4-Benzyl-1*H*-imidazoles with Oxazoline Termini as Histamine H_3 Receptor Agonists

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Received November 9, 2007

Research on the therapeutic applications of the histamine H_3 receptor (H_3R) has traditionally focused on antagonists/inverse agonists. In contrast, H_3R agonists have received less attention despite their potential use in several disease areas. The lower availability of H_3R agonists not only hampers their full therapeutic exploration, it also prevents an unequivocal understanding of the structural requirements for H_3R activation. In the light of these important issues, we present our findings on 4-benzyl-1*H*-imidazole-based H_3R agonists. Starting from two high throughput screen hits (**10** and **11**), the benzyl side chain was altered with lipophilic groups using combinatorial and classical chemical approaches (compounds **12**−**31**). Alkyne- or oxazolino-substituents gave excellent affinities and agonist activities up to the single digit nM range. Our findings further substantiate the growing notion that basic ligand sidechains are not necessary for H_3R activation and reveal the oxazolino group as a hitherto unexplored functional group in H_3R research.

Introduction

The neurotransmitter histamine exerts its physiological actions through four distinct GPCRs[a] known as the histamine H_1, H_2, H_3, and H_4 receptors.[1] The former two have already proven to be excellent drug targets, whereas the latter one was discovered seven years ago[2] and is currently the subject of explorative chemical and pharmacological endeavors.[3,4] The histamine H_3 receptor (H_3R) was discovered by classical pharmacological means in the early 1980s.[5] Evidence gathered over the years has revealed a prominent role of neuronal H_3Rs in modulation of histamine biosynthesis and release as well as in regulation of the release of other neurotransmitters such as dopamine, serotonin, and noradrenaline.[6,7] For about 20 years, all efforts to identify the gene encoding the H_3R were in vain. As a result, drug discovery efforts were seriously frustrated as no recombinant cell lines for HTS approaches were available. A breakthrough came in 1999 when Lovenberg and co-workers reported the cloning of the *c*DNA encoding the H_3R.[8] This breakthrough sparked fierce H_3R research in academia and industry alike.[9,10] Soon, the crucial finding of high H_3R constitutive activity was disclosed, leading to reclassification of H_3R ligands into inverse agonists, antagonists, and agonists.[11,12] Many therapeutic areas in which H_3R may play pivotal roles have since been unraveled, having placed the H_3R in a prominent position within the drug discovery community. More specifically, evidence has been rapidly accumulating that H_3R antagonists or inverse agonists potentially play crucial roles in combating obesity, narcolepsy, allergic rhinitis, cognitive disorders, and others.[10,13–15]

These exciting preclinical studies were to a great extent enabled by many effective antagonists and inverse agonists that emerged from HTS campaigns within pharmaceutical companies following the cloning of the receptor.[16] Similar development of agonists during this quest has been less widely pursued despite early successes with agonists such as **1** (BP 2-94)[17] and **2** (SCH50971)[18,19] in the 1990s (Scheme 1).[20] The main reason for this was undoubtedly the rather limited evidence for potential therapeutic areas of H_3R agonists when compared to antagonists or inverse agonists. Until recently, such evidence has been largely confined to the areas of ischemic arrhythmias, migraine, and asthma.[15] Some of these indications have borne fruit as, in 2006, the agonist N^{α}-methylhistamine (**3**) was the first H_3R ligand ever to complete phase III studies, namely for migraine prophylaxis.[21] However, during the last two years, the list of potential therapeutic areas for H_3R agonists has been expanded with three new interesting areas: obesity,[22] diabetes mellitus,[22] and liver cholestasis.[23] Recent evidence also shows that H_3R-KO mice develop more severe symptoms in experimental allergic encephalomyelitis, the autoimmune model of multiple sclerosis, thereby suggesting that H_3R agonists could be of therapeutic value in multiple sclerosis.[24] It is reasonable to suggest that all this will cause H_3R agonist research to gather more momentum in the near future.

From a fundamental point of view, a serious consequence of the lower availability of agonist studies is that the structural requirements for receptor activation are still not fully understood. Many models for H_3R binding and/or activation involve acid−base interaction of a protonated basic group in the ligand side chain to an aspartate residue in TM3 (D114),[28–30] which is highly conserved in GPCRs for biogenic neurotransmitter amines. Indeed, many known and widely used H_3R agonists have basic groups in their side chains that presumably bind this aspartate residue, see, for example, histamine itself, **3**, RAMH (**4**) and methimepip[25] (**5**) (Scheme 1). However, a handful of reports has challenged this popular hypothesis by disclosing H_3R agonists that have a less basic side chain (i.e., immethridine

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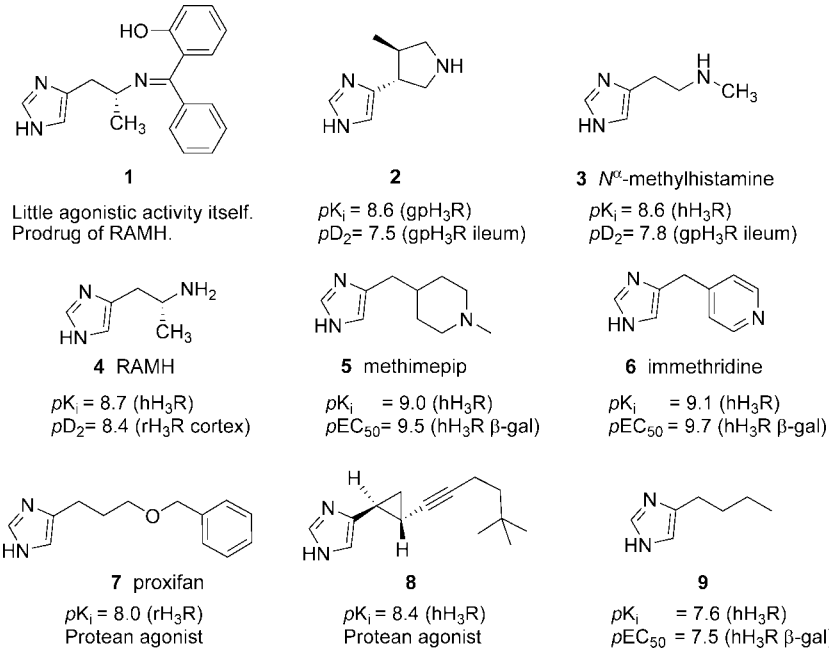
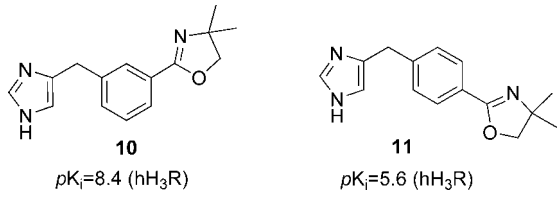
[a] Abbreviations: CADD, computer-assisted computational drug design; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; DAST, diethylaminosulfur trifluoride; DIC, *N*,*N*′-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; GPCR, G protein-coupled receptor; HBA, hydrogen-bond acceptor; HBD, hydrogen-bond donor; HTS, high throughput screen; IBS, imidazole-binding site; KO, knockout; P_1, *N*,*N*-dimethylsulfamoyl; RAMH, *R*-α-methylhistamine; r.t., room temperature; TEA, triethylamine; TFA, trifluoroacetic acid; TM, transmembrane.

