s, 1H), 7.40–7.44 (m, 2H), 7.15 (s, 1H), 3.83 (m, 2H), 3.19 (q, J = 7.1 Hz, 2H), 2.26 (s, 3H), 2.72 (s, 3H). 13C NMR (151 MHz, CD3OD) δ 164.0, 157.8, 155.9, 94.0, 57.4, 43.0, 19.3. HPLC-MS (basic method): tR = 2.0 min, purity: >99%; [M + H]+: 180. HR-MS [M + H]+ calc for C9H14N2O2: 180.1244, found 180.1284.

4-Methyl-6-(3-(methylamino)azetidin-1-yl)pyrimidin-2-amine (13b). A white solid (176 mg, quant.). mp: 220.5 °C. 1H NMR (600 MHz, CD3OD) δ 8.44 (m, 1H), 8.01 (s, 1H), 4.46 (dd, J = 7.3 Hz, 6.2 Hz, 2H), 4.18 (br, 2H), 4.06 (dd, J = 10.6, 6.4 Hz, 2H), 2.28 (s, 3H), 3.19 (q, J = 7.1 Hz, 2H), 2.26 (s, 3H). 13C NMR (151 MHz, CD3OD) δ 164.0, 158.2, 156.4, 142.4, 136.3, 95.8, 55.4, 54.9, 41.7. HPLC-MS (basic method): tR = 1.7 min, purity: 96.1%; [M + H]+: 180. HR-MS [M + H]+ calc for C9H14N2O2: 180.1244, found 180.1284.

4-(3-Ethylamino)azetidin-1-yl)-6-methylpyrimidin-2-amine (13c). 1H NMR (600 MHz, CD3OD/D2O) δ 8.44 (m, 1H), 7.44–7.43 (m, 2H), 7.10 (s, 1H), 3.89–3.90 (m, 1H), 3.23 (s, 3H), 2.79 (s, 3H), 2.10 (t, J = 7.2 Hz, 2H), 2.26 (s, 3H), 1.20 (t, J = 7.2 Hz, 3H). 13C NMR (151 MHz, CD3OD) δ 164.0, 158.2, 156.4, 142.4, 136.3, 95.8, 55.4, 54.9, 41.7. HPLC-MS (basic method): tR = 2.6 min, purity: 95.6%; [M + H]+: 180. HR-MS [M + H]+ calc for C9H14N2O2: 180.1244, found 180.1284.

4-(3-Dimethylamino)azetidin-1-yl)-6-methylpyrimidin-2-amine (13d). Free base 4-methyl-6-(3-(dimethylamino)azetidin-1-yl)pyrimidin-2-amine (160 mg, 0.83 mmol) was obtained from 13b by neutralization with NH4 solution (7 N) in MeOH and filtration of the inorganic salt, followed by evaporation of the solvent. The residue was dissolved in MeOH (5 mL). To this were added formaldehyde (37%, 0.33 mL, 3.99 mmol). The mixture reaction was stirred for 3 h at rt. The reaction mixture was filtered to collect the white solid (250 mg, 53%). 1H NMR (600 MHz, CD3OD) δ 8.18 (s, 1H), 7.26 (s, 1H), 4.46 (dd, J = 11.1, 7.6 Hz, 2H), 4.18 (dd, J = 11.2, 4.5 Hz, 2H), 4.13–4.06 (m, 1H), 2.62 (s, 3H). 13C NMR (151 MHz, CD3OD/D2O) δ 175.2, 163.3, 157.7, 146.0, 136.5, 95.5, 54.9, 49.5, 31.8. HPLC-MS (basic method): tR = 2.1 min, purity: 95.5%; [M + H]+: 180. HR-MS [M + H]+ calc for C9H14N2O2: 180.1244, found 180.1284.

