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Synthesis and evaluation of novel ligands for the histamine H_4 receptor based on a pyrrolo[2,3-*d*]pyrimidine scaffold

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ABSTRACT

Starting from a known H_4R ligand based on a pyrimidine skeleton, a series of novel analogues based on a pyrrolo[2,3-*d*]pyrimidine scaffold have been prepared. Whereas the original pyrimidine congener shows good affinity at hH_4R (K_i = 0.5 μM), its lacks selectivity with a K_i value for the hH_3R of 1 μM. Within the newly synthesized pyrrolo[2,3-*d*]pyrimidines, several congeners show K_i values of less than 1 μM at the hH_4R and show a much improved selectivity profile. Therefore, these series represent an interesting starting point for the discovery of novel hH_4R ligands.

The human histamine 4 (hH_4R) receptor is the newest member of the histamine receptor family. It has been discovered in 2000, independently by several research groups,[1–5] and since then, has attracted much attention from the medicinal chemistry community as promising drug target. The hH_4R is mainly expressed on cells of the immune system (e.g., mast cells, eosinophils, monocytes, dendritic cells, T-cells), although recent reports also show evidence for expression of the hH_4R on non-hematopoietic cells and in the brain. At the cellular level, activation of the hH_4R induces chemotaxis of mast cells and eosinophils and triggers calcium mobilization in mast cells, monocytes and eosinophils. Moreover, the hH_4R modulates the release of various inflammatory mediators.[6] Therefore, the hH_4R is currently exploited as potential drug target for the treatment of various chronic inflammatory and allergic diseases such as inflammatory bowel disease, asthma, rheumatoid arthritis and pruritis.[7,8]

In search for novel hH_4R antagonists, a wide variety of different structural ligands have been discovered. The first non-imidazole H_4R antagonist was JNJ7777120 (Fig. 1), a (piperazinylacyl)indole analogue that displays strong affinity for the hH_4R (K_i = 4 nM).[9] Up to now, it is still considered as the reference antagonist, and this compound has been used in animal models of different inflammatory disorders (mouse allergic airway inflammation, a mouse peritonitis and a rat colitis model).[10] However, the short half-life of this compound prevents its further development. In search for other series of compounds that act as hH_4R antagonists, the 2-amino-pyrimidine scaffold turned out to be a privileged structure. The first patents disclosing 2-amino-pyrimidines as hH_4R antagonists became public in 2005.[11,12] Since then, a wide variety of analogues have been prepared. Overall, it seems that 5- and 6-positions of the pyrimidine scaffold tolerate a wide variety of substituents, while the 2-amino group is mostly essential for hH_4R binding. The *N*-methylpiperazine substituent provides usually one of the most potent analogues, but the *N*-methylpiperazine moiety can be successfully replaced with several analogues. In order to broaden structural variety, a second and third ring has been appended to the pyrimidine ring giving rise to bicyclic and tricyclic analogues, respectively (Fig. 1). Examples of tricyclic analogues include benzothieno[2,3-*d*]pyrimidines, benzofuro[2,3-*d*]pyrimidines[13] and benzo[6,7]cyclohepta[1,2-*d*]pyrimidines.[14] Bicyclic congeners that have been synthesized as hH_4R antagonists are quinazolines,[15] furo[3,2-*d*]pyrimidines,[16] pyrazolo[4,3-*d*]pyrimidines[17] and purines.[18]

However, much to our surprise, the structurally highly related pyrrolo[2,3-*d*]pyrimidine scaffold has not been explored in the hH_4R field (or at least not reported in literature). In this letter, we report our efforts towards the design of novel hH_4R ligands based on a pyrrolo[2,3-*d*]pyrimidine core structure.

The chemistry started off with the synthesis of 2,6-diamino-4-(*N*-methyl-piperazin-1-yl)pyrimidine **2** from 6-chloropyrimidin-2,6-diamine by a simple nucleophilic aromatic substitution reaction (Scheme 1). Although the synthesis of this compound has been described in literature,[19] switching the solvent from 2-methoxyethanol (as originally described) to water greatly increased the yield of the reaction and shortened the reaction time from 16 to 4 h.

