Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2997–3000

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

1,3,4-Trisubstituted Pyrrolidine CCR5 Receptor Antagonists. Part 3: Polar Functionality and Its Effect on Anti-HIV-1 Activity

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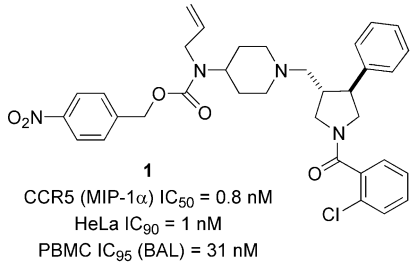
Received 5 April 2002; accepted 19 June 2002

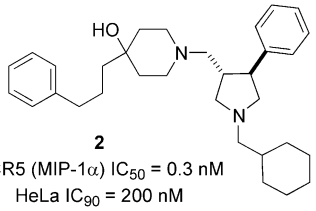
Abstract—Incorporation of acidic functional groups into a lead CCR5 antagonist identified from a targeted combinatorial library resulted in compounds with enhanced anti-HIV-1 activity and attenuated L-type calcium channel affinity.

The β-chemokine receptor CCR5 has been identified as the major co-receptor required by HIV-1 for the entry into monocytes, macrophages and primary T-cells.[1,2] Reports from Takeda,[3] Schering-Plough,[4] and Merck[5] regarding potent, small molecule human CCR5 receptor antagonists possessing the ability to inhibit the replication of HIV-1 in vitro have appeared. CCR5 receptor antagonists represent a potentially new therapy for the treatment of HIV-1 infection and clinical data supporting their use is anxiously awaited.

We recently disclosed the details of some initial structure–activity relationships (SARs) developed for a series of 1,3,4-trisubstituted pyrrolidine CCR5 receptor antagonists exemplified by **1**.[6] Compound **1** has excellent affinity for the human CCR5 receptor, is selective for CCR5 over other chemokine receptors and was demonstrated to have the ability to potently inhibit the entry of HIV-1 into peripheral blood mononuclear cells (PBMCs) via the antagonism of CCR5. While this compound was orally bioavailable in the rat, its high rate of clearance and short half life suggested to us that **1** (or close analogues of **1**) might possess metabolically labile functionality that would negatively impact its long term prospects.



1
CCR5 (MIP-1α) IC_{50} = 0.8 nM
HeLa IC_{90} = 1 nM
PBMC IC_{95} (BAL) = 31 nM



2
CCR5 (MIP-1α) IC_{50} = 0.3 nM
HeLa IC_{90} = 200 nM
PBMC CIC_{95} (BAL) = 580 nM

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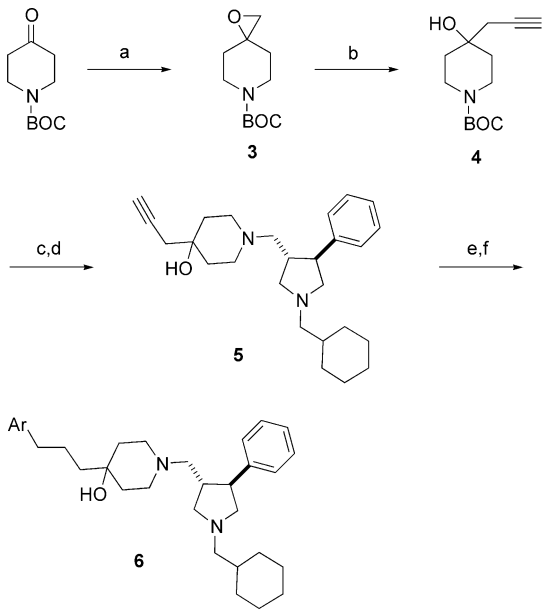
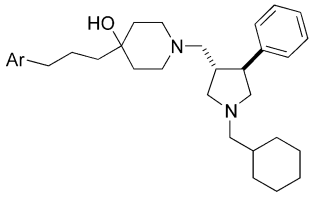
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00605-4



