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Exploration of a new series of CCR5 antagonists: Multi-dimensional optimization of a sub-series containing N-substituted pyrazoles

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ABSTRACT

The introduction of N-substituted pyrazoles in a new series of CCR5 antagonists was shown to substantially increase antiviral activity.

CCR5 is one of the many chemokine receptors involved in the mediation of the inflammation process, In addition to its role as a receptor on inflammatory leucocytes, CCR5 is also the primary co-receptor for macrophage-tropic HIV-1. CCR5 has proven to be an extraordinary target for the pharmaceutical industry, with demonstrated therapeutic applications in HIV-treatment as well as potential applications in various chronic or acute inflammatory diseases.[1]

We recently described a new series of CCR5 antagonists with compounds **1** and **3** being two representatives.[2] This series contains the four pharmacophores found in most of the series reported in the literature: a tertiary basic amine, two hydrophobic groups in the western portion of the molecule (henceforth referred to as the tail), including the tail hydrophobe 1, and an (hetero)aryl in the eastern portion of the molecule (the head). We describe herein, part of our SAR focusing on the head region, specifically the introduction of substituted heteroaryl heads. Early in the project, we screened a wide range of head acyl and sulfonyl groups. From the hundreds of compounds that were prepared, several types stood out, in particular the substituted heteroaryl heads (Fig. 1).

The *N*-phenyl substituted pyrazole **2** was more than fourfold more active than **1** in our HIV antiviral assay (Table 1). It was argued at the time that the increase in activity was lipophilicity-driven (log *D* of 2.92 for **2** vs 1.37 for **1**) and that **2**, ultimately was not a more efficient ligand than **1** when the lipophilicity increase was taken into consideration. However, we observed similar increases in antiviral activity for compounds that contained other substituted heteroaryl heads, and with similar lipophilicity. In particular, compound **4** was threefold more potent than compound **3**, with both compounds having virtually equal measured log *D*s (Table 1).

Taking into consideration this observation, we decided to explore the N-substituted pyrazole heads more exhaustively.

Compounds were prepared according to the general procedure depicted in Scheme 1.

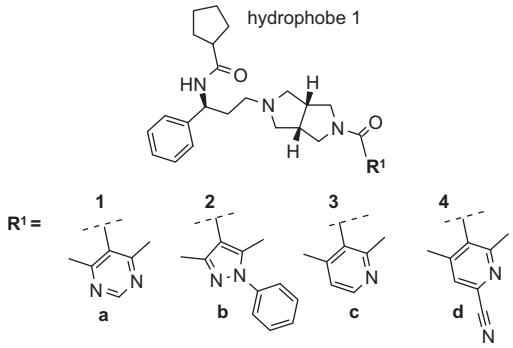

Figure 1. Structures of representative examples of the original series (**1**, **3**) and examples of compounds with substituted heads (**2**, **4**).

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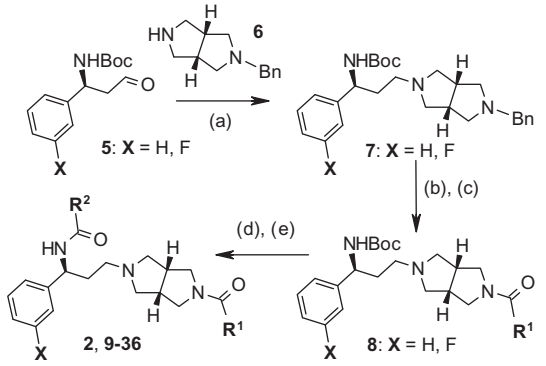
Table 1
Antiviral activity and measured lipophilicity compounds **1**–**4**

Compound	$\mathbf{R^1}$	Antiviral[a] (nM)	log *D*[c]
1	a	6.2[b]	1.37
2	b	<1.5	2.92
3	c	2.1	1.90
4	d	0.7	1.89

[a] Replication inhibition (IC_{50}) of R5-HIV_{NLBal} in JC53-BL cells.
[b] Values are means of at least two experiments.
[c] High throughput log *D* determined at pH 7 using the shake flask method.



