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Discovery of Human CCR5 Antagonists Containing Hydantoins for the Treatment of HIV-1 Infection

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Abstract—A series of hydantoin derivatives has been discovered as highly potent nonpeptide antagonists for the human CCR5 receptor. The synthesis, SAR, and biological profiles of this class of antagonists are described.

The design and synthesis of small-molecule antagonists of chemokine receptors have become a major focus of effort since the discovery that chemokine receptors act as co-receptors in association with CD4 for HIV entry into cells.[1,2] Chemokines are a family of chemotactic cytokines, which are characterized by a distinctive pattern of conserved cysteine residues.[2] They range in size from ~70 to 120 amino acids and are involved in the recruitment and activation of a variety of cell types. Chemokines are divided into four groups (CXC, CC, C, and CX3C), depending on the number and spacing of the conserved cysteine residues. CCR5 is one of the receptors for the CC chemokines (β-chemokines), MIP-1α, MIP-1β, and RANTES, in which two cysteine residues are adjacent. CCR5, a cell-surface 7-transmembrane G-protein coupled receptor, has been identified as a co-receptor for entry of M-tropic HIV viral strains into host cells.[1] Recent human genetic evidence supports CCR5 as a therapeutic target. Individuals homozygous for a 32-base pair deletion in the gene for CCR5 lack this receptor on their cell surfaces and are highly resistant to HIV-1 infection,[3] while infected heterozygous individuals show significantly delayed progression to AIDS.[4] This led to our efforts to identify potent CCR5 antagonists for use as therapeutic agents for the treatment of HIV-1. An extensive screening of the Merck sample collection for compounds with affinity for the CCR5 receptor identified a series of active lead compounds (e.g., **I**) that has already been reported (Fig. 1).[5a]

Modification of the early sulfonamide lead **I**, containing a 1-amino-2-aryl-4-(piperidin-1-yl)butane core structure, resulted in the identification of a variety of potent CCR5 antagonists with low nanomolar binding affinity for CCR5. Recent reports have highlighted the SAR at the indicated four areas of the lead as shown in Figure 2.[5] The main focus of this paper is an investigation of the SAR for the replacement of the sulfonamide group with a hydantoin pharmacophore. Hydantoins have been

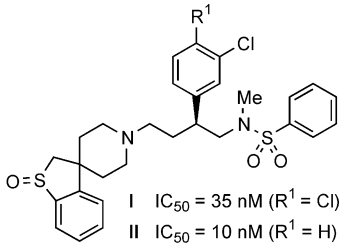

Figure 1. CCR5 antagonist lead compounds.

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reported to be attractive scaffolds in drug design.[6] It features two hydrogen bond acceptors and one donor in the heterocycle, which may be useful in the search for important pharmacophores. In addition to these binding features, hydantoins have three sites of diversity amenable to rapid analogue synthesis. These advantages along with the interesting observation that the activity of amide structure **IV** ($IC_{50}=640$ nM) was considerably improved over the benzoyl analogue **III** led us to incorporate the hydantoin into the benchmark CCR5 antagonist (**II**).[5b]

A general synthesis of hydantoin derivatives of interest is described in Scheme 1.[7] Allylation of 3-chlorobenzyl cyanide **1** followed by reduction with $LAH/AlCl_3$ gave the primary amine **3**, which was coupled to various *N*-Boc amino acids such as D-phenyl alanine, D-phenyl glycine, L-phenyl glycine, D-tryptophan, and so on to give **4**. Boc-deprotection with TFA followed by cyclization with CDI afforded the desired hydantoin **5**. Cleavage of the olefin with ozone or catalytic OsO_4 followed by $NaIO_4$, gave the desired aldehyde **6**, which was used to reductively alkylate a variety of piperidines to give **7**. The diastereomeric mixture **7** was separated by preparative TLC to sort out the more active isomers.

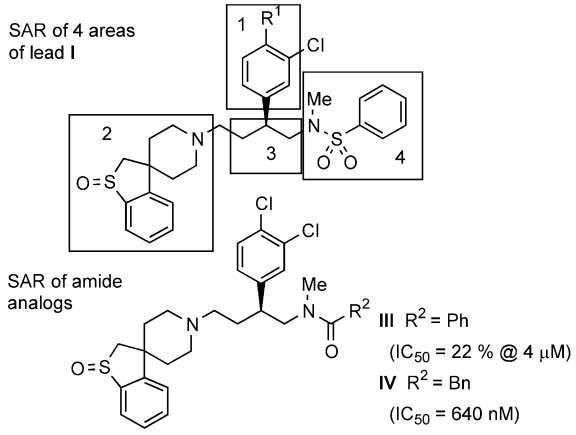
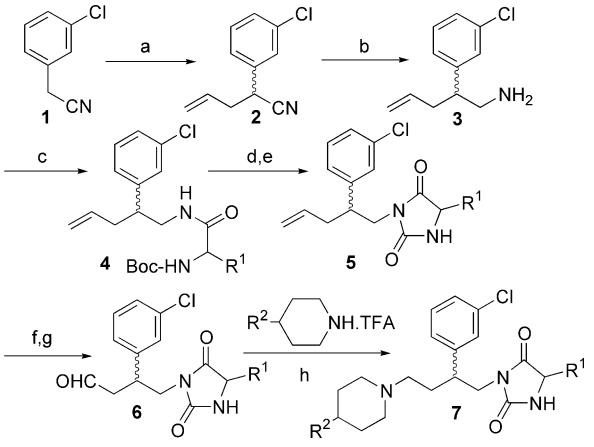