In order to build up the pyrrolo[2,3-*d*]pyrimidine scaffold, the construction of the pyrrole moiety on the preformed pyrimidine

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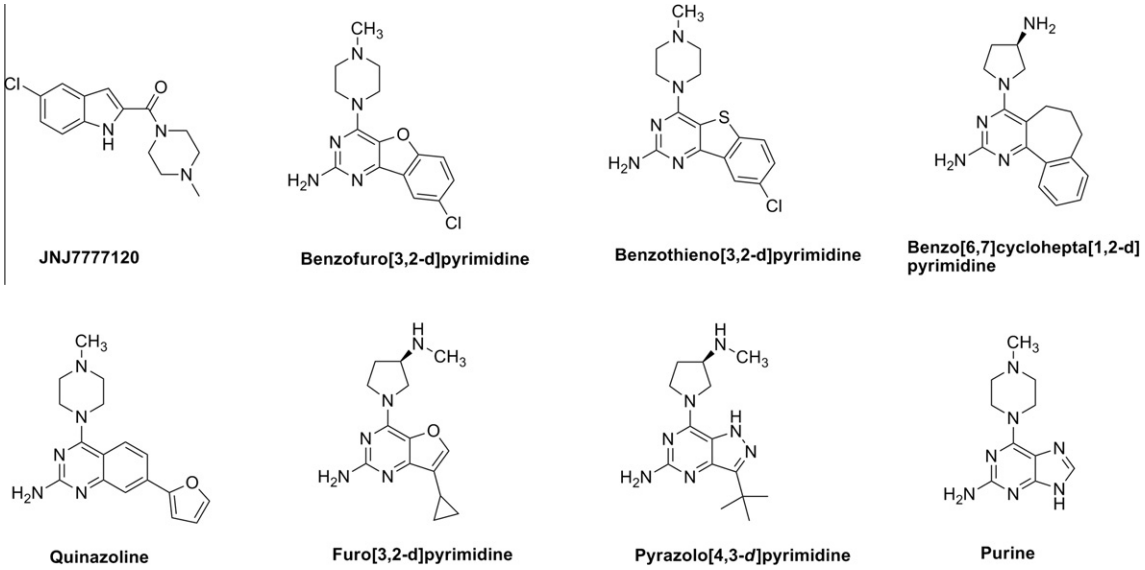
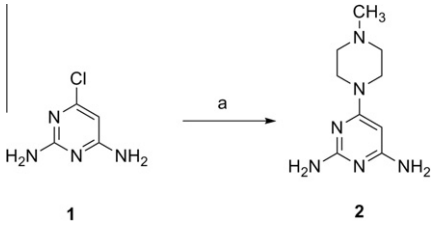
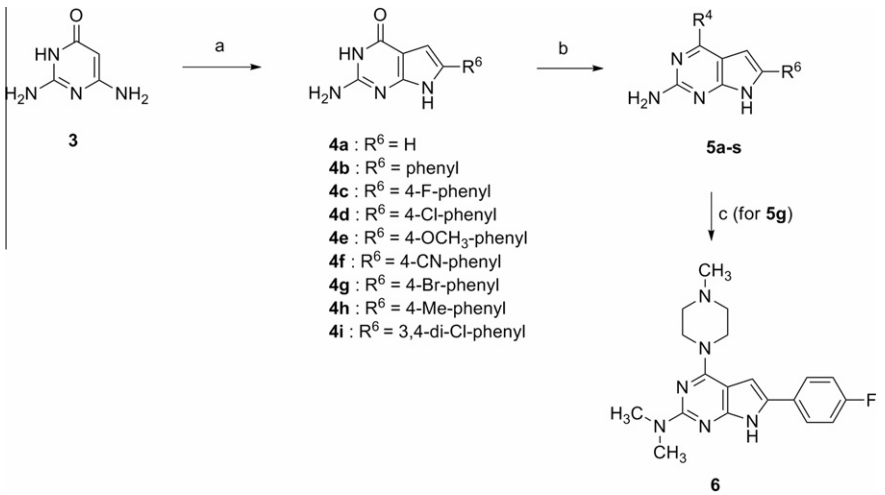


Figure 1. Polycyclic structures of hH_4R ligands.