10.1021/jm7014149 CCC: $40.75
Published on Web 04/24/2008

Scheme 1. Selected H_3R Agonists and Their Affinity/Activity Data as Extracted from the Literature[9,17,19,20,25–27]



Scheme 2. Structures and Affinities of HTS Hits



6)[26] or do not have a basic group in the side chain at all,[27,31–34] an example of which is proxifan (**7**). More remarkably, some of these compounds, i.e., **8** (GT-2331)[33] and **9** (VUF5523),[27] have strictly aliphatic side chains lacking any heteroatom (Scheme 1).

To shed more light on the structural determinants for H_3R activation and in view of the emerging reports on possible therapeutic uses for H_3R agonists, we wish to disclose our findings in the development of rigid H_3R agonists without basic or highly polar groups in the side chain. Starting from two HTS hits (**10** and **11**, Scheme 2), the side chain was systematically altered with predominantly lipophilic substituents (**12**−**31**). This exercise has not only afforded several novel H_3R agonists with affinities and activities in the single digit nM range but has also provided molecular clues on how agonists without a basic side chain are able to activate H_3R. Last, our results also reveal a hitherto unexplored functional group in H_3R research.

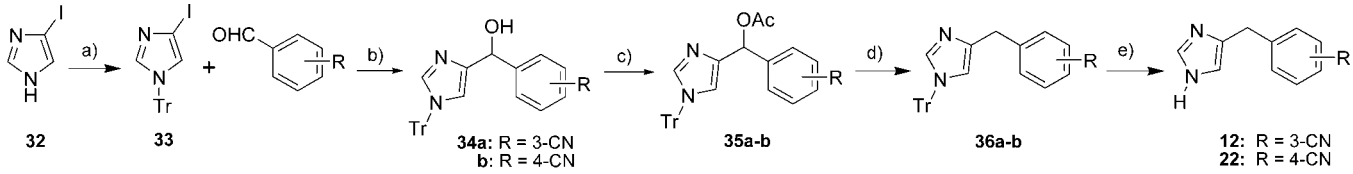
Chemistry

Noncombinatorial Chemistry. Nitrile-substituted compounds **12** and **22** were obtained using a Grignard addition route described by us previously (Scheme 3).[27] 4(5)-Iodo-1*H*-imidazole (**32**) was protected with a trityl group to give **33**. This was reacted with EtMgBr in DCM/Et_2O, and the resulting imidazolyl-Grignard reagent was added to the aldehyde group of the appropriately substituted cyanobenzaldehyde in good yields. The peculiar use of DCM and Et_2O as solvent in the initial Grignard exchange between **33** and EtMgBr is crucial, as more coordinating solvents led to rapid rearrangement of the intermediate 4-imidazolyl Grignard reagent to the undesired 2-isomer.[35] Acetylation of benzylalcohols **34a**,**b** and reduction of the acetate group in **35a**,**b** by hydrogenolysis afforded key intermediates **36a**,**b**, which after acidic deprotection gave **12** and **22**. For the synthesis of bromide-substituted compounds, the potential liability of the arylbromide in the acetate reduction and perhaps in the Grignard exchange prompted the design of an alternative route. Here, the Grignard intermediate was generated from P_1-protected 4-iodoimidazole **37** (see Scheme 4).[26] After a magnesium−copper exchange, the resulting cuprate was reacted with 4-bromo-benzylbromide to install the essential benzylic C−C bond of **38**. Following basic deprotection, target **39** was obtained but only in moderate yield (46%) over two steps. Therefore, in an attempt to improve the yield for the *para* counterpart **23**, we reverted to classical imidazole functionalization on P_1-protected imidazole **40**, as utilized by us before.[36] Key to that approach is the one-pot protection, metalation, and subsequent functionalization of the imidazole nucleus by repeated addition of *n*-BuLi and electrophiles. Acidic deprotection of intermediate **41** afforded target **23** in a yield much improved with respect to that of **39**.

Precursors **23**, **38**, and **39** afforded excellent entry into more elaborate functionalizations of the phenyl groups (Scheme 5). Using standard Suzuki conditions, the bromide group in **38** was exchanged for a 4-pyridyl or phenyl group by means of the boronic acid (for **42a**) or a cyclic boronic diol ester (for **42c**) in moderate to excellent yield. Given the known stability problems of 2-pyridine boronic acid,[37] we performed the C−C coupling for **42b** under Stille conditions using the corresponding stannane **43**. In all cases, final deprotection of the P_1 group to targets **14**−**16** was achieved using KOH in EtOH.

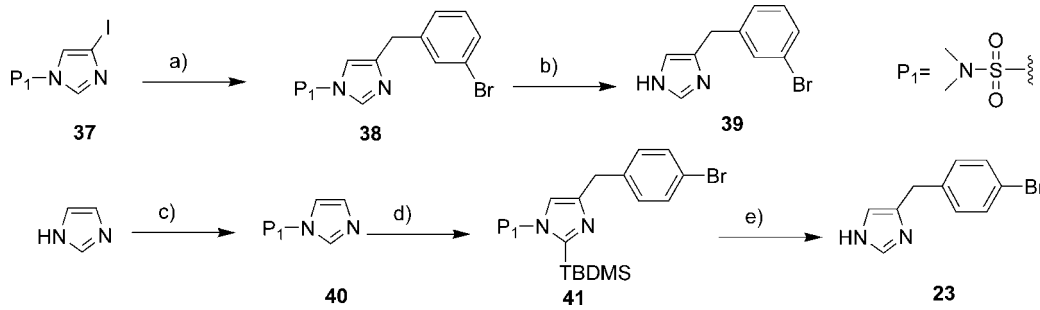
The bromide substituents in **39** and **23** were anticipated to provide excellent handles for alkyne substitution under classical Sonogashira couplings (Pd catalysis, Cu catalysis, TEA). Anticipating that a free imidazole group would be detrimental to this reaction because of its high affinity for Cu ions, we first installed electron-withdrawing groups on the imidazole ring (tosyl or P_1 group) in order to minimize this complexation. Indeed, compounds **44** and **38** underwent smooth Sonogashira couplings with 3,3-dimethyl-1-butyne or phenylacetylene to afford protected alkynes **45** and **46a**,**b**. Subsequent deprotection

Scheme 3. Synthetic Pathways for Compounds **12** and **22**[a]



