Bioorganic & Medicinal Chemistry Letters 20 (2010) 1830–1833

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Evaluation of a 4-aminopiperidine replacement in several series of CCR5 antagonists

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ARTICLE INFO

Article history:
Received 6 January 2010
Revised 28 January 2010
Accepted 1 February 2010
Available online 6 February 2010

Keywords:
5-Amino-3-azabicyclo[3.3.0]octane
4-Aminopiperidine
Replacement
CCR5 antagonists
Chemokine receptor

ABSTRACT

The bicyclic 5-amino-3-azabicyclo[3.3.0]octanes were shown to be effective replacements for the conformationally restricted 4-aminopiperidine ring found in several series of CCR5 antagonists.

In addition to its role as a receptor on inflammatory leucocytes, CCR5 is also the primary co-receptor for macrophage-tropic HIV-1. Since its discovery, 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. Over the last 10 years or so, many CCR5 antagonists chemotypes have been explored.[1] These efforts led to the discovery, optimization and clinical development of several compounds. For example, maraviroc (Selzentry®) was the first CCR5 antagonist approved for the treatment of HIV-1 infection.

We recently described a new template based on the bicyclic 5-amino-3-azabicyclo[3.3.0]octane system (template **1**) and showed it was similar to maraviroc's 3-amino-8-azabicyclo[3.2.1]-octane core system.[2] Since, the 8-azabicyclo[3.2.1]octane system can be seen as a conformationally restricted 4-aminopiperidine ring, we hypothesized that template **1** might also replace the conformationally restricted 4-aminopiperidine ring found in several CCR5 antagonists including vicriviroc from Schering Plough and ICBN-9471 from Incyte (Fig. 1). The alternative template could also be utilized as a replacement in a Roche series, as represented by compound **1**.[3] Compound **1** contains the four pharmacophore elements found in most series of CCR5 antagonists: a tertiary amine, two hydrophobic groups in the western portion of the molecule (henceforth referred to as the tail), and one head heteroaryl group in the eastern portion of the molecule (the head).

Since one of the key interactions between CCR5 and an antagonist involves a salt bridge between the tertiary amine of the antagonist and a glutamic acid in the CCR5 binding site, we wanted to evaluate the possibility of a difference in pK_a between the basic amine of the conformationally restricted 4-aminopiperidine in vicriviroc, INCB-9471, and compound **1** and that of template **1**. The calculated pK_a values of the basic amine of the 4-aminopiperidine ring in vicriviroc, INCB-9471, and compound **1** were, within the margin of error, the same as that of the basic amine of the corresponding compounds containing template **1**.[4] So, we anticipated that the only difference between the two ring systems would be structural and conformational.

Conformational analysis showed a good overlap (Fig. 2) of the lowest energy conformations of a representative example of the key structural features in vicriviroc and INCB-9471 (structure 1) and the corresponding example containing template **1** (structure 2). In particular, both structures presented the basic amine in the same position, with the lone pair in an axial orientation. With the basic nitrogen atoms in perfect alignment, the only difference between the two structures was seen in the distance between atoms 2(2′) and 4(4′). In the case of structure 2, containing template **1**, the distance was longer than in the structure containing the 4-aminopiperidine, structure 1 (6.34 Å and 5.3 Å, respectively).

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doi:10.1016/j.bmcl.2010.02.004

