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New high affinity H_3 receptor agonists without a basic side chain

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Abstract—In this study, we replaced the basic amine function of the known histamine H_3 receptor agonists imbutamine or immepip with non-basic alcohol or hydrocarbon moieties. All compounds in this study show a moderate to high affinity for the cloned human H_3 receptor and, unexpectedly, almost all of them act as potent agonists. Moreover, in the alcohol series, we consistently observed an increased selectivity for the human H_3 receptor over the human H_4 receptor, but none of the compounds in this series possess increased affinity and functional activity compared to their alkylamine congeners. In this new series of compounds VUF5657, 5-(1*H*-imidazol-4-yl)-pentan-1-ol, is the most potent histamine H_3 receptor agonist (pK_i = 8.0 and pEC_{50} = 8.1) with a 320-fold selectivity at the human H_3 receptor over the human H_4 receptor.

1. Introduction

The histamine H_3 receptor was initially discovered as a presynaptic autoreceptor on histaminergic neurons regulating the release and synthesis of histamine in the rat and human brain.[1–3] Nowadays, it is generally accepted that the histamine H_3 receptor also acts as a presynaptic heteroreceptor on several non-histaminergic nerve terminals inhibiting the release of a variety of neurotransmitters in both the central and peripheral nervous system.[4–6] As such, the H_3 receptor is considered to be an important target for the treatment of various CNS-related disorders; ligands for the H_3 receptor have been proposed as potential drugs for the treatment of dementia, attention deficit hyperactivity disorder, narcolepsy and obesity.[7,8]

In order to exploit the interesting therapeutic potential, a wide variety of H_3 receptor agonists and antagonists have been developed in the last decade.[9] All potent H_3 agonists possess an imidazole ring, whereas the alkyl side chain often contains a positively charged amine moiety (see Chart 1 for (*R*)-α-methylhistamine, immepip) or isothiourea moiety (imetit). Initial molecular modelling studies from our laboratory were able to explain H_3 agonism on the basis of the presumed interaction of the positively charged side chain with a putative carboxylate group of the receptor protein.[10] However, it has been shown that a basic group in the imidazole side chain can be omitted.[11]

The cloning of the H_3 receptor cDNA by Lovenberg et al.[12] in 1999 has revealed that the H_3 receptor protein shows all the expected hallmarks of the rhodopsin-class of G-protein coupled receptors (GPCR). As for all GPCRs for aminergic neurotransmitters, the highly conserved aspartate in transmembrane domain three (TM3) is found in the histamine H_3 receptor.[13–15] For various GPCRs, including the histamine H_1 and H_2 receptors,[16–18] this conserved aspartate residue has been convincingly implicated in the binding of protonated side chains found in both agonists and antagonists.[19–23] Moreover, recent computational studies with a 3D-homology model of the histamine H_3 receptor also hypothesized an interaction of the protonated amine of the

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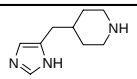
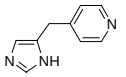
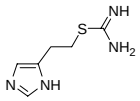
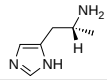
compound	structure	*h*H$_3$ receptor		*h*H$_4$ receptor		selectivity*
		pK_i	pEC$_{50}$	pK_i	pEC$_{50}$	
Immepip[40]		9.3	9.9	7.7	7.2	46
Immethridine[27]		9.1	9.7	6.6	6.0	288
Imetit		9.1[43]	9.7[43]	8.9[44]	8.5[44]	2
(*R*)-α-Methylhistamine		8.4[43]	9.2[43]	6.6[44]	6.0[44]	55

*Selectivity = $K_i(H_4)/K_i(H_3)$.

Chart 1. Binding affinities and functional activities of ligands at the human histamine H_3 and H_4 receptors.

ethylamine side chain of histamine with the conserved Asp^{114} in TM3.[24–26]

Recently, it was observed that replacement of the piperidine ring of immepip by a weakly basic pyridine ring does not affect the binding affinity at the human histamine H_3 receptor. Immethridine exhibits high affinity and agonist activity at the H_3 receptor with a pK_i value of 9.1 and a pEC$_{50}$ value of 9.7 (Chart 1).[27] Surprisingly, immethridine shows an increased selectivity at the H_3 receptor over its closely related H_4 receptor. Since the basicity of the nitrogen atoms in the side chain of immepip and immethridine is different, we questioned whether a basic amine at the side chain is necessary for binding and activation of the human histamine H_3 receptor. This observation opens potential new avenues for selective H_3 receptor agonists. In this study, ligands with different physicochemical properties at the side chain (Fig. 1) were therefore synthesized and tested for their affinity and activity at the human histamine H_3 and its closely related H_4 receptors.

2. Chemistry

The syntheses for all targeted compounds are outlined in Schemes 1–8. The synthesis of **5** was modified from the procedure described by Stark et al.[28] (Scheme 1). Reflux of urocanic acid in methanol saturated with hydrochloric acid gave the methyl ester (**27**) in a quantitative yield. After protection of the imidazole ring of **27** with a triphenylmethyl group, the intermediate **28** was subsequently hydrogenated under a H_2 atmosphere in the presence of a catalytic amount of 5% Pd/C to give **29** in a 75% yield. Reduction of **29** by lithium aluminium hydride in THF gave the alcohol **30** in a 70% yield. Deprotection of the imidazole ring under acidic conditions finally yielded compound **5**.

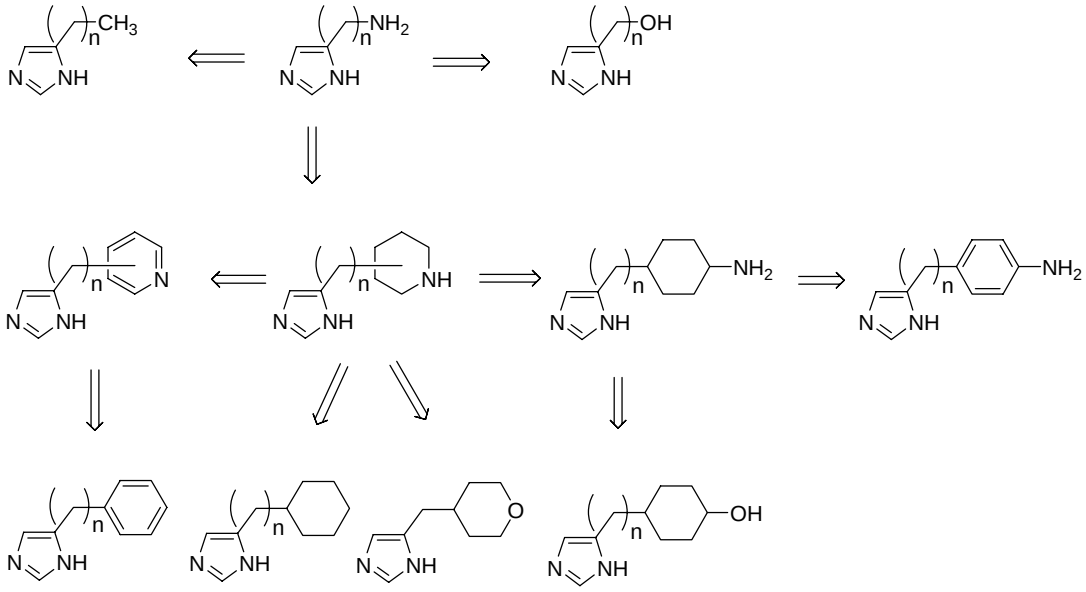
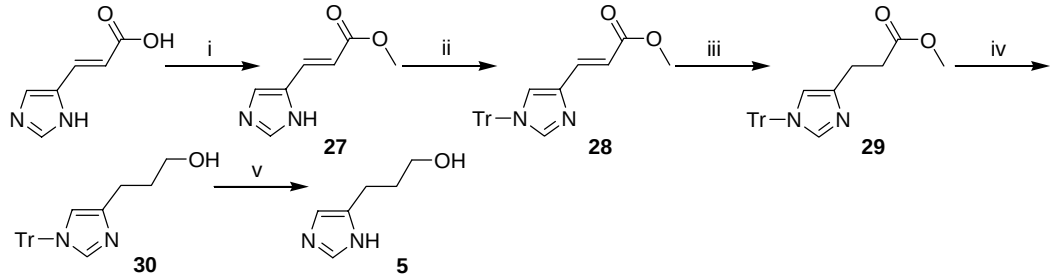
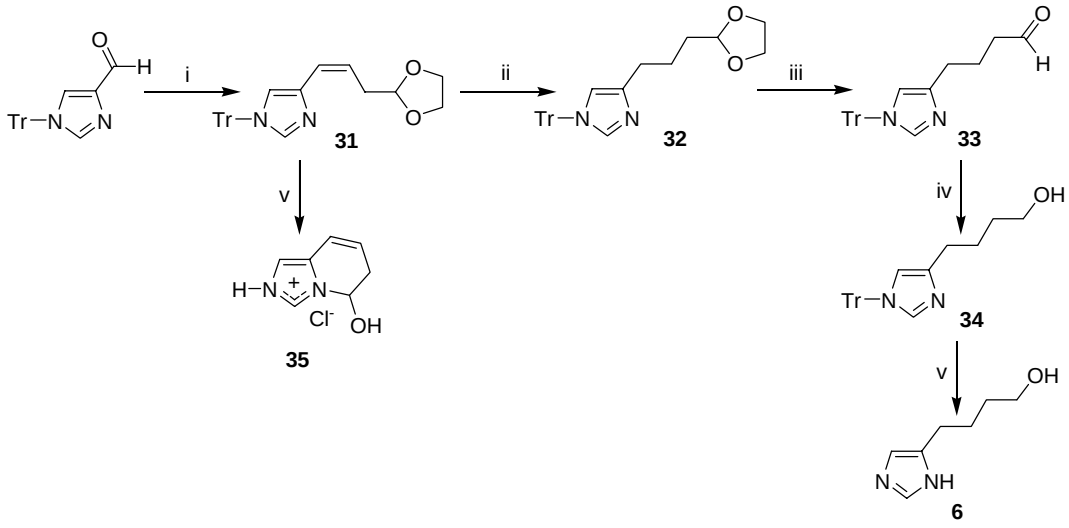


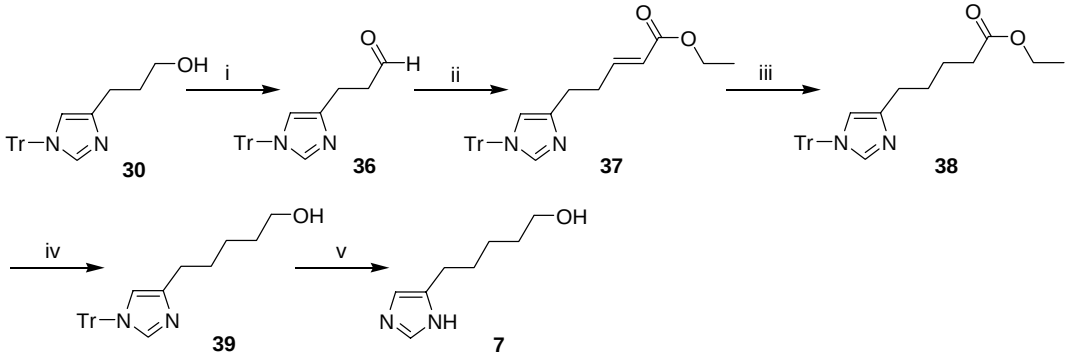
Figure 1. Design of ligands with various physicochemical properties at the side chain.