4-(3-Ethylamino)azetidin-1-yl)-6-methylpyrimidin-2-amine Fumarate (14a). To a solution of carbamate 24a (433 mg, 1.63 mmol) in MeOH (25 mL) was added aq HCl (37%, 0.74 mL, 8.94 mmol). The reaction mixture was stirred at rt overnight. The solvents were removed under reduced pressure. The residue was dissolved in DCM/MeOH (4:1, 25 mL). The pH was adjusted to above 10 with NH4 solution (7 N) in MeOH. The suspension was filtered. The solvents were removed under reduced pressure. The free base (248 mg, 1.50 mmol) was dissolved in MeOH (4 mL), and fumaric acid (88 mg, 0.75 mmol) in MeOH (1 mL) was added. The clear solution was concentrated until a suspension formed. The suspension was cooled overnight at 5 °C. The formed solid was collected by filtration, washed, and dried in vacuo. Dissolving in H2O (5 mL) and freeze-drying gave the title compound as a white fluffy solid (134 mg, 37%). The base-to-fumaric-acid ratio was 1:0.5 based on the 1H NMR peak integration. mp: 222.7–224.1 °C. 1H NMR (600 MHz, CD3OD/D2O) δ 7.62 (d, J = 7.3 Hz, 1H), 6.45 (s, 1H), 6.01 (d, J = 7.3 Hz, 1H), 4.57 (br, 2H), 4.38–4.15 (m, 3H). 13C NMR (151 MHz, CD3OD/D2O) δ 174.7, 162.9, 155.8, 142.4, 136.3, 95.8, 55.4, 54.9, 41.7. HPLC-MS (basic method): tR = 1.7 min, purity: 96.4%; [M + H]+: 180. HR-MS [M + H]+ calc for C9H14N2O2: 180.1244, found 180.1284.
The base-to-fumaric-acid ratio was 1:0.75 based on the $^1$H NMR peak integration. mp: 169.6–169.9 °C. $^1$H NMR (500 MHz, CD$_3$OD/CD$_2$OD) $\delta$ 7.72–7.66 (m, 2H), 6.66 (1H), 6.06 (2J, $\neq$ 7.2 Hz, 2H), 4.54 (2J, $\neq$ 7.2 Hz, 2H), 4.27–4.21 (m, 1H), 3.02–2.95 (m, 2H), 1.72–1.64 (m, 2H), 1.43 (6J, $\neq$ 7.4 Hz, 3H), $^{13}$C NMR (151 MHz, CD$_3$OD/CD$_2$OD) $\delta$ 170.0, 163.6, 156.7, 143.1, 135.8, 95.5, 54.7, 48.6, 47.0, 29.6, 20.9, 13.9. HPLC-MS (basic mode): $t_{R}$ = 3.1 min, purity: 94.6%, [M + $^{+}$H$^+$]: 222. HM-MS [M + $^{+}$H$^+$]$^+$ for C$_{11}$H$_{17}$N$_5$O$_2$: 222.1713, found 222.1705.

