Bioorganic & Medicinal Chemistry Letters 23 (2013) 2663–2670

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A novel series of histamine H_4 receptor antagonists based on the pyrido[3,2-*d*]pyrimidine scaffold: Comparison of hERG binding and target residence time with PF-3893787

Mounir Andaloussi [a], Herman D. Lim [a], Tiffany van der Meer [a], Maarten Sijm [a], Chris B. M. Poulie [a], Iwan J. P. de Esch [a,b], Rob Leurs [a,b], Rogier A. Smits [a,*]

[a] Griffin Discoveries BV, Department of Medicinal Chemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands
[b] VU University Amsterdam, Department of Medicinal Chemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 5 January 2013
Revised 16 February 2013
Accepted 20 February 2013
Available online 1 March 2013

Keywords:
Histamine H_4 receptor
GPCR
Pyrido[3,2-*d*]pyrimidine
hERG
Residence time
PF-3893787

ABSTRACT

In this work we describe the optimization of a lead compound based on the quinazoline template to give a new series of potent pyrido[3,2-*d*]pyrimidines as histamine H_4 receptor antagonists. The pyrido[3,2-*d*]pyrimidine ligands have significantly reduced hERG binding compared to clinical stage compound PF-3893787 while showing good affinities at the human and rodent histamine receptors. The receptor residence time of several of these new compounds was determined for the human H_4R and compared with JNJ7777120 and PF-3893787. The pyrido[3,2-*d*]pyrimidines showed residence times lower than JNJ7777120 but comparable to the residence time of PF-3893787. Overall, the pyrido[3,2-*d*]pyrimidines show an excellent in vitro profile that warrants their further investigation in relevant models of human disease.

The human histamine H_4 receptor (hH_4R) is mainly expressed in various cells of the immune system including eosinophils, T-lymphocytes, dendritic cells, mast cells, and basophils.[1] On the cellular level, H_4R activation induces for example chemotaxis of mast cells and eosinophils and triggers calcium mobilization in mast cells, monocytes and eosinophils.[2] Furthermore, the H_4R modulates the release of various inflammatory mediators. In different animal models, blocking the H_4R with selective antagonists such as JNJ7777120 (**1**, Fig. 1) proved to be beneficial, for example, in OVA-induced allergic airway inflammation, and zymosan-induced peritonitis in the mouse or in a TNDB-induced colitis model in the rat.[3] H_4R antagonists were also shown to be more effective in the attenuation of experimental histamine-induced pruritis than H_1R antagonists.[4,5] All these results indicate that the hH_4R has a distinct pharmacological profile and plays an important role in different inflammatory and allergic disorders. Antagonists are considered as potential drugs for the treatment of diseases like allergic asthma, allergic rhinitis, pruritis, pain, vestibular disorders and inflammatory bowel disease.[3,6]

Recently we reported the fragment-based discovery of a new quinazoline scaffold as a potent H_4R ligand. In that work we synthesized a library of compounds by varying the basic amine on position 4 and screening different halogen, (hetero)aromatic and aliphatic groups at position 7 of the quinazoline (see Fig. 2 for scaffold numbering).[7] We observed that the introduction of aliphatic and heteroaromatic groups on the 7-position gave compounds with excellent H_4R affinity in the low nanomolar range. This series included, among others, 7-(furan-2-yl)-4-(piperazin-1-yl)quinazolin-2-amine (**2**, VUF11489) a potent antagonist of histamine at the H_4R with 255-fold selectivity over the H_3R and good oral bioavailability in the mouse (47%). Unfortunately compounds in this series have considerable affinity for the hERG channel (hERG pK_i of compound **2** is 5.49 in an astemizole radioligand displacement assay) and this remained an issue to address. In literature several strategies to reduce hERG binding are discussed, for example change the steric environment of the basic group (i.e., the piperazine moiety of compound **2**), reduce lipophilicity and introduce polarity.[8] Because we were also interested in finding substituents for the metabolically labile *N*-methylpiperazine and piperazine moieties without losing H_4R affinity, this could potentially be achieved in parallel with a reduction of the hERG affinity. Polarity was to be introduced by replacing the quinazoline ring with a pyrido[3,2-*d*]pyrimidine ring, because we had seen from an in-house fragment-like compound set designed as H_4R ligands that the pyrido[3,2-*d*]pyrimidine scaffold was well tolerated by the H_4R (compare pK_i of compounds **2a** and **2b**, Fig. 2).The furan group is associated with undesired metabolic activation and covalent binding to proteins

* Corresponding author. Tel.: +31 6 41402952.
E-mail address: rogier.smits@griffindiscoveries.com (R.A. Smits).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.02.091

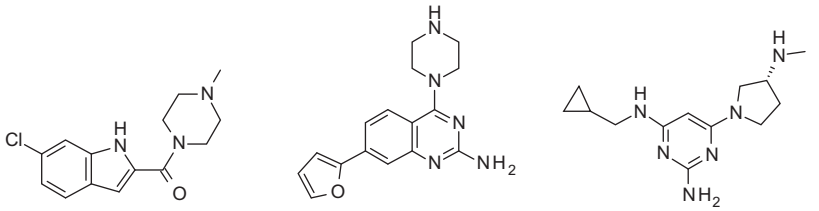


Figure 1. Examples of histamine H_4R receptor ligands.

