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Ligand based design of novel histamine H_4 receptor antagonists; fragment optimization and analysis of binding kinetics

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ABSTRACT

The histamine H_4 receptor is a G protein-coupled receptor that has attracted much interest for its role in inflammatory and immunomodulatory functions. In our search for new H_4R ligands, a low affinity isoquinoline fragment was optimized to 7-(furan-2-yl)-4-(piperazin-1-yl)quinazolin-2-amine (VUF11489), as a new H_4R antagonist. Analysis of its binding kinetics at the human H_4R showed this compound to have a very different dissociative half-life in comparison with reference antagonist JNJ7777120.

Histamine is an endogenous compound with a plethora of pharmacological activities that is mediated by four distinct G protein-coupled receptor subtypes. The H_1R and H_2R have been successfully exploited as drug targets leading to blockbuster drugs for the treatment of allergic conditions such as hay-fever (H_1R) as well as the treatment of peptic ulcers (H_2R).[1] With several compounds currently in clinical trials, the H_3R is the subject of intensive research and has been implicated in a variety of diseases including ADHD, narcolepsy and obesity.[2] The H_4R was independently discovered in 2000 by several groups and is now recognised to play a role in allergic and inflammatory responses, pruritis and the modulation of inflammatory and neuropathic pain.[3–9] The first non-imidazole H_4R antagonist that has been reported is indolecarboxamide JNJ7777120 (**1**, Fig. 1).[10] This highly potent compound (hH_4R K_i = 6 nM, rH_4R K_i = 6 nM) has been used effectively in animal models of inflammatory disease and can be considered the most widely used reference antagonist for H_4R research.[11] A close analogue of **1** that was synthesized in a study of its metabolic and pharmacokinetic parameters is benzimidazole **2**. Despite the more favourable in vitro properties of **2**, these did not translate to an improved in vivo half-life in the rat.[12] Recently, another chemically distinct H_4R antagonist, A-943931 (**3**), was developed by scientists from Abbott Laboratories.[9] This compound combines a high affinity for the H_4Rs of human (K_i = 5 nM), rat (K_i = 4 nM) and mouse (K_b = 6 nM) with a 640-fold selectivity over the hH_3R. A-943931 has an oral bioavailability of 34% in the rat and an in vivo half-life of 2.6 h. A recently described analogue of **3** is A-987306 (**4**). This compound has excellent affinity for the rat (K_i = 4 nM) and human H_4R (K_b = 6 nM) and has 162-fold, selectivity for the hH_4R over the hH_3R.[13] It has improved in vivo pharmacokinetics in the rat with an elimination half-life of 3.7 h and a bioavailability of 26% after oral dosing.

Previously we reported the development of a flexible alignment model for the design of new H_4R ligands based on the structures of JNJ7777120 (**1**) and VUF6884 (**5**, Fig. 1).[14] Using that model, a small series of heterocyclic fragments coupled to an *N*-methylpiperazine group was designed and subsequently evaluated for H_4R affinity to yield several hits with H_4R affinities in the micromolar range. Initially, one of the fragments was optimized for H_4R affinity to yield potent quinoxaline based H_4R ligands (e.g., compound **6**, Fig. 1).[15] In a subsequent study, a scaffold hopping approach was applied by taking a quinazoline fragment to find a series of H_4R ligands that contained numerous very potent analogues, including the potent H_4R inverse agonist VUF10519 (**7**).[16,17]

In this work, a third fragment, isoquinoline **9** (Fig. 2),[15] was taken as a starting point for the development of new histamine

Abbreviations: GPCRs, G protein-coupled receptors; H_1R, histamine H_1 receptor; H_2R, histamine H_2 receptor; H_3R, histamine H_3 receptor; H_4R, histamine H_4 receptor; SAR, structure–activity relationship.

