Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2469–2473

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antagonists of the Human CCR5 Receptor as Anti-HIV-1 Agents. Part 3: A Proposed Pharmacophore Model for 1-[*N*-(Methyl)-*N*-(phenylsulfonyl)amino]-2-(phenyl)-4-[4-(substituted)piperidin-1-yl]butanes

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Received 9 April 2001; accepted 2 July 2001

Abstract—Structure–activity relationship studies directed toward the optimization of (2*S*)-2-(3-chlorophenyl)-1-[*N*-(methyl)-*N*-(phenylsulfonyl)amino]-4-[4-(substituted)piperidin-1-yl]butanes as CCR5 antagonists resulted in the synthesis of the spiro-indanone derivative **8c** ($IC_{50} = 5$ nM). These and previous results are summarized in a proposed pharmacophore model for this class of CCR5 antagonist.

The chemokine receptor CCR5, a member of the seven-transmembrane G-protein coupled family of receptors,[1] has been identified as a primary co-receptor with CD4 by which macrophage tropic HIV-1 virus strains infect their host cells.[2] These CCR5 utilizing HIV-1 strains, now called R5 variants, have been associated with the initial and early phases of HIV-1 infection, although they are generally present throughout the course of the disease AIDS. Individuals homozygous for a 32-base pair deletion in the gene for CCR5, which prevents expression of functional receptor on the cell surface, have been identified as being highly resistant to HIV-1 infection,[3] while infected heterozygous individuals showed significantly delayed progression to AIDS.[4] Given the importance of CCR5 for the establishment, and possible maintenance, of HIV-1 infection in vivo, and the lack of an overt detrimental phenotype in humans that do not express functional CCR5, numerous efforts have been initiated to identify suitable CCR5 antagonists for use as potential therapeutic agents for the treatment of HIV-1 infection.[5–8]

In our first manuscript in this series,[9] the discovery of (2*S*)-2-(3,4-dichlorophenyl)-1-[(*N*-methyl-*N*-phenylsulfonyl)amino]-4-[spiro(2,3-dihydrobenzthiophene-3,4′-piperidin-1′-yl)]butane *S*-oxide (**1**, mixture of *R*- and *S*-sulfoxides) as our initial key lead structure was described, having an $IC_{50} = 35$ nM for inhibition of [^{125}I]-MIP-1α binding to CCR5. Subsequent investigation of the C-2 phenyl revealed that the 3-chlorophenyl derivative **3** had improved CCR5 binding affinity of 10 nM.[10] In addition, the structurally simplified 4-phenylpiperidine derivatives (e.g., **5**, $IC_{50} = 30$ nM) were found to have binding affinities within 3- to 4-fold of the corresponding spiro compounds. Herein, further structure–activity relationship (SAR) studies for the spiro and 4-phenyl series are described and a preliminary pharmacophore model for this class of CCR5 antagonist is proposed.

The synthesis of these modified piperidine derivatives was based on our previously described routes[10–12] (Scheme 1) and utilized (*S*)-(3-(3-chlorophenyl))-4-(*N*-methyl-*N*-phenylsulfonylamino)butanal (**7**). The reductive amination of appropriately substituted piperidines (**6a**) or piperazines (**6b**) using sodium triacetoxyborohydride in dichloroethane (DCE)[13] afforded the coupled products **8a**–**bb** (see Tables 1 and 2) in 55–90% yields.

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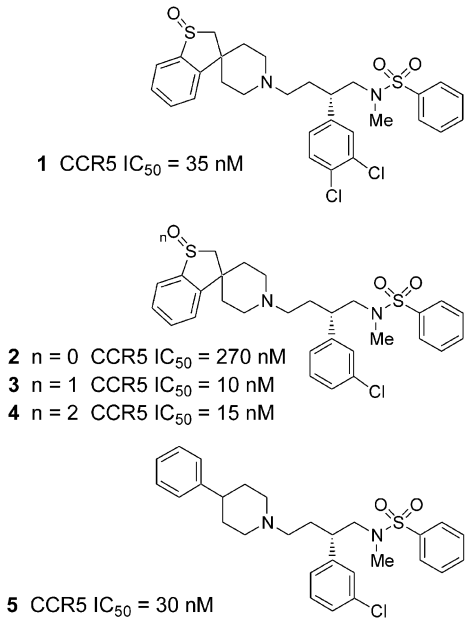
PII: S0960-894X(01)00491-7