Scheme 1. Synthesis of head- and tail-modified bis-pyrrolidine-based CCR5 antagonists. Reagents and conditions: (a) $NaBH(OAc)_3$, CH_2Cl_2, rt, 2 h; (b) H_2 (1 atm), 20% $Pd(OH)_2$, EtOH, rt, 24 h, >95%; (c) $\mathbf{R^1}CO_2H$, EDCI, HOBt, Et_3N (or DIPEA), CH_2Cl_2, rt, overnight, 40–80%; (d) HCl 4 M in dioxane, CH_2Cl_2, rt, 2 h; (e) $\mathbf{R^2}CO_2H$, EDCI, HOBt, Et_3N (or DIPEA), CH_2Cl_2, rt, overnight or $\mathbf{R^2}COCl$, pyridine (or Et_3N), CH_2Cl_2, 0 °C to rt, overnight, 50–80%.

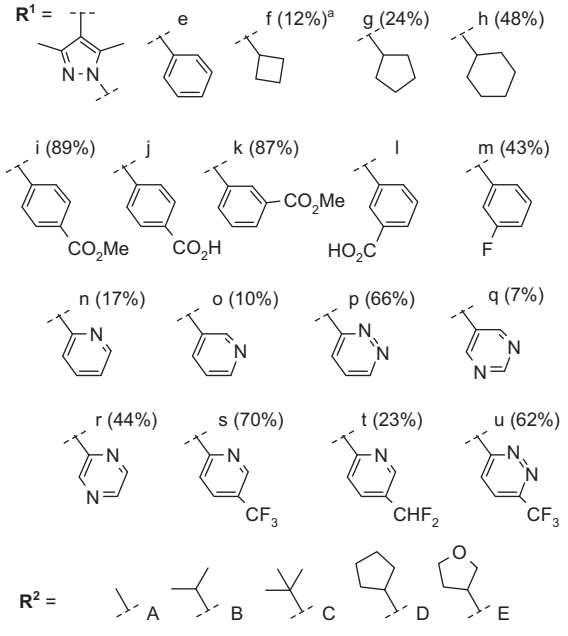
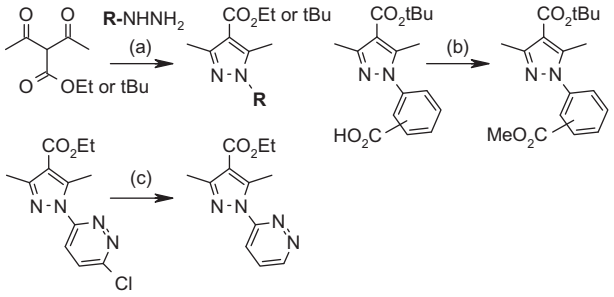
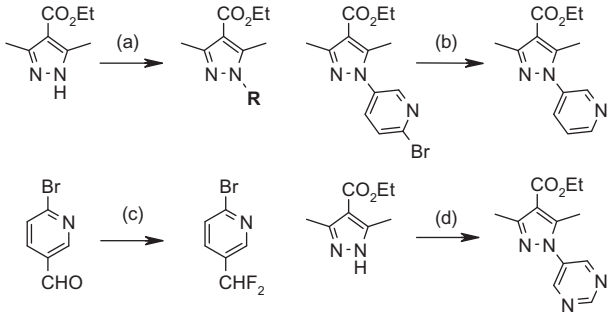


Figure 2. $\mathbf{R^1}$ and $\mathbf{R^2}$ substituents. [a]Percentages in parentheses refer to the un-optimized yields of the corresponding N-substituted pyrazole formation reaction. Pyrazoles R^1CO_2H where R^1 = **e** was commercially available. Pyrazoles R^1CO_2H where R^1 = f, g, h, i, j, k, l, m, n, p, and s were prepared by the de novo preparation of the pyrazole ring. Pyrazoles R^1CO_2H where R^1 = o, q, r, t, and u were prepared by N-alkylation of the pre-formed pyrazole esters.

Aldehydes **5** were prepared according to literature procedures.[3] They were treated with template **6**[4] under reductive N-alkylation reaction conditions to form the orthogonally protected bis-amines



Scheme 2. De novo synthesis of the pyrazole esters. Reagents and conditions: (a) MeOH, H_2O, AcOH, rt, 24 to 48 h, yields in Figure 2; (b) $TMSCHN_2$, MeOH/CH_2Cl_2, rt, 2 h, 32% para, >95% meta; (c) H_2 (1 psi), 10% Pd/C, MeOH/dioxane, rt, 48 h, 22%.