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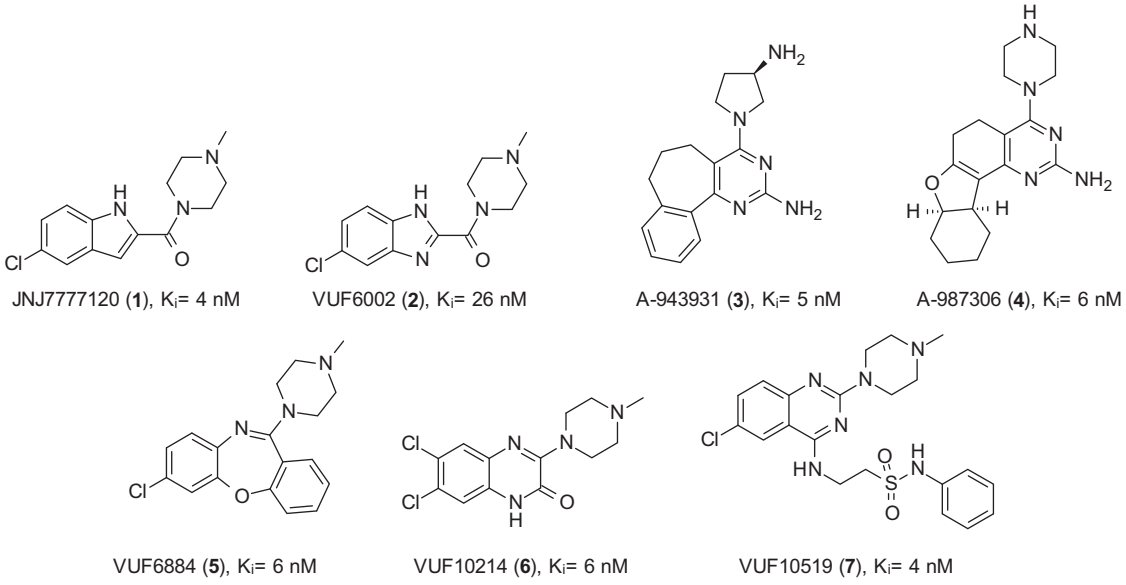


Figure 1. Several potent non-imidazole histamine H_4R receptor ligands.

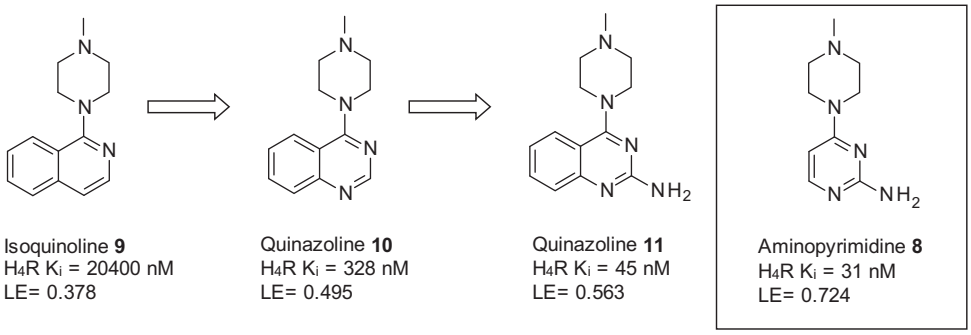
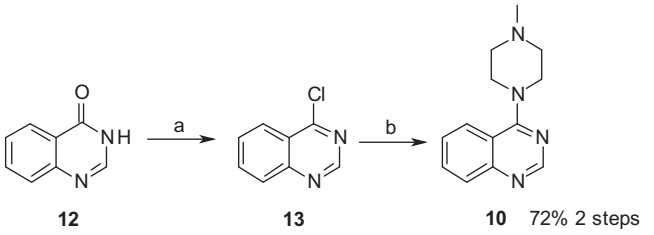


Figure 2. Optimization of isoquinoline fragment **9**. A two-step optimization of isoquinoline **9** gave a 640-fold increase in H_4R binding affinity. LE = Ligand Efficiency (Δg) is calculated as the binding energy per non-hydrogen atom ($\Delta g = \Delta G/N_{\text{non-hydrogen atoms}}$ with $\Delta G = -RT \ln K_i$).[21]

H_4R ligands. Although isoquinoline **9** itself has low H_4R affinity, we hypothesized that the binding mode of this fragment might be similar to that of the much preferred 2-aminopyrimidine scaffold. This 2-aminopyrimidine scaffold has first been described in patent literature by researchers from Bayer Healthcare and has also been extensively studied by research groups from Palau Pharma, UCB Pharma, Johnson and Johnson, Pfizer and Cellzome a.o. (for a review on 2-aminopyrimidines as H_4R ligands we would like to refer to reference nr 18).[18,19]

Consequently, a substantial body of evidence pointed out that the conversion of isoquinoline **9** into a ligand containing a 2-aminopyrimidine moiety was proposed to rapidly lead to novel ligands with good H_4R affinity.