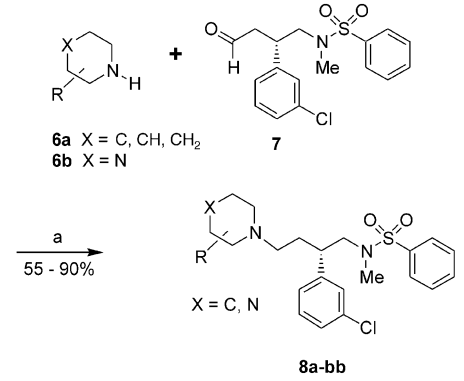


The hydroxy compound **8i** (1:1 mixture of alcohol diastereomers) was prepared from the ketone **8c** by reduction with sodium borohydride in methanol.

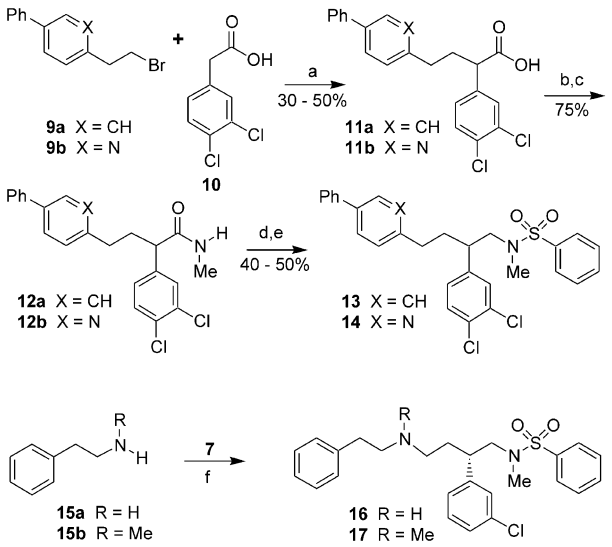
The requisite piperidines and piperazines that were utilized in Scheme 1 were available commercially (**5** and **8l–z,bb**) or were prepared by literature procedures (**2–4**,[10,11] **8a**, **8c, 8j–k**,[14] **8b,d–h**,[11] and **8aa**[15]).

Two non-piperidine derivatives were also prepared by a modified procedure (Scheme 2) in the original 3,4-dichlorophenyl series. Alkylation of 3,4-dichlorophenylacetic acid (**10**) with bromides **9** afforded **11**. Conversion to the amides **12**, reduction and acylation with phenylsulfonyl chloride afforded **13** and **14**. Several open chain compounds of varying length (e.g., **16** and **17**) were prepared by reductive amination of the corresponding amines **15** with **7** as in Scheme 1.

These compounds were then evaluated in a [^{125}I]-MIP-1α based binding assay of CCR5 stably expressed on Chinese hamster ovary (CHO) cells[9,16] (Tables 1 and 2). As previously reported, the sulfide **2** displayed only moderate binding compared to the sulfoxide **3**, while the



Scheme 1. Reagents: (a) HOAc (or DIPEA if HCl salt of **6** is used), $NaBH(OAc)_3$, DCE, rt.

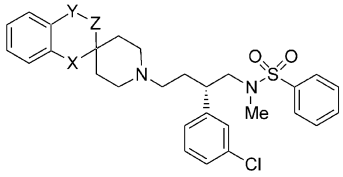


Scheme 2. Reagents: (a) $LiN(SiMe_3)_2$, THF, −70 °C, then **9**, −70 °C to rt; (b) oxalyl chloride, DMF (cat), DCM, rt; (c) $MeNH_2$ (40% aq), THF, 0 °C; (d) DIBAL, DCM, 0 °C to rt; (e) $PhSO_2Cl$, DIPEA, DCM, rt; (f) HOAc, $NaBH(OAc)_3$, DCE, rt.