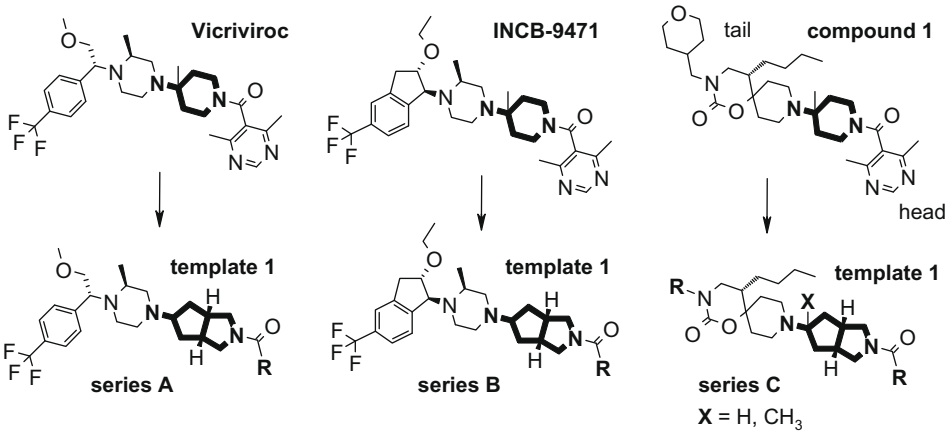


Figure 1. Proposed replacement of the conformationally restricted 4-aminopiperidine in vicriviroc, INCB-9471 and compound **1** with template **1**.

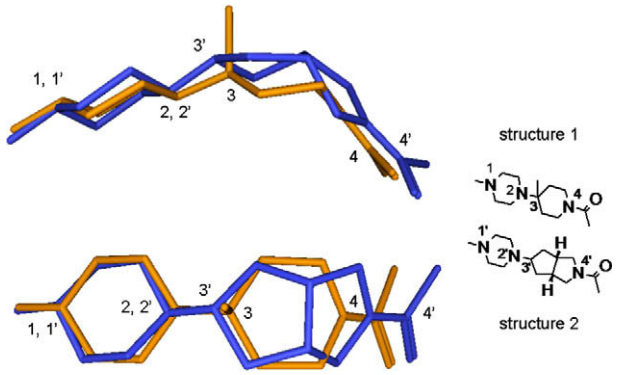


Figure 2. Overlaps of the lowest energy conformations of structure 1 (orange) and structure 2 (blue). (a) Overlaps were performed in MOE[5] using atoms 1, 2, 3, and 4 as anchoring points and were not manually modified. (b) Conformations were generated in Maestro[6] (OPLS-2005 force field, $CHCl_3$ as solvent and distance-dependent dielectric constant of 2 to mimic the hydrophobic nature of the CCR5 binding site) and processed using MOE.

This indicated to us that the head substituent in compounds of series A and B might not be able to overlap optimally with that of the corresponding compounds containing the 4-aminopiperidine template. However, with these encouraging conformational results and keeping in mind the flexibility of the CCR5 receptor, we decided to prepared representative compounds in series A, B, and C. We did not perform a conformational comparison of compounds from series C with compound **1**, but hypothesized that the result would be similar to that we observed in the case of series A and B.

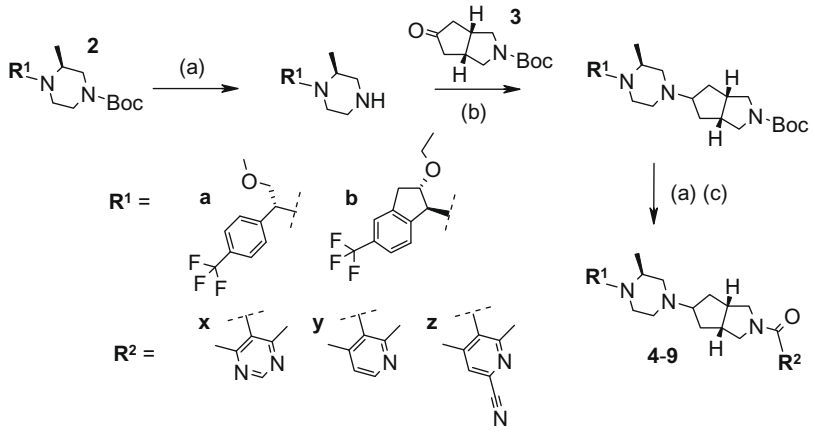
Compounds in series A and B were prepared according to Scheme 1. Intermediate **2a** was prepared according to literature procedures.[7] Intermediate **2b** was prepared according to a slight modification of literature procedures.[8] After hydrolysis of the boc group, the piperazines were coupled with ketone **3**.[2] In both instances, the endo/exo ratio of the products could not be determined due to the complexity of the ^1H NMR spectra of the products created by the presence of several amide rotational isomers. However, the prior observation of the quasi stereospecific behavior of ketone **3** toward reductive N-alkylation suggested that the endo isomer could have been formed predominantly.[2] It is noteworthy to point out as well that during our conformational analysis, we observed that every conformations of structure 2 within 5 kcal/mol of the energy minimum showed the endo conformation. Hydrolysis of the pyrrolidine protecting group and reaction with several carboxylic acids using standard amide coupling procedures provided compounds **4–9**.