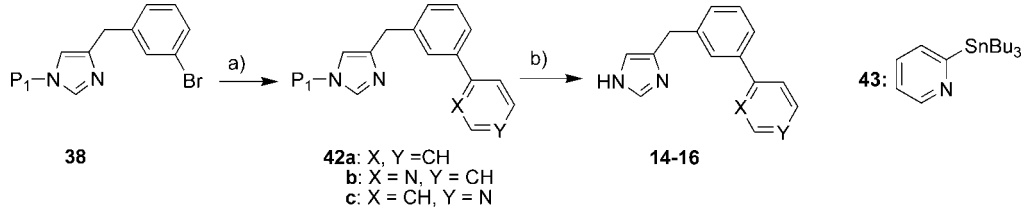
[a] Key: (a) Tritylchloride, TEA, THF, reflux, 1.5 h (64%). (b) [1] EtMgBr, Et_2O, DCM, r.t., 30 min [2] RPhCHO, r.t., 1 d (**34a**: 85%, **34b**: 70%). (c) Ac_2O, DMAP, TEA, DCM, r.t., 1 h (**35a**: 89%, **35b**: 87%). (d) 5% Pd/C, EtOH, H_2, r.t., 3 h (**36a**: 62%, **36b**: 78%). (e) HCl, EtOH, reflux, 1 h (**12**: 79%, **22**: 78%).

Scheme 4. Synthetic Pathway for Intermediate **39** and Compound **23**[a]



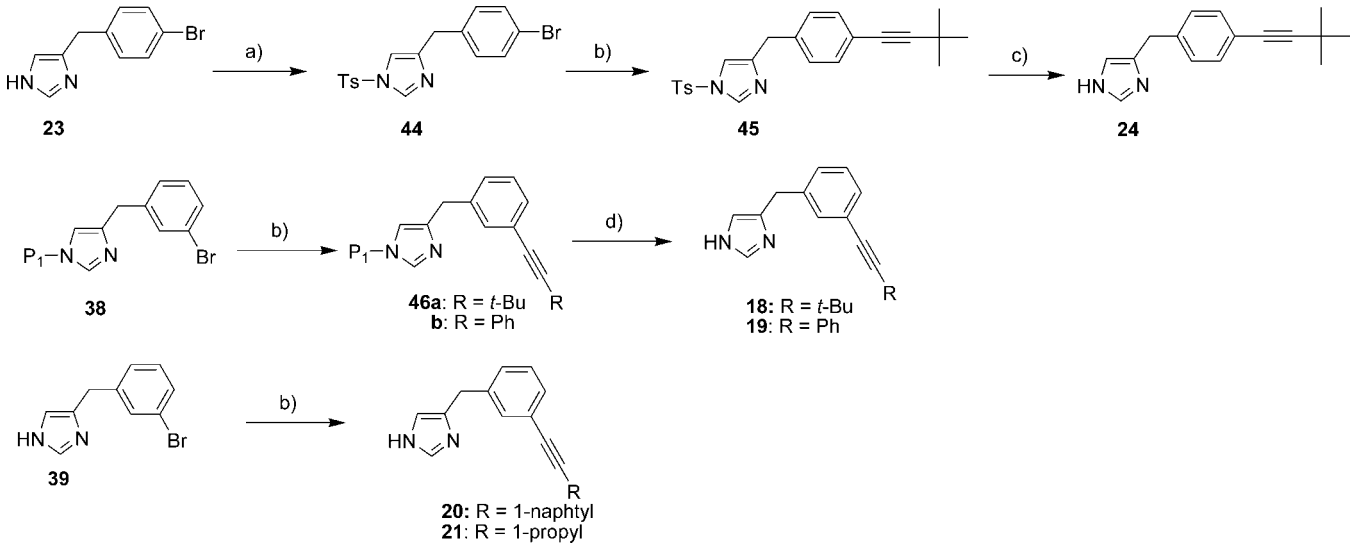
[a] Key: (a) [1] EtMgBr, Et_2O, DCM, r.t., 80 min [2] CuCN.2LiCl [3] 3-$BrPhCH_2Br$, r.t., 3 d (46%). (b) KOH, H_2O, EtOH, reflux, 1 d (quantitative yield). (c) *N*,*N*-dimethylsulfamoylchloride, TEA, DCM, r.t., 1 d (91%). (d) [1] *n*-BuLi, THF, −65 °C, 15 min. [2] *t*-$BuMe_2SiCl$, r.t., 1 h. [3] *n*-BuLi, THF, −65 °C, 30 min. [4] 4-$BrPhCH_2Br$, r.t., 1 d. (e) 30% aq HBr, reflux, 1 d (yield >90% for steps (d) and (e) combined).

Scheme 5. Synthetic Pathway for Compounds **14**−**16**[a]



[a] Key: (a) For **42a,c**: $ArB(OR)_2$, DMF/H_2O, Na_2CO_3, $Pd(PPh_3)_4$, 90 °C, 16 h. For **42b**: 2-tributylstannylpyridine (**43**), DMF, $Pd(PPh_3)_4$, 90 °C, 16 h (**42a**: 91%, **42b**: 30%, **42c**: 26%). (b) KOH, H_2O, EtOH, reflux, 1 d (**14**: 58%, **15**: 98%, **16**: 73%).

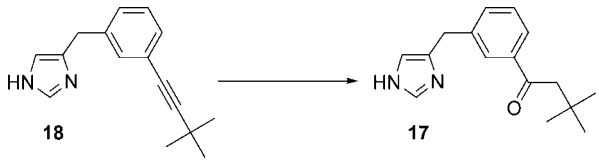
Scheme 6. Synthetic Pathway for Compounds **18**−**21** and **24**[a]



[a] Key: (a) TsCl, DCM, TEA, r.t., 4 d (94%). (b) RC≡CH, TEA, DMF, CuI, $Pd(PPh_3)_4$, 70 °C, 3 h, then: r.t., 1 d (**45**: 49%, **46a**: 56%, **46b**: 39%, **20**: 63%, **21**: 36%). (c) MeOH, 1 M NaOH, heat, 2 h (27%). (d) KOH, H_2O, EtOH, reflux, 1 d (**18**: 36%, **19**: 95%).

of these gave **24**, **18**, and **19** (Scheme 6). To our surprise, we later found that the imidazole group could be left unprotected in the Sonagashira coupling, and this protocol was used to couple bromide **39** with 1-ethynylnaphthalene and 1-pentyne rendering **20** and **21**. An attempt to cleave the *tert*-butyl group in **18** using TFA unexpectedly afforded the ketone **17**, which

Scheme 7. Synthetic Pathway for Compound **17**[a]



[a] Key: TFA, H_2O, 75 °C, 4 h (47%).

