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Inhibition of HIV-1 Infection by Synthetic Peptide Analogues Derived from the NH_2-Terminal Extracellular Region of GPR1

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Abstract—Several shortened peptide analogues of the N-terminal domain of GPR1, an orphan G protein-coupled receptor (GPCR), were prepared and their anti-HIV-1 activities were evaluated. Some of the prepared compounds, especially sulfated derivatives, showed potent inhibitory activity against a broad range of HIV-1, including T cell-tropic, dual cell-tropic and brain-derived (BT) cell-tropic HIV-1 strains.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent in acquired immune deficiency syndrome (AIDS).[1] A recent study has shown that members of the chemokine receptor family of seven-transmembrane G protein-coupled receptors act as essential cofactors for the entry of human immunodeficiency virus type 1 (HIV-1) into cells.[2] The discovery of distinct chemokine receptors explains the differences in cell tropism between viral strains. CXCR4 supports the entry of T cell(T)-tropic HIV-1 strains, whereas CCR5 supports macrophage (M)-tropic HIV-1 strains. It is known that these co-receptors are rich in tyrosine and acidic amino acids at their N-terminal regions, and this contributes to the ability of HIV-1 to fuse with, and enter, target cells.[3] A positively charged region of the V3 loop of gp 120 has been shown to be important for associating with chemokine receptors. Consequently, it can be considered that some of these positively charged residues may directly interact and complement the peptides bearing a sequence of negatively charged acidic amino acids. The Coulomb interaction is the main reason why these chemokine receptors have received much attention as attractive targets for new antiviral therapies.[4] As part of our contribution to this field, we have reported the synthesis of hybrid compounds linked to *N*-carbomethoxycarbonyl-prolyl-phenylalanine (CPF)[5] that mimic CD4 in a previous communication.[6] The compounds can selectively bind to gp120, as peptides mimicking chemokine receptor CCR5, showing significant anti-HIV-1 activity. Recently, it has been found that an orphan G protein-coupled receptor, GPR1 acts as a coreceptor to allow replication of HIV-1 and 2 in brain-derived (BT) cells.[7] For the development of new HIV-1 inhibitors that prevent HIV-1 infection based on a strategy of binding to gp 120, we report here the design and synthesis of peptide analogues of the amino acid sequences at the N-terminal ectodomain of GPR1 having a reduced molecular size but without significant loss of activity. The biological features including anti-HIV-1 activity in various cell cultures are also described.

Since acidic residues and sulfotyrosines in the amino-terminal domain of CCR5 are crucial for viral fusion and entry,[8] we chose two regions of Tyr^{17}–Tyr^{22} and Tyr^{15}–Tyr^{22} that include tyrosine and aspartic acid as target peptide moieties in the amino acid sequences of the N-terminal ectodomain of GPR1.[9] To enhance the selectivity of binding to HIV-1 and hence the anti-HIV-1 activity, hybrid compounds **3** and **9** with CPF were also designed and prepared. The synthesis of hybrid molecule was straightforward and the construction of the covalent linkage between the CPF moiety and the peptide moiety was formed by using spacers derived from *o*-aminophenol (Schemes 1 and 2). A number of reports have implicated a role for sulfate moieties in HIV-1 entry.[10] For the purpose of increasing anti-HIV-1 activity, some sulfated analogues **2**, **4**, **6**, **7**, **8** and **10** were also prepared. The findings of biological assays for compounds **1**–**10** are listed in Tables 1 and 2. As can been seen from the tables, in contrast to the low activity **3**, **5**, **6**, **7** and **9**,