Scheme 3. Alkylation of the preformed pyrazole ester. Reagents and conditions: (a) NaH, DMF, rt, 50 °C or 80 °C, alkylation yields on Figure 2; (b) H_2 (1 atm), 10% Pd/C, EtOH, AcOH, rt, overnight, 53% after recrystallization in MeOH; (c) DAST, Et_2O, CH_2Cl_2, 0 °C to rt, overnight, 53%; (d) CuI, *N*,*N*′-ethylenendiamine, dioxane, reflux, overnight, 7%.

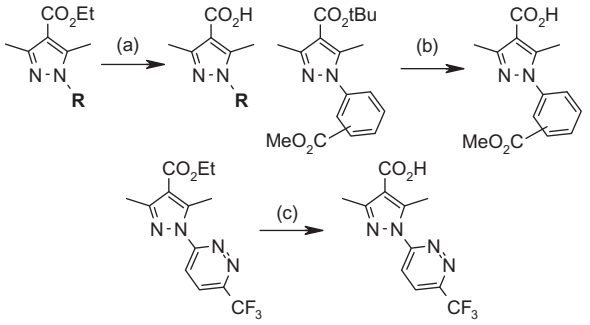
7. The pyrrolidine *N*-benzyl groups were hydrogenolyzed and the pyrrolidine nitrogens were acylated with the appropriate acids $\mathbf{R^1}CO_2H$ (Fig. 2) using standard amidation procedures to give **8**. The tail protecting groups were then hydrolyzed and the primary amines were finally acylated with $\mathbf{R^2}CO_2H$, or $\mathbf{R^2}COCl$ to give compounds **2**, **9–36** (Fig. 2).

The pyrazole esters leading to the formation of acids $\mathbf{R^1}CO_2H$ were either commercially available, like in the case of **e**, or prepared according to two general procedures, de novo formation of the N-substituted pyrazole ring or N-alkylation of the pre-formed pyrazole ester.

The de novo synthesis of the N-substituted pyrazole esters involved reacting ethyl or *t*-butyldiacetoacetate with the appropriate N-substituted hydrazines (Scheme 2).

In the case of **i** and **k**, *t*-butyl diacetoacetate[5] was used, leading to the formation of orthogonally protected diesters, which allowed subsequent chemoselective hydrolysis of the *t*-butyl esters. The hydrazines (free base or hydrochloride salts) used for the preparation of **h**, **m**, **n**, **p**, and **s**, were all commercially available. In the case of **i** and **k**, the products of cyclization with 4- and 3-hydrazinobenzoic acid, respectively, were treated with trimethylsilyldiazomethane to form the methyl esters (Scheme 2). In the case of **p**, 3-chloro-6-hydrazinopyridazine was used and following cyclization, the chlorine atom was hydrogenolyzed under standard reaction conditions (Scheme 2). The hydrazines used for the preparation of pyrazoles **f** and **g** were prepared from the corresponding cycloalkylketones according to literature procedures.[6]

The other approach to the synthesis of the pyrazoles involved N-alkylation of the commercially available 3,5-dimethyl-1H-pyrazole-4-carboxylic acid (Scheme 3). We mostly used conditions in which the pyrazole was deprotonated with sodium hydride in



Scheme 4. Hydrolysis of the pyrazole esters. Reagents and conditions: (a) KOH, $EtOH/H_2O$, 40 °C, overnight; (b) TFA, Et_3SiH, CH_2Cl_2, rt, 2 h; (c) Me_3SnOH, $ClCH_2CH_2Cl$, microwave, 160 °C, 5 h, 37%.

dimethylformamide and then treated with an electrophile such as an haloalkyl or an haloheteroaryl.

The N-alkylation products most of the time precipitated from the reaction mixture and were isolated by filtration. Commercially available chloropyrazine was used for the synthesis of **r**. In the case of **o**, we used the commercially available 2-bromo-5-fluoropyridine. After the N-alkylation reaction, the bromide atom was removed by hydrogenolysis (Scheme 3). In the case of **u**, we used 3-chloro-6-trifluoromethylpyridazine, which was prepared according to literature procedures.[7] In the case of **t**, the electrophile used was 2-bromo-5-difluoromethylpyridine, that was prepared by DAST fluorination of the commercially available 6-bromopyridine-3-carbaldehyde (Scheme 3). For the unreactive 5-bromopyrimidine, N-alkylation using the sodium hydride conditions did not yield any product, so for the preparation of **q**, copper-catalyzed N-alkylation conditions were used instead (Scheme 3).