Scheme 1. Synthetic pathway for **5**. Reagents and conditions: (i) MeOH, HCl (g), Δ, 5 h; (ii) triphenylmethylchloride, THF, TEA, rt, 16 h; (iii) 5% Pd/C, MeOH, 1 atm H_2, 4 h; (iv) $LiAlH_4$, THF, rt, 6 h; (v) 1 N HCl, Δ, 5 h.

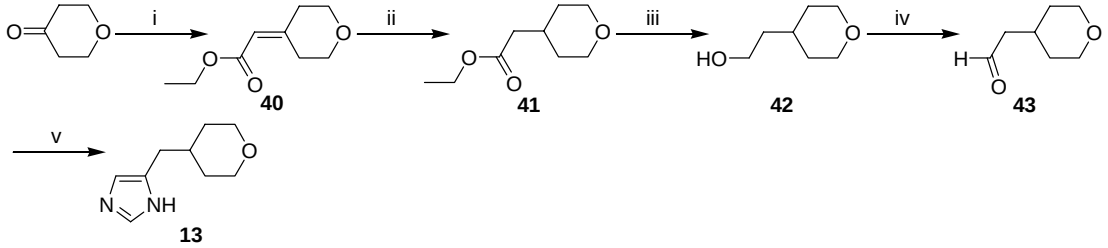


Scheme 2. Synthetic pathway for **6**. Reagents and conditions: (i) *n*-BuLi, diisopropylamine, THF, [2-(1,3-dioxolan-2-yl)ethyl]-triphenylphosphonium bromide, −30 °C → reflux 6 h; (ii) 5% Pd/C, 1 atm H_2, MeOH, 4 h; (iii) acetone, 1 N HCl, 0 °C, 1 h; (iv) $LiAlH_4$, THF, rt, 6 h; (v) 1 N HCl, Δ, 5 h.

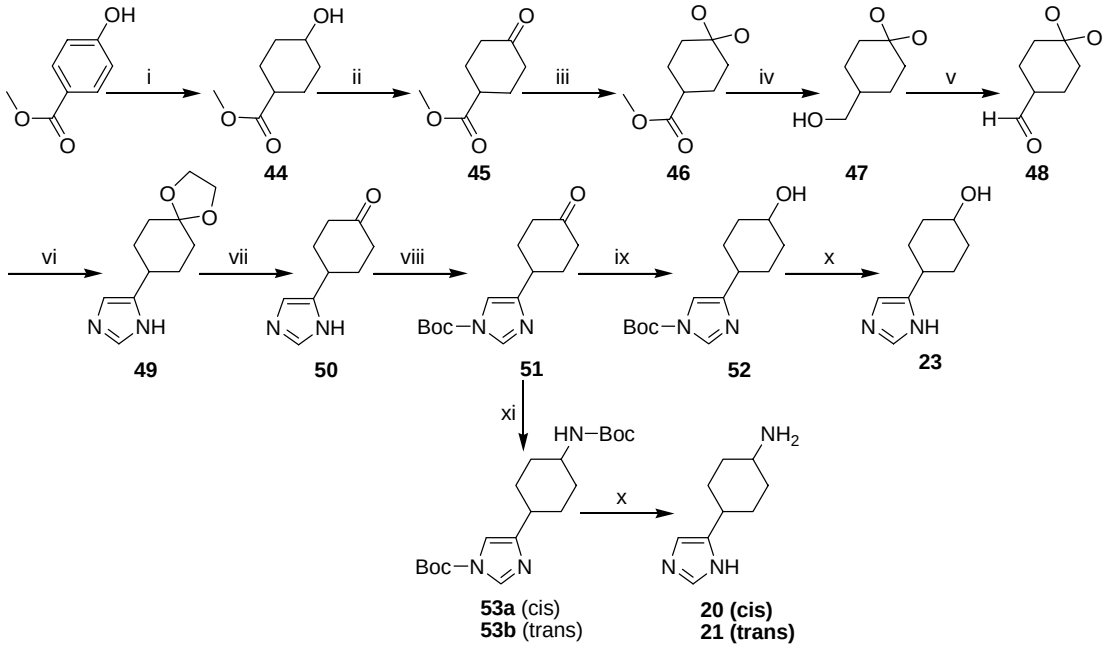


Scheme 3. Synthetic pathway for **7**. Reagents and conditions: (i) oxalyl chloride, DMSO, DCM, TEA, −60 °C to rt, 2 h; (ii) triethyl phosphonoacetate, NaH, THF, 0 °C to rt, 6 h; (iii) 1 atm H_2, 5% Pd/C, MeOH, 4 h; (iv) $LiAlH_4$, THF, rt, 4 h; (v) 1 N HBr, Δ, 3 h.

Compound **6** was synthesized according to Scheme 2; the protected imidazole carboxaldehyde was reacted with [2-(1,3-dioxolan-2-yl)ethyl]-triphenylphosphonium bromide in the presence of LDA to give **31** in a 85% yield. Attempts to hydrolyse the dioxolane ring under aqueous acidic conditions did not result in the expected aldehyde, but we speculate that the cyclic compound **35** was obtained instead. However, the aldehyde **33** was obtained after hydrogenation of the double bond of **31** under a H_2 atmosphere in the presence of 5% Pd/C and subsequently hydrolysis of the dioxolane ring with 1 N HCl in acetone at 0 °C. Reduction of the aldehyde using lithium aluminium hydride yielded the intermediated alcohol **34** in a 75% yield. Deprotection of the imidazole ring gave **6** in a good yield.



Scheme 4. Synthetic pathway for **13**. Reagents and conditions: (i) triethyl phosphonoacetate, NaH, THF, 0 °C to rt, 6 h; (ii) 1 atm H_2, 5% Pd/C, MeOH, 4 h; (iii) $LiAlH_4$, THF, rt, 4 h; (iv) oxalyl chloride, DMSO, DCM, TEA, −60 °C to rt, 2 h; (v) 1—TosMIC, NaCN, EtOH; 2—satd NH_3 in EtOH, 90–110 °C, 16 h.

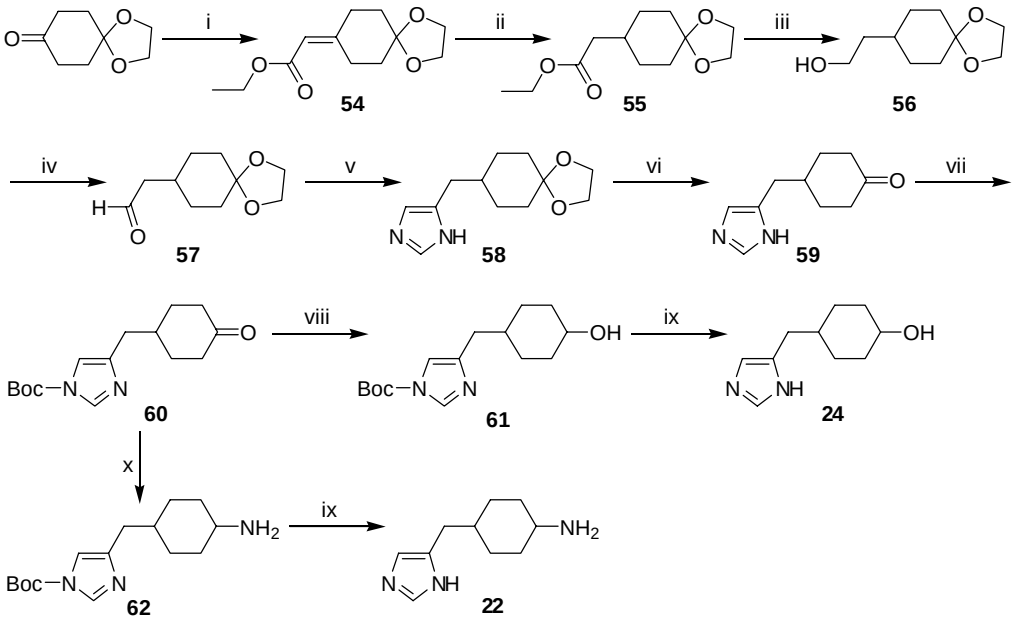


Scheme 5. Synthetic pathway for **20** and **21**. Reagents and conditions: (i) 100 atm H_2, 5% Rh/C, THF, 18 h; (ii) pyridinium chlorochromate (PCC), DCM, Celite, sodium acetate, rt, 4 h; (iii) *p*-TSA, ethylene glycol, toluene, Δ, 18 h; (iv) $LiAlH_4$, ether, rt, 3 h; (v) DMSO, DCM, TEA, −60 °C to rt, 2 h; (vi) 1—TosMIC, NaCN, EtOH; 2—satd NH_3 in EtOH, 90–110 °C, 16 h; (vii) 1 N HCl, rt, 30 min; (viii) di-*tert*-butyldicarbonate, triethylamine, $CHCl_3$, rt, 16 h; (ix) $NaBH_4$, MeOH, rt, 16 h; (x) 1 N HCl, rt, (xi) 1—ammonium acetate, $NaBH_4$, MeOH, rt, 18 h; 2—di-*tert*-butyldicarbonate, triethylamine, $CHCl_3$, rt, 16 h.

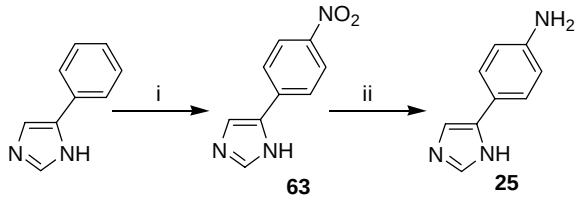
The synthesis of compound **7** followed Scheme 3. The intermediate aldehyde **36** was obtained from the alcohol **30** via a Swern oxidation[29] in a 78% yield. Next, a Wittig reaction of the aldehyde with triethyl phosphonoacetate resulted in the compound **37**. Following the aforementioned procedure for the synthesis for **5**, the targeted alcohol **7** was obtained.

Compound **13** was synthesized according to Scheme 4; a Wittig reaction of tetrahydropyran-4-one with triethyl phosphonoacetate gave **40** in a 88% yield. Following hydrogenation and reduction as described for the synthesis of **5**, the intermediate alcohol **42** was obtained and was subsequently transformed into the aldehyde **43** via a Swern oxidation. Using TosMIC chemistry,[30] the aldehyde of **43** was converted to an imidazole ring to give compound **13** in a 75% yield.