Figure 2. SAR of known CCR5 antagonists.



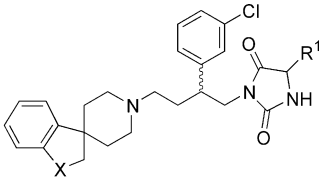
Scheme 1. Reagents: (a) LDA, THF, −78 °C, then allyl bromide, −78 °C to rt; (b) LAH, $AlCl_3$, THF, 0–80 °C; (c) *N*-Boc-amino acid, HOBT, EDC, CH_2Cl_2; (d) TFA, rt; (e) CDI, THF, Hunig's base, reflux; (f) cat OsO_4, 4-methylmorpholine *N*-oxide, *t*-BuOH, H_2O, acetone; (g) $NaIO_4$, THF; (h) $NaBH(OAc)_3$, DIPEA, THF, molecular sieves, rt.

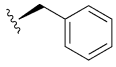
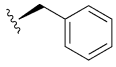
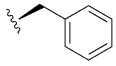
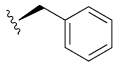
These compounds were then evaluated for CCR5 binding affinity utilizing a ^{125}I-MIP-1α binding assay.[8] Selected compounds were assayed in a PBMC-based viral replication assay as previously described.[9]

Sulfur-containing spiro-amines were not compatible with the hydantoin moiety as shown in Table 1 (**8**–**10**), whereas carbonyl spiropiperidine analogues restored activity (**11** and **12**). The D-phenyl glycine analogue [**12**, (*R*)-configuration, $IC_{50}=606$ nM] was somewhat more potent than the L-phenyl analogue [**13**, (*S*)-configuration, 52% @ 1 μM). Thus, the (*R*)-configuration at this center was held constant throughout our subsequent investigation. Despite a decrease in potency for our initial compounds relative to the lead, the moderate activity of the D-phenylalanine analogue **11** ($IC_{50}=200$ nM) suggested that it might be possible to improve CCR5 potency for this hydantoin pharmacophore with other modifications in the core structure as they were developed in the sulfonamide series.[5e]

Indeed, when the indanone spiropiperidine was replaced with the 4-phenylpiperidine (see Table 2), compound **15** showed similar activity to that of compound **11** ($IC_{50}=250$ vs 200 nM). Removal of the aryl substituent (R^2) resulted in a sharp decrease in the activity (**14**). The hydantoin N–H ($R^3=H$, Table 2) is essential for good activity, when R^2 is Ph or indole (**15**, **18**, and **19**). In other cases, the hydantoin N–H alone does not seem to

Table 1. CCR5 antagonist activity of spiropiperidines

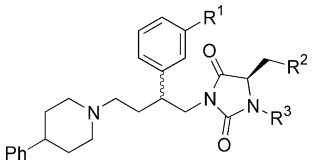


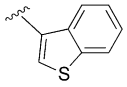
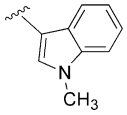
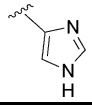
Entry	X	R^1	CCR5[a] IC_{50} (nM)[b]
8	S		24% @ 1.7 μM
9	SO		39% @ 1.7 μM
10	N–SO_2Me		55% @ 1 μM
11	CO		200
12	CO		606
13	CO		52% @ 1 μM

[a]See ref 5a for the procedure.
[b]The IC_{50} results are an average of three independent titrations having calculated standard errors below 15%. The assay-to-assay variation was generally ±2-fold based on the results of the standard compound **II**.

be sufficient to provide good activity (**20**, **21**, and **23**). It was notable that incorporation of D-tryptophan side chain onto the hydantoin gave rise to a separable diastereomeric mixture of indole analogues, **18** and **19**.