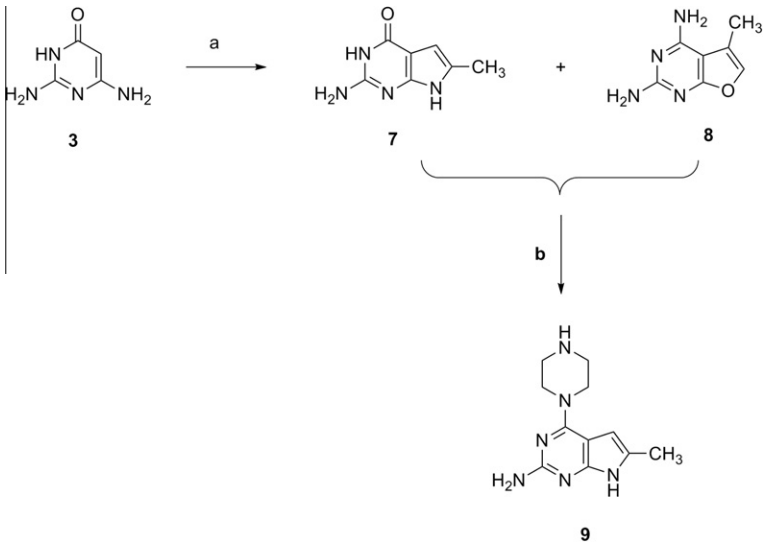


Scheme 1. Synthesis of 2,6-diamino-4-(*N*-methyl-piperazin-1-yl)pyrimidine. Reagents and conditions: (a) *N*-methylpiperazine, H_2O, 4 h, 83%.

ring from **2** was unsuccessful, as only starting material could be recovered. Therefore, the sequence of reactions was changed, as we first constructed the pyrolo[2,3-*d*]pyrimidine scaffold, followed by decoration of the skeleton. Hereto, 2,6-diamino-4-hydroxy-pyrimidine **3** was selected as commercially available starting material. Reaction with a range of α-halo-carbonyl derivatives yielded the desired 2-amino-4-oxo-pyrrolo[2,3-*d*]pyrimidine analogues **4a–h**.[20] All these reactions proceeded in a regiospecific manner, yielding the 6-substituted pyrrolo[2,3-*d*]pyrimidine analogues (Scheme 2). A standard way for derivatisation of the lactam group involves activation of the carbonyl group by halogenation using phosphorus oxychloride. Unfortunately, these harsh conditions only resulted in complex reaction mixtures. Recently, we reported on the use of a convenient phosphonium mediated SNAr reaction for the derivatisation of a lactam functionality.[21] Treatment of **4a–h** with benzotriazol-1-yloxytris(dimethylamino)-phosphoniumhexafluorophosphate (BOP), DBU and a nitrogen nucleophile (piperazine, *N*-alkyl-piperazine, pyrrolidine) leads to the formation of the 4-N-substituted pyrrolo[2,3-*d*]pyrimidine analogues **5a–s**. Although the yield of these reactions was low to moderate (30–70%), its shortness makes it an attractive route. When Boc-protected pyrrolidines were used as nucleophile, a final acidic deprotection step was necessary in order to deliver the desired compounds. Methylation of the exocyclic amino group of compound **5g** was performed by a reductive alkylation reaction using sodium cyanoborohydride and formaldehyde, affording the N,N-dimethylated analogue **6**.