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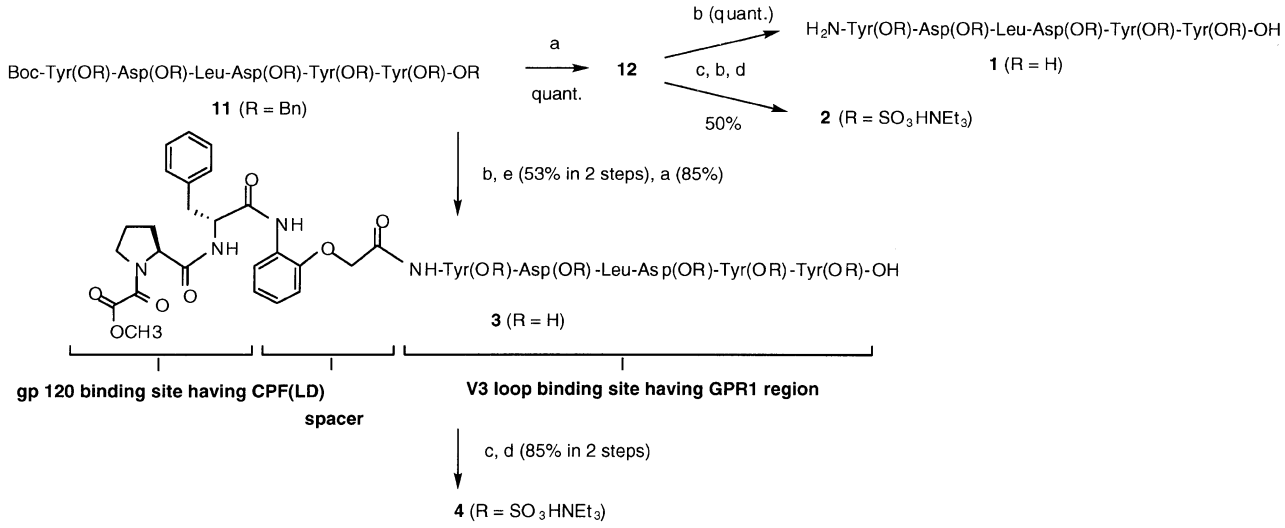
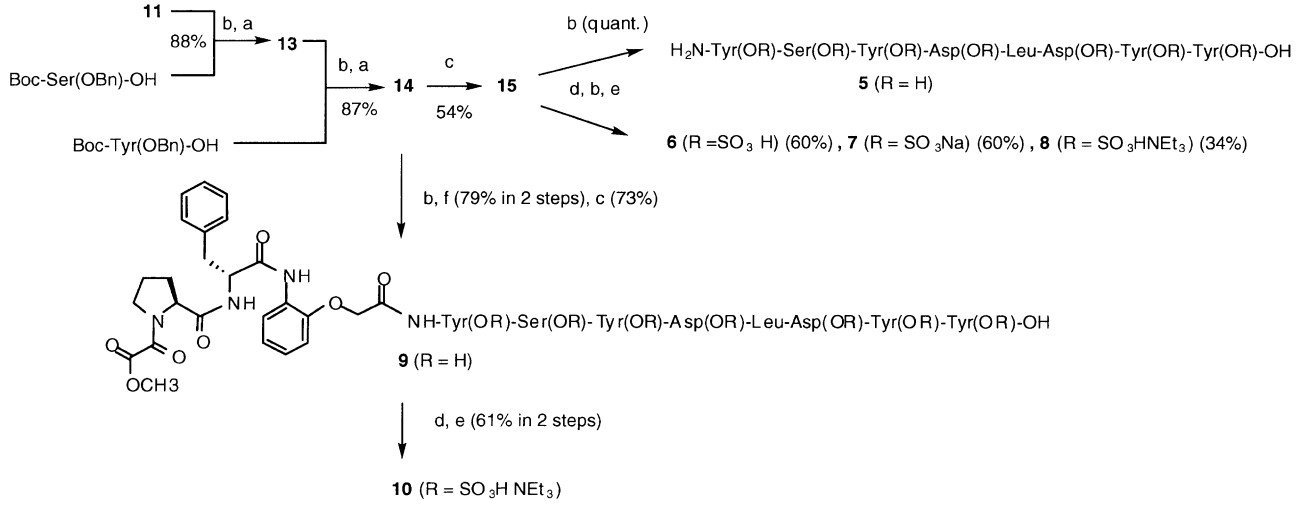
Amino Acid Sequence of *N*-Terminal Region of GPR1