These analogs were tested in a RANTES binding inhibition assay, RANTES being one of the natural chemokine ligands of CCR5, and in a HIV-1 antiviral assay (Table 1).[9] Compounds **4–6** in series A were devoid of binding inhibition and antiviral activity at the highest concentrations tested in the assays, which suggested that the conformationally restricted 4-aminopiperidine ring of this particular series could not be replaced by template **1**.

Compound **7** in series B was active in the binding inhibition assay but inactive in the antiviral assay. Interestingly, compound **8** had the same binding inhibition as compound **7**, but was active in the antiviral assay. This discrepancy could be explained by the accepted mechanism of action of antiviral CCR5 antagonists in which the antagonist binds to the receptor causing a change in conformation which itself prevents interaction of the CCR5 with the HIV-1 gp120 envelop protein. In other words, two CCR5 antagonists could have the same binding inhibition, but different antiviral activities if one causes a better change of receptor conformation than the other. Mechanistically, it is still not clear to us why one antagonist would induce a conformational change leading to a better antiviral activity than another antagonist. Due to the discrepancy between the RANTES binding inhibition assay and the functional antiviral assay, we used the binding inhibition assay as a first line assay, while we only compared compounds with each other based on their functional antiviral activity. Introduction of more potent head groups[10] such as the pyridine and the cyanopyridine increased antiviral activity (e.g., **8** and **9**). This indicated to us that the decrease of potency observed by the introduction of template **1** in INCB-9471 could be compensated for, in this series, by fine-tuning of the head substituent. These results proved that while the introduction of the bicyclic template could lead to divergent SARs from the parent series, further optimization could lead to active compounds.

With these encouraging results, we finally explored the replacement of the conformationally restricted 4-aminopiperidine of compound **1** with template **1**. Compounds **13–20** were prepared according to Scheme 2.

Intermediate **10** was prepared according to literature procedures.[11] After hydrolysis of the protecting group, the piperidine was reacted with ketone **3** to give compound **11**. Introduction of the ipso methyl group in compound **12** was performed in two steps by treating **10** with ketone **3** in the presence of titanium tetraisopropoxide, diethylzinc cyanide, and treatment of the intermediate with methylmagnesium bromide. Here as well, the ratio of endo and exo isomers could not be determined but was assumed to be mostly in favor of the endo isomer. The tail substituents were introduced by alkylation with the appropriate bromide or tosylates. The head substituents were finally introduced after the



Scheme 1. Reagents and conditions. Reagents and conditions: (a) HCl 4 M in dioxane, CH_2Cl_2, rt, 2 h; (b) ketone **3**, $NaBH(OAc)_3$, CH_2Cl_2, rt (69%, 83% over two steps for $\mathbf{R^1}$ = **a**, $\mathbf{R^1}$ = **b**, respectively); (c) $\mathbf{R^2}$–COOH, EDCI, HOBt, DIPEA, CH_2Cl_2, rt.

Table 1
RANTES binding inhibition and antiviral activity of compounds **4–9**

Compounds			Binding[a] (nM)[c]	Antiviral[b] (nM)[c]
	$\mathbf{R^1}$	$\mathbf{R^2}$		
4	**a**	**x**	>500	>625
5	**a**	**y**	>500	>625
6	**a**	**z**	>500	>625
7	**b**	**x**	92	>625
8	**b**	**y**	98	357
9	**b**	**z**	45	59

