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Anti-HIV-1 entry optimization of novel imidazopiperidine-tropane CCR5 antagonists

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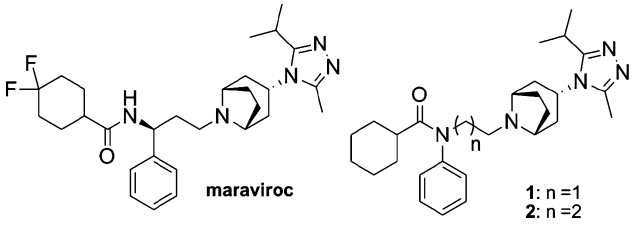
Abstract—A novel series of imidazopiperidine-tropane CCR5 antagonists is described. The series was optimized for anti-HIV-1 potency using a set of phenotypic viral entry assays. This strategy resulted in the identification of several very potent (IC_{50} < 10 nM) inhibitors of HIV-1 entry. One compound (**40**) was further profiled and was found to have attractive selectivity, pharmacokinetic, and antiviral properties.

The chemokine receptor CCR5 has proven to be an exciting target for the pharmaceutical industry in the HIV-1 and inflammation therapeutic areas. CCR5 plays an integral role in the R5-tropic HIV-1 entry process by serving as a critical co-receptor for the viral envelope protein gp120.[1,2] Homozygous individuals with a 32-base pair deletion in the gene encoding CCR5 do not express the functional receptor and are ultimately resistant to R5-tropic HIV-1 infection.[3] These facts have inspired a great amount of research over the past decade to identify anti-HIV-1 therapeutics targeting the CCR5-mediated entry mechanism.[4–8] These efforts have resulted recently in the FDA approval of the first small molecule CCR5 antagonist, maraviroc (Selzentry®),[9,10] for the treatment of HIV-1 infection. Despite this considerable milestone, there is still much interest in the development of second generation CCR5 antagonists with improved properties.

Our chemistry program began with the observation that a wide variety of templates sharing a basic pharmacophore were reported to bind to CCR5 and to possess antiviral properties.[5,6,11] We designed chemical scaffolds that combined the attractive features of reported templates, including maraviroc, with the goal of identifying novel compounds that could be optimized to have superior properties to compounds reported to be in clinical development. Using this approach, compound **1** was identified via a Mip1-β competition binding assay as a modest starting point (IC_{50} = 0.97 μM) for further optimization.[12]



Elongating the *N*-ethyl spacer of **1** by one carbon resulted in **2**, which is a threefold more potent inhibitor of chemokine binding (IC_{50} = 0.31 μM). Modification of the cyclohexylamide moiety of **2** to a urea proved to be quite beneficial, resulting in a 10-fold increase in potency (compound **3** in Table 1). At this stage a series of urea derivatives was made to assess the optimal hydrophobic group at this position (Table 1). Replacement of the cyclohexyl ring with a smaller ring (**4**) or alkyl chain (**5**, **6**) resulted in a slight loss of potency. A phenyl replacement (**7**) was found to be equipotent to **3**; however, replacement with a benzyl group (**8**)

Keywords: CCR5; Chemokine receptor; CCR5 antagonist; HIV-1 entry; Viral entry; Entry inhibitor.
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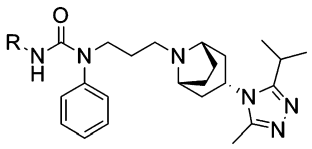
Table 1. IC_{50} values for compounds **3**–**19** in a Mip1-β binding assay



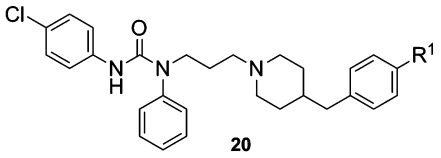
Compound	R	IC_{50}[a] (μM)
3	Cyclohexyl	0.031
4	Cyclopentyl	0.078
5	*n*-Propyl	0.271
6	*i*-Propyl	0.096
7	Phenyl	0.020
8	Benzyl	0.323
9	2-Me-phenyl	0.271
10	3-Me-phenyl	0.029
11	4-Me-phenyl	0.004
12	2-OMe-phenyl	0.381
13	3-OMe-phenyl	0.053
14	4-OMe-phenyl	0.027
15	2-Cl-phenyl	0.100
16	3-Cl-phenyl	0.015
17	4-Cl-phenyl	0.004
18	2,6-Cl-phenyl	0.539
19	2,3-Cl-phenyl	0.048

[a] Values are means of two experiments.

resulted in a 10-fold reduction in potency. *Mono*-substitution at the para position of the phenyl ring in **7** is clearly favored as evidenced by compounds **11**, **14**, and **17** (IC_{50} values of 4, 27, and 4 nM, respectively). It is interesting to note that others have reported the importance of a *para*-substituted phenyl urea group for CCR5 binding (example **20**),[13] thus suggesting a strong pharmacophore overlap with our series. This observation suggested that the triazole moiety most likely interacts with a similar region of the receptor as the benzyl group of **20**.