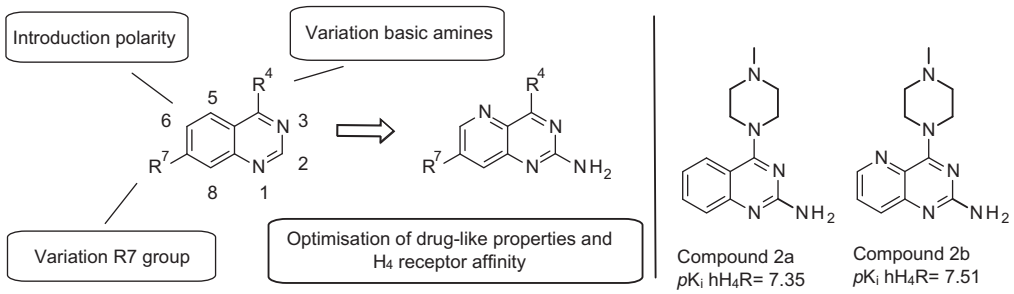
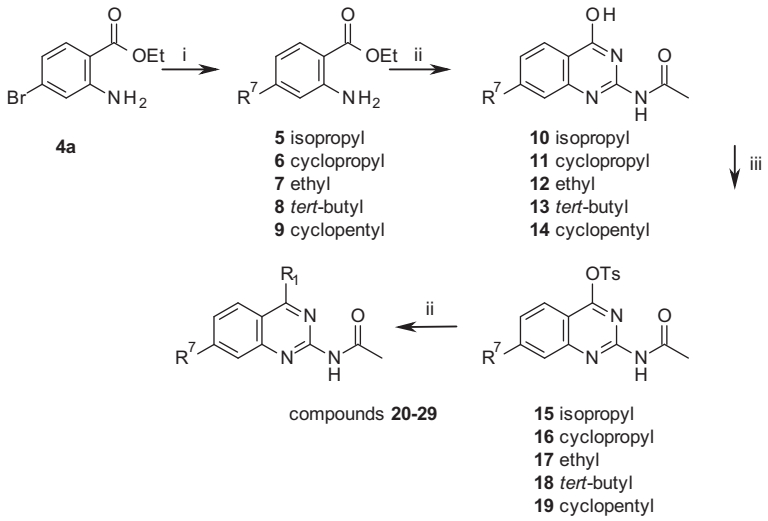


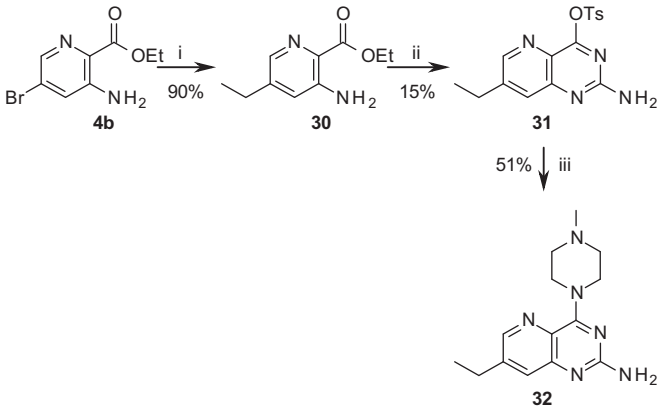
Figure 2. Optimization strategy to improve compound quality of quinazoline-based H_4R antagonists.



Scheme 1. Reagents and conditions: (i) (a) isopropenylboronic acid pinacol ester, $Pd(PPh_3)_4$, K_2CO_3, $PhCH_3$, EtOH, Mw, 130 °C, or (b) cyclopropylboronic acid, PCy_3, $Pd(OAc)_2$, KF, THF, rt. Other building blocks were obtained from commercial suppliers, (c) H_2, Pd/C 5%, EtOH, rt; (ii) (a) chloroformamidine hydrochloride, dimethyl sulfone, sulfolane, 160 °C, (b) acetic anhydride, reflux; (iii) TsCl, K_2CO_3, CH_3CN, Δ; (iv) (a) various (Boc-protected) amines, DIPEA, 1,4-dioxane, rt, (b) dioxane/HCl 2 M, rt.

and was therefore replaced with a saturated hydrocarbon group.[9] This replacement would concomitantly decrease the number of SP2 atoms and improve the drug-like properties.[10] We were also interested in developing new compounds for comparison with PF-3893787 (**3**), the clinical stage compound from Pfizer that has recently been described in the literature.[11] We therefore started this work by exploring the tolerance of the H_4R to various non-aromatic substituents on the 7-position and different amines on the 4-position of the quinazoline moiety (Schemes 1–5). The importance of the 7-position for H_4R binding has been described in our earlier work as well as by researchers from UCB.[7,12]