4-(3-((sec-Butylamino)azetidin-1-yl)pyrimidin-2-amine Fumarate (14g). To a solution of free base 14a (100 mg, 0.60 mmol) in DCM (4 mL) and MeOH (1 mL) were added iso-butylaldehyde (66 μL, 0.66 mmol) and AcOH (34 μL, 0.60 mmol). After 10 min of stirring at rt, NaBH(OAc)$_2$ (201 mg, 0.90 mmol) was added, and the resulting mixture was stirred at rt overnight. The reaction mixture was quenched with 5 M aq NaOH (two drops). The solvents were removed under reduced pressure. The residue was purified by column chromatography (DCM/MeOH/TEA 100:0:0–80:18:2). The selected fractions were collected, and the solvents were removed under reduced pressure. The residue was dissolved in DCM/MeOH (9:1, 10 mL) and washed with satd. aq Na$_2$CO$_3$ solution (10 mL). The aqueous layer was extracted with DCM/MeOH (9:1, 2 × 5 mL). The combined organic phases were dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The free base (25 mg, 0.113 mmol) was dissolved in MeOH (1 mL) and fumaric acid (13 mg, 0.113 mmol) in MeOH (1 mL) was added. After partial evaporation of MeOH and addition of EtOAc (5 mL), a precipitate formed. The suspension was cooled for 1 h at 5 °C. The crystals were filtered, washed with EtOH, and dried in vacuo. Dissolving in H$_2$O (20 mL) and freeze-drying gave the title compound as a white fluffy solid (803 mg, 58%). The base-to-fumaric-acid ratio was 1:1 based on the $^1$H NMR peak integration. mp: 219.0–219.3 °C. H NMR (500 MHz, CD$_3$OD/CD$_2$OD) $\delta$ 2.09–2.02 (m, 4H), 1.86–1.81 (m, 4H), 1.79–1.74 (m, 4H), 1.69–1.64 (m, 4H), 1.58–1.53 (m, 4H), 1.49–1.44 (m, 4H), 1.36–1.31 (m, 4H), 1.29–1.24 (m, 4H), 1.27–1.22 (m, 4H), 1.24–1.19 (m, 4H). $^{13}$C NMR (125 MHz, CD$_3$OD/CD$_2$OD) $\delta$ 174.5, 174.1, 163.2, 156.9, 146.3, 143.8, 137.6, 95.6, 95.5, 56.0, 55.0, 54.9, 46.2, 46.1, 20.9, 19.8 (multiple sets observed). HPLC-MS (basic mode): $t_{R}$ = 3.0 min, purity: 95.6%, [M + $^{+}$H$^+$]: 222. HR-MS [M + $^{+}$H$^+$]$^+$ for C$_{11}$H$_{17}$N$_5$O$_2$: 222.1713, found 222.1715.
NaBH(OAc)₃ (108 mg, 0.68 mmol) was added, and the resulting reaction mixture was quenched with 5 M aq NaOH (two drops). The solvents were removed under reduced pressure. The residue was dissolved in DCM/MeOH (9:1, 20 mL) were added formaldehyde (37%, 70 μL, 0.94 mmol) and AcOH (20 μL, 0.34 mmol). After 10 min of stirring at rt, NaBH(OAc)₃ (402 mg, 1.80 mmol) was added, and the resulting mixture was stirred at rt overnight. The reaction mixture was quenched with 5 M aq NaOH (two drops). The solvents were removed under reduced pressure. The residue was dissolved in DCM/MeOH (9:1, 20 mL). The pH was adjusted to above 10 with NH₄ solution (7 N) in MeOH. The suspension was filtered. The solvents were removed under reduced pressure. The crude free base (25 mg, 0.130 mmol) was dissolved in MeOH (5 mL) and fumaric acid (7.5 mg, 0.065 mmol) in MeOH (2.5 mL) was added. The solvent was evaporated until a suspension formed. The suspension was cooled overnight at 5 °C. The formed solid was collected by filtration, washed, and dried in vacuo. Dissolving in H₂O (5 mL) and freeze-drying gave the title compound as a white fluffy solid (31 mg, 36%). The base-to-fumaric-acid ratio was 1:0.5 based on the 1H NMR peak integration. mp: 229.6 °C. 1H NMR (500 MHz, CDCl₃) δ 7.37 (d, J = 7.0 Hz, 1H), 6.60 (s, 1H), 5.98 (d, J = 7.0 Hz, 1H), 4.34–4.27 (m, 2H), 4.08 (dd, J = 10.8, 4.9 Hz, 2H), 3.62–3.53 (m, 1H), 2.36 (s, 6H). 13C NMR (151 MHz, CDCl₃, CDOD/DO) δ 176.0, 163.2, 157.8, 145.6, 136.7, 95.4, 56.4, 54.4, 41.5. HPLC-MS (basic mode): tᵣ = 2.8 min, purity: >99%, [M + H]⁺: 199. HR-MS [M + H]⁺ calc’d for C₁₀H₁₄N₅O₂: 198.1390, found 198.1388.

4-Chloro-6-isopropylpyrimidin-2-amine (17). Pyrimidin-4(3H)-one (15) (4.87 g, 31.8 mmol) was dissolved in POCl₃ (40 mL, 0.43 mol). The mixture was heated to reflux for 3 h. The solvent was removed under reduced pressure. Ice (150 g) was carefully added to the residue. The pH of the mixture was adjusted to 9–10 with aq NaOH (2.5 M). The mixture was extracted with DCM (3 × 100 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH 10:0–9:1) gave the title compound as an off-white solid (1.41 g, 26%). 1H NMR (500 MHz, CDCl₃) δ 6.53 (s, 1H), 5.33 (br, 2H), 2.77 (hept, J = 6.9 Hz, 1H), 1.22 (d, J = 6.9 Hz, 6H). HPLC-MS (acidic mode): tᵣ = 3.5 min, purity: 96.8%, [M + H]⁺: 172.

4-Chloro-6-ethylpyrimidin-2-amine (18). Pyrimidin-4(3H)-one (16) (6.13 g, 44.1 mmol) was dissolved in POCl₃ (50 mL, 0.54 mol). The mixture was heated to reflux for 3 h. The solvent was removed under reduced pressure. Ice (150 g) was carefully added to the residue. The pH of the mixture was adjusted to 9–10 with aq NaOH (2.5 M). The mixture was extracted with DCM (3 × 10 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH 10:0–9:1) gave the title compound as an off-white solid (3.63 g, 52%). 1H NMR (500 MHz, CDCl₃) δ 6.53 (s, 1H), 5.43 (br, 2H), 2.57 (q, J = 7.6 Hz, 2H), 1.23 (t, J = 7.6 Hz, 3H). HPLC-MS (acidic mode): tᵣ = 3.0 min, purity: >99%, [M + H]⁺: 158.