Once the N-substituted pyrazoles were prepared, the esters were generally hydrolyzed under basic conditions (Scheme 4). In the case of **u**, basic ester hydrolysis conditions led to the hydrolysis of the molecule and none of the acid was recovered from the reaction mixture. However, we were able to hydrolyze the ethyl ester using trimethyltin hydroxide as weak nucleophile (Scheme 4).[8] In the case of **i** and **k**, the *t*-butyl esters were hydrolyzed using trifluoroacetic acid (Scheme 4).

Compounds **19** and **21** were prepared by base hydrolysis of the corresponding methyl esters **18** and **20** (KOH, $EtOH/H_2O$, 40 °C).

The compounds prepared were tested in our R5-HIV-1 antiviral assay[9] and their respective metabolic clearance was measured in vitro, using human liver microsomes (Table 2).

Several SAR trends emerged from the antiviral activity of compounds **9**–**36**.

N-Substitution of the pyrazole with an hydrophobic group led to a substantial increase in antiviral activity (compounds **2** and **22** compared to **1**). The improvement was so substantial that the tail hydrophobe 1 could be trimmed down and still provide compounds that retained good antiviral activity (e.g., compounds **10–12** vs **2**, **16** vs **17**, and **21** vs **22**). It is noteworthy to point out that in our original series bearing unsubstituted heads, we were not able to decrease the size of the tail hydrophobe 1 without abolishing the activity. Also, the boost in activity was so substantial that mildly hydrophilic groups could be tolerated in the tail hydrophobe 1 (e.g., **23** vs **22**, **25** vs **24**, **34** vs **33**, and **36** vs **35**). In our original series with unsubstituted heads, replacement of the optimal cyclopentyl group with a tetrahydrofuran in hydrophobe 1 led to a 20-fold loss in potency (compound **9** vs **1**). These last two observations showed the superiority of the substituted heads over the unsubstituted heads, as far as antiviral activity was concerned, by allowing tail modifications that had hitherto been impossible.

Table 2
HIV-1 antiviral activity, *c* log *P*, and in vitro human metabolic clearance of compounds **1**, **2**, and **9**–**36**

Compound	X	R^1	R^2	Antiviral [a,b] (nM)	*c* log *P*	In vitro metabolic clearance[c]
1	**H**	**a**	**D**	6.2	2.68	—
2	**H**	**e**	**D**	⩽1.5	4.85	—
9	**H**	**a**	**E**	120	1.33	17
10	**H**	**e**	**A**	100	3.48	70
11	**H**	**e**	**B**	8	4.32	—
12	**H**	**e**	**C**	7	4.61	—
13	**F**	**f**	**D**	36	3.96	—
14	**F**	**g**	**D**	16	4.52	—
15	**F**	**h**	**D**	60	5.08	—
16	**H**	**i**	**A**	19	3.39	77
17	**H**	**i**	**D**	<1.2	4.86	—
18	**H**	**j**	**D**	24	2.40	0
19	**H**	**k**	**D**	2	4.86	—
20	**H**	**l**	**D**	13	2.40	3
21	**F**	**m**	**A**	11	3.79	213
22	**H**	**m**	**D**	<1.2	5.01	644
23	**F**	**m**	**E**	2	3.81	331
24	**F**	**n**	**D**	12	3.92	512
25	**F**	**n**	**E**	27	2.57	55
26	**F**	**o**	**D**	7	3.92	—
27	**H**	**p**	**D**	15	2.69	137
28	**F**	**q**	**D**	11	3.00	332
29	**F**	**r**	**A**	⩾625	1.63	—
30	**F**	**r**	**D**	14	3.00	909
31	**F**	**s**	**A**	7	3.41	152
32	**F**	**s**	**E**	<1.2	3.42	343
33	**F**	**t**	**D**	5	4.11	—
34	**F**	**t**	**E**	11	2.76	253
35	**F**	**u**	**D**	5	3.61	—
36	**F**	**u**	**E**	43	2.26	263

[a] Replication inhibition (IC_{50}) of R5-HIV_{NL-BaI} in JC53-BL cells.
[b] Values are means of at least two experiments.
[c] Hydrolysis rate in μL/min/mg of protein. ⩾35 was considered high.