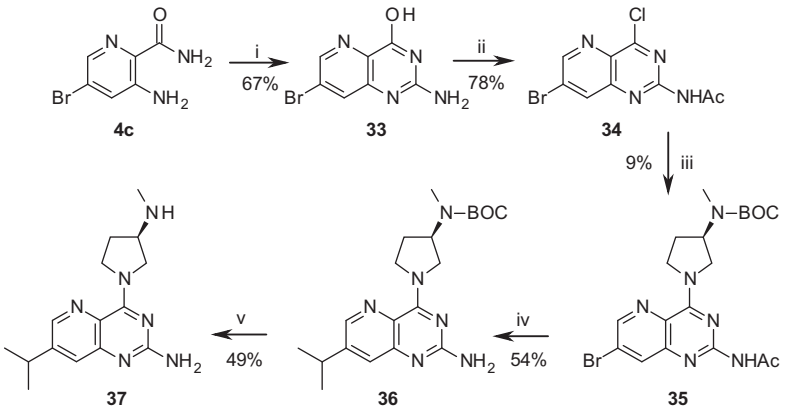
The first quinazoline analogue was synthesized from bromo anthranilic acid **4a** by introducing an isopropylene group in a Suzuki cross-coupling reaction with 2-methyl ethene boronic acid. The double bond was subsequently reduced with hydrogen gas and palladium on activated carbon to get corresponding isopropyl analogue **5** (Scheme 1). For preparation of cyclopropyl analogue **6**, a combination of PCy_3, $Pd(OAc)_2$ and KF was used. Ethyl, *tert*-butyl and cyclopentyl analogues were obtained from commercial sources. Analogues **5**–**9** were then condensed with freshly prepared chloroformamidine hydrochloride and acetylated with acetic anhydride to give the N-protected 4-hydroxyquinazolines **10**–**14**. The 4-position was functionalized by the introduction of a tosyl group, using tosylchloride under reflux with acetonitrile as solvent (compounds **15**–**19**). The conversion to the final products was done by stirring tosylate intermediates **15**–**19** at room temperature in the presence of various amines. The introduction of the different amines could be done either directly or by using their Boc-protected precursors. Piperazine was introduced directly by using 10 equiv and when the reaction mixture was heated at reflux, this



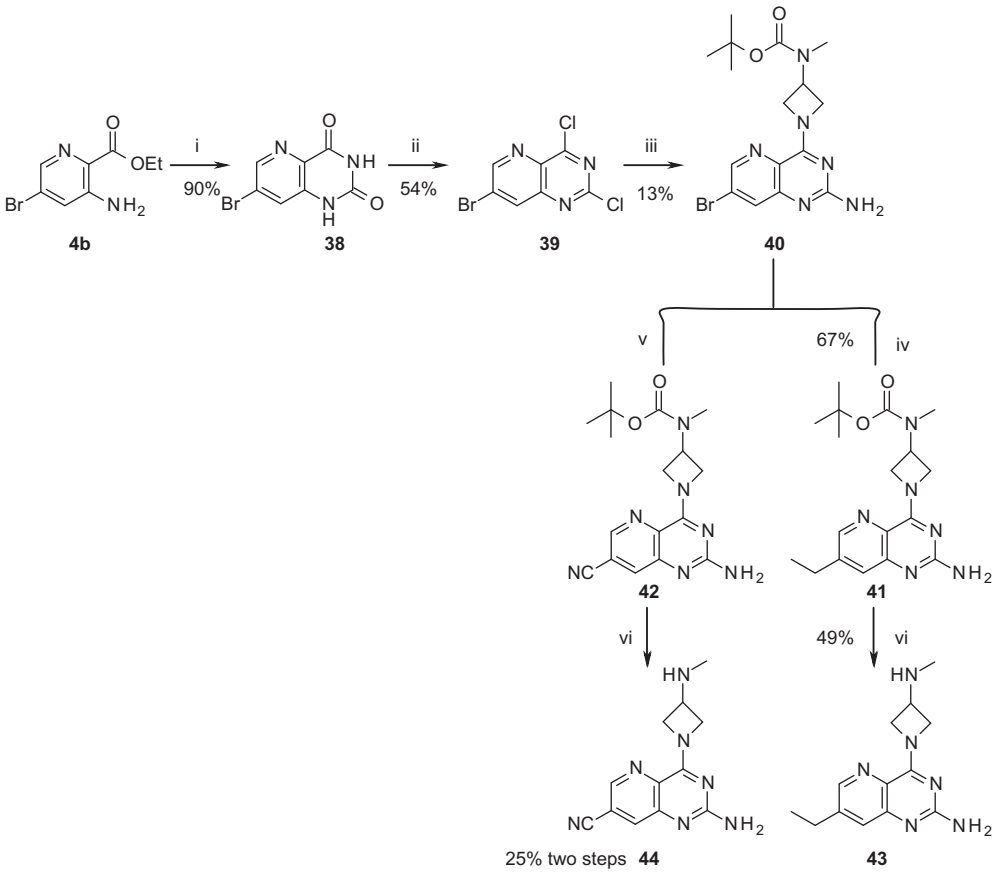
Scheme 2. Reagents and conditions: (i) (a) potassium vinyltrifluoroborate, $Pd(PPh_3)_4$, K_2CO_3, $PhCH_3$/EtOH, Mw, 130 °C, 30 min, (b) H_2, Pd/C 5%, EtOH, rt; (ii) (a) chloroformamidine hydrochloride, dimethyl sulfone, sulfolane, 160 °C, (b) TsCl, K_2CO_3, CH_3CN, Δ; (iii) *N*-methylpiperazine, DIPEA, 1,4-dioxane, rt.