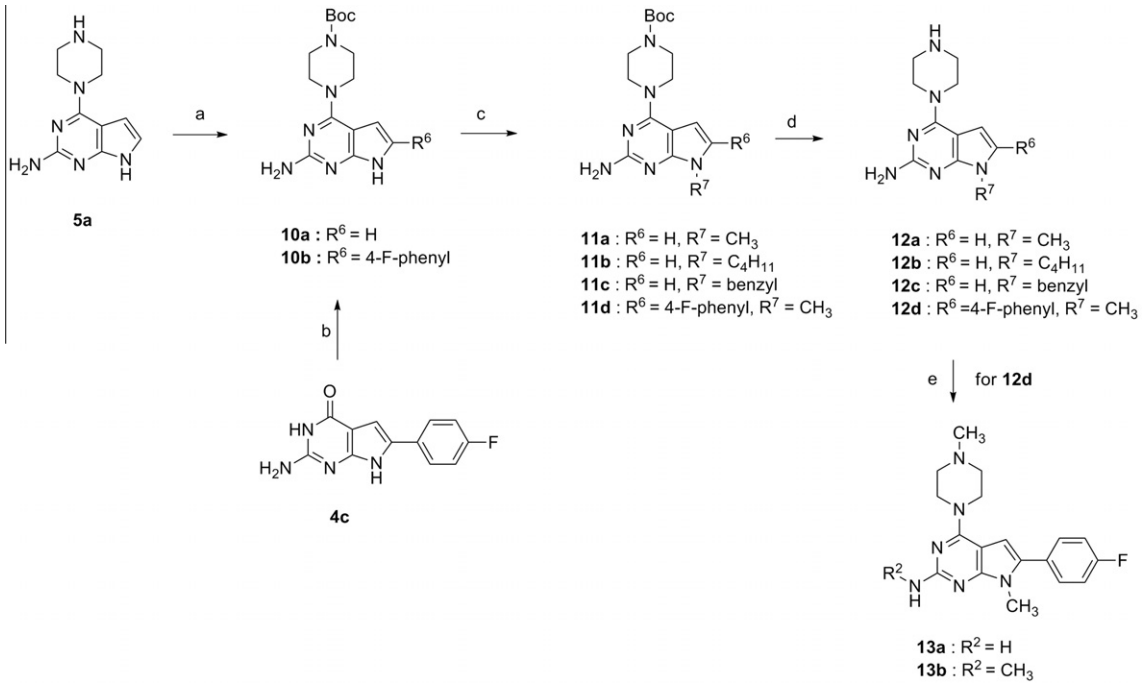
Scheme 2. Synthesis of 6-H and 6-phenyl substituted pyrrolo[2,3-*d*]pyrimidines. Reagents and conditions: (a) R^6COCH_2X (X = Cl or Br), NaOAc, H_2O, dioxane; (b) BOP, DBU, amine, DMF, rt; (c) $NaCNBH_3$, 30% aq formaldehyde, CH_3OH, rt.



Scheme 3. Synthesis of 2-amino-4-*N*-piperazinyl-6-methyl-pyrrolo[2,3-*d*]pyrimidine. Reagents and conditions: (a) chloroacetone, DMF, 60 °C; (b) BOP, DBU, piperazine, DMF, rt.

In contrast to the regiospecific reactions mentioned in Scheme 2, the reaction of **3** with chloroacetone yielded a mixture of two compounds, that is 2-amino-4-hydroxy-6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **7** and 2,4-diamino-5-methyl-furo[2,3-*d*]pyrimidine **8** (Scheme 3).[22] Separation by flash chromatography on silicagel yielded the pure furo[2,3-*d*]pyrimidine derivative **8**, whereas the desired pyrrolo[2,3-*d*]pyrimidine derivative **7** could not be isolated in pure form, but was always contaminated with the furo[2,3-*d*]pyrimidine analogue **8**. Therefore, the mixture was further treated in a one-step BOP-mediated amination reaction, using piperazine as nucleophile, yielding a mixture of 2,4-diamino-5-methyl-furo[2,3-*d*]pyrimidine **8** (which remained unchanged in this reaction) and the desired pyrrolo[2,3-*d*]pyrimidine derivative **9**. At that stage, both compounds could be easily separated affording pure **9**.

For alkylation reactions at the pyrrole nitrogen, compounds **5a** and **5g** were selected as starting materials (Scheme 4). As treatment of **5a** and **5g** with alkyl halides led predominantly to alkylation of the terminal nitrogen atom of the piperazine moiety, protection prior to alkylation was necessary. The *Boc*-protected intermediate **10a** was easily obtained by treatment of the 4-*N*-piperazinyl intermediate **5a** with *Boc* anhydride and **10b** was obtained by treatment of the 4-oxo-pyrrolo[2,3-*d*]pyrimidine **4c** with *N*-*Boc*-piperazine. Although alkylation reactions of these *Boc*-protected intermediates with a suitable alkyl or benzyl halide, predominantly gave the mono-alkylated compound (with an alkyl moiety on the pyrrole nitrogen), the reaction mixture was always contaminated with substantial amounts of the dialkylated compound (alkyl on pyrrole nitrogen and exocyclic amino group). They were, however, easily separated on column, and finally, acidic cleavage