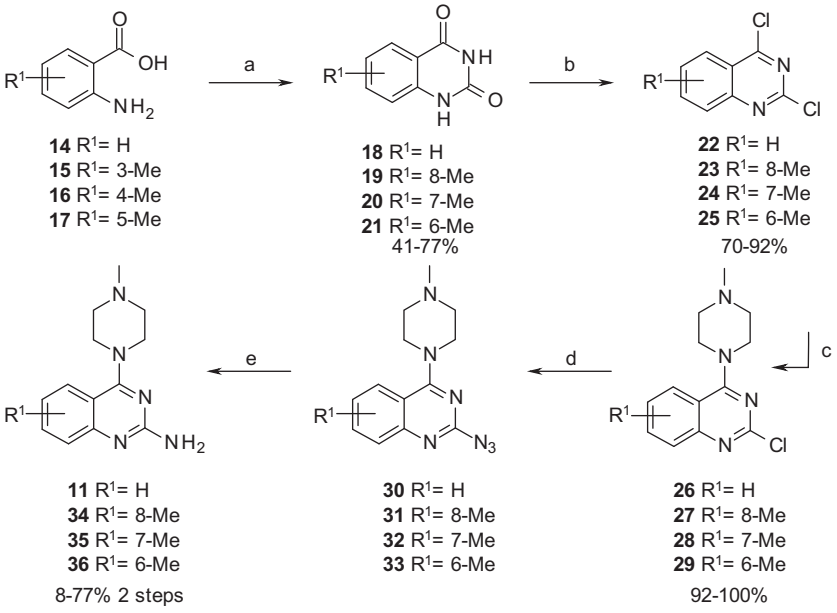
We planned to synthesize compound **10** in addition to 2-aminoquinazoline **11**, to study the effect of introducing a nitrogen atom in the 4-position of compound **9**. Starting from 4-hydroxyquinazoline (**12**, Scheme 1), chlorination with $POCl_3$ gave 1-chloroquinazoline (**13**) that was subsequently coupled to *N*-methylpiperazine to give quinazoline **10**. Anthranilic acids **14**–**17** (Scheme 2) were converted to their corresponding quinazoline-2,4(1*H*,3*H*)-diones (**18**–**21**) by stirring them in molten urea according to a procedure described in literature.[20] Chlorination of quinazolines **18**–**21** with $POCl_3$ in the presence of DIPEA gave 2,4-dichloroquinazolines **22**–**25** that were then selectively substituted at the 4-position to give monosubstituted quinazoline analogues **26**–**29**. The introduction of an amino group at the 2-position was achieved by first



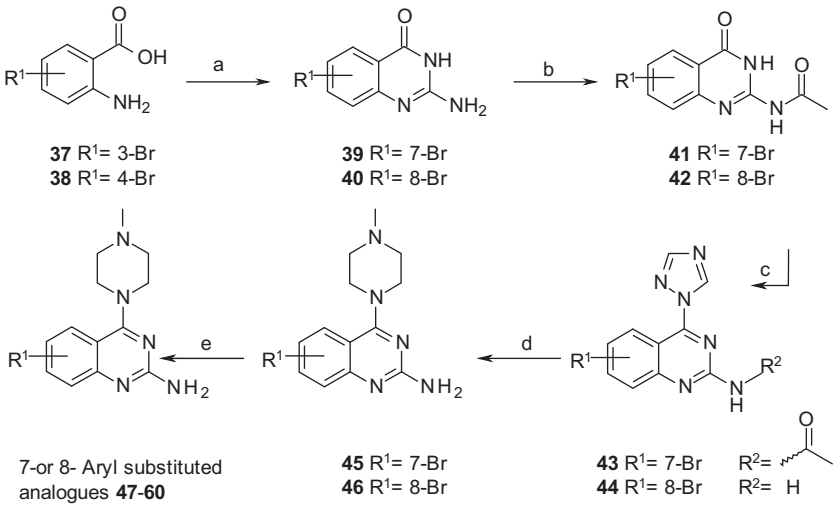
Scheme 1. Reagents and conditions: (a) $POCl_3$, DIPEA, reflux; (b) *N*-methylpiperazine, EtOAc, rt.

introducing an azido group under microwave conditions with sodium azide in *N*-methylpyrrolidone. This was followed by a reduction of the azido substituted quinazolines **30**–**33** with Raney Nickel and hydrogen gas to give 2-amino quinazolines **11** and **34**–**36** in good yield and excellent purity.