Following the procedure outlined in Scheme 5, the compounds **20**, **21** and **23** were synthesized from commercially available methyl-*p*-hydroxybenzoate. Attempts to hydrogenate the benzene ring using 10% Pd/C as catalyst in methanol resulted in hydrogenolysis. However, hydrogenation performed under high pressure (100 atm) of H_2 in THF with a catalytic amount of 5% Rh/C gave the desired product **44** in a quantitative yield. Next, the alcohol **44** was oxidized by pyridinium chlorochromate (PCC) to give the intermediate ketone **45** in a 90% yield. The ketone was converted to the intermediate dioxolane **46** by refluxing with propylene glycol in the presence of *p*-toluenesulfonic acid. The ester **46** was reduced into the alcohol **47** using lithium aluminium hydride, whereafter the alcohol was converted into **48** by a Swern oxidation. Once the aldehyde was formed, it was easily converted into an imidazole ring using TosMIC chemistry to give **50** in a 94% yield. Next, the



Scheme 6. Synthetic pathway for **22** and **24**. Reagents and conditions: (i) triethyl phosphonoacetate, NaH, THF, 0 °C to rt, 6 h; (ii) 1 atm H_2, 5% Pd/C, MeOH, 4 h; (iii) $LiAlH_4$, THF, rt, 4 h; (iv) oxalyl chloride, DMSO, DCM, TEA, −60 °C to rt, 2 h; (v) 1—TosMIC, NaCN, EtOH; 2—satd NH_3 in EtOH, 90–110 °C, 16 h; (vi) 1 N HCl, rt, 30 min; (vii) di-*tert*-butyldicarbonate, triethylamine, $CHCl_3$, rt, 16 h; (viii) $NaBH_4$, MeOH, rt, 16 h; (ix) 1 N HCl, rt; (x) ammonium acetate, $NaBH_4$, MeOH, rt, 16 h.

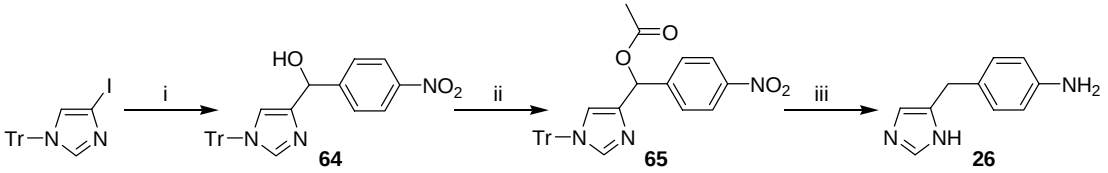


Scheme 7. Synthetic pathway for **25**. Reagents and conditions: (i) fuming HNO_3, H_2SO_4, 0 °C, 2 h; (ii) 5% Pd/C, MeOH, 1 atm H_2, rt, 4 h.

resulting imidazole was treated with di-*tert*-butyldicarbonate to obtain the protected-imidazole intermediate **51** whose ketone can be transformed into **1** the alcohol by reduction using sodium borohydride or **2** the amine by reductive amination using ammonium acetate (as ammonia generator) and sodium triacetoxy borohydride. Since the reduction of the ketone to the alcohol **52** resulted in more than 95% trans-isomer (based on NMR spectra), the compound was treated under mildly acid conditions to give **23**, which was tested for its activity without further isomeric separation. The reductive amination of the ketone gave a mixture of the cis- and trans-isomers (1:3); the resulting mixture was treated with di-*tert*-butyldicarbonate in order to increase the lipophilicity of the molecule to give **53**. The *cis*-(**20**) and *trans*-(**21**) isomers were successfully separated by column chromatography.

Compounds **22** and **24** were synthesized according to Scheme 6. Following the aforementioned procedure for the synthesis of **13** but using 1,4-dioxa-spiro[4,5]decan-8-one as starting material, the intermediate dioxolane **58** was obtained in a moderate yield. Hydrolysis of the dioxolane ring under mildly acidic conditions provided the ketone **59**, which was subsequently treated with di-*tert*-butyldicarbonate to give **60**. Using the procedures described above for the synthesis of **20**, **21** and **23**, the ketone **60** was converted into the alcohol **61** and the amine **62**. After deprotection of **61** and **62** under mildly acidic conditions, compounds **22** and **24** were obtained as racemic mixtures and tested for their activities without further separation.

Compound **25** was prepared following Scheme 7. Nitration of the commercially available 4-phenyl-imidazole, using fuming nitric acid and concentrated sulfuric acid, gave **63** in 45% yield. Next, the nitro group was reduced under an atmosphere of H_2 to give the product **25** in a quantitative yield.



Scheme 8. Synthetic pathway for **26**. Reagents and conditions: (i) EtMgBr, DCM, 4-nitrobenzaldehyde, rt, 16 h; (ii) DMAP, DCM, acetic anhydride, Δ, 6 h; (iii) 1—10% Pd/C, MeOH, 1 atm H_2, rt, 4 h; 2—1 N HBr, Δ, 5 h.

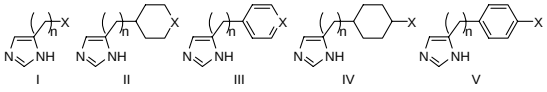
As shown in Scheme 8, compound **26** was prepared from protected 4-iodo-imidazole. Treatment of the iodo compound with ethyl magnesium bromide and 4-nitrobenzaldehyde gave the alcohol **64** in a 85% yield. Acetylation of the alcohol using acetic anhydride resulted in the intermediate **65**, from which the acetoxy group was removed by hydrogenation under an atmosphere of H_2 in the presence of 10% Pd/C. In this step, the nitro group was simultaneously reduced to an aniline. After deprotection in acidic conditions, derivative **26** was obtained in a good yield.

3. Results and discussion

Previously, it was observed that replacement of the piperidine ring of the potent and moderately selective H_3 agonist immepip (**11**, Table 1) with a less basic pyridine ring leads to the highly potent H_3 agonist immethridine (**16**), which exhibits an improved (300-fold) selectivity at the human H_3 receptor over the H_4 receptor.[27] A decreased basicity of the side chain is apparently well tolerated for H_3 receptor agonism, whereas the requirements for H_4 receptor activity seem to be more stringent.[27] As a result, increased selectivity at the human histamine H_3 receptor might be obtained by alterations of the side chain.

In this study, we evaluated two new series of compounds, in which the basic nitrogen function of histamine homologues was replaced with a non-basic alcohol or methyl moiety. As can be seen in Table 1, these compounds (**5**–**10**) still retained moderate to high H_3 receptor affinities, which were not dramatically lower compared to their respective histamine homologues. In the new series of non-basic H_3 receptor agonists VUF5657 (**7**), the

Table 1. Affinities and functional activities of various ligands on the human histamine $H_{3/4}$ receptors



Compound	VUF	Structure	X	*n*	hH_3		hH_4		Selectivity[e]
					pK_i ± SEM[a]	pEC_{50} ± SEM[b]	α[c]	pK_i ± SEM[d]	
1	Histamine[f]	I	$-NH_2$	2	8.0 ± 0.1	8.0 ± 0.0	1.0	7.8 ± 0.0	1.5
2	8326[f]	I	$-NH_2$	3	7.3 ± 0.0	7.4 ± 0.2	0.9	7.3 ± 0.1	0.7
3	Imbutamine[f]	I	$-NH_2$	4	8.4 ± 0.0	8.1 ± 0.1	0.9	7.8 ± 0.1	3.6
4	Impentamine[f]	I	$-NH_2$	5	8.3 ± 0.1	8.3 ± 0.1	0.9	6.4 ± 0.1	72.0
5	5698	I	–OH	3	6.5 ± 0.1	6.1 ± 0.1	0.9	5.6 ± 0.1	7.8
6	5793	I	–OH	4	7.4 ± 0.0	7.3 ± 0.3	1.3	6.0 ± 0.3	23.0
7	5657	I	–OH	5	8.0 ± 0.0	8.1 ± 0.1	1.3	5.5 ± 0.0	316.0
8	5523	I	$-CH_3$	3	7.6 ± 0.0	7.5 ± 0.1	1.1	6.2 ± 0.1	26.0
9	5524	I	$-CH_3$	4	7.6 ± 0.1	7.3 ± 0.3	1.2	6.7 ± 0.1	8.9
10	5466	I	$-CH_3$	5	7.5 ± 0.1	7.6 ± 0.4	0.9	6.8 ± 0.0	3.9
11	Immepip[f]	II	–NH–	1	9.3 ± 0.0	9.9 ± 0.0	1.0	7.7 ± 0.0	46.0
12	4929[f]	II	–NH–	2	7.7 ± 0.1	7.9 ± 0.1	0.6	6.4 ± 0.0	19.0
13	5870	II	–O–	1	6.7 ± 0.1	6.9 ± 0.1	0.9	5.2 ± 0.1	34.0
14	5653	II	$-CH_2-$	1	7.4 ± 0.1	7.4 ± 0.2	1.4	6.2 ± 0.1	17.0
15	5654	II	$-CH_2-$	2	6.8 ± 0.0	n.d.[i]	n.d.	6.4 ± 0.0	2.2
16	Immethridine[g]	III	–N–	1	9.1 ± 0.0	9.7 ± 0.1	0.9	6.6 ± 0.0	288.0
17	5889[g]	III	–N–	2	8.2 ± 0.0	8.2 ± 0.0	0.9	5.9 ± 0.0	191.0
18	5511	III	–CH–	1	6.4 ± 0.0	n.d.	n.d.	6.7 ± 0.1	0.6
19	5512	III	–CH–	2	6.1 ± 0.0	n.d.	n.d.	6.1 ± 0.2	1.6
20	5803 (cis)	IV	$-NH_2$	0	7.0 ± 0.0	7.6 ± 0.3	1.3	7.7 ± 0.3	0.2
21	5804 (trans)	IV	$-NH_2$	0	7.4 ± 0.0	7.9 ± 0.1	1.1	6.5 ± 0.3	7.9
22	5805	IV	$-NH_2$	1	7.2 ± 0.0	7.4 ± 0.1	0.5 [h]	6.4 ± 0.0	6.6
23	5796	IV	–OH	0	5.4 ± 0.1	n.d.	n.d.	<4	n.d.
24	5798	IV	–OH	1	6.8 ± 0.1	6.3 ± 0.2	1.1	5.2 ± 0.1	38.0
25	5801	V	$-NH_2$	0	6.0 ± 0.0	n.d.	n.d.	5.8 ± 0.1	1.5
26	5802	V	$-NH_2$	1	6.1 ± 0.0	n.d.	n.d.	5.7 ± 0.1	2.3

[a] The pK_i values were measured by [^{3}H]-N^{α}-methylhistamine binding to membranes of SK-N-MC cells expressing the human H_3 receptor in the presence of the ligand.

[b] The pEC_{50} values were determined by the inhibition of the cAMP-stimulated β-galactosidase transcription in SK-N-MC cells stably expressing the human H_3 receptor. Results are presented as mean ± SEM of at least three independent experiments.

[c] α = intrinsic activity, as the ratio of the maximum response of each ligand to the maximum response of histamine.

[d] The pK_i values were measured by [^{3}H]-histamine for H_4 binding to membranes of SK-N-MC cells expressing the human H_4 receptor in the presence of the ligand. Results are presented as mean ± SEM of at least three independent experiments.

[e] Selectivity = $K_i(H_4)/K_i(H_3)$.

[f] See Ref. 27.

[g] See Ref. 26.

[h] Inverse agonism.