Scheme 4. Synthesis of alkylated pyrrolo[2,3-*d*]pyrimidine analogues. Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMF, rt; (b) BOP, DBU, *N*-Boc-piperazine, DMF, rt; (c) R^7X (X = I or Br), NaH, THF, rt; (d) TFA/CH_2Cl_2, rt; (e) $NaCNBH_3$, 30% aq formaldehyde, CH_3OH, rt.

of the *Boc* group afforded a series of 7-N-substituted pyrrolo[2,3-*d*]pyrimidine analogues **12a–d**. Reductive amination of compound **12d** with formaldehyde led to the *N*-methyl-piperazinyl product **13a** as major compound, whereas the double methylated product **13b** (i.e. methylation of the exocyclic amino group and the piperazine nitrogen) was isolated as a minor side product.

All compounds synthesized were evaluated for their affinities at hH_4R and in selected cases for the human histamine H_3 receptor (hH_3R). For these in vitro determinations radioligand competition binding assays were used as previously described in Tomasch et al.[23] In brief, determination of hH_4R affinity was performed with crude membrane preparations of Sf9 cells transiently expressed hH_4R in full length and co-expressed with G-Protein subunits $G_{\alpha i/o}$ and $G_{\beta 1\gamma 2}$. [^{3}H]Histamine was used as radioligand and JNJ7777120 (10 μM concentration) for the determination of non specific binding. For the determination of human H_3 receptor affinity crude membrane preparations of HEK293 cells stably expressing the hH_3R in full length were used. [^{3}H]N^{α}-Methylhistamine was used as radioligand and 10 μM Pitolisant was used for the determination of non specific binding. All determinations were performed in four different concentrations ranging from 0.1 to 100 μM in triplicates in at least two independent experiments. All binding data analysis was performed with GraphPad Prism™ (Version 3.02).

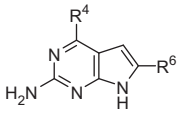
Compound **2** was previously evaluated for its functional activity in hH_4R transfected cell lines with a Ca^{2+} Influx FLIPR Assay.[17] As starting point for our internal drug discovery program, this compound was evaluated for its binding affinity towards the hH_4R in a radioligand competition binding assay, whereby compound **2** displays a K_i value of 0.53 μM. The appendage of a pyrrole ring to the pyrimidine ring afforded the pyrrolo[2,3-*d*]pyrimidine analogue **5a**, which lost all affinity for the hH_4R, displaying a K_i value of 45.92 μM (Table 1). In order to restore activity, additional substituents were introduced on the pyrrole moiety. The presence of a small methyl group at position 6 of the pyrrolo[2,3-*d*]pyrimidine scaffold (compound **9**) led to a greatly improved binding affinity (K_i = 2.67 μM). The insertion of a phenyl at position 6 yielded compound **5b** with comparable activity as the 6-methyl analogue **9**. The presence of a piperazinyl (**5b**) or 4-*N*-piperazinyl (**5c**) moiety at 4-position of the skeleton led only to marginal differences in binding affinity.

In order to probe into the optimal substitution pattern on the aromatic ring, a series of analogues was prepared, carrying halogens, electron-withdrawing and electron-donating substituents on the phenyl ring (Table 2, compounds **5d**–**5j**). Most of these analogues do show activity in the hH_4R binding assay, displaying K_i values between 2.64 and 8.99 μM, with only the presence of a 3,4-dichlorophenyl ring (compound **5j**) leading to a drastic decrease in affinity (K_i = 36.12 μM).

A series of analogues was prepared to study the effect of the size of the alkyl group on the piperazinyl moiety (Table 3). Whereas hydrogen (**5k**), methyl (**5d**), and ethyl groups (**5l**) all have comparable hH_4R binding affinities, increasing the size towards an isopropyl group (compound **5m**) led a drastic loss of affinity (K_i = 48.01 μM). It confirms the trend observed in other series of hH_4R antagonists, that sterically demanding substituents are not tolerated.