Given the potency of **11** in the chemokine binding assay, this compound was tested in a luciferase-reporter phenotypic viral entry assay to assess its antiviral properties.[14] The compound was evaluated using a panel of viral stocks pseudotyped with envelop sequences derived from five different viral isolates representing a diversity of viral subtypes. Maraviroc was also tested as a control compound. The results of these studies are shown in Table 2.

Despite the potent effects of **11** in the CCR5 binding experiments, this compound was determined to be 5- to 10-fold less potent than our target profile for viral entry inhibition. Chemokine binding assays are often used for optimizing the potency of a chemical series in terms of receptor antagonism. However, chemokine inhibition often does not correlate well with viral entry inhibition. Many potent inhibitors of chemokine binding have been identified that have modest to no antiviral activity (data not shown). This fact highlights the subtle differences in the allosteric modes of inhibition for chemokine binding versus gp120 binding. Most CCR5 antagonists are believed to bind in the transmembrane region of the GPCR resulting in conformational changes in the extracellular loops of the receptor.[5] These conformational changes may differentially affect the binding of the various chemokines and gp120 to the receptor. Given the disconnect often observed between chemokine and viral entry inhibition, we chose to utilize viral entry assays for optimization of antiviral potency.



20

The SAR reported around **20** suggested that a polar substituent at R^1 (such as SO_2Me) greatly enhances the antiviral properties of this series.[13] Given the potential overlap of the benzyl group of **20** with the triazole moiety of our series we decided to modify the triazole group to incorporate a polar side chain that could potentially occupy the same space as that found in the case of R^1 in **20**. The initial results of this effort are shown in Table 3.

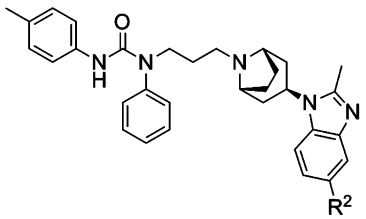
A benzimidazole scaffold was first utilized in an attempt to replace the triazole ring. It was hypothesized that this system would closely mimic the *para*-substituted benzyl group of **20**. A previous report in the literature on a related chemical series suggested that replacement of the 3-isopropyl-5-methyltriazole system with 2-methylbenzimidazole maintains antiviral potency.[15] The endo geometry of the tropane-benzimidazole was suggested to be preferred since the sterics of this system force the tropane ring into a boat conformation, thus positioning the imidazole ring in a similar orientation

Table 2. Viral entry inhibition, IC_{50}[a] (μM)

Compound	Virus				
	JRCSF	ASM80	Ba-L	97-ZA-003	RU570
11	0.118	0.058	0.048	0.029	0.028
Maraviroc	0.005	0.006	0.004	0.008	0.002

[a] Values are means of two experiments.

Table 3. Viral entry inhibition, IC_{50}[a] (μM)



21: $R^2 = H$
22: $R^2 = SO_2Me$

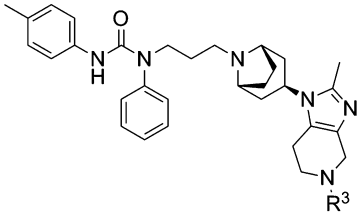
Compound	Virus				
	JRCSF	ASM80	Ba-L	97-ZA-003	RU570
21	0.019	0.022	0.050	0.015	0.018
22	>10,000	>10,000	>10,000	>10,000	>10,000

[a] Values are means of two experiments.

as the triazole ring in the exo geometry.[15] The endo benzimidazole derivative **21** proved to be slightly more potent than **11** in the viral entry assays. However, the sulfone derivative **22** was found to be inactive, indicating that the polar group was not oriented in the proper space. To increase the flexibility at the R^2 position a substituted imidazopiperidine scaffold was utilized. Precedent existed in the patent literature for the use of substituted imidazopiperidines as components of tropane-based CCR5 antagonists.[16,17] Substitution of the imidazopiperidine group with a variety of polar groups (compounds **23**–**26**) resulted in very potent inhibitors of viral entry as indicated in Table 4. The importance of this polar group was further evidenced by the much reduced potency of imidazo-*N*-methylpiperidine **27**. Further exploration of amides at the R^3 position indicated that a variety of hydrophobic groups were tolerated adjacent to the carbonyl moiety (compounds **28**–**34**). Similar SAR trends at R^3 were found in the imidazo-sulfonamide, carbamate, and urea series (data not shown).