excess amine was also able to remove the acetyl group to directly give the deprotected 2-aminoquinazoline. The 3-aminomethyl pyrrolidine and 3-aminomethyl azetidine groups were introduced by using (*R*)-3-(*N*-Boc-*N*-methylamino)pyrrolidine and 3-Boc-3-methylamino azetidine respectively and the subsequent removal of the acetyl group by heating the reaction mixture with a saturated aqueous Na_2CO_3 under microwave irradiation. Removal of the Boc-protecting group was finally done by stirring the Boc-protected amines in a 2 M solution of HCl in dioxane at room temperature. To build the pyrido[3,2-*d*]pyrimidin-2-amine scaffold we started our synthetic pathway by reacting ethyl 3-amino-5-bromopicolinate (compound **4b**, Scheme 2) with potassium vinyltrifluoroborate under Suzuki conditions. This reaction gave the vinyl compound which was immediately reduced under a hydrogen atmosphere in ethanol to yield the desired ethyl 3-amino-5-ethylpicolinate (**30**). The treatment of (**30**) with chloroformamidine hydrochloride, dimethyl sulfone, and sulfolane at 160 °C, allowed the formation of the corresponding 2-aminopyrimidine. To perform the amination at position 4 of the 2-aminopyridopyrimidine, we introduced a tosyl group by treating the corresponding reagent with tosyl chloride and potassium carbonate. Finally, the treatment of intermediate **31** with *N*-methylpiperazine yielded the corresponding methylpiperazine compound (**32**) (Scheme 2). Because of the low overall yield described in Scheme 2 and the complication that diversity on the 7-position needed to be introduced early on in the synthesis, we decided to perform a Suzuki reaction at a later stage of our synthetic route. Therefore, we initiated a synthesis by the condensation of the 3-amino-5-bromopicolinamide (**4c**) with chloroformamidine hydrochloride, dimethyl sulfone and sulfolane, at 165 °C to give 2-amino-7-bromopyrido[3,2-*d*]pyrimidin-4-ol (**33**, Scheme 3). To perform a selective chlorination at the C-4 carbon atom using $POCl_3$ and DIPEA in toluene at reflux, protection of the 2-amino group with an acetyl group was needed (compound **34**). Then the corresponding chlorinated compound was treated directly with *N*,*N*-methyl-Boc-pyrrolidine, and DIPEA in 1,4-dioxane at room temperature to yield pyrrolidine analogue (**35**). Compound **35** was then reacted with isopropenylboronic acid pinacol ester in the presence of tetrakis(triphenylphosphine)palladium(0) and potassium carbonate to form the corresponding olefin. This was then reduced to isopropyl analogue **36** by treatment with palladium 5% on carbon under a hydrogen atmosphere at room temperature using ethanol as the solvent. To get the target compound (**37**), Boc-deprotection using a 4 M HCl solution in 1,4-dioxane was performed. Although our synthetic plan described in Scheme 3 allowed us to prepare target compound (**37**), the yield for the synthesis of compound (**35**) was very low (9%). To increase the yield of reacting the basic amine with the pyrido[3,2-*d*]pyrimidine scaffold, we used pyrido[3,2-*d*]pyrimidine dione **38** as starting material (Scheme 4). This starting material was then converted to dichloro analogue **39**, which was stirred overnight in a mixture of *N*,*N*-methyl-Boc-azetidine, DIPEA, and 1,4-dioxane. The direct amination on position 2 was done by using a saturated solution of ammonia in ethanol to give the corresponding aminopyridopyrimidine (**40**) in only 13% yield (two steps).

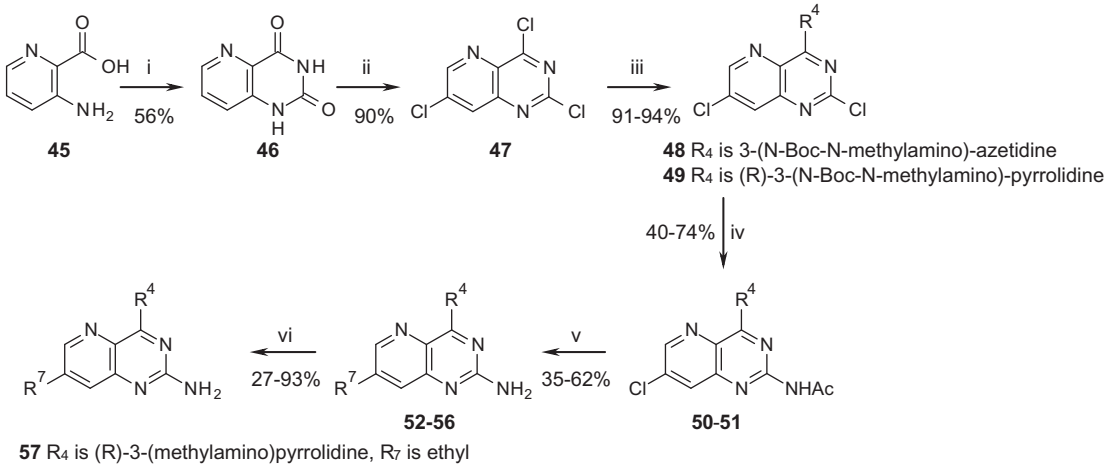
While the introduction of the ethyl group in intermediate **41** was performed according to the procedures used for the isopropyl group in intermediate **36**, the nitrile analogue was synthesized by reacting the bromine compound with $Zn(CN)_2$ and tetrakis(triphenylphosphine)palladium(0) at 150 °C. The Boc-deprotection was performed using the same conditions reported in Scheme 2 to give compound **44**. To circumvent the problem of a low yield of the amination reaction using intermediate **39**, we performed the synthesis of 2,4,7-trichloropyridopyrimidine (**47**) in two steps from 3-aminopicolinic acid (**45**) by a urea condensation, followed by the introduction of the three chlorine atoms on positions 2, 4 and 7 of pyrido[3,2-*d*]pyrimidine dione (**46**) according to a previously reported procedure.[13] With compound **47** in hand, the first substitution was done at the 4-position with basic amine R4 (pyrrolidine or azetidine analogue) and DIPEA in 1,4-dioxane at room temperature to give **48** and **49**. The Buchwald–Hartwig reaction was employed in the synthesis of



Scheme 3. Reagents and conditions: (i) chloroformamidine hydrochloride, dimethylsulfone, sulfolane, 165 °C; (ii) Ac_2O, Δ; (iii) (a) $POCl_3$, DIPEA, $PhCH_3$, Δ, (b) (*R*)-3-(*N*-Boc-methylamino)pyrrolidine, DIPEA, 1,4-dioxane, rt; (iv) (a) isopropenylboronic acid pinacol ester, $Pd(PPh_3)_4$, K_2CO_3, $PhCH_3$, EtOH, Mw, 130 °C, (b) H_2, Pd/C 5%, EtOH, rt. The acetyl group was removed in the Suzuki reaction through the presence of excess base; (v) HCl (4 M) in 1,4-dioxane, 1,4-dioxane, rt.