Several bromine substituted analogues of **11** were prepared by a different procedure than the one described in Scheme 2. Anthranilic acids **37** and **38** (Scheme 3) were reacted with freshly prepared chloroformamidine in a mixture of molten dimethylsulfone and sulfolane according to a procedure described in literature.[21] The obtained 2-aminoquinazolin-4(3*H*)-ones **39** and **40** were then treated with acetic anhydride to give their acetylated products **41** and **42**. In the following step **41** and **42** were treated with $POCl_3$ at



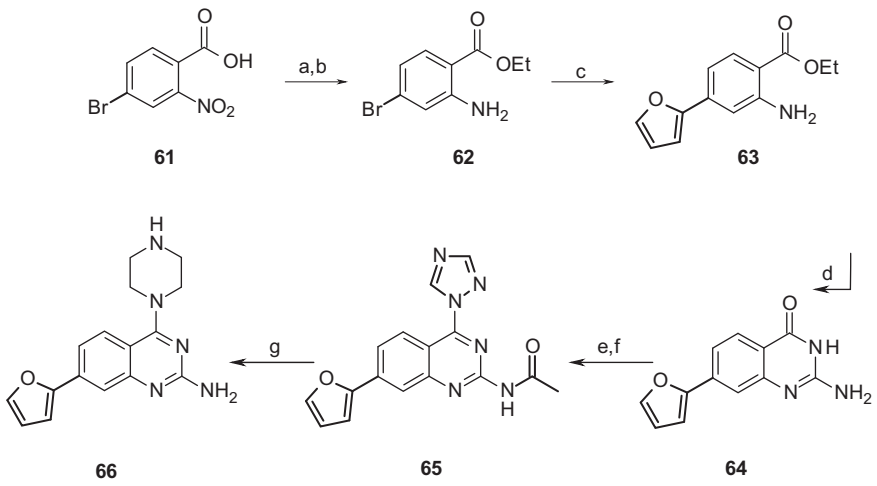
Scheme 2. Reagents and conditions: (a) urea, 160–180 °C, aq NaOH, HCl; (b) DIPEA, $POCl_3$, reflux; (c) EtOAc, DIPEA, *N*-methylpiperazine, rt; (d) *N*-methylpyrrolidone, NaN_3, microwave; (e) THF, H_2, Raney Nickel, 1 atm, rt.



Scheme 3. Reagents and conditions: (a) dimethylsulfone, sulfolane, chloroformamidine hydrochloride, ~165 °C; (b) Ac_2O, reflux; (c) acetonitrile, DIPEA, $POCl_3$, 1,2,4-triazole, rt; (d) dioxane, *N*-methylpiperazine, reflux; (e) EtOH/toluene, 2 M Na_2CO_3, $Pd(PPh_3)_4$, $ArB(OH)_2$, microwave, 120 °C. The yields for these reaction steps are given in the experimental section and are mostly crude yields of compound often containing significant amounts of reagent.

room temperature and reacted in situ with excess triazole to yield 4-triazolo substituted quinazolines **43** and **44**.[21] After work up, it was found that the acetyl group of compound **44** was lost during the reaction, while the acetyl group of **43** remained unaffected. This observation indicated a remarkable difference in chemical behavior between the 7- and 8-bromo intermediates. The substitution of the triazole moiety of **43** and **44** with *N*-methylpiperazine in dioxane to give 7- and 8-bromo analogues **45** and **46** proceeded smoothly with a concomitant loss of the acetyl group of **43**. The procedures eventually gave desired compounds **45** and **46** in high purity after crystallization from isopropanol. Quinazolines **45** and **46** were subsequently used in a Suzuki coupling reaction with a variety of boronic acids to give 7- and 8-aryl substituted quinazolines **47**–**60**. Nitro benzoic acid **61** (Scheme 4) was esterified with ethanol and subsequently reduced to its corresponding aniline (**62**) with zinc in acetic acid. Intermediate **62** was then coupled to 2-furylboronic acid under Suzuki cross-coupling conditions to give **63** that was the ring-closed with chloroformamidine HCl to give quinazoline **64**. The introduction of an acetyl group on the 2-amino group with acetic anhydride gave the crude amide that was substituted with a 1,2,4-triazole group. This triazole analogue (**65**) was converted to compound **66** by refluxing in dioxane in the presence of excess piperazine.