tert-Butyl (1-(2-Amino-6-isopropylpyrimidin-4-yl)azetidin-3-yl)carbonate (21a). A microwave vial charged with chloroform 17 (400 g, 2.33 mmol), carbamate 28a (402 mg, 3.23 mmol), and dioxane (10 mL) was heated for 120 min at 150 °C under microwave irradiation. The reaction mixture was diluted with water (20 mL) and extracted with DCM (3 × 20 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH/TFA 100:0–0:98:2) gave the title compound as a white fluffy solid (30 mg, 44%). The base-to-fumaric-acid ratio was 1:1 based on the 1H NMR peak integration. mp: 229.6–229.8 °C. 1H NMR (600 MHz, CDCl₃/CDOD/DO) δ 7.74 (d, J = 7.1 Hz, 1H), 6.64–6.55 (m, 2H), 6.10 (dd, J = 7.2, 4.0 Hz, 1H), 4.62–4.51 (m, 2H), 4.50–4.41 (m, 2H), 4.41–4.29 (m, 1H), 3.28–3.13 (m, 4H), 1.40–1.27 (m, 2H). 13C NMR (151 MHz, CDCl₃/CDOD/DO) δ 174.1, 173.8, 163.3, 157.0, 156.9, 144.1, 140.0, 136.6, 95.6, 95.5, 53.9, 52.7, 45.6, 45.4, 9.3, 9.2 (multiple sets observed). HPLC-MS (basic mode): tᵣ = 3.0 min, purity: >99%, [M + H]⁺: 222. HR-MS [M + H]⁺ calc’d for C₁₀H₁₂N₂O₂: 221.7133, found 221.7222.

4-Pyrrolidin-1-ylazetidin-1-ylpyrimidin-2-amine Fumarate (14k). To a solution of free base 14k (100 mg, 0.60 mmol) in MeCN (20 mL) were added 1,4-diiodobutane (0.095 mL, 0.72 mmol) and K₂CO₃ (166 mg, 1.20 mmol). The resulting mixture was heated at reflux for 16 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography (DCM/MeOH/TFA 100:0–0:80:18.2). The selected fractions were collected, and the solvents were removed under reduced pressure. The residue was dissolved in DCM/MeOH (9:1, 10 mL) and washed with satd. aq Na₂CO₃ solution (10 mL). The aqueous layer was extracted with DCM/MeOH (9:1, 2 × 5 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH/TFA 100:0–90:9:1) gave the title compound as a yellow oil (280 mg, 50%). 1H NMR (250 MHz, CDCl₃) δ 5.50 (s, 1H), 5.03 (br, 2H), 4.79 (br, 2H), 4.20 (t, J = 8.6 Hz, 2H), 4.07–
tert-Butyl (1-(2-Amino-6-isopropylpyrimidin-4-yl)azetidin-3-yl)-(ethyl)carbamate (21c). Carbamate 27c (1.02 g, 2.78 mmol) was dissolved in MeOH/EtOH (10:10 mL) and reacted with H₂ gas under atmospheric pressure using Pd/C (5%, 0.60 g) overnight at rt. The mixture was filtered over Celite, and the filtrate was concentrated in vacuo. The resulting yellowish oil (900 mg, a mixture of intermediate and diphenylmethane) was used in the next step without further purification. A microwave vial charged with chloride 19 (100.697 mmol), carbamate 28a (1.20 g, 6.97 mmol), DIPEA (1.23 mL, 6.99 mmol), and dioxane (20 mL) was heated for 60 min at 150 °C under microwave irradiation. The reaction mixture was diluted with water (40 mL) and extracted with DCM (3 × 40 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH 10:0–9:1) gave the title compound as a yellowish solid (726 mg, 36%). ¹H NMR (300 MHz, CDCl₃) δ 5.50 (s, 1H), 4.99 (br, 1H), 4.78 (br, 2H), 4.58 (br, 1H), 4.30 (t, J = 8.3 Hz, 2H), 3.83–3.73 (m, 2H), 2.20 (s, 3H), 1.45 (s, 9H). HPLC-MS (acidic mode): tf = 2.7 min, purity: 95.5%, [M + H]⁺: 280.