Scheme 4. Reagents and conditions: (i) urea, 160 °C, 0.2 M NaOH; (ii) $POCl_3$, DIPEA, $PhCH_3$, Δ; (iii) (a) 3-Boc-3-methylaminoazetidine, DIPEA, 1,4-dioxane, rt, (b) NH_3/EtOH, Mw, 150 °C; (iv) (a) potassium vinyltetrafluoroborate, $Pd(PPh_3)_4$, $PhCH_3$, EtOH, Mw, 130 °C, (b) H_2, Pd/C 5%; (v) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, Mw, 150 °C; (vi) HCl (2 M) in 1,4-dioxane, rt.

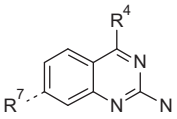


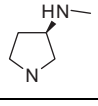
Scheme 5. Reagents and conditions: urea, 160 °C, 0.2 M NaOH; (ii) PCl_5, $POCl_3$, Mw, 160 °C; (iii) (a) corresponding amine, DIPEA, 1,4-dioxane, rt; (iv) $Pd(OAc)_2$, xantphos, K_2CO_3, $AcNH_2$, Mw, 130 °C; (v) (a) cyclopentene boronic acid or isopropylene boronic acid, potassium vinyl tetrafluoroborate, $Pd(PPh_3)_4$, $PhCH_3$, EtOH, Mw, 130 °C or potassium cyclpropyl trifluoroborate, $Pd(OAc)_2$, *n*-$BuPAd_2$, $PhCH_3$, H_2O, Mw, 130 °C for the 7-cyclopropyl substituted analogues, (b) H_2, Pd/C 5%, EtOH, rt; (vi) HCl (2 M) in 1,4-dioxane, rt.

compounds **50** and **51**. The introduction of the alkyl group was carried out by a Suzuki reaction followed by hydrogenation with palladium 5% on carbon and hydrogen gas. Cyclopropyl analogues **59** and **61** were synthesized directly with a different palladium source and ligand $Pd(OAc)_2$/*n*-$BuPAd_2$, using potassium cyclopropyl trifluoroborate.[14]

Interestingly, having an excess of base in the Suzuki reaction (3 equiv) and heating at 130 °C led to the concomitant

Table 1
H_4R SAR of quinazoline analogues substituted with aliphatic substituents on the 7-position



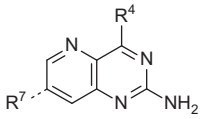
Compound no.	R_4	R_7	*h*H_4R pK_i ± SEM	*h*H_3R pK_i ± SEM	K_i H_3R/H_4R	*h*ERG pK_i ± SEM
VUF11489 **2**			7.82 ± 0.05[a]	5.47 ± 0.05[a]	223	5.49 ± 0.11[a]
20			7.91 ± 0.17[a]	6.48 ± 0.05[a]	27	5.47 ± 0.04[a]
21			8.47 ± 0.04[a]	6.80 ± 0.06[b]	47	6.08 ± 0.03[a]
22			7.45 ± 0.11[b]	6.07 ± 0.06[b]	24	n.d.
23			7.82 ± 0.03[b]	6.31 ± 0.10[b]	32	n.d.
24			7.94 ± 0.02[a]	6.81 ± 0.07[b]	13	5.33 ± 0.07[a]
25			8.15 ± 0.02[a]	6.16 ± 0.07[b]	98	5.44 ± 0.09[a]
26			7.83 ± 0.06[a]	6.66 ± 0.04[a]	15	5.82 ± 0.06[a]
27			7.95 ± 0.09[a]	6.21 ± 0.083[a]	89	5.61 ± 0.08[a]
28			7.77 ± 0.07[a]	6.22 ± 0.20[b]	56	5.56 ± 0.03[b]
29			7.66 ± 0.04[a]	6.75 ± 0.03[b]	8	n.d.

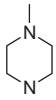
[a] Measured by displacement of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4R or H_3R. pK_i's are calculated from at least three independent measurements as the mean ± SEM, $n \geqslant 3$ unless mentioned otherwise.
[b] $n = 2$. n.d. = not determined.

deprotection of the acetamide on position 2 to give compounds **52**–**56**. Finally, to perform the Boc-deprotection, 4 M HCl solution in 1,4-dioxane was used to give target compounds **57**–**61**.