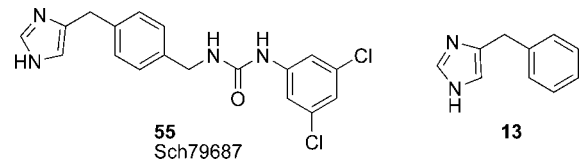
was also included in our assays (Scheme 7). While hydration of alkynes with aqueous acid is known to be a sluggish process in the absence of coreagents such as Hg salts, we assume it is the electron donating character of both the *tert*-butyl and the phenyl substituent that allows this hydration to proceed relatively mildly through stabilization of the intermediate vinyl cation.[38]

The noncombinatorial synthesis of compounds **10** and **26** involved preparation of a Grignard reagent from an appropriately substituted arylbromide **47a**,**b** followed by a subsequent addition to *N*-trityl-protected imidazole-carbaldehyde **48** (Scheme 8). Treatment of the intermediate alcohols **49a**,**b** with H_2/Pd under acidic conditions induced both reduction of the alcohol and cleavage of the trityl-protecting group to arrive at the target compounds.

Combinatorial Chemistry. Hit optimization of oxazoline **10** was done by a combinatorial chemistry approach. A total of 42 amino alcohols were converted into their corresponding oxazolines or oxazines as outlined in Scheme 9 for representative members **27**−**31**. Nitrile **36a** was hydrolyzed to the carboxylic acid **50**, which, without purification, was carried on to an acidic deprotection step rendering key precursor **51**. The imidazole group was again protected, this time using the more stable 4-methoxyphenylsulfonyl group, and the resulting molecule **52** was coupled to a pentafluorophenol-derivatized resin to give an activated carboxylic acid linked to solid support (**53**). Aminolysis of **53** with a variety of different amino alcohols afforded a library of amides (general structure **54**), which were subsequently cyclized using DAST[39] and deprotected with base to deliver the desired library.

Results and Discussion

HTS Hits and Design Strategy. At the start of this project, a HTS of the UCB compound database was initiated using a [^{3}H]-N^α-methylhistamine displacement assay. Two of the hits that emerged from this screen were compounds **10** and **11** (Scheme 2). These compounds have moderate to high affinity for hH_3R (pK_i hH_3R = 8.4 and 5.6, respectively). Their structures are composed of an imidazole ring linked to a dimethyloxazoline unit through a benzyl linker, i.e., are based on a 4-benzyl-1*H*-imidazole template. Compound **10** behaves as an efficient agonist on hH_3R (vide infra). Two interesting observations can be made with regard to the structure of this agonist. First, although the 4-benzyl-1*H*-imidazole template has occasionally been explored in a systematic H_3R SAR study,[40] the resulting lead compound (**55**) was reported to behave as an antagonist on hH_3R (human saphenous vein assay and guinea pig ileum electrical field stimulated contraction assay).[41] In fact, only one H_3R agonist with a 4-benzyl-1*H*-imidazole core is known, i.e., **13**.[27] Second, the oxazoline unit (a cyclic imidate) is not frequently encountered in medicinal chemistry. Its claim to fame is the use as a protecting group in organic synthesis. We launched a project in which both the 4-benzyl-1*H*-imidazole and oxazoline moieties were explored as a means to achieve efficient agonistic activity on hH_3R. Toward this end, we followed two main approaches: (A) replacement of the oxazoline ring by structurally distinct groups to explore the general propensity of substituted 4-benzyl-1*H*-imidazoles to activate H_3R and (B) substitution of the oxazoline ring with additional substituents or replacement by the closely related oxazines.



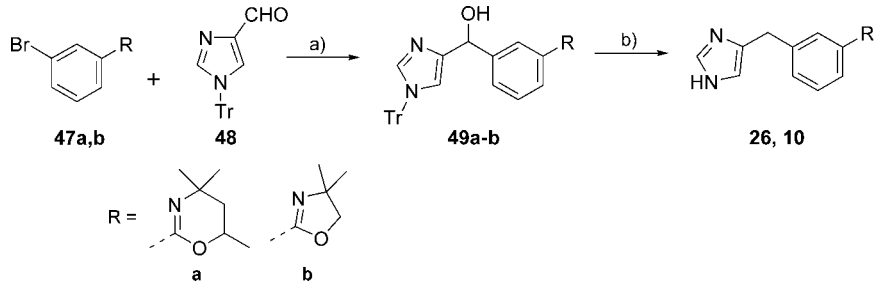
Approach A: Exploring the 4-Benzyl-1*H*-imidazole Core. The oxazoline substituent in hit **10** was replaced by other functional groups to get a better understanding of what variations are tolerated on the general 4-benzyl-1*H*-imidazole agonist core. This explorative strategy involved substituents such as nitriles, phenyls, and pyridines. We also included a series of compounds in which the benzyl linker was extended by one acetylene unit to further limit the flexibility of the side chain. Such a double-constraint strategy was used in optimizing H_3R affinity in the GT-2331 series, where a tandem cyclopropane and acetylene constraint worked excellently.[42,43] H_3R affinity was assayed using radiolabeled [^{3}H]-N^α-methylhistamine displacement on membranes from CHO cells stably expressing the H_3R. Compounds with pK_i values over 6.5 were also tested for their functional effects (G_i-mediated inhibition of cAMP production).

The effects of the *meta* substituents are presented in Table 1. A cyano-substituent (**12**) did not improve upon the affinity of unsubstituted compound **13**. However, attachment of a phenyl or 2-pyridyl group (**14** and **15**) increased the affinity equally by about 0.5 log unit with respect to that of **13**, while the 4-pyridyl group (as in **16**) is significantly less tolerated than the 2-pyridyl or phenyl group. All this indicates that the pyridine N-atom in **15** plays no important role in receptor affinity and that a more exposed polar group (as in **16**) is actually disfavored. It is of interest to note that this trend is the opposite of what has been observed in the immethridine series (**6**), which differs in that it lacks the middle phenyl group.[26,27] That is, replacement of the pyridine N-atom in immethridine by a CH group led to an almost 1000-fold drop in affinity. This opposite trend is a strong indication that the aryl side chain of the present compounds is targeting a different, more lipophilic pocket of the hH_3R than the side chain from immethridine. In line with this hypothesis was the affinity observed for **17**, where the incorporation of a lipophilic 3,3-dimethyl-1-butanoyl group was beneficial.

Gratifyingly, the double-constraint approach using the alkyne-extended system proved successful in the *meta* system. A variety of lipophilic endgroups comprising a *tert*-butyl, phenyl, or propyl group (**18**, **19**, **21**) was able to push the hH_3R affinity in the single digit nM range. Increasing the size of the group, however, was detrimental: a dramatic 1000-fold drop in affinity was observed for the 1-naphthyl derivative **20** with respect to phenyl derivative **19**. Taken together, these findings clearly indicate that the benzylalkyne linker in conjunction with a moderately sized lipophilic group induces efficient recognition of the postulated lipophilic pocket to achieve high H_3R affinities.