sulfone **4** had only slightly less affinity (IC_{50} = 270, 10, and 15 nM for **2**, **3**, and **4**, respectively).[10] An initial survey of other known spiro piperidines was undertaken to understand the beneficial binding affinities of the sulfoxide and sulfone moieties, whether due to a hydrogen bond acceptor interaction or a simple polar effect. As anticipated, the spiro-indane **8a** (IC_{50} = 180 nM) was about the same as the sulfide **2**. The observation that **8a** was significantly poorer than the simple 4-phenyl compound **5** indicated that the phenyl ring is not optimally disposed in **8a** and that the sulfoxide and sulfone moieties have a substantial interaction with the receptor which more than compensates for this deficiency. While the spiro-indoline **8b** did show somewhat improved binding over **8a** (IC_{50} = 50 and 180 nM), the best spiro piperidine found was the ketone **8c** (IC_{50} = 5 nM), which now directs the carbonyl oxygen into the plane of the phenyl ring rather than out of the plane as with **3** and **4**. This improved binding was also reflected in a PBMC-based antiviral assay in which **8c** was 8-fold better than **3** in a side-by-side assay[17] (IC_{95} = 1500 vs 12,500 nM[18]). Surprisingly, the lactams **8d** and **8e**, in which the oxygen is similarly disposed as **8c**, but would be expected to be a better hydrogen bond acceptor, were poorer inhibitors (see below), as were both orientations of the six-member lactams **8f** and **8g**. Extension of the sulfonyl as in the sulfonamide **8h** was detrimental (IC_{50} = 12% I@1000 nM). Reduction of the ketone of **8c** to the alcohol derivative **8i** (1:1 mixture of alcohol isomers) indicated that a hydroxy at this position was at best only marginally beneficial compared to **8a** (IC_{50} = 100 vs 180 nM).

Other arrangements of the spiro linkage were not advantageous, for example, the 2-spiro-benzofuran **8j** and the spiro-pyran **8k** (Table 1), although the phenyl substituted spiro derivative **8l** and the non-spiro urea **8m** (Fig. 1) did have moderate activity (IC_{50} = 300 and 90 nM). The importance of the substitution being exclusively at the 4-postion was verified with the 3,4-

Table 1. Structures and activities for spiro piperidine derivatives



Compd	X	Y-Z	CCR5[a] IC_{50} (nM)[b]
2	—[c]	$-SCH_2-$	270
3	—	$-S(O)CH_2-$	10[d]
4	—	$-S(O)_2CH_2-$	15
8a	—	$-CH_2CH_2-$	180[d]
8b	—	$-NHCH_2-$	50
8c	—	$-C(O)CH_2-$	5[d,e]
8d	—	–C(O)NH–	45[d]
8e	—	–C(O)N(Me)–	100[d]
8f	—	$-C(O)NHCH_2-$	35
8g	—	$-NHC(O)CH_2-$	35
8h	—	$-N(MeSO_2)CH_2-$	12%[f]
8i	—	$-CH(OH)CH_2-$	100
8j	$-CH_2-$	–O–	260
8k	–O–	$-C(O)CH_2-$	24%[f]

[a]See refs 9 and 14 for a description of the binding assay.
[b]The IC_{50} values are an average of three independent titrations having calculated standard errors usually less than 15%. The assay-to-assay variation was generally ±2-fold or less based on the results for the standard compound **3**.
[c]The X in these structures is a single bond.
[d]The average of two or more separate experiments.
[e]The IC_{50} for compound **8c** was at least 2-fold better than **3** in two separate head-to-head experiments.
[f]Percent inhibition at 1000 nM; this was highest concentration used.

fused piperidines **8n** and **8o** and the 3-phenyl isomer **8p** (IC_{50} = 250 nM, 32% and 40%@1000 nM, respectively). Thus, in light of these findings and the fact that the simple 4-phenyl derivative **5** was within 3-fold in activity to **3**, alternative non-spiro structures were further investigated.

The role of the basic piperidine was first investigated with the corresponding less basic piperazines[19] **8q** and **8r** which both showed greatly diminished binding compared to **5** (IC_{50} = 700 and 2400 vs 30 nM, Table 2). The biphenyl **13** and 4-phenylpyridine **14** (see Scheme 2) were completely inactive (IC_{50}'s > 10,000 nM). Several basic, but non-piperidine structures, as exemplified by the open chain compounds **16** and **17**, were also all inactive (IC_{50}'s > 10,000 nM). Thus, with this scaffold the piperidine subunit seemed to be required in terms of a critical N^+–H salt interaction with the receptor as well as proper orientation of the 4-phenyl substituent.

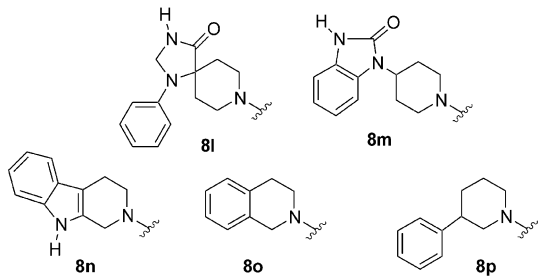
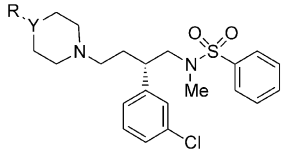


Figure 1. Structures of alternative piperidine analogues.