As mentioned in the introduction, VUF11489 was one of the H_4R ligands previously made in the quinazoline series that showed a favourable selectivity over the H_3R and had a pK_i of 7.82.[6] Nevertheless it contained a furan group and considerable hERG affinity, which we considered a significant liability. Replacing the furan by a cyclopentyl group on position 7 of the quinazoline heterocycle provided compound **20** with a comparable pK_i of 7.91. The replacement of the piperazine group of **20** with a 3-aminomethylazetidine group gave an increase in H_4R binding of about 0.5 log units (compound **21**). This finding shows that it was indeed possible to replace both the furan group and the piperazine moiety while maintaining and even increasing H_4R affinity. Both *t*-butyl substituted analogues **22** and **23** however showed lower affinities, indicating that the *t*-butyl group at the 7-position is less well tolerated than the cyclopentyl group. The combination of a cyclopropyl group with both amines (compounds **24** and **25**) gave a slight improvement of H_4R affinity compared with the *t*-butyl analogues. If the cyclopropyl group is replaced with an ethyl group a similar affinity is found (compare compounds **24** and **25** with compounds **26** and **27**). It was found that the combination of a (*R*)-*N*-methylpyrrolidin-3-amine moiety, common to many known H_4R ligands

Table 2
H_4R SAR of pyrido[3,2-*d*]pyrimidine analogues with reduced hERG binding



Compound no.	R_4	R_7	*h*H_4R pK_i ± SEM	*h*H_3R pK_i ± SEM	K_i H_3R/H_4R	*h*ERG pK_i ± SEM
PF-3893787	Structure in Figure 1		7.99 ± 0.08[a]	6.73 ± 0.07[a]	22	5.46 ± 0.22[b]
32			8.03 ± 0.05[a]	6.67 ± 0.09[b]	23	4.57 ± 0.08[a]
43			7.97 ± 0.10[a]	6.11 ± 0.12[a]	72	4.46 ± 0.20[b]
57			8.04 ± 0.12[a]	5.82 ± 0.02[a]	166	4.36 ± 0.22[b]
44			7.27 ± 0.04[a]	5.02 ± 0.02[b]	186	<4[a]
58			8.19 ± 0.09[a]	6.82 ± 0.09[a]	23	<4[a]
59			8.28 ± 0.09[a]	6.20 ± 0.05[a]	120	<4[a]
60			7.60 ± 0.03[a]	6.08 ± 0.05[a]	33	4.89 ± 0.15[b]
61			7.99 ± 0.06[a]	6.00 ± 0.06[b]	98	<4[a]
37			7.91 ± 0.11[a]	6.84 ± 0.01[b]	12	n.d.[a]

[a] Measured by displacement of [^{3}H]histamine binding using membranes of HEK cells transiently expressing the human H_4R or H_3R. pK_is are calculated from at least three independent measurements as the mean ± SEM, $n \geqslant 3$ unless mentioned otherwise.
[b] $n = 2$. n.d. = not determined.

also gave potent compounds when combined with an ethyl or cyclopropyl group (compounds **28** and **29**).[15,16] The H_3R affinity of this series of H_4R ligands was determined as well and it was found that the compounds have pK_i's between **5** and **7** for this receptor with cyclopentyl and cyclopropyl analogues **21** and **25** having the highest affinity of pK_i 6.80 and 6.81, respectively.

The compounds were also evaluated for their ability to displace [^{3}H]astemizole from the hERG channel and some compounds were found to bind this off target with considerable affinity. PF-3893787 was also found to have significant affinity for the hERG channel (pK_i 5.46 Tables 1 and 2). The hERG affinity of this compound series is not significantly reduced in comparison with VUF11489 by changing the substituents on the 7-position or the steric environment around the basic center and, as mentioned in the introduction, we prepared a series of pyrido[3,2-*d*]pyrimidines with (cyclo)aliphatic substituents on the 7-position and various amines on the 4-position (Fig. 2, Table 2). These new pyrido[3,2-*d*]pyrimidine analogues all maintained good affinities, with the only exception being the 7-cyano analogue (compound **44**) that has an affinity of pK_i 7.27. This SAR indicates that for this scaffold various combinations of aliphatic substituents with aminergic moieties are well tolerated by the H_4R. Like the quinazoline series, the pyrido[3,2-*d*]pyrimidine analogues also have considerable affinity for the H_3R, yet good H_4R selectivity >100-fold was found, in particular for analogues **57**, **59** and **61**. Interestingly, almost all analogues based on the pyrido[3,2-*d*]pyrimidine scaffold were found to have a much lower affinity for the hERG channel than the compounds from the quinazoline series, having a hERG K_i lower than 10 μM which is a good improvement in comparison with VUF11489 and PF-3893787. The introduction of an additional nitrogen atom in a closely related tricyclic aminopyrimidine scaffold developed by Savall et al. also had a strong detrimental effect on the ability of those compounds to bind the hERG channel, thereby greatly improving the quality of the physicochemical and pharmacological properties.[16]

Considering the prominent species differences that are found histamine H_4R orthologues and the possible complications this may pose in preclinical development, several analogues from the

Table 3
Affinity of quinazoline and pyrido[3,2-*d*]pyrimidine analogues at the histamine receptor subtypes[a]

Compound no.	hH_1R $K_i \pm SEM$	hH_2R $pK_i \pm SEM$	hH_3R $pK_i \pm SEM$	hH_4R $pK_i \pm SEM$	mH_4R $pK_i \pm SEM$	rH_4R $pK_i \pm SEM$
PF-3893787	n.d.	n.d.	6.73 ± 0.07	7.99 ± 0.08	7.26 ± 0.07	7.26 ± 0.10
43	4.47 ± 0.12	4.85 ± 0.05	6.11 ± 0.12	7.97 ± 0.10	7.30 ± 0.12	7.15 ± 0.16
57	4.95 ± 0.03	5.50 ± 0.07	5.82 ± 0.02	8.04 ± 0.12	7.07 ± 0.08	6.86 ± 0.12
58	4.78 ± 0.16	5.55 ± 0.04	6.82 ± 0.09	8.19 ± 0.09	7.71 ± 0.17[b]	7.56 ± 0.01[b]
59	4.68 ± 0.08	n.d.	6.20 ± 0.05	8.28 ± 0.09	7.82 ± 0.18	7.53 ± 0.16

[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. pK_i values are calculated from at least three independent measurements as the mean ± SEM ($n \geqslant 3$), unless mentioned otherwise.
[b] $n = 2$. n.d. = not determined.