[i] n.d. = not determined.

hydroxyl analogue of impentamine (**4**) is the most potent agonist with a pK_i value of 8.0 and a pEC_{50} value of 8.1 at the human H_3 receptor. Although the H_3 receptor affinity of **7** was not increased compared to impentamine **4** (Table 1), the H_4 receptor affinity of **7** was considerably lower (Table 1), resulting in an increased selectivity (320-fold) at the human histamine H_3 receptor over the H_4 receptor compared to impentamine (Table 1). On the other hand, a replacement of the side-chain amine with a methyl group leads to ligands with both reasonable H_3 and H_4 receptor affinity. For instance, compound **10** shows good affinity (pK_i = 7.5) for the human H_3 receptor and does not show a considerable decrease in H_4 receptor affinity as observed for the related hydroxy compound **7**. Consequently, compared to impentamine (**4**) compound **10** shows a decreased selectivity for the human H_3 receptor over the H_4 receptor (Table 1).

Previously, it has indeed been reported that high H_3 receptor affinity could be obtained without charged or basic groups in the side chain[31–33] and the alkyl imidazoles **8**–**10** previously proved to be interesting H_3 receptor antagonists, when tested on the guinea-pig intestine.[34] Interestingly, compounds **5**–**10** all acted as full agonists at the human H_3 receptor in the recombinant test system used in the present study (Table 1). This finding resembles previous observations with other H_3 receptor ligands. With the cloning of the H_3 receptor gene by Lovenberg et al.[12] and the availability of recombinant cellular test systems we characterized the histamine homologues imbutamine and impentamine and the thiourea burimamide as potent and effective agonists at the human H_3 receptor[35] (Table 1), although we previously had also identified these compounds as potent H_3 antagonists at guinea-pig intestinal preparations.[36] In view of the surprising finding of H_3 agonism of the alkyl imidazoles **8**–**10**, we also investigated in this study the pharmacological characteristics of the acetylene-based H_3 antagonist (1*S*,2*S*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole (GT-2331, Fig. 2A).[37,38] Previously, in vitro studies on the guinea-pig ileum identified GT-2331 as a potent histamine H_3 antagonist. GT-2331 shows high affinity for the cloned human H_3 receptor stably expressed in SK-N-MC cells (pK_i = 8.1). Moreover, as found for the alkyl imidazoles, GT-2331 also acts as an effective full agonist (pEC_{50} = 7.7, α = 1.0) at the human H_3 receptor (Fig. 2B), confirming recent results by others.[43] The agonist actions of GT-2331 are competitively antagonized by clobenpropit, which, as reported previously[35], shows no agonist effects at the human H_3 receptor. Schild plot analysis of the obtained data yields the expected pA_2 value of 9.5 for the H_3 antagonist (Fig. 2B).

In view of the fact that the amine functions of imbutamine and impentamine are apparently not prerequisites for potent H_3 receptor agonism, we also examined the basic function in the side chain of the potent H_3 agonists immepip (**11**) and immethridine (**16**). Replacement of the piperidine nitrogen of immepip **11** or its homologue VUF4929 (**12**) with an oxygen or carbon resulted in strong reductions in H_3 and H_4 receptor affinity (Table 1). The pyridine analogue **17** displays a 400-fold lower affinity at both the human H_3 and H_4 receptors (H_3: pK_i = 6.7; H_4: pK_i = 5.2) compared to immepip. Interestingly, the cyclohexyl analogue **14** shows a significantly better H_3 receptor affinity (pK_i = 7.4) compared to the pyridine compound **17** (Table 1). Like with the related impentamine analogues, the non-basic immepip analogues **13** and **14** also act as full H_3 receptor agonists (Table 1).

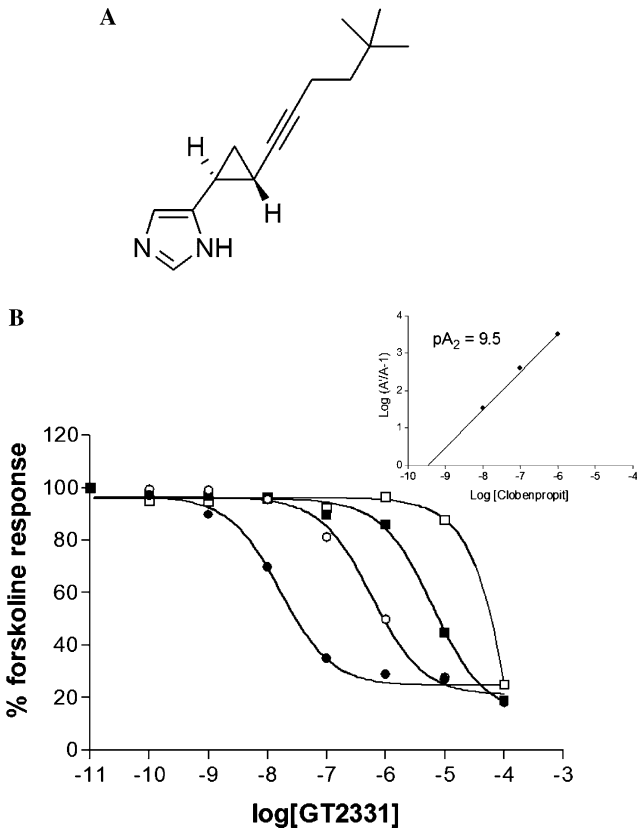


Figure 2. (A) Chemical structure of (1*S*,2*S*)-(+)-4-[2-(5,5-dimethylhex-1-ynyl)-cyclopropyl]-1*H*-imidazole, GT-2331. (B) Agonistic action of GT-2331 at the human H_3 receptor and its competitive antagonism by clobenpropit. The human H_3 receptor was stably expressed in SK-N-MC neuroblastoma cells, which were co-transfected with the β-galactosidase gene under the control of cAMP-responsive elements. H_3 agonism was measured as the inhibition of forskolin (10 μM)-induced expression of β-galactosidase. The actions of GT-2331 were measured in the absence (●) or presence of 10 (○), 100 (■) or 1000 nM (□) of clobenpropit. The inset shows the transformation of the data by Schild plot analysis, yielding a pA_2 value of 9.5 for clobenpropit.

As shown in Table 1, the phenyl-based immethridine analogues **18** and **19** possess a much lower affinity at the human H_3 receptor compared to immethridine (**16**) or its longer homologue **17**. Interestingly, no major differences in affinity for the H_4 receptor were observed between **18** and immethridine (**16**) or **19** and **17** (Table 1). These data indicate that the H_3 receptor binding site is very efficiently targeted by the piperidine and pyridine rings of immepip and immethridine, respectively. These moieties contribute mainly to high H_3 receptor affinity and do not seem to be crucial for H_3 receptor activation.

To further examine the role of a basic amine in the side chain, we incorporated cyclohexylamines (**20**–**22**), cyclo-

hexylalcohols (**23** and **24**) or anilines (**25** and **26**) and tested these compounds for their affinities and effects at the human H_3 and H_4 receptors. The cyclohexylamines **20**–**22** can be seen as rigidified imbutamine (**3**) or impentamine (**4**) analogues and lose approximately one log unit affinity at the human H_3 receptor. Interestingly, the *cis*-(**20**) and *trans*-(**21**) isomers both act as agonists at the H_3 receptor and do not show any major difference in their interaction with the human H_3 receptor (Table 1 and Fig. 3A). In contrast, at the human H_4 receptor the cis-isomer (**20**) shows high affinity ($pK_i = 7.7$), is as effective as histamine or immepip at the H_4 receptor ($pEC_{50} = 7.7$, $\alpha = 1.1$) and displays a 30-fold higher affinity compared to the trans-isomer **21** (Fig. 3B, $pK_i = 6.5$, $pEC_{50} = 6.0$, $\alpha = 1$). As such these two isomers (**20** and **21**) can be regarded as interesting tools for the human H_4 receptor. An extension of the spacer length between the imidazole ring and the cyclohexylamine does not show improved affinity for the H_3 or H_4 receptor (Tables 1 and 2), but interestingly, the longer homologue **22** behaves as a partial inverse agonist at the human H_3 receptor. Rigidification of the side chain of impentamine **4** apparently not only causes a decrease in affinity, but also causes **22** to prefer to bind to the inactive form of the H_3 receptor. Replacement of the side-chain nitrogen of the cyclohexylamines (**20**–**22**) with an alcohol (**23** and **24**) resulted in strongly decreased affinity at both human histamine H_3 and H_4 receptors (Table 1). Also the replacement of the cyclohexylamine with an aniline resulted in strongly decreased affinities at both the human histamine H_3 and H_4 receptors (Table 1).

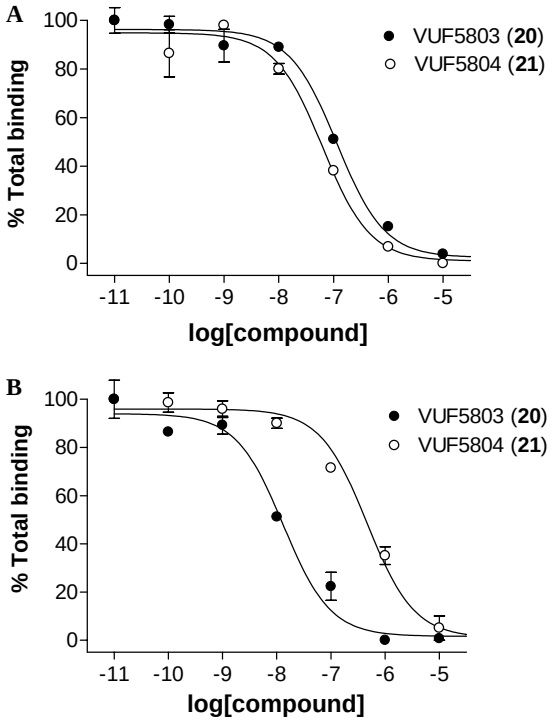


Figure 3. Comparison of the affinities between VUF5803 (**20**) and VUF5804 (**21**) at the human histamine H_3 (**3A**) and H_4 receptors (**3B**) indicates binding preference of the cis-isomer (VUF5803) for the human H_4 receptor.

4. Conclusion

By changing the nature of the side chain of potent H_3 receptor agonists like imbutamine and immepip a variety of interesting new tools for histamine H_3 and H_4 receptors have been identified. VUF5657 (**7**), a hydroxyl analogue of impentamine (**4**), was identified as a potent H_3 receptor agonist with a 320-fold selectivity for the human histamine H_3 receptor over the H_4 receptor. This selectivity equals the selectivity of the recently reported selective H_3 agonist immethridine[27] but VUF5657 shows somewhat lower H_3 receptor affinity.

VUF5803 and VUF5804, the cis- and trans-isomers of the cyclohexylamine analogue of immepip, show interesting differences in activities at the H_4 receptor and may become valuable tools in the H_4 receptor field. Various alkylimidazoles and the related GT-2331 were efficacious H_3 receptor agonists, despite the fact that these molecules lack any polar group in the side chain. Most models for aminergic G-protein coupled receptors, including the H_3 receptor, assume that the amine moiety of the agonists interacts with a conserved aspartate residue in transmembrane domain three.[19,25,26] Our results give further evidence that replacement of the amine group of H_3 receptor agonists with non-basic groups does not always lead to a significant loss in affinity and agonist activity at the human H_3 receptor.