Scheme 1. Reagents and conditions: (a) $(CH_3)_3SOI$, NaH, DMSO, 50 °C (53%); (b) trimethylsilylacetylene, LiHMDS, $LiClO_4$, THF, then K_2CO_3, MeOH (94%); (c) 1 M HCl, MeOH; (d) 1-cyclohexylmethylene-3-(*R*)-formyl-4-(*S*)-phenyl pyrrolidine, DIEA, $NaB(OAc)_3H$, CH_2Cl_2 (65%, two steps); (e) Ar-Br, cat. $Pd(OAc)_2$, cat. Ph_3P, NaOAc, DMF, 60 °C; (f) H_2, 10% Pd/C, MeOH (40–65%, two

A targeted combinatorial chemistry library based in part on the 1,3,4-trisubstituted pyrrolidine scaffold was designed with the goal of identifying synergisms among known and novel CCR5 pharmacophores in these molecules.[7] This led to the identification of a new pyrrolidine lead analogue (**2**) which has excellent affinity for CCR5, but more modest anti-HIV-1 activity than **1**. While we thought that **2** might also have appreciable ion channel affinity (a common characteristic of many lipophilic, basic ligands for G-protein coupled receptors), its structural simplicity relative to **1** made it an attractive starting point for further investigations. We wish to describe herein our discovery that the incorporation of appropriate acidic functional groups into **2** can have a dramatic effect on the anti-HIV-1 properties of these molecules.

Table 1. Inhibition of [^{125}I]-MIP-1α binding[a] by pyridine analogues of **2**



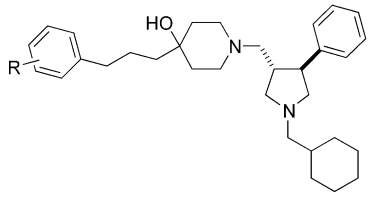
Compd	R	CCR5 IC_{50} (nM)
2	Ph-	0.3
7	2-Pyridyl	7
8	3-Pyridyl	6
9	4-Pyridyl	12

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean for $n=$ three determinations. SD were generally ±20% of the average. See ref 8 for assay protocol.

We initially envisioned preparing a series of analogues of **2** possessing a varied array of functional groups in place of the pendant phenyl ring (Scheme 1). In practice, commercially available N-BOC-4-piperidinone was reacted with dimethyloxosulfonium methylide to provide epoxide **3**. Epoxide ring opening with lithio trimethylsilylacetylene followed by treatment of the quenched reaction mixture with potassium carbonate in methanol afforded terminal alkyne **4** in high yield. Acid deprotection of **4** followed by reductive amination gave **5**, which was a versatile, late-stage intermediate for the preparation of analogues of **2**. Palladium catalyzed cross-coupling of **5** with aryl halides followed by alkyne reduction provided the analogues in Tables 1–3.

All analogues were tested for their ability to displace [^{125}I]-MIP-1α from the CCR5 receptor expressed on

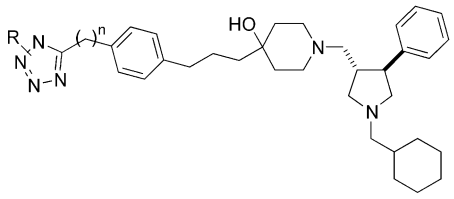
Table 2. Inhibition of [^{125}I]-MIP-1α binding[a] and anti-HIV-1 infectivity[b] activity of carboxy analogues of **2**



Compd	R	CCR5 IC_{50} (nM)	HeLa IC_{90} (nM)
2	-H	0.3	200
10	2-CO_2H	100	>1000
11	3-CO_2H	3	>1000
12	4-CO_2H	10	1000
13	3-CH_2CO_2H	6	300
14	4-CH_2CO_2H	5	100
15	4-$(CH_2)_2CO_2H$	2	40

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean for $n=$ three determinations. SD were generally ±20% of the average. See ref 8 for assay protocol.
[b]Inhibition (CIC_{90}) over a 48 h period of a single HIV-1 (BAL) infection cycle in HeLa Magi cells expressing both CXCR4 and CCR5. See ref 9 for assay protocol.