tert-Butyl (1-(2-Amino-6-methylpyrimidin-4-yl)azetidin-3-yl)-(methyl)carbamate (23b). A microwave vial charged with chloride 19 (287 mg, 2.00 mmol), carbamate hydrochloride 28b (445 mg, 2.00 mmol), DIPEA (0.70 mL, 4.00 mmol), and dioxane (4 mL) was heated for 60 min at 150 °C under microwave irradiation. The reaction mixture was diluted with water (15 mL) and extracted with DCM (3 × 10 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH 10:0–9:1) gave the title compound as a colorless oil (501 mg, 46%). ¹H NMR (300 MHz, CDCl₃) δ 5.58 (s, 1H), 4.99 (br, 1H), 4.78 (br, 2H), 4.58 (br, 1H), 4.31 (t, J = 8.3 Hz, 2H), 3.85–3.73 (m, 2H), 2.46 (q, J = 7.6 Hz, 2H), 1.45 (s, 9H), 1.20 (t, J = 7.6 Hz, 3H). HPLC-MS (acidic mode): tf = 3.0 min, purity: 94.5%, [M + H]⁺: 294.

tert-Butyl (1-(2-Amino-6-ethylpyrimidin-4-yl)azetidin-3-yl)-(ethyl)carbamate (23c). Carbamate 27c (1.02 g, 2.78 mmol) was dissolved in MeOH/EtOH (10:10 mL) and reacted with H₂ gas under atmospheric pressure using Pd/C (5%, 0.60 g) overnight at rt. The mixture was filtered over Celite, and the filtrate was concentrated in vacuo. The resulting yellowish oil (900 mg, a mixture of intermediate and diphenylmethane) was used in the next step without further purification. A microwave vial charged with chloride 19 (101 mg, 0.71 mmol), carbamate hydrochloride (287 mg, 1.00 mmol), DIPEA (0.123 mL, 0.71 mmol), and dioxane (3 mL) was heated for 90 min at 150 °C under microwave irradiation. The reaction mixture was diluted with water (10 mL) and extracted with DCM (3 × 10 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH 10:0–9:1) gave the title compound as a colorless oil (79 mg, 35% over two steps, extrapolated). ¹H NMR (300 MHz, CDCl₃) δ 6.03 (br, 2H), 5.46 (s, 1H), 5.06 (br, 1H), 4.73 (br, 1H), 4.25 (t, J = 8.7 Hz, 2H), 4.17–4.05 (m, 2H), 3.33 (q, J = 7.0 Hz, 2H), 2.76 (hept, J = 6.9 Hz, 1H), 1.46 (δ, 9H), 1.23 (d, J = 6.9 Hz, 6H), 1.14 (t, J = 7.0 Hz, 3H). HPLC-MS (acidic mode): tf = 3.6 min, purity: >99%, [M + H]⁺: 294.

tert-Butyl (1-(2-Amino-6-ethylpyrimidin-4-yl)azetidin-3-yl)-(methyl)carbamate (22b). A microwave vial charged with chloride 18 (500 mg, 3.17 mmol), carbamate 28b (546 mg, 3.17 mmol), DIPEA (0.55 mL, 3.17 mmol), and dioxane (11 mL) was heated for 45 min at 150 °C under microwave irradiation. The reaction mixture was diluted with water (50 mL) and extracted with DCM (3 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH 10:0–9:1) gave the title compound as a colorless oil (250 mg, 27%). ¹H NMR (300 MHz, CDCl₃) δ 5.49 (s, 1H), 4.99 (br, 1H), 4.78 (br, 2H), 4.58 (br, 1H), 4.31 (t, J = 8.3 Hz, 2H), 3.85–3.73 (m, 2H), 2.46 (q, J = 7.6 Hz, 2H), 1.45 (s, 9H), 1.20 (t, J = 7.6 Hz, 3H). HPLC-MS (acidic mode): tf = 3.0 min, purity: 94.5%, [M + H]⁺: 294.