Structurally related diamines frequently used in other H_4R drug design programs were also incorporated onto the pyrrolo[2,3-*d*]pyrimidine scaffold (Table 4). It seems that for the 6-unsubstituted pyrrolo[2,3-*d*]pyrimidine analogues, the *N*-methyl-homopiperazinyl substituent (analogue **5n**) shows better binding affinity than its 4-*N*-piperazinyl counterpart (compound **5a**). In the 6-phenyl, as well as in the 6-(4-fluorophenyl) series, the 4-*N*-Me-homopiperazinyl (compounds **5o** and **5p**) substituent show comparable affinity at hH_4R, when compared to their 4-*N*-Me-piperazinyl congeners (compounds **5c** and **5d**). Both optically pure enantiomers of 3-amino-pyrrolidine were coupled to the pyrrolo[2,3-*d*]pyrimidine scaffold. Both isomers show good affinity in the hH_4R binding assay, with the (*S*)-isomer **5r** (K_i = 0.98 μM) being slightly higher affine

Table 1
SAR of 6-substituted pyrrolo[2,3-*d*]pyrimidines at hH_4R



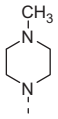
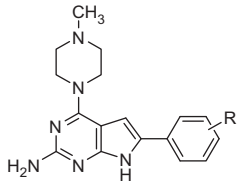
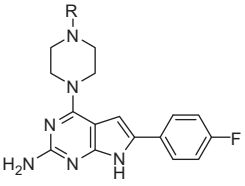
Compd.	R^4	R^6	hH_4R K_i (μM)	*c*Log*P*	LE
5a		H	45.92	0.58	0.37
9		CH_3	2.67	1.08	0.45
5b		Phenyl	1.41	2.68	0.36
5c		Phenyl	5.40	3.25	0.31

Table 2
SAR of 6-phenyl pyrrolo[2,3-*d*]pyrimidines at hH_4R



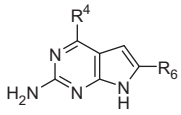
Compd.	R	hH_4R K_i (μM)	*c*Log*P*	LE
5d	4-F	2.64	3.40	0.32
5e	4-Cl	3.28	3.97	0.31
5f	4-OCH_3	3.32	3.05	0.30
5g	4-CH_3	3.73	3.75	0.31
5h	4-CN	7.32	2.70	0.28
5i	4-Br	8.99	4.12	0.29
5j	3,4-di-Cl	36.12	4.56	0.24

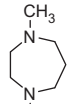
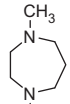
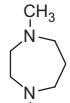
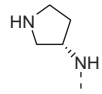
Table 3
SAR of 6-(4-fluorophenyl)pyrrolo[2,3-*d*]pyrimidines at hH_4R



Compd.	R	hH_4R K_i (μM)	*c*Log*P*	LE
5k	H	0.39	2.82	0.38
5d	Methyl	2.64	3.40	0.32
5l	Ethyl	4.07	3.93	0.29
5m	Isopropyl	48.01	4.24	0.28

Table 4
Replacements for the piperazinyl moiety



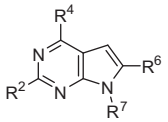
Compd.	R^4	R^6	hH_4R K_i (μM)	*c*Log*P*	LE
5n		H	8.72	1.14	0.38
5o		Phenyl	1.41	3.23	0.33
5p		4-F-phenyl	2.93	3.38	0.30
5q		4-F-phenyl	1.71	2.41	0.34
5r		4-F-phenyl	0.98	2.41	0.36
5s		4-F-phenyl	5.95	3.15	0.31

than the corresponding (*R*)-distomer **5s** (K_i = 1.71 μM). When the 3-amino-pyrrolidine moiety was coupled via its exocyclic amino group to the pyrrolo[2,3-*d*]pyrimidine skeleton (yielding compound **5s**), a fivefold decrease in binding affinity (K_i = 5.95 μM) was observed.

