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Studies on the structure–activity relationship of 1,3,3,4-tetra-substituted pyrrolidine embodied CCR5 receptor antagonists. Part 1: Tuning the N-substituents

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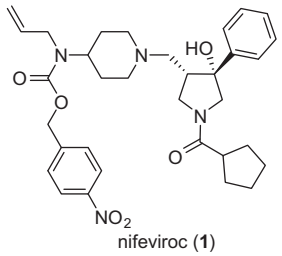
ABSTRACT

A novel series of CCR5 antagonists has been identified, utilizing the lead, nifeviroc, which were further modified based on bioisosteric principles. Lead optimization was pursued by balancing potential toxicity and potency. Potent analogues with low toxic properties were successfully developed by formation of urea and amide bonds at the nitrogen at position 4- of the pyrrolidine ring.

Human immunodeficiency virus (HIV) infects millions of people worldwide and causes the fatal disease Acquired Immune Deficiency Syndrome (AIDS). Although highly active antiretroviral therapy (HAART), a combination of drugs targeting HIV-1 protease, reverse transcriptase and integrase, has been successful in suppressing viral replication and in reducing HIV-1-associated mortality, issues relating to the development of viral resistance and the long term toxicity of the component drugs remain prominent.[1] Accordingly it seems urgent to discover new classes of drugs for therapeutic intervention with better efficacy and safety profiles.[2]

The discovery that chemokine receptors function as HIV-1 co-receptors has provided us novel targets for controlling HIV-1 infection.[3,4] Although several chemokine receptors have been implicated in HIV entry in various experimental systems, CCR5, a member of the G-protein-coupled receptor (GPCR) superfamily, is the principal co-receptor for the R5 HIV-1 strains that is most commonly transmitted between individuals and predominates during the early years of infection.[5,6] With the combination of high-throughput screening and medicinal chemistry-based structure optimization, a considerable number of potent CCR5 antagonists with excellent in vitro anti-viral activities have been discovered.[7–11]

Our efforts have led to the discovery of a potent CCR5 antagonist, nifeviroc (**1**), featuring a 1,3,3,4-tetra-substituted pyrrolidine template (Fig. 1). This compound Antagonizes RANTES-induced calcium elevation and GTPγS binding in CCR5-expressing CHO cells with IC_{50} values of 3.5 nM and 2.9 nM, respectively. It also showed excellent in vitro anti-HIV activity in PBMC cells (EC_{50} <0.4 nM).[12] However, further development of **1** could be limited the undesir-



nifeviroc (**1**)

Figure 1. Structure of nifeviroc.

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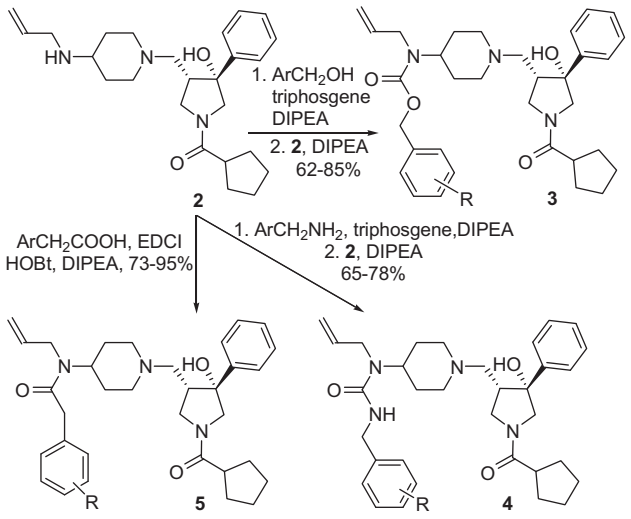


Scheme 1.

able aromatic nitro-moiety, a possible mutagenic and carcinogenic functionality.[13–15] Secondly, the high hERG affinity (IC_{50} <1.0 μM) of **1** suggested a narrow safety window. In a continuation of our work, a series of analogues of **1** were prepared aimed at eliminating any potential toxicity liability of the aromatic nitro group and decreasing hERG channel affinity.

As depicted in Scheme 1, we accessed further analogues of **1** via the key intermediate **2**.[12] A round of carbamates **3** was prepared by reacting substituted benzyl alcohols with triphosgene and then trapping the resulting chloro-formates with **2**. Urea analogues **4** were assembled by condensation of **2** with carbamic chlorides generated in situ from benzyl amines with triphosgene. Finally we then extended this series by replacing the carbamate linkage with an amide linker by coupling **2** with substituted or unsubstituted phenylacetic acids to afford the amides **5**.