tert-Butyl (1-(2-Amino-6-ethylpyrimidin-4-yl)azetidin-3-yl)-(ethyl)carbamate (22c). Carbamate 27c (1.02 g, 2.78 mmol) was dissolved in MeOH/EtOH (10:10 mL) and reacted with H₂ gas under atmospheric pressure using Pd/C (5%, 0.60 g) overnight at rt. The mixture was filtered over Celite, and the filtrate was concentrated in vacuo. The resulting yellowish oil (900 mg, a mixture of intermediate and diphenylmethane) was used in the next step without further purification. A microwave vial charged with chloride 18 (95 mg, 0.60 mmol), crude intermediate (221 mg), DIPEA (0.105 mL, 0.60 mmol), and dioxane (5 mL) was heated for 90 min at 150 °C under microwave irradiation. The reaction mixture was diluted with water (10 mL) and extracted with DCM (3 × 10 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography (DCM/MeOH 10:0–9:1) gave the title compound as a yellowish oil (92 mg, 42% over two steps, extrapolated). ¹H NMR (300 MHz, CDCl₃) δ 5.54 (s, 1H), 4.83 (br, 3H), 4.24 (t, J = 8.5 Hz, 2H), 4.12–3.96 (m, 2H), 3.36 (q, J = 7.1 Hz, 2H), 2.49 (q, J = 7.6 Hz, 2H), 1.48 (s, 9H), 1.29–1.11 (m, 6H). HPLC-MS (acidic mode): tf = 3.3 min, purity: >99%, [M + H]⁺: 322.
oil (501 mg, 46%). 1H NMR (300 MHz, CDCl3) δ 7.84 (d, J = 5.9 Hz, 1H), 5.63 (d, J = 5.8 Hz, 1H), 4.22 (t, J = 5.8 Hz, 2H), 4.09–3.98 (m, 2H), 2.93 (s, 3H), 1.47 (s, 9H). HPLC-MS (acidic mode): tR = 2.8 min, purity: >999%, [M + H]+: 328.

tert-Butyl (1-(2-Aminopyrimidin-4-yl)azetidin-3-yl)(ethyl)carbamate (27c). A solution of carbamate (2.09 g, 5.49 mmol) in MeOH (100 mL) was passed through an H-cube cartridge, and the combined organic phases were dried over Na2SO4, filtered, and concentrated in vacuo. The title compound was obtained as a white solid (2.09 g, 63%). 1H NMR (300 MHz, CDCl3) δ 7.84 (d, J = 5.9 Hz, 1H), 5.63 (d, J = 5.8 Hz, 1H), 4.22 (t, J = 5.8 Hz, 2H), 4.09–3.98 (m, 2H), 2.93 (s, 3H), 1.47 (s, 9H). HPLC-MS (acidic mode): tR = 3.9 min, purity: 99.6%, [M + H]+: 367.

tert-Butyl (1-(2-Aminopyrimidin-4-yl)azetidin-3-yl)(propyl)carbamate (27d). A solution of carbamate (2.09 g, 5.49 mmol) in MeOH (100 mL) was passed through an H-cube cartridge, and the combined organic phases were dried over Na2SO4, filtered, and concentrated in vacuo. The title compound was obtained as a white solid (2.09 g, 63%). 1H NMR (300 MHz, CDCl3) δ 7.84 (d, J = 5.9 Hz, 1H), 5.63 (d, J = 5.8 Hz, 1H), 4.22 (t, J = 5.8 Hz, 2H), 4.09–3.98 (m, 2H), 2.93 (s, 3H), 1.47 (s, 9H). HPLC-MS (acidic mode): tR = 3.9 min, purity: 99.6%, [M + H]+: 367.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.9b01462.

Nepholometry results of 14d; best-scored docking poses of 14d; functional assay on H2R and H4R; effect of 14d on CYP3A4, CYP2D6, and CYP2C9 activities; sociability effect of 14d in the social recognition test in mice; HPLC-MS chromatogram and spectra of 14d (PDF) Biochemical data of 1, 11a to 14k (XLS)

Molecular formula strings (CSV)

MD simulations of the model where the nPr group of 14d is pointing toward the extracellular vestibule (Movie 1)
MD simulations of the model where the nPr group of 14d is directed toward the intracellular half of the receptor (Movie 2) (MPG)

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Notes
The authors declare no competing financial interest.

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■ Abbreviations

α, intrinsic activity compared to histamine; cAMP, cyclic adenosine monophosphate; CRE, cAMP response element; DCM, dichloromethane; DipeA, N,N-disopropyllethylenimine; FLIPR, fluorometric imaging plate reader; GPCR, G protein-coupled receptor; GTPSγ, guanosine 5′-O-[γ-thio] triphosphate; IFF, interaction fingerprint; i.p., intraperitoneal; mp, melting point; MD, molecular dynamics; NAMH, Nα-methylhistamine; NMP, N-methyl-2-pyrrolidone; SAR, structure–activity relationship; satq. aq, saturated aqueous; S.D., standard deviation; S.E.M., standard error of mean; SFR, structure–function relationship; rt, room temperature; TEA, triethylamine; THF, tetrahydrofuran; μW, microwave reaction

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