As mentioned earlier in the introduction, it was hypothesized that the isoquinoline fragment (**9**) could be rapidly optimized to an H_4R ligand with good affinity in only two steps (Fig. 2). The first step was the introduction of an additional nitrogen atom at the isoquinoline four position to afford quinazoline **10**. The second step was the introduction of an amino group on the two position to yield a benzofused 2-aminopyrimidine or 2-aminoquinazoline (compound **11**). Compound **11**, like compound **8**, contains the 2-aminopyrimidine group that gives high H_4R affinity when



Scheme 4. Reagents and conditions: (a) EtOH, H_2SO_4, reflux; (b) Zn, AcOH, MeOH, 0 °C–rt; (c) 2-furylboronic acid, 2 M Na_2CO_3, tetrakis $Pd(PPh_3)_4$, toluene, microwave 120 °C; (d) dimethylsulfone, sulfolane, chloroformamidine hydrochloride, ~165 °C; (e) Ac_2O, reflux; (f) acetonitrile, DIPEA, $POCl_3$, 1,2,4-triazole, rt; (g) piperazine, dioxane, reflux. The yields for these reaction steps are given in the experimental section and are mostly crude yields of compound often containing significant amounts of reagent.

combined with a basic amine such as *N*-methylpiperazine on the 4-position. Compound **8** was synthesized as a reference fragment because it is currently the most widely used structural element in H_4R drug discovery efforts.[9,18,19] The high ligand efficiency of fragment **8** (LE = 0.724) makes it a very attractive starting point for drug development efforts and optimization for H_4R affinity.[22] If quinazoline **11** would indeed have appreciable H_4R affinity, then a subsequent optimization round could quickly lead to highly potent compounds. Preparation and evaluation of compounds **10** and **11** quickly confirmed that they indeed had improved H_4R affinity. In fact, a very substantial increase in H_4R affinity of about 640-fold was observed going from fragment **9** to quinazoline **11**. In addition, quinazoline **11** maintained a good LE of 0.563 that could allow for an efficient optimization of the desired compound properties.

Quinazoline **11** was then decorated with hydrophobic substituents to fill a hydrophobic pocket that was identified on the basis of a flexible alignment model of compound **11** with the two 2-aminopyrimidine antagonists described by scientists from Abbott (compounds **3** and **4**, Fig. 3).[9,13] The model in Figure 3 suggests that substituents on the 7- or 8- position could occupy a hydrophobic pocket that is addressed by **3** and **4**. Based on this hypothesis, a series of 7- and 8- substituted quinazolines was prepared to target the identified pocket with hydrophobic substituents and improve H_4R binding (Table 1). During the course of these studies several

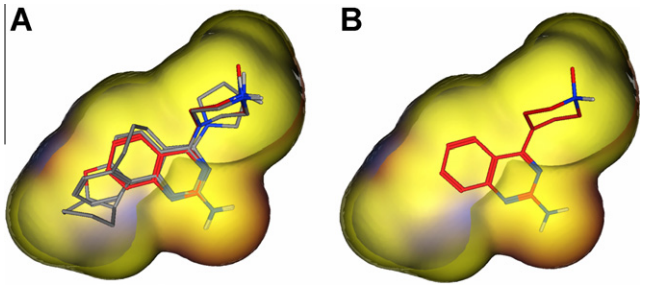
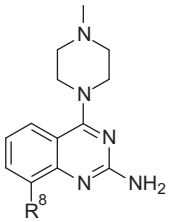


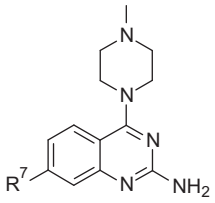
Figure 3. (**A**) Flexible alignment model of compound **11** (in red) and histamine H_4R antagonists **3** and **4** (in grey). The calculated van der Waals surface represents the H_4R active site to which the compounds bind. The hydrophobic surface is colored yellow, the polar surface blue and the mild polar surface red. (**B**) Substitution of the quinazoline 7- (grey arrow) or 8-position (white arrow) with lipophilic substituents can fill the hydrophobic pocket and increase H_4R affinity of compound **11**.