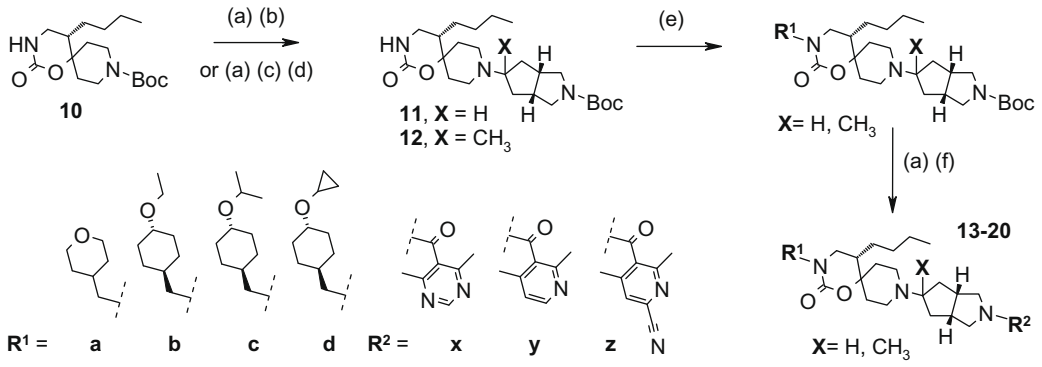
[a] Binding inhibition (IC_{50}) of [^{128}I]-RANTES to CCR5-expressing CHO cells.
[b] Replication inhibition (IC_{50}) of R5 HIV_{NLBal} in JC53-BL cells.
[c] Values are means of at least two experiments.

hydrolysis of the pyrrolidine protecting group and reaction with the appropriate carboxylic acids using standard amidation reaction conditions.

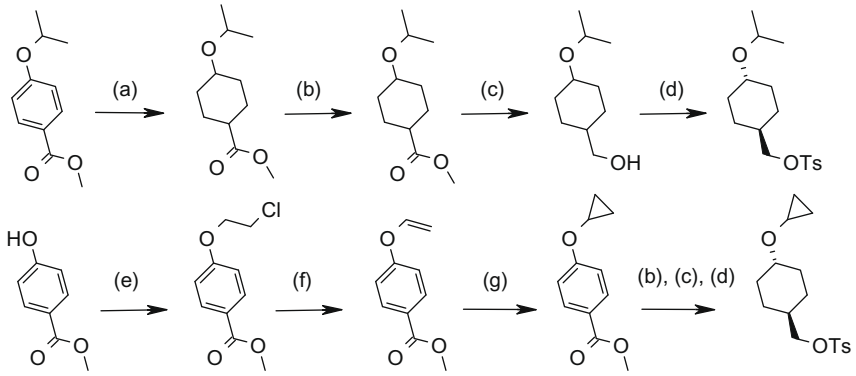
The bromide $\mathbf{R^1}$–Br in which $\mathbf{R^1}$ = **a** was prepared according to literature procedures.[10] The tosylate $\mathbf{R^1}$–OTs in which $\mathbf{R^1}$ = **c** was prepared from the commercially available methyl 4-isopropoxybenzoate (Scheme 3). The phenyl ring was hydrogenolyzed using rhodium to give a 9/1 mixture of cis and trans 4-alkoxy cyclohexyl methyl carboxylates which were equilibrated under basic condition to give a 1/1 mixture of isomers. The ester was reduced using lithium aluminium hydride and the primary alcohol was treated with tosyl chloride. At this stage, the cis and trans isomers could be separated using silica gel chromatography. The tosylate $\mathbf{R^1}$–OTs in which $\mathbf{R^1}$ = **b** was prepared the same way using methyl 4-ethoxybenzoate as starting material. As far as $\mathbf{R^1}$ = **d** was concerned, direct alkylation of methyl 4-hydroxybenzoate with cyclopropylbromide did not provide any cyclopropoxybenzoate, so the cyclopropyl was introduced via a Simmons–Smith reaction of the vinylic ether.