In another round of optimization, structural variation was introduced on the pyrrole nitrogen (Table 5). For the 6-unsubstituted pyrrolo[2,3-*d*]pyrimidine analogues, the introduction of a small methyl group (compound **12a**; K_i = 36.35 μM) did not have any effect on the binding affinity, when compared to the pyrrolo[2,3-*d*]pyrimidines, bearing a free NH group (compound **5a**; K_i = 45.92 μM). However, insertion of bulkier substituents such as a *n*-butyl (affording analogue **12b**) or benzyl (furnishing derivative **12c**), increased the affinity for the hH_4R (K_i values of 8.13 and 25.22 μM, respectively), when compared to the analogue **5a** (K_i = 45.92 μM). On the other hand, for the 6-(4-fluorophenyl) analogues, methylation of the pyrrole nitrogen led to a drastic decrease in binding affinity. This is clear when the binding affinity data are compared between compounds **5k** (K_i = 0.39 μM) and its corresponding methylated analogue **12d**, which is endowed with a K_i value of 44.15 μM. Similarly, *N*-methylation of pyrrolo[2,3-*d*]pyrimidine analogue **5d** (K_i = 2.64 μM) led to a 30-fold loss in binding affinity (**13a**; K_i = 67.87 μM). It indicates that for this series, having a hydrogen bond donor at that position may be important for affinity.

Methylation of the 2-amino group (**6**; K_i = 10.45 μM), leads to a fivefold decrease in activity, when compared to its 2-amino counterpart (**5d**; K_i = 2.64 μM). A more pronounced effect is observed when the exocyclic amino group, as well as the pyrrole NH moiety, are both methylated as in compound **13b**, giving rise to a complete loss in activity, displaying a K_i value of more than 100 μM. These observations are in agreement with previous SAR studies that

Table 5
SAR of alkylated pyrrolo[2,3-*d*]pyrimidines at hH_4R



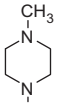
Compd.	R^2	R^4	R^6	R^7	hH_4R K_i (μM)	*c*Log*P*	LE
6	$N(CH_3)_2$		4-F-phenyl	H	10.45	4.32	0.26
12a	NH_2		H	CH_3	36.35	0.53	0.36
12b	NH_2		H	*n*-butyl	8.13	2.12	0.35
12c	NH_2		H	benzyl	25.22	2.30	0.28
12d	NH_2		4-F-phenyl	CH_3	44.15	2.78	0.25
13a	NH_2		4-F-phenyl	CH_3	67.87	3.35	0.23
13b	$NHCH_3$		4-F-phenyl	CH_3	>100	4.06	0.21

Table 6
Affinity profile of selected compounds

Compd.	hH_4R K_i (μM)	hH_3R K_i (μM)	*c*Log*P*	LE
2	0.53	1.08	0.42	0.57
5k	0.39	84.66	2.82	0.38

demonstrated the essential contribution of the exocyclic amino group on hH_4R binding.

An important way of assessing the 'drug-likeness' of compound series is the determination of their lipophilicity. The *c*Log*P* values of all analogues were calculated. Although there are a number of cases where binding affinity increases with additional lipophilic substituents (e.g. compare compounds **5a** and **5b**), this is not always the case (e.g. analogues **5k**/**5m, 5d/5j, 5n/5o**). It indicates that the increased binding affinity within this series is not due to aspecific lipophilic effects.

In order to assess the effectiveness of a compound optimization process, ligand efficiency (LE) is a valuable concept.[24] Throughout this paper, the LE values (in kcal/mol) have been calculated for all derivatives made. It has been stated that a minimum LE of 0.3 is required in a hit or lead compound to be useful. The original hit **2** had an LE value of 0.57, whereas. compound **5k**, the result of this optimization process, is endowed with a LE index of 0.38, and therefore still suitable as lead compound.

In conclusion, starting from a known H_4R ligand based on a pyrimidine skeleton, a series of analogues based on a

pyrrolo[2,3-*d*]pyrimidine scaffold have been prepared. Whereas the original pyrimidine congener **2** shows good affinity at hH_4R (K_i = 0.53 μM), its lacks selectivity with a K_i value for the hH_3R of 1 μM (Table 6). Within the newly synthesized pyrrolo[2,3-*d*]pyrimidines, several congeners (e.g. compound **5k**) show K_i values of less than 1 μM at the hH_4R and show an improved preference profile (for compound **5k**: hH_3R K_i = 84.66 μM). A pyrrolo[2,3-*d*]pyrimidine scaffold has not been described for hH_4R ligand and is, therefore, representing an interesting starting point for further medicinal chemistry work on this structural novel hit.

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