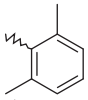
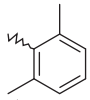
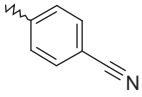
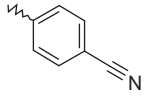
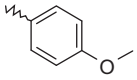
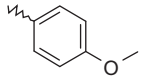
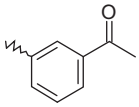
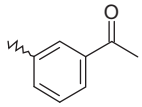
patent applications appeared that described 5,6,7,8-tetrahydroquinazolines and quinazolines with substituents that were well tolerated on both the 7- and 8-positions.[19,23,24] The introduction of a methyl substituent on the 6-position of aminopyrimidine **11** gave a drop in H_4R affinity of about 20-fold, adding to the evidence that the 7- and 8-positions might be preferred. Compound **34** that has an 8-methyl substituent also showed somewhat decreased H_4R binding, whereas 7-methyl substituted compound **35** was essentially equipotent to its unsubstituted analogue **11**. A similar effect was seen when a bromine atom was introduced on the 7- and 8-positions (compare compounds **45** and **46** with **11**). The introduction of a phenyl group on either the 7- or 8-position gave compounds with good H_4R affinity. 8-Phenyl analogue **47** was equipotent to compound **11** and a slight increase in H_4R binding was observed for 7- phenyl compound **48**. Although no increase in H_4R binding was seen, compounds **47** and **48** demonstrate that a substituent of considerable size is tolerated at both the 7- and 8-positions. In an attempt to constrain the rotational flexibility of the phenyl rings, two methyl groups were introduced on the ortho positions of **47** and **48** to give 2,6-dimethylphenyl analogues **49** and **50**. In both cases a drop in affinity was observed, about sevenfold for compound **49** (compare with **47**) and fivefold for compound **50** (compare with **48**). The introduction of an electron withdrawing or electron donating substituents on the 3- or 4-positions of the phenyl rings of **47** and **48** did not lead to significantly improved affinity (compounds **51**–**56**) and even proved to be very detrimental for compounds **51**, **53** and **55**. The introduction of a 3-furyl group on the 8-position gave a drop in H_4R binding affinity of about threefold (compound **57**). However, the introduction of this same substituent on the 7-postion gave an increase in affinity, leading to compound **58** with an H_4R affinity of 6 nM. The replacement of the oxygen atom of **58** with a sulfur atom (compound **59**) or moving the oxygen atom from the 3- to the 2- position (compound **60**) was allowed, resulting in potent H_4R ligands with a respective K_i of 4 and 5 nM. This SAR study at the H_4R reveals that substitution of the 7-position with various substituents is preferred over substitution of the 8-position for all of the quinazolines in Table 1.

Because literature reports that the *N*-methylpiperazine moiety is a metabolically unstable group we also synthesized the demethylated analogue of **60**, compound **66**.[9] This compound was found to have good in vitro metabolic stability (Table 2) prompting further investigation of its PK profile in vivo. The administration of

Table 1
SAR of 7- and 8-substituted quinazolines





No.	R^8	H_4R K_i ± SEM[a]	No.	R^7	H_4R K_i ± SEM[a]
1	JNJ7777120	4	**2**	VUF6002	26
	Histamine	9 ± 1		Thioperamide	79 ± 15
11	H	32 ± 6	**36**	[b]	649 ± 35
34	CH_3	91 ± 4	**35**	CH_3	24 ± 2
46	Br	111 ± 15	**45**	Br	17 ± 1
47		32 ± 6	**48**		19 ± 2
49		221 ± 45	**50**		90 ± 9
51		2333 ± 253	**52**		29 ± 2
53		945 ± 553	**54**		55 ± 2
55		676 ± 187	**56**		15 ± 1
57		109 ± 26	**58**		6 ± 0.4
			59		4 ± 0.8
			60		5 ± 0.4

[a] Measured by displacement of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4R or H_3R. K_i's are calculated from at least three independent measurements as the mean ± SEM.
[b] R^7 = H, R^6 = methyl.

Table 2
In vitro and in vivo metabolic stability of compound **66**

No.	Species	Microsomal stability[a]	$F_{p.o.}$ (mouse)[b]	$T_{1/2}$ (h)
66	Human	96%		
	Mouse	80%	47%	6.87
	Rat	83%		

[a] Percentage remaining after a 60 min incubation with liver microsomes (performed at Cerep, France).
[b] Study performed by ChemPartner (Shanghai, PRC).

Table 3
Affinity of selected analogues at the histamine receptor subtypes

No.	H_4R K_i ± SEM[a]	H_3R K_i ± SEM	H_2R % displacement[b]	H_1R K_i ± SEM
58	6 ± 0.4	188 ± 36	54%	7373 ± 974
59	4 ± 0.8	63 ± 17	74%	2800 ± 550
60	5 ± 0.4	98 ± 13	51%	6422 ± 848
66	16 ± 2.3	3408 ± 424	n.d.	10,500 ± 840

[a] Measured by displacement of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4R, H_3R or H_1R.[25] K_i's are calculated from at least three independent measurements as the mean ± SEM.
[b] % displacement of cimetidine from the H_2R at 10 μM performed in duplicate (Cerep, France).

66 to mice showed a good oral bioavailability of 47% with an in vivo half-life of 6.8 h.