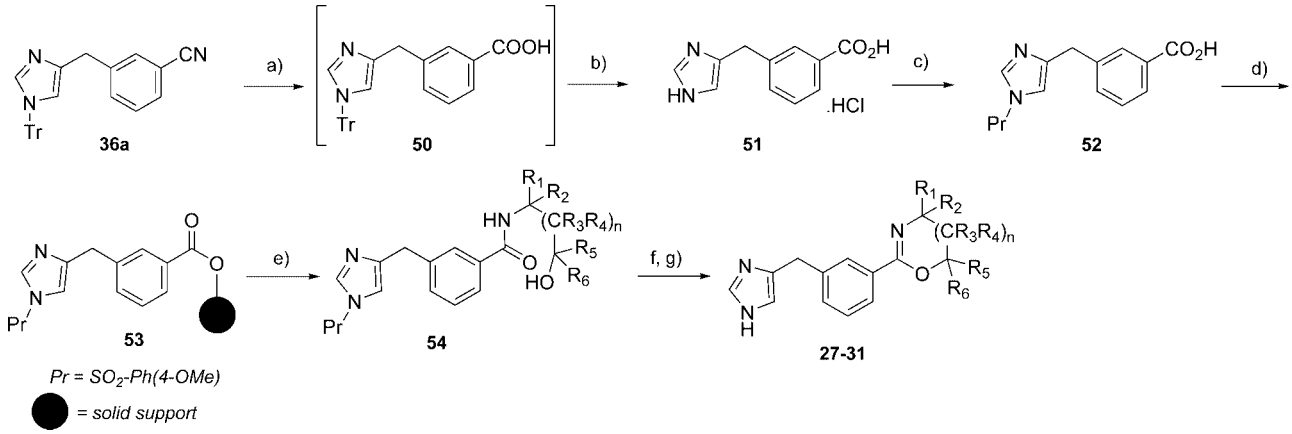
The 4-benzyl-1*H*-imidazole-containing ligands described by Schering−Plough[40,41] (i.e., **55**) all contain substituents on the *para* position. For purposes of comparison, we selected a concise set of compounds from Table 1 and tested the corresponding *para*-substituted compounds (Table 2). The large positional effect observed between our hits **10** and **11** proved to be a general effect: *meta* substitution is also preferred over *para*

Scheme 8. Representative Synthetic Pathway for Compounds **10** and **26**[a]



[a] Key: (a) [1] Mg, THF [2] aldehyde **48**, r.t., 0.5−13 h (**49a**: 71%, **49b**: 57%). (b) 80−135 psi H_2, 10% Pd/C, AcOH, 66 °C, 4 h (**26**: 65%, **10**: 19%).

Scheme 9. Representative Combinatorial Synthetic Pathway for Compounds **27**−**31**[a]



[a] (a) KOH, EtOH/H_2O, reflux, 18 h. (b) 1.0 M HCl, reflux, 1 h (55% over steps (a) and (b)). (c) 4-Methoxyphenylsulfonyl chloride, TEA, DCM, 0 °C−r.t., overnight (28%). (d) PS-TFP resin, DIC, DMAP, DCM/DMF, r.t., 18 h. (e) Amino alcohol $H_2NC(R_1R_2)(CR_3R_4)_nC(R_5R_6)OH$, DCM, DMF, r.t., 18 h. (f) DAST, DCM, r.t., 1.5 h. (g) [1] 1 N NaOH, MeOH, r.t., overnight. [2] Preparative LC-MS.

substitution in two other tested couples (**24** vs **18**, **22** vs **12**). In fact, *para* substitution with neither a small bromide, amino, or cyano substituent nor with a *tert*-butylacetylene group improved at all upon the affinity of unsubstituted compound **13**.

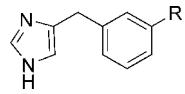
Approach B: Exploring the Oxazoline Substituent. Taking into account that a *meta*-positioned oxazoline unit leads to a highly efficient compound with unique structural properties, we unleashed a combinatorial chemistry approach to optimize the oxazoline substructure and explore the general effect of a *meta*-positioned cyclic imidate substituent (Scheme 10). This was achieved by preparing compounds containing oxazolines (general structure **56a**) or their six-membered counterparts (oxazines, general structure **56b**).

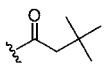
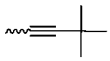
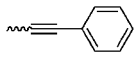
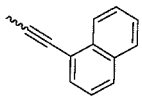
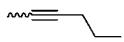
A total of 42 amino alcohols were selected based on their chemical diversity and with the aid of a pharmacophore model.[44] Of the library of 42 oxazolines and oxazines prepared and tested, six compounds had appreciable affinity for hH_3R (Table 3), while the others displayed inferior pK_i values, for example, compound **31**. It appears that dimethylsubstitution at C1 (see Scheme 10 for enumeration) is most beneficial for affinity, as indicated by **10** and **26** having excellent pK_i values of 8.44 and 8.90, respectively. A further increase in substituent size at C1, as in **27**, leads to a reduction in affinity. Likewise, monosubstitution at C1 with larger substituents (**29** and **30**) as well as disubstitution at C2 (**28** and **31**) are less favored. Of overall importance here is that the discussed six hits consist of both five-membered oxazolines and six-membered oxazines containing strictly lipophilic substituents. This suggests that the presence of a *meta*-positioned cyclic imidate moiety and of a small, suitably oriented lipophilic unit close to the N-atom on this imidate ring are both beneficial for appreciable affinity. Interestingly, when comparing approaches A and B, the cyclic imidate group can be considered a somewhat rigid linker between the phenyl group and a lipophilic end group.

Functional Activities on hH_3R and gpH_3R. While exploring the effect of structural modifications on the affinities of the ligands, we also closely monitored functional activities of our compounds at the G_i-coupled H_3R. Initial one-point screening of ligand activities in approach A was accomplished by determining inhibition of forskolin-stimulated cAMP production (see Tables 1 and 2). This screen revealed that, without exception, all compounds measured were agonists on the hH_3R. The same conclusion was reached for the compounds for approach B listed in Table 3, albeit that a different type of screen was used (GTPγS assay, see Table 4). Selected candidates from both approaches A and B were investigated in more detail for their functional properties on hH_3R (Table 4). All compounds display pEC_{50} values (GTPγS assay) that correspond acceptably with their pK_i values. The highest functional activities were observed for compounds **10**, **18**, and **26**, having excellent pEC_{50} values of 7.8, 8.1, and 8.7, respectively. The full dose−response curves for these three compounds (Figure 1) indicate that all behave as full agonists on hH_3R ($\alpha \approx 1$). Clearly, the compounds described here represent a significant addition to the growing list of efficient hH_3R agonists that lack basic side chains.[27,31–34]

Compounds **26**, **18**, **30**, and **17** were also tested on guinea pig H_3R (gpH_3R) by measuring their inhibitory effect on electrically evoked contractile response of isolated guinea pig intestine. The twitch contraction observed on the guinea pig isolated ileal myenteric plexus in response to electrical field stimulation is principally due to the release of acetylcholine from