Table 4
[^{35}S]GTPγS assay

Compound no.	hH_4R $pIC_{50} \pm SEM$[a]	pK_b[b]
PF-3893787	7.27 ± 0.06	8.46 ± 0.06
43	6.95 ± 0.07	8.14 ± 0.07
57	6.90 ± 0.07	8.08 ± 0.09
58	7.01 ± 0.14	8.20 ± 0.14
59	7.15 ± 0.10	8.33 ± 0.10

Antagonism against 100 nM histamine at the human H_4R.
[a] $n \geqslant 5$.
[b] pK_b is determined with the Cheng and Prusoff equation, wherein the EC_{50} of histamine is 6.95 nM.

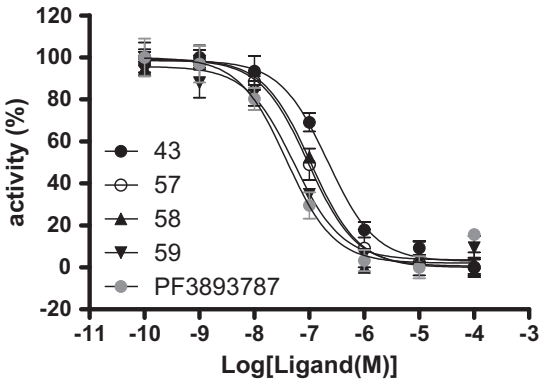


Figure 3. Antagonism at the human H_4R. The compounds dose dependently inhibit 100 nM histamine-induced activity of the human H_4R in [^{35}S]GTPγS assay.

pyrido[3,2-*d*]pyrimidine series were selected for further profiling at the different histamine receptor subtypes and rodent H_4 receptors (Table 3). It was found that the compounds have varying selectivities between 186- and 12-fold for the hH_4R over the other human histamine receptor subtypes. All compounds also retained good affinity for the rat and mouse H_4R with only compound **57** having an affinity that is significantly lower than that of histamine itself for the species orthologues (histamine pK_i is 7.1 ± 0.1 for the mH_4R and 7.0 ± 0.1 for the rH_4R).[17] The selected compounds were also evaluated in a [^{35}S]GTPγS assay for their functional activities at the hH_4R. In this assay, the compounds show their ability to antagonize 100 nM histamine-induced activity at the hH_4R (Table 4, Fig. 3). The calculated pK_b values, derived from pIC_{50} values with the Cheng–Prusoff equation, approximate the pK_i values of the corresponding compounds as determined with the radioligand displacement assay.[18]

Recently we have described the use of compound residence time as a parameter to take into consideration when optimizing ligands for the hH_4R.[2] The widely used H_4R ligand JNJ7777120 is a compound that shows efficacy in many animal models. Interestingly, JNJ7777120 often has the best efficacy in those models in comparison with compounds from other structural classes, even despite its short in vivo pharmacokinetic elimination half-life.[19] We have also speculated that the efficacy of JNJ7777120 may be the result of its relatively long residence time at the various H_4R receptors. Residence time is considered to be an important parameter to consider in the lead optimization process and we therefore studied the residence time of compounds **43**, and **57**–**59** to compare with the clinical stage compound PF-3893787 and JNJ7777120 (Table 5). When comparing the dissociation constant and associated pK_D from Table 5 with the pK_i determined in the radioligand binding assay we find closely matching values. It should be taken into consideration that compounds with long residence times may not be detected in standardized radioligand binding assays, because these assays may not have an incubation time that gives and accurate estimation of the compounds' affinity at equilibrium. In the case of longer residence times, compound affinities may therefore be underestimated. We found that none

Table 5
Binding kinetics at the human H_4R determined in competitive kinetic [^{3}H]histamine binding assay[a]

Compound no.	k_{on}[b]	k_{off}[c]	pK_D[d]	τ (min)
PF-3893787	2.00 ± 0.23	0.052 ± 0.01	7.60 ± 0.15	15.0 ± 3.9
JNJ7777120	3.64 ± 0.43	0.017 ± 0.01	8.40 ± 0.10	62.0 ± 13.6
VUF11489	5.46 ± 1.99	0.193 ± 0.04	7.39 ± 0.08	4.10 ± 0.8
43	7.89 ± 2.95	0.077 ± 0.05	8.07 ± 0.13	14.0 ± 8.4
57	5.68 ± 2.72	0.108 ± 0.02	7.67 ± 0.15	6.60 ± 1.1
58	9.93 ± 0.13	0.102 ± 0.00	7.99 ± 0.01	6.80 ± 0.1
59	6.18 ± 4.76	0.055 ± 0.02	7.87 ± 0.32	13.7 ± 3.8

[a] Data were analyzed with Graphpad Prism 5.0, $n = 3$ unless mentioned otherwise.
[b] Data expressed as $10^6\ M^{-1}\ min^{-1}$.
[c] Data expressed as $M^{-1}\ min^{-1}$.
[d] pK_D obtained from $K_D = k_{off}/k_{on}$.

of the compounds has a residence time longer than the **62** minutes found for JNJ7777120. However, compounds **43**, **59** and PF-3893787 all have residence times about fourfold lower than the reference antagonist JNJ7777120 but fourfold higher than lead compound VUF11489. It has been reported that longer residence times can lead to improved compound efficacy and target selectivity in vivo.[20] However, no preclinical data that describes the efficacy of PF-3893787 in various disease models has been disclosed so far. Therefore we aim to use the reference compounds and novel pyrido[3,2-*d*]pyrimidines described in this work to further study the relevance of target residence time for H_4R drug development and select a candidate compound for further development.