5. Experimental section

5.1. Materials

Reagents were obtained from commercial suppliers and used without further purification. Solvents used were of either AR or HPLC grade. Dry THF and DCM were obtained from distillation over lithium aluminium hydride and calcium hydride, respectively. Compounds **1**–**4**,[36] **8**–**12**[27,36] and **16**–**19**[27] were obtained according to the literature procedures. GT-2331 Perceptin®, (1*S*,2*S*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole was synthesized as described earlier.[42] Thin-layer chromatography was carried out on Merck Kieselgel 60 F_{254} on aluminium sheets and preparative flash chromatography was performed on J. T. Baker Kieselgel 60 under pressure. Melting points were determined with an Electrothermal IA9200 apparatus. ^{1}H spectra were recorded on a Bruker AC-200 spectrometer with the residual undeuterated solvent peak as reference.

6. Methods

6.1. 3-(1*H*-Imidazol-4-yl)-acrylic acid methyl ester hydrochloride (27)

Urocanic acid (1 g, 7.25 mmol) was dissolved in MeOH (50 mL) and HCl gas was bubbled through the solution for 10 min. The resulting solution was heated at reflux temperature for 5 h and the solvent was evaporated to obtain **27** as a white solid (1.35 g, 100%). ^{1}H NMR

(D_2O): δ 8.87 (s, 1H), 7.84 (s, 1H), 7.66 (d, 1H, J = 17.2 Hz), 6.62 (d, 1H, J = 17.2 Hz), 3.88 (s, 3H).

6.2. 3-(1-Trityl-1*H*-imidazol-4-yl)-acrylic acid methyl ester (28)

Compound **27** (1.25 g, 6.63 mmol) was suspended in THF (50 mL) and triethylamine (2.3 mL, 16.6 mmol) was added to the suspension at 0 °C (ice bath). Triphenylmethyl chloride (2.1 g, 7.53 mmol) dissolved in THF (50 mL) was added dropwise and the mixture was stirred at 0 °C for 1 h followed by 16 h at room temperature. The resulting solution was concentrated under reduced pressure and the residue was dissolved in DCM (50 mL). The solution was washed with water (3 × 50 mL) and the organic layer was dried over sodium sulfate. After evaporation of the solvent under reduced pressure, the product (**28**) was recrystallised from EtOAc to give a white solid. (1.96 g, 75%). ^{1}H NMR ($CDCl_3$): δ 7.55 (d, 1H, J = 17.2 Hz), 7.48 (s, 1H), 7.15–7.36 (m, 15H), 7.09 (s, 1H), 6.60 (d, 1H, J = 17.2 Hz), 3.80 (s, 3H).

6.3. 3-(1-Trityl-1*H*-imidazol-4-yl)-propionic acid methyl ester (29)

Compound **28** (1.5 g, 3.81 mmol) and 5% Pd/C (150 mg) were added to MeOH (25 mL) and the mixture was hydrogenated under a hydrogen atmosphere for 4 h. The suspension was filtered through a Celite-packed column and the filtrate was evaporated under reduced pressure to give **29** as a white solid (1.5 g, 99%). ^{1}H NMR ($CDCl_3$): δ 7.15–7.40 (m, 16H), 6.67 (s, 1H), 3.49 (s, 3H), 2.65 (t, 2H, J = 7.8 Hz), 2.10 (t, 2H, J = 7.8 Hz).

6.4. 3-(1-Trityl-1*H*-imidazol-4-yl)-propan-1-ol (30)

Compound **29** (1.25 g, 3.16 mmol) was added to a suspension of $LiAlH_4$ (0.24 g, 6.32 mmol) in dry THF (25 mL) and the resulting mixture was heated at reflux temperature for 6 h. Slowly, saturated sodium carbonate (5 mL) was added and stirred until all $LiAlH_4$ was destroyed. The mixture was filtered through a Celite-packed column and the filtrate was concentrated under reduced pressure. DCM (25 mL) was added and the resulting solution was washed with saturated sodium carbonate (3 × 50 mL) and dried over sodium sulfate. Concentration in vacuo gave **30** as a white solid (0.81 g, 70%). ^{1}H NMR ($CDCl_3$): δ 7.15–7.40 (m, 16H), 6.59 (s, 1H), 3.74 (t, 2H, J = 7.5 Hz), 2.69 (t, 2H, J = 7.5 Hz), 1.87 (quint, 2H, J = 7.5 Hz).

6.5. 3-(1*H*-Imidazol-4-yl)-propan-1-ol hydrobromide (5)

Compound **30** (0.5 g, 1.36 mmol) was dissolved in 1 N HBr (5 mL) and heated at reflux for 5 h. The mixture was washed with ether (3 × 25 mL) and the aqueous layer was concentrated under reduced pressure. The product was recrystallised from EtOH/ether to give a light yellow solid (0.13 g, 45%). ^{1}H NMR (D_2O): δ 8.54 (s, 1H), 7.21 (s, 1H), 3.62 (t, 2H, J = 7.2 Hz), 2.74 (t, 2H, J = 7.2 Hz), 1.92 (quint, 2H, J = 7.2 Hz). APCI/MS, m/z 127.7 $[M+1]^+$. Anal. ($C_6H_{11}N_2OBr \cdot 1.5H_2O$) C, H, N. Mp 83.1–84.0 °C.

6.6. 4-(3-[1,3]Dioxolan-2-yl-propenyl)-1-trityl-1*H*-imidazole (31)

Diisopropylamine (0.8 mL, 5.9 mmol) was added to dry THF (50 mL) and the solution was cooled to −30 °C. *n*-BuLi (3.7 mL, 1.6 M in *n*-hexane) was added dropwise and stirred at −30 °C for 15 min. Finely powdered [2-(1,3-dioxolan-2-yl)ethyl]-triphenylphosphonium bromide (2.6 g, 5.9 mmol) was added in one portion and the resulting mixture was stirred at 0 °C for 1 h. A solution of trityl-imidazol-4-yl carboxaldehyde (2 g, 5.9 mmol) in THF (50 mL) was added dropwise and the mixture was heated at reflux for 6 h. Saturated sodium carbonate (50 mL) was added, the organic solvent was separated and evaporated under reduced pressure. The residue was dissolved in DCM (50 mL) and washed with water (3 × 50 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (ether) to give **31** as a light yellow solid (2.1 g, 85%). ^{1}H NMR ($CDCl_3$): δ 7.39 (s, 1H), 7.15–7.35 (m, 15H), 6.79 (s, 1H), 6.36 (d, 1H, J = 11.7 Hz), 5.57 (dt, 1H, J = 11.7 and 7.1 Hz), 4.96 (t, 1H, J = 4.8 Hz), 3.79–3.92 (m, 4H), 2.87 (m, 2H).

6.7. 4-(3-[1,3]Dioxolan-2-yl-propyl)-1-trityl-1*H*-imidazole (32)

Compound **31** (2.0 g, 4.7 mmol) and 25 mg of 5% Pd/C were added to MeOH (25 mL). The mixture was hydrogenated under a hydrogen atmosphere at room temperature for 4 h and filtered through a Celite-packed column. The filtrate was concentrated under reduced pressure and recrystallised from EtOH to give **32** as a yellow solid (2.0 g, 100%). ^{1}H NMR ($CDCl_3$): δ 7.52 (s, 1H), 7.15–7.35 (m, 15H), 6.55 (s, 1H), 4.82 (t, 1H, J = 4.5 Hz), 3.79–3.94 (m, 4H), 2.64 (t, 2H, J = 7.5 Hz), 1.69 (m, 4H).

6.8. 4-(1-Trityl-1*H*-imidazol-4-yl)-butyraldehyde (33)

Compound **32** (1.8 g, 4.25 mmol) was dissolved in acetone (25 mL) and the solution was cooled in an ice bath. 1 N HCl was slowly added until the solution was pH 3. The resulting solution was stirred at 0 °C for 1 h and saturated sodium carbonate (25 mL) was added. The mixture was concentrated under reduced pressure, DCM (25 mL) was added to the residue and the organic layer was washed with water (3 × 25 mL), dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc) to give **33** as a light yellow solid (1.5 g, 95%). ^{1}H NMR ($CDCl_3$): δ 9.70 (s, 1H), 7.15–7.35 (m, 16H), 6.51 (s, 1H), 2.56 (t, 2H, J = 7.4 Hz), 2.42 (t, 2H, J = 7.4 Hz), 1.96 (quint, 2H, J = 7.4 Hz).

6.9. 4-(1-Trityl-1*H*-imidazol-4-yl)-butan-1-ol (34)

Using the procedure described for the synthesis for **30**. The title compound (**34**) was obtained from **33** (1.25 g,

3.29 mmol) as a light yellow solid (0.94 g, 75%). ^{1}H NMR ($CDCl_3$): δ 7.15–7.35 (m, 16H), 6.52 (s, 1H), 3.75 (t, 2H, J = 7.5 Hz), 2.70 (t, 2H, J = 7.5 Hz), 1.72 (m, 4H).

6.10. 5-Hydroxy-5,6-dihydro-2H-imidazo[1,5-*a*]pyridin-4-ium (35)

NMR (D_2O): δ 8.82 (s, 1H), 7.30 (s, 1H), 6.65 (d, J = 11.4 Hz, 1H), 6.20 (m, J = 11.4, 1H), 6.10 (m, 1H), 2.75–2.85 (m, 2H). MS (m/z): 138.11 $[M+1]^+$.

6.11. 4-(1*H*-Imidazol-4-yl)-butan-1-ol hydrochloride (6)

Compound **33** (0.75 g, 1.96 mmol) was dissolved in 1 N HCl (5 mL) and heated under reflux for 5 h. The mixture was washed with ether (3 × 25 mL) and the aqueous layer was concentrated under reduced pressure. The product was recrystallised from EtOH/ether to give a light yellow solid (0.14 g, 40%). ^{1}H NMR (D_2O): δ 8.52 (s, 1H), 7.23 (s, 1H), 3.71 (t, 2H, J = 7.6 Hz), 2.79 (t, 2H, J = 7.6 Hz), 1.55–1.89 (m, 4H). APCI/MS, m/z 141.2 $[M+1]^+$. Anal. ($C_7H_{13}N_2OCl{\cdot}0.7H_2O$) C, H, N. Mp 89.8–91.2 °C.

6.12. 3-(1-Trityl-1*H*-imidazol-4-yl)-propionaldehyde (36)

A solution of oxalyl chloride (0.6 mL, 6.8 mmol) in dry DCM (50 mL) was cooled to −60 °C under a nitrogen atmosphere. A solution of dimethylsulfoxide (1.0 mL, 14.1 mmol) in DCM (50 mL) was added dropwise and subsequently stirred for 15 min at −60 °C. Next, a solution of **29** (2 g, 5.4 mmol) in dry DCM (50 mL) was added dropwise and stirred for 45 min at −60 °C. Subsequently, triethylamine (2.5 mL, 17.8 mmol) was added and the mixture was warmed to room temperature. The reaction mixture was washed with water (3 × 100 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure. After the purification by flash chromatography (EtOAc), the product (**36**) was obtained as a light yellow solid (1.54 g, 78%). ^{1}H NMR ($CDCl_3$): δ 9.7 (s, 1H), 7.1–7.3 (m, 16H), 6.5 (s, 1H), 2.8–2.9 (m, 4H).