Table 1. Affinities and Activities of *Meta*-Substituted 4-Benzyl-1*H*-imidazole on hH_3R (Approach A)

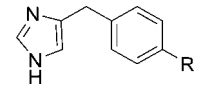


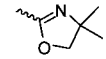
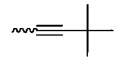
Cmpd	R	pK_i +/- SEM[a]	Activity +/- SEM[b]
10		8.40 +/- 0.01	95.0 +/- 0.2
12	CN	6.81 +/- 0.13	79.5 +/- 2.0
13	H	6.79 +/- 0.18	72.9 +/- 1.4
14		7.48 +/- 0.08	73.5 +/- 1.0
15		7.39 +/- 0.02	85.2 +/- 4.2
16		6.37 +/- 0.04	- [c]
17		7.49 +/- 0.06	38.3 +/- 6.6
18		8.35 +/- 0.01	88.8 +/- 0.9
19		8.74 +/- 0.10	93.3 +/- 0.7
20		5.76 +/- 0.02	- [c]
21		8.50 +/- 0.05	94.2 +/- 2.9
	clobenpropit	9.47 +/- 0.08	-62.8 +/- 14.2
	immepip	9.39 +/- 0.10	83.9 +/- 2.0

[a] Displacement of [^{3}H]-N^{α}-methylhistamine on membranes from CHO cells stably expressing the hH_3R ($n = 3$, SEM shown). [b] Defined as the percentage of reduction in cAMP levels in CHO cells stably expressing the human H_3R induced by treatment of 10^{-5} M of compound relative to untreated cells ($n = 3$, SEM shown). [c] Not determined. Reference values for H_3R inverse agonist clobenpropit and for H_3R agonist immepip are also given.

the cholinergic nerve terminals.[45,46] In this model, potent H_3R agonists such as RAMH, immethridine, immepip, or imetit induce a concentration-dependent decrease of the electrically induced twitch contraction through the activation of the histaminergic H_3 presynaptic receptor[47–50] present on this preparation ($pD_2 = 7.2$, 7.5, 8.0, and 7.9, respectively).[26] Under the same conditions, increasing concentrations of an H_3R antagonist such as thioperamide induced a rightward shift without any decrease of the maximal amplitude of the concentration−response curve to RAMH, suggesting the existence of a competitive inhibition between thioperamide and RAMH on the histamine H_3 receptors present on this preparation ($pA_2 = 7.6 \pm 0.07$ ($n = 30$), Schild plot slope of 1.07). A similar result was obtained with increasing concentrations of compounds **30** and **17** (not shown). The potencies (pA_2 values) of these compounds amount to 5.65 ± 0.05 ($n = 22$) and 6.02 ± 0.06 ($n = 12$), respectively, implying at least 40-fold lower potencies than thioperamide. Increasing concentrations of compounds **26** and **18** induced a rightward shift as well with, however, a significant decrease of the maximal amplitude of the concentration−response curve to RAMH (Figure 2). This noncompetitive behavior is not due to residual agonist activity of these compounds, as up to $10^{-6.5}$ M, neither **18** nor **26** decreases twitch contraction by itself. The pA_2 values of **26** and **18** amount to 8.4 ± 0.10 ($n = 16$) and 7.1 ± 0.06 ($n = 11$), respectively, and as such these compounds are equipotent or even 6-fold more potent than thioperamide.

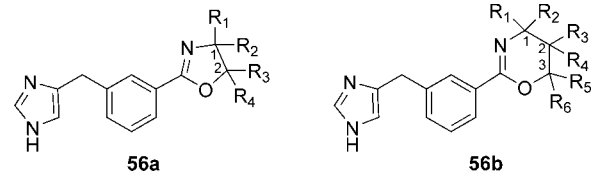
Table 2. Affinities and Activities of *Para*-Substituted 4-Benzyl-1*H*-imidazole on hH_3R (Approach A)



Cmpd	R	pK_i +/- SEM[a]	Activity +/- SEM[b]
11		5.60 +/- 0.13	- [c]
13	H	6.79 +/- 0.18	72.9 +/- 2.0
22	CN	6.09 +/- 0.09	37.7 +/- 5.5
23	Br	6.38 +/- 0.02	33.6 +/- 2.4
24		6.27 +/- 0.06	30.1 +/- 7.2
25 [d]	NH_2	6.59 +/- 0.04	42.0 +/- 3.2

[a] Displacement of [^{3}H]-N^{α}-methylhistamine on membranes from CHO cells stably expressing the hH_3R ($n = 3$, SEM shown). [b] Defined as the percentage of reduction in cAMP levels in CHO cells stably expressing the human H_3R induced by treatment of 10^{-5} M of compound relative to untreated cells ($n = 3$, SEM shown). [c] Not determined. [d] Reported by us before.[27] Reference values for H_3R inverse agonist clobenpropit and for H_3R agonist immepip are shown in Table 1.

Scheme 10. General Structures of the 42 Library Members Prepared by Combinatorial Chemistry[a]

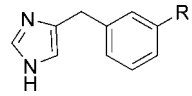


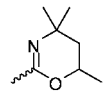
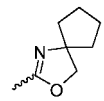
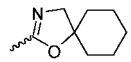
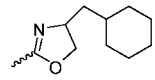
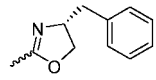
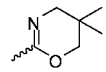
56a **56b**

[a] Enumeration of the imidate ring carbons is also depicted.

The antagonist behavior observed on the guinea pig ileal myenteric plexus contrasts with the agonist profile observed in the GTPγS assay. However, such differences are not uncommon to occur. In fact, Harper et al. very recently disclosed a novel type of assay which predicts that certain imidazole-containing H_3R ligands, previously classified as antagonists based on isolated tissue bioassays, may possess residual agonism efficacy.[51] Indeed, discrepancies between activity assays on hH_3R and gpH_3R have already been reported for a wide variety of H_3R imidazole-based agonists. This list includes compounds with a basic side chain such as imbutamine,[27] impentamine,[12,27] and a three-carbon chain homologue of the agonist imetit[12] as well as agonists without a basic side chain as exemplified by **8**,[27,52] *n*-alkyl substituted imidazoles (**9**),[27] and burimamide.[27] These results together with our current findings further strengthen Harper's notion. Discrepancies in functional activities of H_3R

Table 3. Affinities of the Best Library Compounds (Approach B)



Cmpd	R	pK_i +/- SEM[a]
10		8.44 +/- 0.04
26		8.90 +- 0.04
27		7.40 +/- 0.10
28		7.25 +/- 0.05
29		6.15 +/- 0.05
30		7.05 +/- 0.07
31		5.60 +/- 0.06

[a] Displacement of [^{3}H]-N^{α}-methylhistamine on membranes from CHO cells stably expressing the hH_3R. $n = 2-5$, SEM is shown. Reference values for H_3R inverse agonist clobenpropit and for H_3R agonist immepip are shown in Table 1.