As far as the pyrazole N-substituent were concerned, an aryl or an heteroaryl group was preferred over a cycloalkyl group (e.g., compounds **13**–**15** vs **2**, **24**, or **26**). Also, it was observed that electron-withdrawing groups on the aryl substituent further increased the antiviral activity. This was more obvious when the tail hydrophobe 1 was un-optimal, such as a methyl group (e.g., compounds **16** and **21** vs **10**) as the combined effect of this SAR trend with the optimal cyclopentyl tail hydrophobe 1 led to compounds with un-comparable antiviral activities below detection levels (e.g., compounds **17** and **22** vs **2**). Albeit not as good as an hydrophobic electron-withdrawing substituent, even the highly hydrophilic electron-withdrawing carboxylic acid was tolerated (e.g., compounds **18** and **20**). The antiviral potency increase brought by the introduction of *N*-heteroaryl groups was about 10-fold lower than that of an *N*-aryl group (e.g., compounds **24**–**28**, and **30** vs **2**). However, electron-withdrawing hydrophobic groups on the heteroaryl group could, to a certain extend, modulate the loss in antiviral activity observed when switching from an aryl to an heteroaryl pyrazole N-substituent (compound **32** vs **25**, **33** vs **24**, and **35** vs **27**).

Most of the compounds prepared were more lipophilic than compound **1**, as estimated by their *c* log *P*s. So, it still appeared that the boost in antiviral activity of this series of N-substituted pyrazoles was lipophilicity-driven. However, **18** and **20**, both bearing a carboxylic acid were less lipophilic than **1** and were still very active in the antiviral assay. This indicated to us that there was more to the increase in activity than a simple lipophilicity effect. From binding inhibition experiments (data not shown), we observed that the effect of the N-substituted pyrazoles on the binding inhibition was not as dramatic as it was observed for the antiviral activity (i.e., activity fold increases were disproportionally higher for the antiviral assay than for the binding inhibition assay). This observation could seem to indicate that the N-substituted pyrazoles did not increase antiviral activity solely via an increase in binding energy. In order to explain the increase in antiviral potency, we hypothesized that the N-substituted pyrazole induced a more efficient allosteric rearrangement of the CCR5 receptor which has been shown to be required for antiviral activity after the binding of the antagonist.[10] From the findings described above, we concluded that as far as the *N*-pyrazole substituent was concerned, electron-poor aromatics were best accommodated in the head CCR5 binding site. We believe that the presence of nitrogen atoms in the aromatic ring led to a decrease in antiviral activity boost because they induced a polarization of the electron density of the ring. Electron density calculations suggested that substitution with an electron-withdrawing substituent delocalized some of the negative partial charge from the nitrogen atoms to the substituent. This led to an overall 'softer' electrostatic charge distribution in the aromatic ring, thus counter-balancing the detrimental effect of the nitrogen atoms.

As we improved our understanding of the SAR trends of the N-substituted pyrazoles, we soon realized that we were limited to the exploration of a very small region of space as far as multi-dimensional optimization was concerned. Indeed while aryl pyrazole N-substituents offered a substantial boost in antiviral activity, the best groups led to compounds with extremely high in vitro human clearance, and projected in vivo clearances higher than hepatic blood flow. From this set of compounds, **18** and **20** had the lowest metabolic clearance (0 and 3 μL/min/mg of protein, respectively). However the presence of the carboxylic acid functionality led to an unsurmountable decrease in intrinsic permeability.

We assumed that by lowering the lipophilicity of the compounds, we would decrease first pass metabolism. We thus focused on compounds that contained heteroaryl pyrazole N-substituents. Our efforts to reach the best compromise between optimal activity and lower *c* log *P* in the heteroaryl N-substituted pyrazole series led to the preparation of **34** (antiviral IC_{50} = 11 nM, *c* log *P* = 2.76). Unfortunately, **34** also showed low stability in our human liver microsome assay (253 μL/min/mg of protein). Metabolite identification failed to attribute specific oxidative pathways to the N-substituted pyrazole heads. We believe that instead of contributing directly to the metabolic instability, such heads generated molecules that were better recognized by the cytochrome P450 enzymes.

Unable to associate potent antiviral activity with good metabolic stability we abandoned our work on the pyrazole sub-series.

We described herein a sub-series of CCR5 antagonists in which the use of N-substituted pyrazole heads substantially increased antiviral activity. SAR analysis showed that the best pyrazole substituents for activity were electron-poor aromatic systems. Such groups, however, provided metabolically unstable compounds.

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