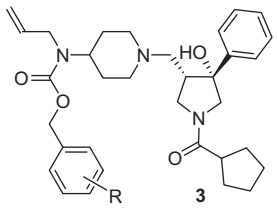
With all the designed compounds in hand, we evaluated their ability to inhibit RANTES-stimulated [^{35}S]-GTPγS binding to CCR5-expressing CHO cell membranes, and antiviral activity in R5-tropic HIV-1 Ba-L infected peripheral blood mononuclear cells (PBMCs) with 10% FBS. The biological data for the carbamates **3** is summarized in Table 1. We have previously reported that deletion of the nitro group or introduction of electron-donating groups resulted in a dramatic loss of antiviral activity, with different effects on excellent binding inhibition (compare **1**, **3a** with **3b** and **3c**).[12] In contrast, in this study we found that the addition of electron-withdrawing groups in the 4-position of the terminal phenyl group (**3d**, **3e**, **3f**, **3g**) restored excellent antiviral potency (EC_{50} <3 nM), although incorporation of these groups was detrimental to CCR5 binding inhibition. Substitution with halogens was accompanied by a slight reduction in potency (**3h**, **3i**, **3j**). Unfortunately, all these new analogues displayed similar hERG channel affinity potency, except for methylsulfonyl analogue **3f** which showed a moderate drop of hERG affinity at 1 μM.

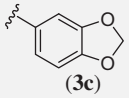
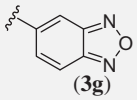
It can be seen from Table 1 that IC_{50}'s measured in the assay that uses RANTES as the ligand for the CCR5 receptor do not follow the same trends as the EC_{50} measured in the whole cell assay where the GP120 protein on the virus itself is the ligand.

In order to study the effects of the carbamate linker on the potency in more depth, and to decrease the potential toxicity further, we developed a series of urea analogues. From the results of Table 2, we found that the urea compounds (except **4a**) showed slightly less hERG affinity than the corresponding carbamates, while showing excellent binding inhibition and antiviral activity. Among this series of compounds, **4e** matched the potency as the lead, nifeviroc. However, it maintained high hERG affinity. Of note, **4i** and **4j** showed weak hERG binding activity, but also had excellent potency.

Next we turned our attention to the amide series **5**. As indicated in Table 3, a wide range of *para*-substituents on the terminal phenyl group featuring an amide linker were examined and most led to a drop in antiviral potency, although good CCR5 binding inhibition was retained (**5a–g**). However, similar to **3f** the methylsulfonyl analogue (**5h**) displayed encouraging binding and antiviral

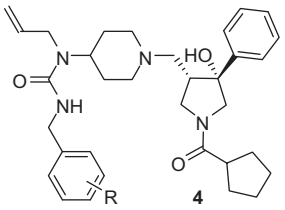
Table 1
Biological data for carbamates **3**



R	IC_{50}[a] (nM)	EC_{50}[b] (nM)	hERG binding[c]	hERG binding[d]
4-NO_2 (**1**)	2.9	<0.4	62.0	98.0
H (**3a**)	8.75	78	—	—
4-OMe (**3b**)	6.22	>100	—	—
 (**3c**)	122	>100	—	—
4-CF_3 (**3d**)	>30	2	80.8	104.6
4-OCF_3 (**3e**)	6.15	2.5	87.9	100.8
4-SO_2Me (**3f**)	37.3	<0.4	44.1	96.3
 (**3g**)	113.6	<0.4	72.6	102.2
4-F (**3h**)	220.6	18	70.2	101.9
4-Cl (**3i**)	1.09	2.8	76.2	97.9
4-Br (**3j**)	20.2	65	—	—

[a] SPA GTPγS assay.
[b] Antiviral activity in PBMC.
[c] % Inhibition at 1 μM.
[d] % Inhibition at 10 μM.

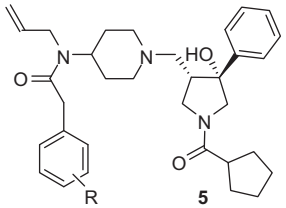
Table 2
Biological data for ureas **4**



R	IC_{50}[a] (nM)	EC_{50}[b] (nM)	hERG binding[c]	hERG binding[d]
H (**4a**)	37.7	>100	34.7	84.1
4-F (**4b**)	1.71	30	40.6	82.4
4-Cl (**4c**)	2.66	1.54	50.3	97.8
4-NO_2 (**4d**)	3.2	3	37.2	83.1
4-CF_3 (**4e**)	1.09	<0.4	51.1	104
4-OCF_3 (**4f**)	6.15	2.5	87.9	100.8
2,4-F_2 (**4g**)	1.62	16	—	—
3,4-F_2 (**4h**)	1.69	8	—	—
4-SO_2NHCH_3 (**4i**)	2.93	2.8	7.3	19.9
4-$SO_2N(CH_3)_2$(**4j**)	5	0.9	13.1	73.9

[a] SPA GTPγS assay.
[b] Antiviral activity in PBMC.
[c] % Inhibition at 1 μM.
[d] % Inhibition at 10 μM.