6.13. 5-(1-Trityl-1*H*-imidazol-4-yl)-pent-2-enoic acid ethyl ester (37)

NaH (60% dispersed in mineral oil, 0.18 g, 4.5 mmol) was suspended in THF (25 mL) and cooled in an ice bath. A solution of triethyl phosphonoacetate (0.9 mL, 4.5 mmol) in THF (25 mL) was added dropwise. The mixture was stirred at room temperature for 30 min and a solution of **36** (1.50 g, 4.1 mmol) in THF (25 mL) was added dropwise. The resulting mixture was stirred for additional 6 h at room temperature, cooled to 0 °C and water (100 mL) was added. The mixture was extracted with ether (3 × 50 mL), the combined ether layers were dried over sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography (EtOAc) yielded **37** as a light yellow solid (1.35 g, 75%). ^{1}H NMR ($CDCl_3$): δ 7.39 (s, 1H), 7.15–7.35 (m, 15H), 6.76 (dt, 1H, J = 14.5 and 7.6 Hz), 6.52 (s, 1H), 5.61 (d, 1H, J = 14.5 Hz), 4.15 (q, 2H, J = 7.5 Hz), 2.62 (t, 2H, J = 7.6 Hz), 2.45 (m, 2H), 1.21 (t, 3H, J = 7.5 Hz).

6.14. 5-(1-Trityl-1*H*-imidazol-4-yl)-pentanoic acid ethyl ester (38)

Using the procedure described for the synthesis for **29** but **37** (1.25 g, 2.86 mmol) was used. The title compound (**38**) was obtained as a light yellow solid (1.25 g, 100%). ^{1}H NMR ($CDCl_3$): δ 7.15–7.35 (m, 16H), 6.54 (s, 1H), 4.08 (q, 2H, J = 7.5 Hz), 2.65 (t, 2H, J = 7.4 Hz), 2.28 (t, 2H, J = 7.4 Hz), 1.52–1.84 (m, 4H), 1.20 (t, 3H, J = 7.5 Hz).

6.15. 5-(1-Trityl-1*H*-imidazol-4-yl)-pentan-1-ol (39)

Using the procedure described for the synthesis for **30** but **38** (1.25 g, 2.85 mmol) was used. The title compound (**39**) was obtained as a light yellow solid (0.9 g, 80%). ^{1}H NMR ($CDCl_3$): δ 7.15–7.35 (m, 16H), 6.55 (s, 1H), 3.64 (t, 2H, J = 7.5 Hz), 2.62 (t, 2H, J = 7.5 Hz), 1.52–1.84 (m, 4H), 1.41 (m, 2H).

6.16. 5-(1*H*-Imidazol-4-yl)-pentan-1-ol hydrochloride (7)

Using the procedure described for the synthesis for **6** but **39** (0.9 g, 2.28 mmol) was used. The title compound (**7**) was recrystallised from EtOH/ether to give a light yellow solid (0.23 g, 52%). ^{1}H NMR (D_2O): δ 8.49 (s, 1H), 7.15 (s, 1H), 3.56 (t, 2H, J = 7.8 Hz), 2.72 (t, 2H, J = 7.8 Hz), 1.52–1.84 (m, 4H), 1.38 (m, 2H). APCI/MS, m/z 155.2 $[M+1]^+$. Anal. ($C_8H_{15}N_2OCl{\cdot}0.3H_2O$) C, H, N. Mp 94.5–95.8 °C.

6.17. (Tetrahydropyran-4-ylidene)-acetic acid ethyl ester (40)

Using the procedure described for the synthesis for **37** but tetrahydropyran-4-one (1 g, 10 mmol) of was used. The title compound (**40**) was obtained as a light yellow oil (1.5 g, 88%). ^{1}H NMR ($CDCl_3$): δ 5.66 (s, 1H), 4.12 (q, 2H, J = 7.1 Hz), 3.72 (m, 4H), 3.00 (m, 2H), 2.32 (m, 2H), 1.24 (t, 3H, J = 7.1 Hz).

6.18. (Tetrahydropyran-4-yl)-acetic acid ethyl ester (41)

Using the procedure described for the synthesis for **38** but **40** (1.5 g, 8.8 mmol) was used. The title compound (**41**) was obtained as a light yellow oil (1.5 g, 100%). ^{1}H NMR ($CDCl_3$): δ 4.14 (q, 2H, J = 7.1 Hz), 4.08 (m, 2H), 3.38 (m, 2H), 2.20 (d, 2H, J = 7.2 Hz), 1.99 (m, 1H), 1.55 (m, 2H), 1.24–1.41 (m, 5H).

6.19. 2-(Tetrahydropyran-4-yl)-ethanol (42)

Using the procedure described for the synthesis for **39** but **41** (1.5 g, 8.8 mmol) was used. The title compound (**42**) was obtained as a colourless oil (0.77 g, 67%). ^{1}H NMR ($CDCl_3$): δ 3.95 (m, 2H), 3.68 (t, 2H, J = 7.1 Hz), 3.44 (m, 2H), 1.30–1.62 (m, 7H).

6.20. (Tetrahydropyran-4-yl)-acetaldehyde (43)

Using the procedure described for the synthesis for **36** but **42** (0.75 g, 5.8 mmol) was used. The title compound (**43**) was obtained as a light yellow oil (0.55 g, 75%). ^{1}H

NMR ($CDCl_3$): δ 9.77 (s, 1H), 3.94 (m, 2H), 3.40 (m, 2H), 2.40 (d, 2H, J = 7.0 Hz), 2.30 (m, 1H), 1.37–1.63 (m, 4H).

6.21. 4-(Tetrahydropyran-4-ylmethyl)-1*H*-imidazole hydrochloride (13)

Finely powdered sodium cyanide (21.1 mg, 0.4 mmol) was added in one portion to a stirred suspension of tosylmethyl isocyanide (TosMIC) (0.92 g, 4.7 mmol) and **43** (0.55 g, 4.3 mmol) in absolute ethanol (25 mL). The reaction mixture became clear and the solution was stirred for additional 2 h. The solvent was evaporated under reduced pressure and $CHCl_3$ (25 mL) was added. The resulting mixture was filtered and the filtrate was concentrated in vacuo to give a brown oil, which was transferred to a stainless steel bomb. A saturated solution of ammonia in ethanol (50 mL) was added and the mixture was heated at 90–110 °C for 16 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in DCM (50 mL) and washed with 10% sodium carbonate solution (50 mL) and water (2 × 50 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to give a dark brown oil which was subsequently treated with activated charcoal. Filtration and purification using flash chromatography (MeOH) gave the title compound (**13**) as a light-yellow oil (0.54 g, 75%). ^{1}H NMR (D_2O): δ 8.53 (s, 1H), 7.22 (s, 1H), 3.96 (m, 2H), 3.45 (m, 2H), 2.68 (d, 2H, J = 7.0 Hz), 1.90 (m, 1H), 1.59 (m, 2H), 1.30 (m, 2H). APCI/MS, *m/z* 167.2 $[M+1]^+$. Anal. ($C_9H_{15}N_2OCl{\cdot}0.5H_2O$) C, H, N. Mp 142.9–144.2 °C.

6.22. 4-Hydroxy-cyclohexanecarboxylic acid methyl ester (44)

In a stainless steel bomb, methyl *p*-hydroxybenzoate (10 g, 65.8 mmol) and 5% Rh/C (1 g) were suspended in THF (100 mL). The suspension was hydrogenated under 100 atm hydrogen for 18 h. The mixture was filtered through a Celite-packed column and the filtrate was concentrated under reduced pressure. The crude product was purified by bulb-to-bulb distillation (80–100 °C, 1 mmHg) to give the product (**44**) as a colourless oil (10.2 g, 98%). ^{1}H NMR ($CDCl_3$): δ 3.78 (m, 1H), 3.65 (s, 3H), 2.21–2.50 (m, 2H), 1.10–1.95 (m, 8H).

6.23. 4-Oxo-cyclohexanecarboxylic acid methyl ester (45)

Celite (10 g), sodium acetate (1.5 g, 19.0 mmol) and pyridinium chlorochromate (20.5 g, 94.8 mmol) were suspended in DCM (200 mL). Compound **44** (10 g, 63.2 mmol) was added dropwise and the mixture was stirred at room temperature for 4 h. The resulting mixture was filtered by suction through a column packed with silica gel (75 g) and the silica gel was washed thoroughly with ether (250 mL). After concentration of the filtrate under reduced pressure, the product (**45**) was obtained as a colourless oil (8.9 g, 90%) and was used in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 3.71 (s, 3H), 2.78 (m, 1H), 1.89–2.55 (m, 8H).

6.24. 1,4-Dioxa-spiro[4.5]decane-8-carboxylic acid methyl ester (46)

Compound **45** (8.5 g, 54.4 mmol), ethylene glycol (3.8 mL, 68.0 mmol) and *p*-toluenesulfonic acid monohydrate (1.1 g, 5.8 mmol) were dissolved in toluene (250 mL) and heated at reflux in Dean–Stark apparatus for 18 h. The reaction mixture was concentrated under reduced pressure and 50% $NaHCO_3$ solution (200 mL) was added. The resulting mixture was extracted with ether (3 × 200 mL) and the combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by bulb-to-bulb distillation (138–145 °C, 15 mmHg) to give the product (**46**) as a colourless oil (9.8 g, 90%). ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 4H), 3.68 (s, 3H), 2.29 (m, 1H), 1.40–2.0 (m, 8H).

6.25. (1,4-Dioxa-spiro[4.5]dec-8-yl)-methanol (47)

Using the procedure described for the synthesis for **30** but **46** (9.5 g, 47.5 mmol) was used. The title compound (**47**) was obtained as a colourless oil (7.8 g, 95%). ^{1}H NMR ($CDCl_3$): δ 3.96 (s, 4H), 3.48 (d, 2H, J = 7.5 Hz), 1.77 (m, 4H), 1.47 (m, 4H), 1.29 (m, 2H).

6.26. 1,4-Dioxa-spiro[4.5]decane-8-carbaldehyde (48)

Using the procedure described for the synthesis for **37** but **47** (7.5 g, 43.6 mmol) was used. The title compound (**48**) was obtained as a light yellow oil (7.2 g, 97%). ^{1}H NMR ($CDCl_3$): δ 9.69 (s, 1H), 3.96 (s, 4H), 2.27 (m, 1H), 1.50–2.05 (m, 8H).

6.27. 4-(1,4-Dioxa-spiro[4.5]dec-8-yl)-1*H*-imidazole (49)

Using the procedure described for the synthesis for **13** but **48** (7.0 g, 41.1 mmol) was used. The title compound (**49**) was obtained as a light yellow oil (6.4 g, 75%). ^{1}H NMR ($CDCl_3$): δ 7.60 (s, 1H), 6.79 (s, 1H), 3.96 (s, 4H), 2.72 (m, 1H), 2.09 (m, 2H), 1.55–1.90 (m, 6H).