The most potent analogues in these series (**58**–**60** and **66**) were evaluated for their affinity for the other histamine receptor subtypes (Table 3). Low affinity was found for the H_1R and H_2R subtypes and a 225-fold selectivity over the H_3R affinity was found for 3-furyl analogue **66**. The functional behavior of compounds **58**–**60** and **66** at the H_4R was evaluated in a [^{35}S]GTPγS binding assay (Fig. 4). All four analogues were found to effectively antagonize histamine at the human H_4R with corresponding calculated K_is of 4, 3, 5 and 7 nM for compounds **58**, **59**, **60** and **66** (Table 4). The histamine receptor subtype affinities at mouse and rat receptors was determined to see whether the selectivity and high affinity of **66** would be maintained across the species (Table 5).

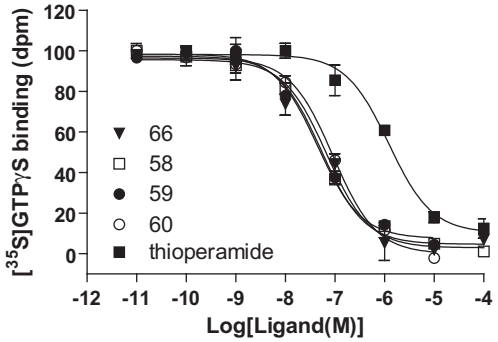


Figure 4. Pharmacological characterization of analogues **58**-**60** and **66**. Potency (IC_{50}) of compounds **58**-**60** and **66** in [^{35}S]GTPγS binding mediated by the human H_4R expressed in HEK 293T cells (the assay was performed in the presence of 100 nM of histamine). Data are given in Table 3 and are expressed as mean ± SEM of three independent experiments.

Table 4
Potency of H_4R antagonism for analogues **58**–**60** and **66** at the human H_4R

No.	H_4R IC_{50} ± SEM[a]	H_4R K_i ± SEM[a]
Thioperamide	977 ± 68	63 ± 4
58	47 ± 2	4 ± 1
59	46 ± 7	3 ± 1
60	83 ± 6	5 ± 1
66	106 ± 32	7 ± 2

[a] Calculated on the basis of an EC_{50} for a histamine of 7 nM and a GTPγS assay histamine concentration of 100 nM.

Table 5
Affinity of compound **66** at various histamine receptor orthologues[a]

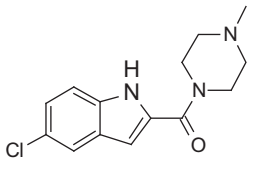
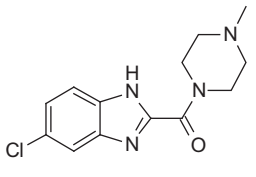
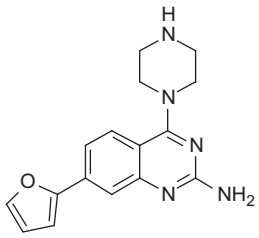
Receptor	Human	Mouse	Rat
H_1R	10,500 ± 840	3300 ± 1500	3600 ± 1800
H_3R	9200 ± 1200	1070 ± 230	2560 ± 275
H_4R	16 ± 2.3	23 ± 6	174 ± 47

[a] Measured by displacement of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H4R, H3R or H1R. Ki's are calculated from at least three independent measurements as the mean ± SEM

Although the affinities for the rat and mouse H_1R remained close to that of the human H_1R, significant affinity differences of about 10-fold were found between the human and mouse H_3R and human and rat H_4R.

Considering the growing awareness important property in lead optimization, because it plays a pivotal role in PK/PD and compound efficacy, we also measure binding kinetics for selected compounds.[26] Therefore, we studied the binding kinetics (k_{off} value) of **66** and two H_4R antagonists (JNJ7777120 (**1**) and VUF6002 (**2**)) at the human H_4R (Table 6).[27] For calculation of the k_{off} of the unlabeled ligand, k_{on} and k_{off} values of [^{3}H]histamine has to be determined experimentally. Experiments with multiple concentrations of [^{3}H]histamine in a binding association assay (Fig. 5) result in k_{on} values for histamine of 7.77 ± 0.71 $M^{-1}min^{-1}$ and k_{off} of 0.083 ± 0.01 min^{-1} or $T_{½}$ of 11.21 min. The K_d value derived from the k_{on} and k_{off} values is 10.7 ± 1.4 nM, which is very close to the value determined in the saturation binding assay, 9 nM. The k_{off} value of H_4R ligands was determined by measuring the association of [^{3}H]histamine to the hH_4R in the absence and the presence of competing ligands (Fig. 6). Significant differences in k_{off} values are observed for these ligands with a k_{off} value of 62 min for compound **1** that is twofold and 15-fold longer than compounds **2** and **66** respectively (Table 6). It is interesting to note that the dissociation of **2** is quite a bit faster than **1**, when the only structural

Table 6
k_{off} values of H_4R ligands

Compound		*n*	k_{off}[a] (min^{-1})	$T½$[b] (min)
	(**1**)	8	0.009 ± 0.001	62.0 ± 13.6
	(**2**)	5	0.056 ± 0.008	25.6 ± 11.5
	(**66**)	4	0.193 ± 0.041	4.1 ± 0.8

[a] Data were analyzed with Graphpad Prism 5.0. (Graphpad Software Inc., USA).
[b] $T_{½} = \ln 2/k_{off}$.