Compounds **13–20** were tested in the RANTES binding inhibition and antiviral assays (Table 2).[9]

As it was observed earlier, introduction of template **1** in this series led to a decrease in antiviral activity (e.g., compound **13** compared to compound **1**). Interestingly the benefit of having the ipso methyl observed in the SAR optimization that led to the identification of compound **1**[3] did not translate in this particular series, with compound **15** being virtually inactive in the antiviral assay. In this series as well, the decrease in potency could be compensated by the introduction of a more potent head (compounds **14** and **16**), by the introduction of a different tail (compound **17**), or by the combination of both (compound **18**). Compound **18** was the most active of the series in the antiviral assay, with an IC_{50} of 4 nM. It is noteworthy to point out the outstanding boost of potency observed by the introduction of the cyanopyridine head. Indeed, while compound **15** was virtually inactive in the antiviral assay ($IC_{50} \geqslant 625$ nM), compound **17** had an antiviral IC_{50} of 19 nM. Unfortunately, we observed that the introduction of the cyanopyridine head led to a decrease in metabolic stability (data not shown), so we decided to focus on increasing potency by changing the tail substituent while keeping the pyrimidines head, our most metabolically stable substitutent.[5] Switching the tail substituent from a tetrahydropyranyl (compound **13**) to a 4-ethoxycyclohexyl (compound **18**) increased potency about 14-fold.



Scheme 2. Synthesis of compounds in series C. Reagents and conditions: (a) HCl 4 M in dioxane, CH_2Cl_2, rt, 2 h; (b) ketone **3**, $NaBH(OAc)_3$, CH_2Cl_2, rt (69% over two steps); (c) ketone **3**, $Ti(O^iPr)_4$, Et_2AlCN, CH_2Cl_2, rt; (d) MeMgBr, THF, rt (37% over two steps); (e), $\mathbf{R^1}$–Br or $\mathbf{R^1}$–OTs, NaH, DMF, rt; (f) $\mathbf{R^2}$–COOH, EDCI, HOBt, DIPEA, CH_2Cl_2, rt.



Scheme 3. Synthesis of tosylates $\mathbf{R}^1$–OTs. Reagents and conditions: (a) Rhodium 5% on alumina, MeOH, H_2, 55 psi, rt, 70%; (b) MeONa/MeOH, 50 °C, 65%; (c) $LiAlH_4$, THF, 0 °C, >95%; (d) TsCl, Et_3N, DCM, 0 °C, rt, 41% cis, 48% trans; (e) 2-chloroethyl *p*-toluenesulfonate, K_2CO_3, DMF, 60 °C, 95%; (f) *t*-BuOK, THF, −15 °C, 16% (also isolated 14% of the *t*-butyl ester and 50% of a mixture of *t*-butyl and methyl esters); (g) CH_2I_2, Et_2Zn, CCl_3CO_2H, DCE, −15 °C to rt, 62% .

Table 2
RANTES binding inhibition and antiviral activity of compounds **13**–**20**

Compounds				Binding[a] (nM)[c]	Antiviral[b] (nM)[c]
	$\mathbf{R}^1$	$\mathbf{R}^2$	**X**		
1	—	—	—	4	6
13	**a**	**x**	**H**	14	262
14	**a**	**z**	**H**	15	9
15	**a**	**x**	$\mathbf{CH_3}$	25	⩾625
16	**a**	**z**	$\mathbf{CH_3}$	61	18
17	**b**	**x**	**H**	44	19
18	**b**	**y**	**H**	34	4
19	**c**	**x**	**H**	23	12
20	**d**	**x**	**H**	27	12

[a] Binding inhibition (IC_{50}) of [^{128}I]-RANTES to CCR5-expressing CHO cells.
[b] Replication inhibition (IC_{50}) of R5 HIV_{NLBal} in JC53-BL cells.
[c] Values are means of at least two experiments.

Further exploration of the ether alkyl substituent did not significantly improve potency (compounds **19** and **20** compared to compound **18**). This indicated to us that we had reached the maximum optimization potential of this series. Compound **17** was about two-fold more potent than compounds **19** and **20** in the binding inhibition assay, but was 27-fold less potent in the antiviral assay. This discrepancy showed that slight modifications in the tail substitution pattern could have a dramatic effects on antiviral activity in the absence of significant change in the ability to bind to the receptor.

In conclusion, as far as functional activity is concerned, the 5-amino-3-azabicyclo[3.3.0]octane bicyclic system was shown to be an acceptable replacement for the conformationally restricted 4-aminopiperidine system found in several series of CCR5 antagonists. After the introduction of the bicyclic replacement, further SAR optimization led to the identification of active new series of CCR5 antagonists.

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