In search of a minimum structure for the piperidine, both the 4-unsubstituted compound **8s** and the 4-*t*-butyl **8t** were found to be inactive (27% and 40% I@1000 nM), thus, substitution on the phenyl ring of **5** was undertaken (Table 2). The 2-methyl **8u** was a poorer inhibitor, again indicating that the phenyl perpendicular to the plane of the piperidine was not optimal, although this was mostly overcome with the 2-MeO derivative **8v**. The 3-trifluoromethyl **8w** and 4-chloro **8x** derivatives were also less active, although the 4-fluoro **8y** was at least equipotent to **5**. Thus, it appeared that substitution on this ring is limited and that there is an apparent steric interaction with 4-piperidine substituents which linearly extend much further than a phenyl. Extension of the phenyl ring as a benzyl or phenylethyl (**8z** and **8aa**) again reduced activity, although further homologation to the less rigid 3-phenylpropyl **8bb** restored binding potency comparable to the best spiro derivative (**8bb** and **8c**, IC_{50} = 5 nM). However, the antiviral activity of **8bb** was not improved over **3** (IC_{95} = 50,000 vs 25,000 nM for **3** in the same assay[18]).

Table 2. Structures and activities for non-spiro piperidine derivatives



Compd	R	Y	CCR5[a] IC_{50} (nM)[b]
5	C_6H_5-	–CH–	30
8q	C_6H_5-	–N–	700
8r	$2\text{-}MeC_6H_4-$	–N–	2400
8s	H	–CH–	27%[c]
8t	*t*-Bu	–CH–	40%[c]
8u	$2\text{-}MeC_6H_4-$	–CH–	400
8v	$2\text{-}MeOC_6H_4-$	–CH–	70
8w	$3\text{-}CF_3C_6H_4-$	–CH–	120
8x	$4\text{-}ClC_6H_4-$	–CH–	200
8y	$4\text{-}FC_6H_4-$	–CH–	25
8z	$C_6H_5CH_2-$	–CH–	250
8aa	$C_6H_5CH_2CH_2-$	–CH–	65
8bb	$C_6H_5CH_2CH_2CH_2-$	–CH–	5

[a]See refs 9 and 14 for a description of the binding assay.
[b]The IC_{50} values are an average of three independent titrations having calculated standard errors usually less than 15%. The assay-to-assay variation was generally ±2-fold or less based on the results for the standard compound **3**.
[c]Percent inhibition at 1000 nM.

Based on the above results, a tentative pharmacophore model for inhibition of MIP-1α binding to CCR5 was developed (Fig. 2). In our first report,[9] the critical role of the *N*-methyl-*N*-phenylsulfonylamino had been established in that the N–H phenylsulfonamide and corresponding benzamide derivatives were much less active. Thus, the conformation of this portion must be limited to conformations not readily accessible by the later derivatives. The sulfonamide oxygens may also directly interact with the receptor or point towards a hydrophilic area since the corresponding ether derivative was much less active (41% I@1000 nM). Since the

4-position on the phenylsulfonyl moiety was shown to be compatible with a wide range of substitution (but not the 2- and 3-positions), the phenyl appears to lay in an extended channel. The stereochemistry of the C-2 phenyl had also been determined to be stereospecific for the (*S*) configuration. The requirement for the C-2 phenyl and its substituent preferences were previously discussed[10] wherein substitution was shown to be limited to small, nonpolar moieties at the 3-position (H, Cl, F, and Me), although 4-methyl was not detrimental. Thus, the phenyl appears to be in a small, lipophilic area. The need for the piperidine nitrogen, assumed to be protonated at the pH of the testing medium, implies an important salt interaction with the receptor. The piperidine is also required for proper orientation of the 4-substitution.