We have developed a series of new H_4R ligands based on the pyrido[3,2-*d*]pyrimidine scaffold from a lead-series containing a quinazoline moiety and managed to greatly reduce the hERG binding of those compounds. In parallel we succeeded to introduce various basic amines to create a series of highly potent compounds

with excellent in vitro profiles at the various histamine receptor subtypes. Although JNJ777120 remains the compound with the longest known residence time at the hH_4R of 62 min, we were able to find several compounds with extended residence times comparable to that of clinical candidate PF-3893787. Further work on this novel compound series will focus on its characterization in vivo and the selection of an optimized lead for further development.

Acknowledgments

The authors would like to thank Maikel Wijtmans, Andreas Ehlers, Hans Custers and Andrea van de Stolpe for their unrelenting support and fruitful discussions. This work was supported by COST action BM0806.

References and notes

1. Hofstra, C. L.; Deasi, P. J.; Thurmond, R. L.; Ping, W.; Leung, F. *J. Pharm. Exp. Ther.* **2003**, *305*, 1212.
2. Thurmond, R. L.; Gelfland, E. W.; Dunford, P. J. *Nat. Rev. Drug Disc.* **2008**, *7*, 41.
3. Smits, R. A.; Leurs, R.; de Esch, I. J. P. *Drug Discov. Today* **2009**, *12*, 628.
4. Gutzmer, R.; Geschwandtner, M.; Rossbach, K.; Mommert, S.; Werfel, T.; Kietzmann, Baeumer W *Front. Biosci. (Schol Ed.)* **2011**, *3*, 985.
5. Dunford, P. J.; Williams, K. N.; Desai, P. J.; Karlsson, L.; McQueen, D.; Thurmond, R. L. *J. Allergy Clin. Immunol.* **2007**, *119*, 176.
6. Desmadryl, G.; Gaboyard-Niay, S.; Brugeaud, A.; Travo, C.; Saleur, A.; Dyhrfjeld-Johnsen, J.; Wersinger, E.; Chabbert, C. *Br. J. Pharmacol.* **2012**, *167*, 905.
7. Smits, R. A.; Lim, H. D.; van der Meer, T.; Kuhne, S.; Bessembinder, K.; Zuiderveld, O. P.; Wijtmans, M.; de Esch, I. J. P.; Leurs, R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 461.
8. Shagufta, D. G.; Kaasse, E.; de Vries, H.; Brussee, J.; Nalos, L.; Rook, M. B.; Vos, M. A.; van der Heyden, M. A. G.; Ijzerman, A. P. *ChemMedChem* **2009**, *10*, 1722.
9. Neuwirth, C.; Mosesso, P.; Pepe, G.; Fiore, M.; Malfatti, M.; Turteltaub, K.; Dekant, W.; Mally, A. *Mol. Nutr. Food Res.* **2012**, *56*, 1363.
10. Walters, P. W.; Green, J.; Weiss, J. R.; Murcko, M. A. *J. Med. Chem.* **2011**, *54*, 6405.
11. Mowbray, C. E.; Bell, A. S.; Clarke, N. P.; Collins, M.; Jones, R. M.; Lane, C. A.; Liu, W. L.; Newman, S. D.; Paradowski, M.; Schenk, E. J.; Selby, M. D.; Swain, N. A.; Williams, D. H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6596.
12. Raphy, G. U.S. Patent US20100298289, 2010.
13. Tikad, A.; Aksirra, M.; Massip, S.; Leger, J.-M.; Jarry, C.; Guillaumet, G.; Routier, S. *Eur. J. Org. Chem.* **2012**, *24*, 4523.
14. Molander, G. A.; Gormisky, P. E. *J. Org. Chem.* **2008**, *73*, 7481.
15. Examples of compounds with different basic amines including the pyrrolidine group are manifold. Various reviews give an overview of preferred roups. (a) Marson, C. M. *Chem. Rev.* **2001**, *111*, 7121; (b) Engelhardt, H.; Smits, R. A.; Leurs, R.; Haaksma, E.; de Esch, I. J. P. *Curr. Opin. Drug Discov. Devel.* **2009**, *9*, 132.
16. Savall, B. M.; Gomez, L.; Chavez, F.; Curtis, M.; Meduna, S. P.; Kearney, A.; Dunford, P.; Cowden, J.; Thurmond, R. L.; Grice, C.; Edwards, J. P. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6577.
17. Lim, H. D. Ligand-Receptor Interactions of the Histamine H4 Receptor, Doctoral Thesis, VU University Amsterdam, The Netherlands, 2009.
18. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
19. Venable, J. D.; Cai, H.; Chai, W.; Dvorak, C. A.; Grice, C. A.; Jablonowski, J. A.; Shah, C. R.; Kwok, A. K.; Ly, K. S.; Pio, B.; Wei, J.; Desai, P. J.; Jiang, W.; Nguyen, S.; Ling, P.; Wilson, S. J.; Dunford, P. J.; Thurmond, R. J.; Lovenberg, T. J.; Karlsson, L.; Carruthers, N. J.; Edwards, J. P. *J. Med. Chem.* **2005**, *48*, 8289.
20. Copeland, R. A.; Pompliano, D. L.; Meek, T. D. *Nat. Rev. Drug Disc.* **2006**, *5*, 730.