Table 4. Functional Properties of Selected Compounds as Measured by the Effect of the Compound on [^{35}S]GTPγS Binding to Membranes Expressing H_3R Receptors[a]

cmpd	pEC_{50} ± SEM
10	7.8 ± 0.33
14	6.7 ± 0.11
17	7.1 ± 0.20
18	8.1 ± 0.05
23	6.2 ± 0.23
26	8.7 ± 0.10
30	7.1 ± 0.30

[a] $n = 2-6$, SEM is shown.

ligands are largely assay-dependent and seem to be consistent throughout structurally diverse types of imidazole-containing molecules.

Pharmacophore Modeling. A CADD approach was used to explain the observed binding and functional properties of our compounds, which are all relatively rigid and have no basic groups except for the imidazole. The key observation is that addition of an additional hydrophobic side chain is tolerated and in some cases increases the affinity toward the hH_3R, all indicative of an important interaction with a lipophilic pocket. Interestingly, several molecular modeling studies reveal two distinct lipophilic pockets available for antagonist binding.[28,44,53,54] For our investigations on this matter, the starting geometries of H_3R ligands, among which the well-known antagonist **57** (ciproxifan) and antagonist/partial agonist **58** (GR175737)[33,55] were generated and partially optimized using Sybyl6.8 (structures shown in Figure 3a). Conformational spaces were generated using our internal procedure CONFEX as has been described previously.[56] The conformational spaces generated were used as starting points to find common pharmacophoric features using DISCOtech and FlexS (Sybyl6.8). For clarity, only **57** and **58** are represented in Figure 3b. Compounds **10** and **18** were subsequently fitted onto the generic pharmacophore to ensure maximal coverage of the pharmacophoric features. Comparing the pharmacophoric characteristics of **10** and **18** with

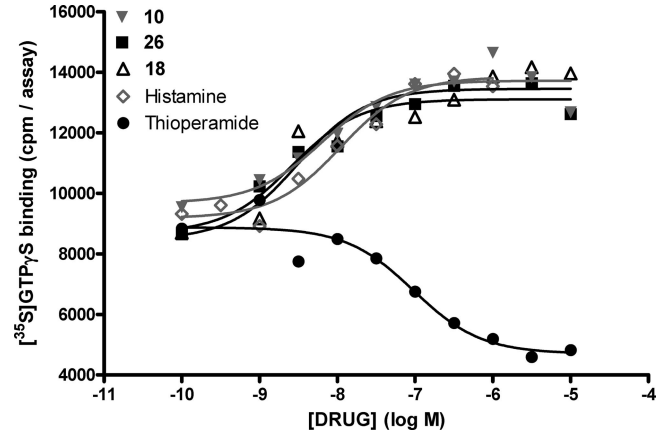


Figure 1. Full curves for functional activity on hH_3R of compounds **10**, **26**, and **18** as well as for reference agonist histamine and reference antagonist thioperamide. $n = 2-6$.

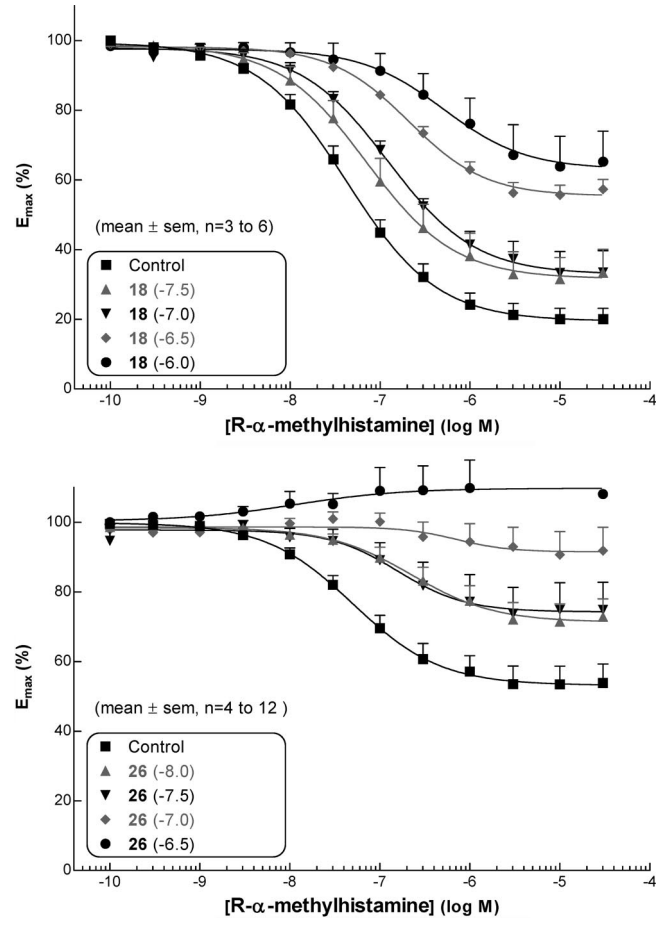


Figure 2. Effect of compounds **18** and **26** on guinea pig isolated paced ileal myenteric plexus stimulated with RAMH. Shown on the *y*-axis is the E_{max} value, which represents the maximum inhibitory effect of contraction. The inset shows the tested compound and the log value of the applied concentration.

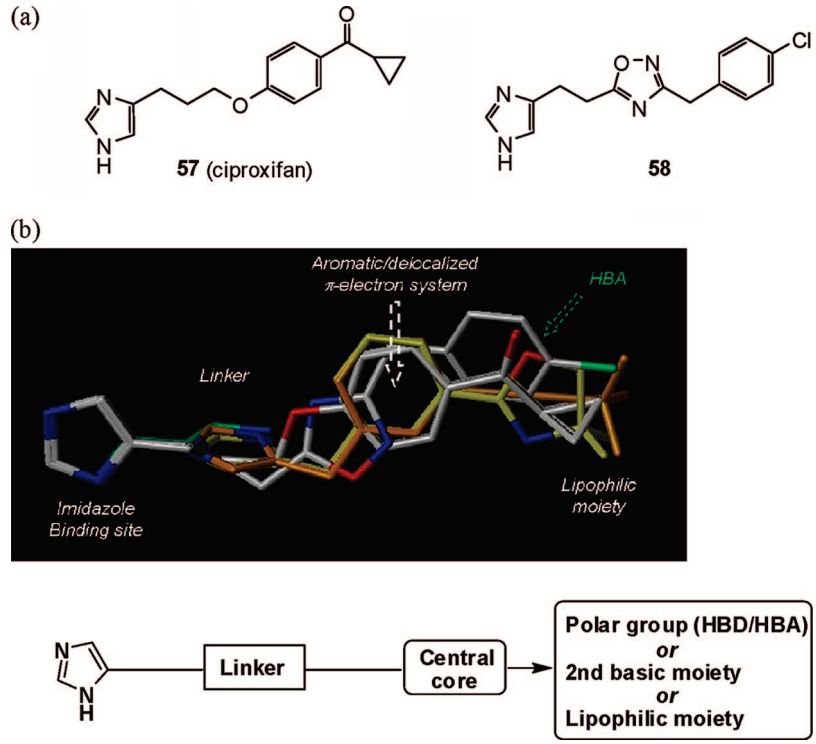


Figure 3. (a) Structures of **57** and **58**. (b) Superposition of compounds **10** and **18** to a generic pharmacophore model for imidazole-based H_3R antagonists showing **57** and **58** colored by atom. The pharmacophore features the presence of a basic moiety (IBS), an aromatic system as well as an additional HBA and lipophilic moiety. Compound **10** and **18** are represented with the carbon atoms colored in yellow and orange, respectively. Histamine is represented with the carbon atoms colored in green. For clarity, all hydrogen atoms have been omitted. Also shown is a 2D schematic representation.