6.28. 4-(1*H*-Imidazol-4-yl)-cyclohexanone (50)

Compound **49** (6.2 g, 29.8 mmol) was dissolved in 1 N HCl (150 mL) and stirred at room temperature for 1 h. The resulting mixture was washed with $CHCl_3$ (3 × 150 mL) and subsequently basified with sodium carbonate until pH ≈ 12 and extracted with $CHCl_3$ (3 × 150 mL). The combined $CHCl_3$ layers were dried over sodium sulfate and concentrated under reduced pressure. Purification with flash chromatography (MeOH) gave the product **50** as a light yellow oil (4.6 g, 94%). ^{1}H NMR ($CDCl_3$): δ 7.61 (s, 1H), 6.82 (s, 1H), 3.12 (m, 1H), 2.20–2.55 (m, 6H), 1.80–1.95 (m, 2H).

6.29. 4-(4-Oxo-cyclohexyl)-imidazole-1-carboxylic acid *tert*-butyl ester (51)

Compound **50** (4.5 g, 27.4 mmol) and triethylamine (7.7 mL, 54.8 mmol) were dissolved in $CHCl_3$ (100 mL). The solution was cooled in an ice bath and a solution of di-*tert*-butyldicarbonate (6.6 g, 30.1 mmol) in $CHCl_3$ (100 mL) was added dropwise. The mixture was stirred

at room temperature for 16 h and washed with saturated sodium carbonate (3 × 200 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The product **51** was recrystallised from ether as a light yellow solid (5.1 g, 70%). ^{1}H NMR ($CDCl_3$): δ 8.02 (s, 1H), 7.13 (s, 1H), 3.07 (m, 1H), 2.30–2.55 (m, 6H), 1.80–2.05 (m, 2H), 1.62 (s, 9H).

6.30. 4-(4-Hydroxy-cyclohexyl)-imidazole-1-carboxylic acid *tert*-butyl ester (52)

Compound **51** (2.0 g, 7.5 mmol) was dissolved in MeOH (50 mL) and sodium borohydride (0.3 g, 7.9 mmol) was slowly added. The mixture was stirred at room temperature for 16 h and concentrated under reduced pressure. Saturated sodium carbonate (50 mL) was added and the resulting mixture was washed with THF (3 × 50 mL). The combined organic layers were dried over sodium sulfate and concentrated. After flash chromatography (MeOH), the product **52** was obtained as a light yellow solid (1.3 g, 65%). ^{1}H NMR ($CDCl_3$): δ 7.98 (s, 1H), 7.10 (s, 1H), 3.85 and 3.67 (m, 1H (cis and trans)), 2.65–2.85 (m, 1H), 2.00 (m, 4H), 1.65–1.85 (m, 2H), 1.62 (s, 9H), 1.40 (m, 2H).

6.31. 4-(1*H*-Imidazol-4-yl)-cyclohexanol hydrobromide (23)

Compound **52** (1.0 g, 3.8 mmol) was added to 1 N HBr (5 mL) and stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the product **23** was recrystallised from EtOH/ether as a light yellow solid (0.4 g, 45%). ^{1}H NMR (D_2O): δ 8.47 and 8.50 (s, 1H (cis and trans)), 7.12 and 7.20 (s, 1H (cis and trans)), 3.63 and 3.95 (m, 1H (cis and trans)), 2.68–2.85 (m, 1H), 2.00 (m, 2H), 1.65–1.85 (m, 2H), 1.40 (m, 4H). APCI/MS, *m/z* 167.6 $[M+1]^+$. Anal. ($C_9H_{15}N_2OBr{\cdot}1.0H_2O$) C, H, N. Mp 167.2–169.0 °C.

6.32. 4-(4-*tert*-Butoxycarbonylamino-cyclohexyl)-imidazole-1-carboxylic acid *tert*-butyl ester (53a and b)

Compound **51** (2.0 g, 7.5 mmol), ammonium acetate (5.8 g, 75.0 mmol) and sodium borohydride (0.3 g, 7.9 mmol) were added to MeOH (50 mL). The mixture was stirred at room temperature for 16 h and concentrated under reduced pressure. Saturated sodium carbonate (50 mL) was added and the resulting mixture was washed with THF (3 × 50 mL). The combined organic layers were dried over sodium sulfate and concentrated. The residue was dissolved in $CHCl_3$ (50 mL) and triethylamine (2.1 mL, 15.0 mmol) was added. The mixture was cooled in an ice bath and a solution of di-*tert*-butyldicarbonate (1.6 g, 7.5 mmol) in $CHCl_3$ (50 mL) was added dropwise. The mixture was stirred at room temperature for 16 h and washed with saturated sodium carbonate (3 × 500 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The products **53a** and **b** were separated by flash chromatography (*n*-hexane:DCM:EtOH (saturated with NH_3) 7.5:2:0.5) as a light yellow solid (0.5 and 0.8 g, respectively). ^{1}H NMR ($CDCl_3$): compound **53a** (cis) δ 7.97 (s, 1H), 7.04 (s, 1H), 4.64 (br, 1H), 3.75 (m, 1H), 2.61 (m, 1H), 1.65–2.00 (m, 4H), 1.62 (s, 9H), 1.43 (s, 9H), 1.15–1.40 (m, 4H). Compound **53b** (trans) δ 7.95 (s, 1H), 7.00 (s, 1H), 4.41 (br, 1H), 3.47 (m, 1H), 2.48 (m, 1H), 2.10 (m, 4H), 1.57 (s, 9H), 1.42 (s, 9H), 1.05–1.40 (m, 4H).

6.33. 4-(1*H*-Imidazol-4-yl)-cyclohexylamine dihydrobromide (20 and 21)

Using the procedure described for the synthesis for **23**. Compounds **53a** or **b** (0.5 g, 1.4 mmol) of were used, and the title products **20** and **21** were obtained, respectively, as light yellow solids (0.20 and 0.25 g, respectively). ^{1}H NMR (D_2O): compound **20** (cis) δ 8.56 (s, 1H), 7.26 (s, 1H), 3.43 (m, 1H), 3.09 (m, 1H), 1.96 (m, 6H), 1.68 (m, 2H). APCI/MS, *m/z* 166.3 $[M+1]^+$. Anal. ($C_9H_{17}N_3Br_2{\cdot}0.5H_2O$) C, H, N. Mp 241.2–242.4 °C. Compound **21** (trans) δ 8.53 (s, 1H), 7.18 (s, 1H), 3.23 (m, 1H), 2.78 (m, 1H), 2.13 (m, 4H), 1.49 (m, 4H). APCI/MS, *m/z* 166.6 $[M+1]^+$. Anal. ($C_9H_{17}N_3Br_2{\cdot}0.3H_2O$) C, H, N. Mp > 300 °C.

6.34. (1,4-Dioxa-spiro[4.5]dec-8-ylidene)-acetic acid ethyl ester (54)

Using the procedure described for the synthesis for **40** but 1,4-Dioxa-spiro[4.5]decan-8-one (10.0 g, 64.0 mmol) of was used. The title compound (**54**) was obtained as a light yellow oil (13.8 g, 95%). ^{1}H NMR ($CDCl_3$): δ 5.62 (s, 1H), 4.14 (q, 2H, J = 7.3 Hz), 3.98 (s, 4H), 2.95 (m, 2H), 2.33 (m, 2H), 1.72 (m, 4H), 1.21 (t, 3H, J = 7.3 Hz).

6.35. (1,4-Dioxa-spiro[4.5]dec-8-yl)-acetic acid ethyl ester (55)

Using the procedure described for the synthesis for **41** but **54** (13.0 g, 57.4 mmol) was used. The title compound (**55**) was obtained as a light yellow oil (13.2 g, 100%). ^{1}H NMR ($CDCl_3$): δ 4.14 (q, 2H, J = 7.5 Hz), 3.98 (s, 4H), 2.24 (d, 2H, J = 7.4 Hz), 1.45–1.95 (m, 9H), 1.25 (t, 3H, J = 7.5 Hz).

6.36. 2-(1,4-Dioxa-spiro[4.5]dec-8-yl)-ethanol (56)

Using the procedure described for the synthesis for **42** but **55** (13.0 g, 57.0 mmol) was used. The title compound (**56**) was obtained as a colourless oil (10.0 g, 95%). ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 4H), 3.67 (t, 2H, J = 7.8 Hz), 1.20–1.95 (m, 11H).

6.37. (1,4-Dioxa-spiro[4.5]dec-8-yl)-acetaldehyde (57)

Using the procedure described for the synthesis for **43** but **56** (9.0 g, 48.4 mmol) was used. The title compound (**57**) was obtained as a light yellow oil (8.0 g, 90%). ^{1}H NMR ($CDCl_3$): δ 9.75 (s, 1H), 3.98 (s, 4H), 2.45 (d, 2H, J = 7.8 Hz), 1.30–1.98 (m, 9H).

6.38. 4-(1,4-Dioxa-spiro[4.5]dec-8-ylmethyl)-1*H*-imidazole (58)

Using the procedure described for the synthesis for **44** but **57** (8.0 g, 43.3 mmol) was used. The title compound

(**58**) was obtained as a light yellow oil (6.8 g, 70%). ^{1}H NMR ($CDCl_3$): δ 7.52 (s, 1H), 6.70 (s, 1H), 3.89 (s, 4H), 2.56 (d, 2H, J = 7.2 Hz), 1.73 (m, 4H), 1.25–1.49 (m, 5H).

6.39. 4-(1*H*-Imidazol-4-ylmethyl)-cyclohexanone (59)

Using the procedure described for the synthesis for **50** but **58** (6.8 g, 30.4 mmol) was used. The title compound (**59**) was obtained as a light yellow oil (5.2 g, 95%). ^{1}H NMR ($CDCl_3$): δ 7.57 (s, 1H), 6.79 (s, 1H), 2.54 (d, 2H, J = 7.5 Hz), 1.95–2.35 (m, 7H), 1.30–1.45 (m, 2H).

6.40. 4-(4-Oxo-cyclohexylmethyl)-imidazole-1-carboxylic acid *tert*-butyl ester (60)

Using the procedure described for the synthesis for **51** but **59** (5.0 g, 28.0 mmol) was used. The title compound (**60**) was obtained as a light yellow oil (5.8 g, 75%). ^{1}H NMR ($CDCl_3$): δ 8.05 (s, 1H), 7.13 (s, 1H), 2.57 (d, 2H, J = 7.5 Hz), 2.39 (m, 4H), 2.10 (m, 3H), 1.67 (s, 9H), 1.40–1.65 (m, 2H).

6.41. 4-(4-Hydroxy-cyclohexylmethyl)-imidazole-1-carboxylic acid *tert*-butyl ester (61)

Using the procedure described for the synthesis for **53** but **60** (2.9 g, 10.4 mmol) was used. The title compound (**61**) was obtained as a light yellow oil (2.1 g, 70%). ^{1}H NMR ($CDCl_3$): δ 8.05 (s, 1H), 7.10 (s, 1H), 3.65 and 4.01 (m, 1H (cis and trans)), 2.45 and 2.50 (d, 2H (cis and trans), J = 7.5 Hz), 1.89 (m, 2H), 1.70 (m, 2H), 1.62 (s, 9H), 0.95–1.60 (m, 5H).