Table 2. CCR5 antagonist activity of phenylpiperidines



Entry	R^1	R^2	R^3	CCR5[a] IC_{50} (nM)[b]
14	Cl	H	H	43% @ 10 μM
15	Cl	Ph	H	250
16	Cl	Ph	Me	52% @ 1 μM
17	Cl	Ph	Bn	40% @ 1 μM
18[c]	Cl	Indol-3-yl	H	25
19[d]	Cl	Indol-3-yl	H	80
20	Cl		H	67% @ 1 μM
21	Cl		H	43% @ 1 μM
22	H	Indol-3-yl	H	71% @ 1 μM
23	H		H	25% @ 1 μM

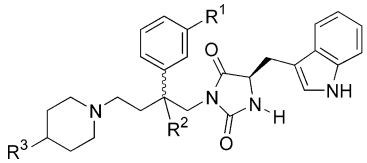
[a]See ref 5a for the procedure.
[b]The IC_{50} results are an average of three independent titrations having calculated standard errors below 15%. The assay-to-assay variation was generally ±2-fold based on the results of the standard compound **II**.
[c]Higher R_f isomer.
[d]Lower R_f isomer.

Because the (*S*)-stereochemistry at the benzylic position was already established in other active CCR5 antagonists,[5] the stereochemistry at the benzylic position in the more potent diastereomer **18** was assumed to be (*S*). From the decreased activity of **20** and **21**, it became apparent that the indole N–H is another essential pharmacophore. Deletion of the 3-chloro- moiety (R^1, Table 2) resulted in a substantial loss of binding activity (**22**). Replacement of the indole moiety with a smaller imidazole was also unsatisfactory (**23**).

Two further important modifications to the original structure have been highlighted recently: (1) addition of a methyl group at the benzylic position[5e] and (2) the incorporation of a 4-CBZ carbamate moiety in place of the 4-phenyl in the piperidine.[5d] These two groups were then combined in the indole-substituted hydantoin structure **18** (Table 3). This combination produced remarkably potent compounds with low nanomolar IC_{50} values (2–5 nM). Potency enhancement resulting from a benzylic methyl group was not observed for the phenyl piperidine series (**24** vs **25**), whereas either a *meta*-chloro on the central phenyl or a methyl group at the benzylic position alone increased the binding affinity slightly in the carbamate series (**28**, **29**, **32**, and **33**). However, an additive effect of these two was not observed. Compounds **30** and **34** that combined these two substitutions showed a sharp decrease in the binding affinity over the *des*-chloro and/or *des*-methyl examples.

The increase in antiviral activity was ca. 2-fold over the compound **27**, when the modifications were made (**28**, **29**, and **32**). The viral spread assay results for selected carbamate compounds containing the indole moiety indicate that the high potency in the CCR5 binding assay did not translate into high potency in the viral spread assay. While initial studies suggest that optimization of

Table 3. CCR5 antagonist activity and antiviral activity of indole series



Entry	R^1	R^2	R^3	CCR5[a] IC_{50} (nM)[b]	Antiviral activity IC_{95} (nM)
24	H	H	Ph	71% @ 1 μM	ND[c]
25	H	Me	Ph	80% @ 1 μM	ND
26	Cl	Me	Ph	100	ND
27	H	H	CBZ-N-(Et)	13	25,000
28[d]	Cl	H	CBZ-N-(Et)	2 (22)[e]	12,500
29	H	Me	CBZ-N-(Et)	4	12,500
30	Cl	Me	CBZ-N-(Et)	77	ND
31	H	H	CBZ-N-(Allyl)	7	>3,000
32	Cl	H	CBZ-N-(Allyl)	3	12,500
33	H	Me	CBZ-N-(Allyl)	5	>3000
34	Cl	Me	CBZ-N-(Allyl)	32	ND

[a]See ref 5a for the procedure.
[b]The IC_{50} results are an average of three independent titrations having calculated standard errors below 15%. The assay-to-assay variation was generally ±2-fold based on the results of the standard compound **II**.
[c]ND, not determined.
[d]Higher R_f diastereomer.
[e]IC_{50} of lower R_f diastereomer. The rest of the above examples are diastereomeric mixtures.

antiviral activity may be possible by further modification around the hydantoin and tryptophan areas, the origin of this discordant result is still under investigation.

Compounds **27**–**29** and **31**–**34** were all found to be selective CCR5 antagonists versus other chemokine receptors, such as CCR1, CCR2, CCR3, CCR4, and CXCR4 ($IC_{50} > 1000$ nM).

In summary, starting with lead **I** as a design template, a series of hydantoin-based CCR5 receptor antagonists was synthesized and evaluated for their binding potency as well as antiviral activity. Replacement of the sulfonamide with a hydantoin moiety was possible. Aromatic amino acid derivatives were found to be active in the binding assay with the D-tryptophan derivative **28** being the most potent compound. When coupled with carbamates, benzylic methyl, or 3-chloro, antiviral activity increased in our series (Table 3). This suggests that efficient inhibition of HIV-1 replication may be possible by further modification of potent hydantoin-based CCR5 antagonists, blocking entry of M-tropic HIV viral strains into the host cells effectively.

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