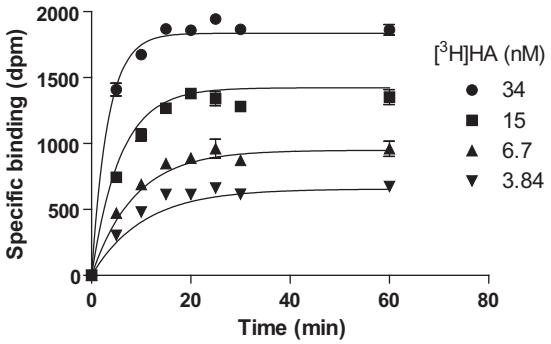


Figure 5. Association of different concentrations of [^{3}H]histamine with the hH_4R.

difference between the two reference compounds is an aromatic nitrogen atom. Interestingly, compound **1** is a compound with a short in vivo half-life that shows robust efficacy is a range of animal models. Although it is tempting to speculate that this is a direct result of its slow dissociation from the H_4R this hypothesis needs further investigation, including the in vivo evaluation of for example compound **66**.

In a previous study, a H_4R pharmacopore model was used to design a focused set of fragments. In this work, one of these fragments was rapidly optimized in two steps to give 4-(4-methylpiperazin-1-yl)quinazolin-2-amine (**11**) with good H_4R affinity. A flexible alignment model of this compound with two aminopyrimidine H_4R antagonists reported in literature indicates the possibility to decorate compound **11** on the 7- or 8-positions to fill a hydrophobic pocket and improve ligand binding affinity. Following this observation, the introduction of aliphatic and aromatic groups on the 7-position indeed gave compounds with excellent H_4R affinity in the low nanomolar range. This series includes, among others, 7-(furan-2-yl)-4-(piperazin-1-yl)quinazolin-2-amine (VUF11489, **66**) a potent antagonist of histamine at the H_4R with 255-fold selectivity over the H_3R and good oral bioavailability in the mouse.

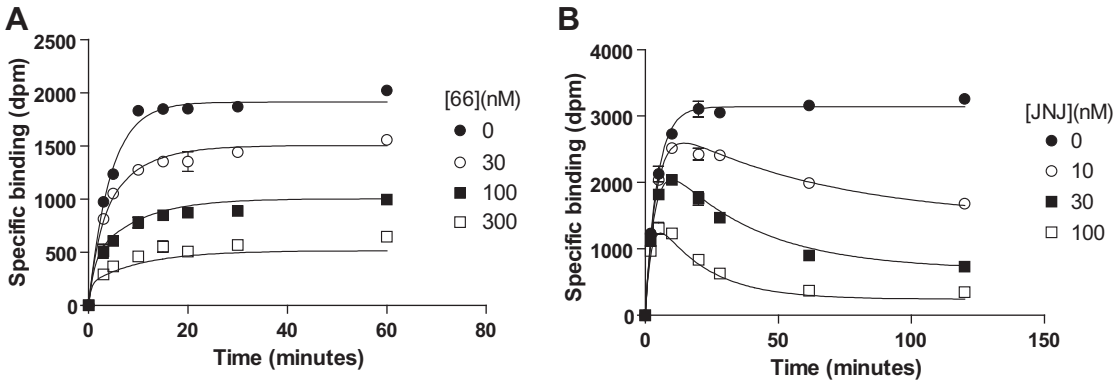


Figure 6. Association of [^{3}H]histamine with the hH_4R in the absence and presence of competing H_4R ligands. The presence of competitive ligand will reduce the bound [^{3}H]histamine. The kinetic constants of competitive ligands were determined using the Motulsky–Mahan equation.[24] Panel A shows a rapidly dissociating compound (**66**) and panel B shows the slowly dissociating H_4R antagonist JNJ7777120.

Analysis of the binding kinetics of this compound and two reference H_4R antagonists gave large variations in receptor dissociation rates that may offer new avenues for in vitro compound optimization for in vivo efficacy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.104.

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