Efforts were also made to explore modifications of the imidazole methyl group of **23** (R^4) as well as R^5 and R^6 phenyl substitutions, as shown in Table 5. Interestingly, replacement of the methyl group at R^4 with a hydrogen (compound **35**) resulted in a complete loss of antiviral activity. This result emphasized the importance of this position in influencing critical conformational aspects of the tropane ring. The ethyl derivative **36** was equipotent to **23**; however, the isopropyl derivative **37** was much less potent. Fluoro-substitution at R^5 appeared to be tolerated in the meta position (**38**) but negatively impacted viral entry inhibition at the para position (**39**), resulting in a 10-fold loss in potency. In many cases a methyl group at R^6 served as a site of metabolic oxidation. This group could be replaced with several more metabolically stable groups while maintaining potent antiviral activity as evidenced by analogs **40**–**42**.

Table 4. Viral entry inhibition, IC_{50}[a] (μM)

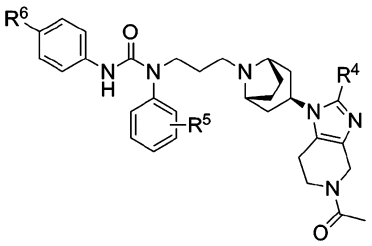


23: R^3 = COMe
24: R^3 = SO_2Me
25: R^3 = COOMe
26: R^3 = CONHMe
27: R^3 = Me
28: R^3 = COnPr
29: R^3 = COiPr
30: R^3 = COtBu
31: R^3 = COcyPr
32: R^3 = COcyBu
33: R^3 = COcyPent
34: R^3 = COPh

Compound	Virus				
	JRCSF	ASM80	Ba-L	97-ZA-003	RU570
23	0.009	0.016	0.028	0.009	0.005
24	0.004	0.004	0.005	0.003	0.002
25	0.012	0.014	0.025	0.012	0.008
26	0.007	0.008	0.021	0.005	0.004
27	0.291	0.426	1.127	0.264	0.102
28	0.007	0.010	0.018	0.007	0.004
29	0.007	0.012	0.021	0.007	0.004
30	0.002	0.003	0.005	0.002	0.002
31	0.004	0.004	0.006	0.003	0.002
32	0.005	0.004	0.006	0.003	0.002
33	0.003	0.005	0.008	0.003	0.002
34	0.007	0.007	0.019	0.006	0.005

[a] Values are means of two experiments.

Table 5. Viral entry inhibition, IC_{50}[a] (μM)



35: R^4 = H; R^5 = H; R^6 = Me
36: R^4 = Et; R^5 = H; R^6 = Me
37: R^4 = iPr; R^5 = H; R^6 = Me
38: R^4 = Me; R^5 = 3-F; R^6 = Me
39: R^4 = Me; R^5 = 4-F; R^6 = Me
40: R^4 = Me; R^5 = H; R^6 = CF_3
41: R^4 = Me; R^5 = H; R^6 = F
42: R^4 = Me; R^5 = H; R^6 = Cl

Compound	Virus				
	JRCSF	ASM80	Ba-L	97-ZA-003	RU570
35	>10,000	>10,000	>10,000	>10,000	>10,000
36	0.014	0.014	0.056	0.010	0.010
37	0.100	0.272	0.761	0.121	0.051
38	0.009	0.011	0.062	0.017	0.007
39	0.115	0.120	1.048	0.116	0.090
40	0.003	0.005	0.008	0.005	0.002
41	0.004	0.006	0.009	0.005	0.002
42	0.007	0.013	0.016	0.009	0.004

[a] Values are means of two experiments.

Compound **40** was selected for further evaluation. The pharmacokinetic properties of **40** in rat are shown in Table 6 and Figure 1. Overall, the compound has an attractive PK profile with 52% oral bioavailability and a terminal half-life of 10.7 h. Given the reported pharmacokinetic challenges with clinically advanced CCR5 antagonists,[18] we were quite encouraged by these results. Compound **40** was screened for selectivity in a panel of chemokine receptor binding assays and was found to potently inhibit Mip1-β binding to CCR5 (IC_{50} = 1 nM), but showed no significant inhibition in similar assays for CCR1, CCR2, CCR3, CXCR2, and CXCR4 when tested up to a concentration of 10 μM. Compound **40** was also screened in a panel of binding assays representing 26 off-target GPCRs and was found for each receptor to have at least 1000-fold selectivity for CCR5. In a [^{35}S] GTP-γS CCR5 functional assay, **40** was determined to have no agonist activity but was found to be a potent functional antagonist (IC_{50} = 0.4 nM). Finally, the antiviral properties of **40** were evaluated in a 7-day PBMC assay (15% FBS) using an R5-tropic HIV-1_{Ba-L} strain. Compound **40** was found to potently inhibit viral replication with an IC_{90} of 6 nM.