Table 3. Inhibition of [^{125}I]-MIP-1α binding[a] and anti-HIV-1 infectivity[b] activity of tetrazole analogues of **2**



Compd	R	*n*	CCR5 IC_{50} (nM)	HeLa IC_{90} (nM)
2			0.3	200
16	-H	0	0.2	4
17	-H	1	1	100
18	1-CH_3	0	0.2	100
19	2-CH_3	0	0.4	>300

[a]Displacement of [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. Data are reported as mean ± SD for $n=$ three determinations. SD were generally ±20% of the average. See ref 8 for assay protocol.
[b]Inhibition (CIC_{90}) over a 48 h period of a single HIV-1 (BAL) infection cycle in HeLa Magi cells expressing both CXCR4 and CCR5. See ref 9 for assay protocol.

Chinese Hamster Ovary (CHO) cell membranes.[8] The results obtained for a series of analogues in which the pendant phenyl ring of **2** was replaced with a pyridine is summarized in Table 1. None of the pyridine isomers had CCR5 affinity comparable to **2** and their was no distinct preference for the position of the nitrogen in pyridine ring.

More promising results were obtained with carboxylate derivatives of **2** (Table 2). Based on the results of the CCR5 binding assay, carboxy substitution at the 3- or 4-position of the phenyl ring was clearly favored over that at the 2-position (compare **11** and **12** to **10**). Moreover, homologation of these analogues was potency enhancing, affording analogues (**13**–**15**) with single digit nanomolar IC_{50}'s in the MIP-1α binding assay and potency comparable to or exceeding lead analogue **2** in their ability to inhibit a single HIV-1 infection cycle in a CCR5-expressing HeLa Magi cell line.[9]

The SAR developed for piperidine pharmacophores as part of the investigations into 2-aryl-4-(piperidin-1-yl)butanamine CCR5 antagonists suggested that the pendant phenyl ring of these current compounds would tolerate substitution with more sterically demanding groups.[5] Replacing the carboxy group of **12** with the bioisosteric C-linked tetrazole afforded an analogue (**16**) that had subnanomolar affinity for CCR5 in the ligand competition assay and was 50-fold more potent than **2** in the HeLa cell assay Table 3). The loss in anti-infectivity potency seen in the HeLa cell assay with homologueue **17** and *N*-methyl regioisomers **18** and **19** demonstrates that the acidic nature of the tetrazole is an important factor in blocking the HIV-1 fusion process from occuring. The discordance seen here between compound activities in the ligand competition and anti-infectivity assays indicates that care must be taken in interpreting the results of the former to be predictive of a compound's potential as an anti-HIV-1 agent.[10]

Compound **16** was found to have significantly attenuated affinity for the L-type calcium channel as compared to **2**.[11] If this property can be considered to be a marker for potential off-target activities, then zwitterionic compounds analogous to **16** could provide benefits beyond enhanced antiviral potency. Unfortunately, the rat pharmacokinetic profile **16** was found to be far from desirable; the high rate of clearance ($Cl_p = 61$ mL/min/kg), short half-life ($t_{1/2} = 20$ min) and neglible oral bioavailablity precluded further the pursuit of **16** as a candidate for further development.

In conclusion, while it is not clear how the acidic tetrazole contributes to the enhanced antiviral activity of **16** (one possible explanation is that the acidic or zwitterionic nature of this compound can inhibit or limit the accessibility of receptor states necessary for fusion), determining the effect of the incorporation of comparable functionality into other CCR5 antagonist templates was viewed to be worthwhile based on the results reported herein. Efforts in this area have been initiated and reports on these pursuits will follow in due course.

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