Table 3
Biological data for amides **5**



R	IC_{50}[a] (nM)	EC_{50}[b] (nM)	hERG binding[c]	hERG binding[d]
H (**5a**)	3.5	>100	44.6	92
4-OMe (**5b**)	14.6	>100	40.7	88.7
4-NO_2 (**5c**)	5.3	10	68.9	96.1
4-F (**5d**)	11	>100	43.9	91.5
4-Cl (**5e**)	3.6	>100	40.1	94.8
4-Br (**5f**)	17.8	>100	—	—
4-CF_3 (**5g**)	149.2	70	—	—
4-SO_2Me (**5h**)	4	34	23.9	71.1
4-SO_2NH_2 (**5i**)	2.1	8	14.4	16.6
4-SO_2NHCH_3 (**5j**)	1.53	0.8	18.2	34.1
4-$SO_2N(CH_3)_2$ (**5k**)	3.32	<0.4	20.3	98.6

[a] SPA GTPγS assay.
[b] Antiviral activity in PBMC.
[c] % Inhibition at 1 μM.
[d] % Inhibition at 10 μM.

Table 4
Anti-HIV activity and pharmacokinetics for **5j**

Anti-HIV activity (PBMCs) EC_{50} (nM)	
HIV-1 006 (R5)	<0.3
HIV-1 599 (X4)	>1000
HIV-1 593 (X4/R5)	>1000
Rat pharmacokinetics[a]	
Cl_p (L/h/kg)	7.26
V_{dss} (L/kg)	7.80
C_{max} (μg/L)	1403
$t_{1/2}$ (h)	0.77
%F	1.7
In vitro pharmacokinetics	
P_e[b] ($\times 10^6$ cm/s)	0.07
P_{app}[c] ($\times 10^6$ cm/s)	4.73 (A–B)
	51.9 (B–A)
E_H[d]	0.97

[a] Average data generated after 10 mg/kg po and 5 mg/kg IV in $n = 3$ animals/dose.
[b] As measured by the parallel artificial membrane permeability assay and values are represented as mean ± SD (n = four wells).
[c] CACO-2 P_{app} obtained from 4 transwells.
[d] Incubation with isolated human liver microsomes see Obach, *Drug Metab. Dispos.* **1999,** *27*, 1350.

activity with attenuated hERG affinity. These findings prompted us to prepare the sulfonamides analogues **5i–k** and interestingly, **5i** hardly inhibited the hERG channel at 1 and 10 μM and kept excellent potency (EC_{50} = 8 nM). Introduction of a mono-methyl sulfonamide (**5j**), and dimethyl-sulfonamide (**5k**) increased slightly hERG channel affinity, but also improved the antiviral activity. The key finding from this survey was that a hydrophilic group in the terminal phenyl, such as SO_2Me, SO_2NH_2, SO_2NHCH_3 or $SO_2N(CH_3)_2$ detuned hERG activity.

A recent review[16] has noted that polar or electronegative groups in the para position of phenyl rings may increase binding, possibly by interaction with Thr623 and Ser624 of the selectivity filter region of the hERG protein.

The pharmacokinetics and the ability of **5j** to inhibit HIV replication in various strains was examined (Table 4). Consistent with **5j** being an antagonist of CCR5, potent activity against R5 tropic strains (HIV-1 006) was observed (EC_{50} <0.8 nM). However with X4 tropic (HIV-1 599) or X4/R5 dual tropic (HIV-1 593) strains antiviral activity was lacking (EC_{50} >1000 nM).

In rats, **5j** showed rapid plasma clearance, possibly by phase 1 metabolism as predicted by exposure of **5j** to isolated human liver microsomes. Additionally systemic exposure appears limited with PAMPA assays reveling low permeability consistent with a low PO C_{max} (0.031 μM) and CACO-2 data suggesting **5j** maybe a substrate for efflux transporters.

In summary, SAR studies around the carbamate linker and substituents on the left phenyl portions of nifeviroc have led to the identification of 4-sulfamoyl analogues, **4i**, **4j**, **5j** and **5k** as highly potent CCR5 antagonists with significantly excellent antiviral activity, very weak hERG affinity and lacking the undesirable aromatic nitro functionality. Further SAR and pharmacokinetic optimization on this series will be the subject of future publications.

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