Table 6. Pharmacokinetic properties of **40** in rat (mean values, n = 3)

Parameter	IV	PO
Dose (mg/kg)	5	30
C_{max} (μg/ml)	0.86	0.54
AUC (μg h/ml)	2.89	8.99
Clearance (ml/min/kg)	28.8	—
Half-life (h)	10.7	7.2
% F	—	52

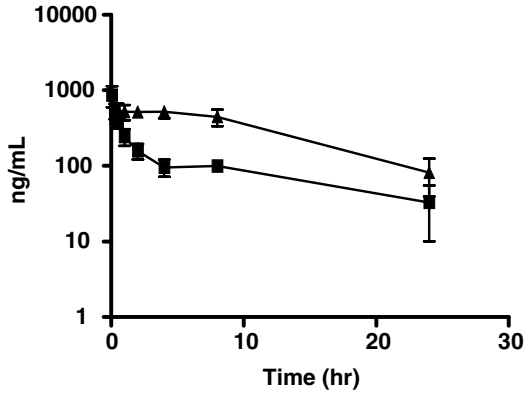


Figure 1. Concentration versus time for 30 mg/kg po (▲) and 5 mg/kg IV (■) doses of **40** in rat (mean values ± SEM, $n = 3$).

In summary, a novel series of substituted imidazopiperidine-tropane CCR5 antagonists was identified. Viral entry assays were used to optimize the antiviral properties of this chemical series and many potent inhibitors of viral entry were identified. One such compound, **40**, was further profiled and was shown to have attractive pharmacokinetic, selectivity, and antiviral properties. Further studies with **40** and other members of the imidazopiperidine-tropane series are ongoing.

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References and notes

1. Kwong, P. D.; Wyatt, R.; Robinson, J.; Sweet, R. W.; Sodroski, J.; Hendricks, W. A. *Nature* **1998**, *393*, 648.
2. Wu, L.; Gerard, N. P.; Wyatt, R.; Choe, H.; Parolin, C.; Ruffing, N.; Borsetti, A.; Cardoso, A. A.; Desjardin, E.; Newman, W.; Gerard, C.; Sodroski, J. *Nature* **1996**, *384*, 179.
3. Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C. M.; Saragosti, S.; Lapouméroulie, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y. J.; Smyth, R. J.; Collman, R. G.; Doms, R. W.; Vassart, G.; Parmentier, M. *Nature* **1996**, *382*, 722.
4. Barber, C. G. *Curr. Opin. Invest. Drugs* **2004**, *5*, 851.
5. Wieslaw, K.; Bifulco, N.; Yang, H.; Boone, L.; DeAnda, F.; Watson, C.; Kenakin, T. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 2663.
6. Wieslaw, K.; Peckham, J.; Duan, M.; Kenakin, T.; Jenkinson, S.; Gudmundsson, K.; Piscitelli, S.; Feldman, P. *Curr. Med. Chem. Anti-Infect. Agents* **2005**, *4*, 133.
7. Shaheen, F.; Collman, R. *Curr. Opin. Infect. Dis.* **2004**, *17*, 7.
8. Meanwell, N.; Kadow, J. *Curr. Opin. Drug Discov. Devel.* **2003**, *6*, 451.
9. Dorr, P.; Westby, M.; Dobbs, S.; Griffin, P.; Irvine, B.; Macartney, M.; Mori, J.; Rickett, G.; Smith-Burchnell, C.; Napier, C.; Webster, R.; Armour, D.; Price, D.; Stammen, B.; Wood, A.; Perros, M. *Antimicrob. Agents Chemother.* **2005**, *49*, 4721.
10. Meanwell, N.; Kadow, J. *Curr. Opin. Invest. Drugs* **2007**, *8*, 669.
11. Debnath, A. *J. Med. Chem.* **2003**, *46*, 4501.
12. CCR5 binding potency was evaluated by monitoring inhibition of [^{125}I]Mip-1β binding to membranes from CHO-K1 cells stably expressing recombinant human CCR5.
13. Imamura, S.; Kurasawa, O.; Nara, Y.; Ichikawa, T.; Nishikawa, Y.; Iida, T.; Hashiguchi, S.; Kanzaki, N.; Iizawa, Y.; Baba, M.; Sugihara, Y. *Bioorg. Med. Chem.* **2004**, *12*, 2295.
14. Coakley, E.; Petropoulos, C.; Whitcomb, J. *Curr. Opin. Infect. Dis.* **2005**, *18*, 9.
15. Wood, A.; Armour, D. *Prog. Med. Chem.* **2005**, *43*, 239.
16. Basford, P; Stephenson, P; Taylor, S; Wood, A. PCT International Patent Application WO 2003084954, 2003.
17. Stupple, P. PCT International Patent Application WO 2005033107, 2005.
18. Walker, D.; Abel, S.; Comby, P.; Muirhead, G.; Nedderman, A.; Smith, D. *Drug Metab. Dispos.* **2005**, *33*, 587.