[Met-Glu-**Asp**-Leu-**Glu**-**Glu**-Thr-Leu-Phe-**Glu**-**Glu**-Phe-**Glu**-Asn-**Tyr**^{15}-Ser-**Tyr**^{17}-**Asp**-Leu-**Asp**-**Tyr**-**Tyr**^{22}-Ser-Leu-**Glu**-Ser-Cys-]



Scheme 1. Synthesis of Tyr^{17}–Tyr^{22} region of GPR1: (a) $Pd(OH)_2/C$, H_2; (b) 4 N HCl–dioxane; (c) SO_3–pyridine; (d) NEt_3; (e) CPF–$NHC_6H_4OCH_2CO_2H$, WSC HCl, HOBt, NMM.



Scheme 2. Synthesis of Tyr^{15}–Tyr^{22} region of GPR1: (a) WSC HCl, HOBt, NMM; (b) 4 N HCl–dioxane; (c) $Pd(OH)_2/C$, H_2; (d) SO_3–pyridine; (e) NEt_3; (f) CPF–$NHC_6H_4OCH_2CO_2H$, WSC HCl, HOBt, NMM.

compounds **1**, **2**, **4**, **8** and **10** showed significantly higher anti-HIV-1 activity in the syncytium assay,[11] which is an effective method for examining the effects of compounds on the early steps of HIV-1 infection. It is interesting to note that compound **1** has inhibitory activity despite being a non-sulfated peptide. The sulfated compounds **8** and **10** corresponding to positions 15–22 in the N-terminal region of GPR1 showed potent inhibitory effects against a broad range of HIV-1, including T cell-tropic (IIIB), dual cell-tropic (GUN1WT), and BT cell-tropic (GUN1V) strains by indirect immunofluorescence assay (IFA).[12] No significant cytotoxicity of the compounds was observed in our study at 1000 μg/mL.

Synthesized peptide analogues are attractive candidates as new lead compounds for the development of chemokine receptor-directed anti-HIV-1 drugs.

Table 1. The anti-HIV-1 activities of **1**–**10**[a]

Compd	1000[b]	200	50	0 (μg/mL)
1	4	62	188	n.t.[c]
2	0	52	193	215
3	55	100	155	192
4	12	88	182	n.t.
5	133	252	228	n.t.
6	124	165	231	n.t.
7	191	210	235	n.t.
8	4	52	258	n.t.
9	121	222	215	n.t.
10	1	66	219	n.t.

[a]C8166/GUN1WT syncytium formation assay. Number of syncytia formed were counted.
[b]No significant cytotoxicity of the compounds at 1000 mg/mL was observed.
[c]Not tested.

Table 2. The anti-HIV-1 activities of **1**–**10**[a]

Compd	IFA[b]			
	CCR5		CXCR4	GPR1
	GUN1WT[c]	IIIB[d]	GUN1WT[e]	GUN1V[f]
1	180	350	66	42
2	65	15	55	25
3	100	150	120	100
4	55	80	60	35
5	>1000	>1000	>1000	660
6	>1000	>1000	>1000	>1000
7	>1000	>1000	>1000	660
8	18	10	42	4.5
9	>1000	>1000	>1000	660
10	10	10	42	4.1

[a]No significant cytotoxicity of the compounds at 1000 μg/mL was observed.
[b]IC_{50} (μM) of each peptide was determined by IFA in comparison with the number of foci induced by each HIV-1 strain without peptides.
[c]NP-2/CD4/CCR5 cells were used for infection with HIV-1 (GUN1WT).
[d]NP-2/CD4/CXCR4 cells were used for infection with HIV-1 (IIIB strain).
[e]NP-2/CD4/CXCR4 cells were used for infection with HIV-1 (GUN1WT).
[f]NP-2/CD4/GPR1 cells were used for infection with HIV-1 (GUN1V).

References and Notes

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