6.42. 4-(1*H*-Imidazol-4-ylmethyl)-cyclohexanol hydrobromide (24)

Using the procedure described for the synthesis for **23** but **61** (2.0 g, 7.1 mmol) was used. The title compound (**24**) was obtained as a light yellow solid (0.6 g, 35%). ^{1}H NMR (D_2O): δ 8.52 (s, 1H), 7.19 (s, 1H), 3.57 and 3.92 (m, 1H (cis and trans)), 2.60 (d, 2H, J = 7.5 Hz), 1.97 (m, 2H), 1.78 (m, 2H), 1.62 (m, 1H), 1.00–1.45 (m, 4H). APCI/MS, m/z 181.2 $[M+1]^+$. Anal. ($C_{10}H_{17}N_2OBr{\cdot}0.5H_2O$) C, H, N. Mp 167.2–168.9 °C.

6.43. 4-(4-Amino-cyclohexylmethyl)-imidazole-1-carboxylic acid *tert*-butyl ester (62)

Compound **61** (2.9 g, 10.4 mmol), ammonium acetate (8.0 g, 104.0 mmol) and sodium borohydride (0.4 g, 10.6 mmol) were added to MeOH (50 mL). The mixture was stirred at room temperature for 16 h and concentrated under reduced pressure. Saturated sodium carbonate (50 mL) was added and the resulting mixture was washed with THF (3 × 50 mL). The combined organic layers were dried over sodium sulfate and concentrated. After flash chromatography (MeOH), the product (**62**) was obtained as a light yellow oil (2.0 g, 70%). ^{1}H NMR ($CDCl_3$): δ 8.12 (s, 1H), 7.15 (s, 1H), 2.76 and 3.08 (m, 1H (cis and trans)), 2.52 (m, 2H), 1.68–1.87 (m, 2H), 1.60 (s, 9H), 1.45 (m, 3H), 0.90–1.25 (m, 4H).

6.44. 4-(1*H*-Imidazol-4-ylmethyl)-cyclohexylamine dihydrobromide (22)

Using the procedure described for the synthesis for **23** but **62** (1.0 g, 3.6 mmol) was used. The title compound (**22**) was obtained as a light yellow oil (0.5 g, 41%). ^{1}H NMR (D_2O): δ 8.50 (s, 1H), 7.17 (s, 1H), 3.09 and 3.38 (m, 1H (cis and trans)), 2.60 (d, 2H, J = 7.0 Hz), 1.96 (m, 2H), 1.74 (m, 2H), 1.60 (m, 1H), 1.38 (m, 2H), 1.11 (m, 2H). APCI/MS, m/z 180.6 $[M+1]^+$. Anal. ($C_{10}H_{19}N_3Br_2$) C, H, N. Mp > 300 °C.

6.45. 4-(4-Nitro-phenyl)-1*H*-imidazole (63)

4-Phenyl-imidazole (0.5 g, 3.47 mmol) was dissolved in concentrated sulfuric acid (5 mL) at 0 °C and fuming nitric acid (0.15 mL) was added. The mixture was stirred for 2 h and ice cubes (ca. 25 g) were added. The resulting mixture was stirred for 10 min and filtered. The residue was washed with water (25 mL). The filtrate was cooled in an ice bath and basified with 10% sodium hydroxide solution until the pH was basic. The yellow suspension was filtered and the remained solid was washed with water (50 mL). The product (**63**) was purified by flash chromatography (EtOAc) as a yellow solid (0.3 g, 45%). ^{1}H NMR ($CDCl_3$): δ 8.00 (d, 2H, J = 7.8 Hz), 7.72 (d, 2H, J = 7.8 Hz), 7.47 (s, 1H), 7.29 (s, 1H).

6.46. 4-(1*H*-Imidazol-4-yl)-phenylamine dihydrobromide (25)

Compound **63** (0.25 g, 1.32 mmol), 5% Pd/C (25 mg) and 1 N HBr (5 mL) were added to MeOH (25 mL). The mixture was hydrogenated under hydrogen atmosphere at room temperature for 4 h and filtered through a Celite-packed column. The filtrate was concentrated under reduced pressure and recrystallised from EtOH to give **25** as a yellow solid (0.27 g, 65%). ^{1}H NMR (D_2O): δ 8.79 (s, 1H), 7.82 (m, 3H), 7.53 (d, 2H, J = 7.8 Hz). APCI/MS, m/z 160.3 $[M+1]^+$. Anal. ($C_9H_{11}N_3Br_2$) C, H, N. Mp > 300 °C.

6.47. (4-Nitro-phenyl)-(1-trityl-1*H*-imidazol-4-yl)-methanol (64)

1-Trityl-4-iodo-imidazole (2 g, 4.58 mmol) was dissolved in DCM (50 mL), ethylmagnesium bromide (3.0 M in THF, 1.6 mL) was added and the resulting mixture was stirred at room temperature for 45 min. 4-Nitrobenzaldehyde (0.76 g, 5.04 mmol) dissolved in DCM (50 mL) was added dropwise and stirred at room temperature for 16 h. The mixture was washed with saturated sodium carbonate solution (3 × 50 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent and recrystallisation from EtOAc yielded **64** as a pale yellow solid (1.8 g, 85%). ^{1}H NMR ($CDCl_3$): δ 8.14 (d, 2H, J = 7.8 Hz), 7.56 (d, 2H, J = 7.8 Hz), 7.41 (s, 1H), 7.04–7.32 (m, 15H), 6.60 (s, 1H), 5.83 (s, 1H).

6.48. Acetic acid (4-nitro-phenyl)-(1-trityl-1*H*-imidazol-4-yl)-methyl ester (65)

Compound **64** (1.5 g, 3.25 mmol) and 4-dimethylaminopyridine (DMAP, 0.04 g, 0.33 mmol) were dissolved

in DCM (50 mL). The solution was cooled in an ice bath and acetic anhydride (0.5 mL, 5 mmol) was added dropwise and stirred for 1 h and heated under reflux for 6 h. After cooling to the room temperature, saturated sodium carbonate (50 mL) was added. The organic layer was separated, washed with water (3×50 mL), dried over sodium sulfate and evaporated under reduced pressure. The product (**65**) was recrystallised from EtOAc as a light yellow solid (1.55 g, 95%). ^{1}H NMR ($CDCl_3$): δ 8.12 (d, 2H, $J = 7.8$ Hz), 7.56 (d, 2H, $J = 7.8$ Hz), 7.43 (s, 1H), 7.04–7.35 (m, 15H), 6.77 (s, 1H), 6.72 (s, 1H), 2.14 (s, 3H).

6.49. 4-(1*H*-Imidazol-4-ylmethyl)-phenylamine dihydrobromide (26)

Compound **65** (1.25 g, 2.48 mmol) and 10% Pd/C (0.15 g) were added to MeOH (25 mL). The mixture was stirred under a hydrogen atmosphere at room temperature for 4 h and filtered through a Celite-packed column. HBr (5 mL) was added and the solution was concentrated under reduced pressure. The title compound (**26**) was recrystallised from EtOH/ether as a light yellow solid (0.45 g, 55%). ^{1}H NMR (D_2O): δ 8.61 (s, 1H), 7.42 (m, 4H), 7.25 (s, 1H), 4.17 (s, 2H). APCI/MS, *m/z* 174.2 $[M+1]^+$. Anal. ($C_{10}H_{13}N_3Br_2$) C, H, N. Mp > 300 °C.

7. Pharmacology

Radioligand displacement studies were performed as follows; SK-N-MC cells, a human neuroblastoma cell line, stably expressing the human histamine $H_{3(445aa)}$ or H_4 receptor[12,39] were grown at 37 °C in a humidified atmosphere with 5% CO_2 in minimum essential medium eagle (EMEM), supplemented with foetal calf serum (10%, v/v), penicillin (50 IU/ml), non-essential amino acids, L-glutamine (2 mM), streptomycin (50 μg/ml) and sodium pyruvate (50 μg/ml) in the presence of G418 (600 μg/ml). H_3 and H_4 receptor affinity was determined by [^{3}H]-N^{α}-methylhistamine (82.0 Ci/mmol, NEN Dupont, Boston MA, USA) and [^{3}H]-histamine (23.2 Ci/mmol, NEN Dupont, Boston MA, USA) binding as described.[40] Binding data were analyzed using Graphpad Prism 3.0; K_i values were calculated from the IC_{50} according to the Cheng and Prusoff equation.[41]

The functional experiments were performed with SK-N-MC cells, stably co-expressing the human H_3 or H_4 receptor and the β-galactosidase gene under the control of cAMP-responsive elements. In these experiments, cells were stimulated for 6 h with forskolin and histamine receptor ligands, whereafter the cells were lysed and β-galactosidase activity was measured colourimetrically as described previously.[27]

Appendix A

Compound	VUF	Mp (°C)	APCI/MS $[M+1]^+$	Formula	Elemental analysis (C, H, N)
5	5698	83.1–84.0	127.7	$C_6H_{11}N_2OBr{\cdot}1.5H_2O$	Calcd (30.8, C; 6.0, H; 12.0, N) Anal. (30.94, C; 5.85, H; 12.2, N)
6	5793	89.8–91.2	141.2	$C_7H_{13}N_2OCl{\cdot}0.7H_2O$	Calcd (44.4, C; 7.7, H; 14.8, N) Anal. (44.26, C; 7.83, H; 14.6, N)
7	5657	94.5–95.8	155.2	$C_8H_{15}N_2OCl{\cdot}0.3H_2O$	Calcd (48.8, C; 8.0, H; 14.2, N) Anal. (48.87, C; 8.06, H; 14.2, N)
13	5870	142.9–144.2	167.2	$C_9H_{15}N_2OCl{\cdot}0.5H_2O$	Calcd (51.1, C; 7.6, H; 13.2, N) Anal. (50.97, C; 7.48, H; 13.2, N)
20	5803	241.2–242.4	166.3	$C_9H_{17}N_3Br_2{\cdot}0.5H_2O$	Calcd (32.2, C; 5.4, H; 12.5, N) Anal. (32.28, C; 5.35, H; 12.6, N)
21	5804	>300	166.6	$C_9H_{17}N_3Br_2{\cdot}0.3H_2O$	Calcd (32.5, C; 5.3, H; 12.6, N) Anal. (32.47, C; 5.44, H; 12.5, N)
22	5805	>300	180.6	$C_{10}H_{19}N_3Br_2$	Calcd (35.2, C; 5.6, H; 12.3, N) Anal. (35.02, C; 5.58, H; 12.3, N)
23	5796	167.2–169.0	167.2	$C_9H_{15}N_2OBr{\cdot}1.0H_2O$	Calcd (40.8, C; 6.5, H; 10.6, N) Anal. (40.67, C; 6.43, H; 10.5, N)
24	5798	167.2–168.9	181.2	$C_{10}H_{17}N_2OBr{\cdot}0.5H_2O$	Calcd (44.5, C; 6.7, H; 10.4, N) Anal. (44.45, C; 6.63, H; 10.4, N)
25	5801	>300	160.3	$C_9H_{11}N_3Br_2$	Calcd (33.7, C; 3.5, H; 13.1, N) Anal. (33.76, C; 3.32, H; 13.0, N)
26	5802	>300	174.2	$C_{10}H_{13}N_3Br_2$	Calcd (35.8, C; 3.9, H; 12.5, N) Anal. (36.02, C; 3.78, H; 12.5, N)

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