those of **57** and **58** suggests that one of the hydrophobic pockets could be available for binding by agonists such as **10** and **18** as well (Figure 3b). The latter two compounds share the aromatic, delocalized π-electron system as well as an additional polar group and peripheral lipophilic moiety found in various H_3R antagonists.[57] The model predicts that filling of the hydrophobic pocket by the peripheral lipophilic group of **10** and **18** places their imidazole rings in overlap with the primary amine of histamine, which is located near the highly conserved and crucial aspartate D114 residue (Figure 3b). Consequently, while the imidazole ring of histamine has been shown to interact with E206 at the IBS, we suggest that our constrained agonists, which only contain one basic moiety, interact through their imidazole moiety with D114 instead. Indeed, the existence of only one specific imidazole binding site for all imidazole-containing histamine H_3R ligands and the role of the interaction between the imidazole ring and E206 are challenged by mutagenesis experiments, indicating that E206A mutation does not significantly modify the K_D value of iodoproxyfan,[30] which suggests the possibility of alternative binding modes. We therefore hypothesize that the alternative imidazole−D114 interactions, observed for **10** and **18**, allow for receptor activation in analogy to receptor activation through D114 by a basic group of many known dibasic agonists (vide supra). More specifically, the interaction of the agonists with D114 is suggested to have a pronounced effect on the conformation of this residue, which has already been associated with receptor activation.[28]

Receptor Selectivity and Cytochrome P_{450} Binding. The three high-affinity ligands **17**, **18**, and **26** were evaluated further for their selectivity on the full panel of histamine receptors. None showed appreciable inhibition of H_1R or H_2R (<20% and <40% inhibition at 10 μM, respectively). However, all three exhibit significant affinity for the human H_4 receptor (Table 5).

Table 5. Additional Pharmacological Data on Selected Compounds[a]

cmpd	hH_4R (pK_i)[b]	CYP2D6 (IC_{50}, μM)	CYP3A4 (IC_{50}, μM)
11	17 ± 1%[c]	0.543 ± 0.008	2.522 ± 0.079
17	8.25 ± 0.05	1.926 ± 0.012	1.104 ± 0.027
18	8.25 ± 0.05	1.785 ± 0.044	1.860 ± 0.049
19	n.d.	0.488 ± 0.007	2.763 ± 0.142
21	n.d.	0.644 ± 0.015	1.639 ± 0.093
26	8.08 ± 0.03	0.292 ± 0.003	0.873 ± 0.022

[a] Left column: affinity of test compounds for hH_4R ($n = 2-4$, SEM is shown). Right columns: inhibition of cytochrome P_{450} isoforms CYP2D6 and CYP3A4 by test compounds as established by dextromethorphan *O*-demethylase and midazolam 1′-hydroxylase assays, respectively. $n = 11$, SEM is shown. [b] Displacement of [^{3}H]-histamine on membranes from CHO cells stably expressing the hH_4R. [c] Percentage inhibition of [^{3}H]-histamine binding at 10 μM test compound.

Such moderate H_3R/H_4R selectivity levels are regularly encountered with imidazole-containing ligands, as these two receptors share the highest homology within the histamine receptor family.[58,59] Furthermore, in recent years, it has become clear that another potential liability of many imidazole-containing compounds is their binding to cytochrome P_{450} isoforms.[60,61] This may induce distorted metabolism and/or drug−drug interactions. A clear illustration of the latter is the case of H_3R imidazole−ligands ciproxifan and thioperamide, which cause significant drug−drug interactions in rats when coadministered with the antipsychotic drugs risperidone and haloperidol, likely through cytochrome P_{450} interactions.[62] We tested a selected set of compounds on two important isoforms of cytochrome P_{450}: CYP2D6, which is estimated to be involved in the metabolism of 30% of marketed drugs despite its low abundancy of ca. 2%,[61] and CYP3A4, which is the most abundant isoform and involved in the metabolism of ca. 60% of all clinically relevant drugs. Unfortunately, both isoforms were potently inhibited by our test set (Table 5) with IC_{50} values ranging from

0.5−2.8 μM. This is very likely a result of the presence of the imidazole ring.

Despite the undesired H_4R- and cytochrome P_{450}-binding of the compounds, both approaches A and B have left us with some interesting H_3R agonists as exemplified by the highly potent oxazoline-derivative **26** ($pEC_{50} = 8.7$). These compounds may fulfill a valuable role as tools in H_3R research.

Conclusion

In the current study, we have systematically explored the use of a 4-benzyl-1*H*-imidazole core and an oxazoline side chain in pursuit of hH_3R agonists. Classical synthetic means as well as combinatorial chemistry approaches led to the preparation of 55 compounds. *Meta* substitution of the phenyl group was generally preferred over *para* substitution. The most potent compounds possess an alkyne moiety (**18**, **21**) or a cyclic imidate moiety (**10**, **26**) with a peripheral lipophilic group. Interestingly, all compounds proved to be agonists for the hH_3R. Yet, a second basic unit in the side chain, often associated with H_3R agonism, was lacking in all these compounds. We rationalized these binding and functional results by molecular modeling studies, which reveal that the peripheral lipophilic group of the agonists occupies a hydrophobic pocket previously known to play a role in antagonist binding. This in turn positions the imidazole group near aspartate residue D114, allowing receptor activation.

The clinical potential of the present compounds is unfortunately compromised by substantial binding to cytochrome P_{450} isoforms and moderate selectivity for hH_3R over hH_4R. Our findings nevertheless contribute valuable lessons about the exact determinants for hH_3R affinity and activity and provide a framework for explaining H_3R agonism by an ever growing list of ligands not having any polar groups in the side chain. Such a framework will prove useful in responding to the steadily increasing list of therapeutic indications for H_3R agonists. Most notably, the oxazoline unit represents an unexplored type of structure in H_3R agonist research and opens new venues in the H_3R antagonist area as well.[63]

Supporting Information Available: Synthesis and spectral characterization of all compounds, all procedures for biological measurements, and selected copies of spectra for key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM7014149