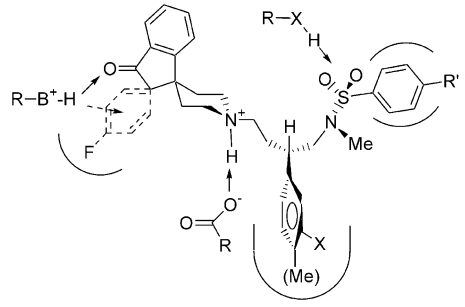


Figure 2. Proposed CCR5 pharmacophore model for **8c** and **8y** (hashed lines). The orientation of the three substituents on the central methine carbon is a low energy conformation based on the SAR and modeling of **8c** and several other compounds in this series.[20]

Figure 3 shows three possible conformations of the spiro piperidine subunit. The role of the spiro structure in combination with the activity of the simple 4-phenyl compound **5** initially seemed to imply that structure **A**, and not **B**, was the active conformation and that the sulfoxide provided an additional beneficial receptor interaction, thus the observed 3-fold improvement in binding activity ($IC_{50} = 10$ vs 30 nM for **3** and **5**). The effect of the sulfoxide stereochemistry, and thus the absolute stereochemistry for the sulfoxide interaction, was not determined since the two diastereomers were not separated. The observed improvement with ketone **8c** was again consistent with this model and might have resulted from a more optimal hydrogen bonding orientation for the oxygen.

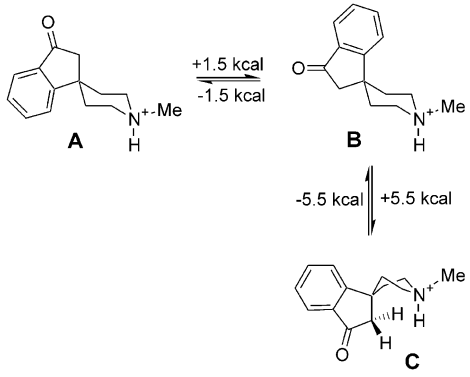


Figure 3. Structures and relative energies of alternative piperidine conformations. **A** and **B** are the two chair conformations and **C** is a twist boat conformation.

However, the results from the indane **8a** and the 2-methylphenyl analogue **8r** compared to **5**, as well as the poor activity of the lactams, seemed to contradict this simple assumption and led to consideration of other possible motifs (Fig. 3). Conformer **C** was initially of interest since the phenyl would still occupy the same space as the equatorial 4-phenyl of **5** and might explain the poorer activity of the lactams. Conformational analysis of the piperidine moiety[21] (as the protonated *N*-Me derivative) yielded structures **A**–**C** and afforded the indicated relative conformational energies. However, this analysis showed that **C** was significantly higher in energy than either **A** or **B** (5–7 kcal/mol) and thus might not be energetically accessible. Nevertheless, structures **A** and **B** were found to have little (<2 kcal) conformational energy difference. Similar analysis for the lactam **8d** also shed some light on its poorer activity in that there is a larger energy difference between its corresponding conformers **A** and **B** ($+3.2$ kcal), thus significantly deceasing its relative abundance. The observation that **8a** and **8r** were significantly less active than **5** might also be rationalized by a beneficial interaction of the phenyl with the same receptor residue when not forced perpendicular to the piperidine (see Fig. 2, hashed line phenyl ring). Also noteworthy is the observation that the more flexible 3-phenylpropyl **8bb** might advantageously place the phenyl in a similar *beta* orientation, but further removed from the piperidine ring. Thus, as indicated in Figure 2, conformer **B** is the preferred model at this time and was the subject of further investigation.[22]

Thus, the results presented here offer a detailed description of a pharmacophore model for inhibition of MIP-1α binding to CCR5 with this type of antagonist scaffold, which has been demonstrated to be capable of preventing the infection of PBMC cells by R5 strains of HIV-1. A more complete description of a CCR5 binding model incorporating these and related compounds will be described in the near future.

Acknowledgements

We would like to thank Elizabeth Roth and William Schleif for performing the PBMC assays on compounds **8c** and **8bb**.

References and Notes

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18. The results of the PBMC assay were highly variable with IC_{95} values for **3** (used as the standard in each assay) ranging from 1500 to 25,000 nM.
19. Piperidines are generally more basic than the corresponding piperazines as evident from the pK_a's for *N*-methylpiperidine and *N*,*N*-dimethylpiperazine (10.1 and 8.1, respectively).
20. Conformations for **8c** were generated using an in-house distance geometry program (Kearsley, S. K., unpublished results) and energy minimized using a distance-dependent dielectric constant of 78 and the MMFFs forcefield.[23] The pharmacophore model was created by generating a consensus overlay of several compounds in this series using MEGA-SQ.[24]
21. For the piperidine moiety, the conformational analysis was performed starting from both chair structures as depicted by **A** and **B**. Different ring conformations were generated and minimized as described above[20] to afford conformers **A**–**C** with their indicated relative energies.
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