カーを有する二価型リガンドの開発. 日本ケミカルバイオロジー学会 第 6 回年会. 東京, 2011 年 5 月 23-25 日.

70) 大橋南美, 野村 渉, 鳴海哲夫, 奥田善章, 伊倉貞吉,伊藤暢聡, Nancy E. Lewin, 糸谷恭子, Peter M. Blumberg, 玉村啓和. 環境応答性蛍光基を活用した PKC リガンドの orthogonal screening methods. 日本ケミカルバイオロジー学会 第 6 回年会. 東京, 2011 年 5 月 23-25 日.

71) 鳴海哲夫, 清家俊輔, 玉村啓和, Jeffrey W. Bode. 新規アミノ酸モノマーの設計と合成: 縮合剤を用いないα-ペプチド合成への展開. 第9回次世代を担う有機化学シンポジウム. 東京, 2011年5月27-28日.

72) 鳴海哲夫, 新井啓之, 野村 渉, 玉村啓和, 吉村和久, 原田恵嘉, 松下修三. HIV 外被タンパク質 gp120 の構造変化を誘起する低分子 CD4 ミミックの構造活性相関研究. 創薬懇話会. 岡山, 2011 年 7 月 6-7 日

73) 野村 渉, 田中智博, 増田朱美, 鳴海哲夫, 玉村啓和. 2 価結合型リガンドの新規デザインによる CXCR4 の細胞表面における機能解析. 第 5 回バイオ関連化学シンポジウム, 筑波, 2011 年 9 月 12-14 日.

74) 野村 渉, 田中智博, 青木 徹, 相馬 晃, 相川春夫, 鳴海哲夫, 玉村啓和. 2 価型 CXCR4 リガンドの開発と受容体結合機能の解析. 第 48 回ペプチド討論会. 札幌, 2011 年 9 月 27-29 日.

75) 野村 渉, 堤 浩, 阿部清一朗, 森 あつみ, 鳴海哲夫, 相川春夫, 玉村啓和. ロイシンジッパー構造を基にした青色蛍光を発するタグープローブシステム. 第 48 回ペプチド討論会. 札幌, 2011 年 9 月 27-29 日.

76) 野村 渉, 橋本知恵, 中原 徹, 大矢亜紀, 宮内浩典, 大庭賢二, 鳴海哲夫, 相川春夫, 駒野 淳, 山本直樹, 玉村啓和. HIV ワクチンを指向した gp41 の動的構造変化を模倣した抗原ペプチドの開発研究.第 48 回ペプチド討論会. 札幌, 2011 年 9 月 27-29 日.

77) 新井啓之, 鳴海哲夫, 野村 渉, 原田恵嘉, 吉村和久, 松下修三, 玉村啓和. HIV 外被タンパク質 gp120 の構造変化を誘起する低分子 CD4 ミミックの構造活性相関研究. 第 55 回日本薬学会関東支部大会. 東京, 2011 年 10 月 8 日.

78) 尾崎太郎, 浦野恵美子, 鳴海哲夫, 野村 渉, Kasthuraiah Maddali, Yves Pommier, 山本直樹、駒野淳, 玉村啓和. HIV タンパク質 Vpr を基にしたインテグラーゼ阻害剤の構造活性相関研究. 第 55 回日本薬学会関東支部大会. 東京, 2011 年 10 月 8 日.

79) 宍戸美華, 鳴海哲夫, 相川春夫, 玉村啓和. N-(ベンゾイルオキシ)スルホンアミドによるα-β-不飽和エノンの アジリジン化反応. 第 55 回日本薬学会関東支部大会. 東京, 2011 年 10 月 8 日.

80) 清家俊輔, 鳴海哲夫, 相川春夫, 玉村啓和. クロロアルケン型ジペプチドイソスターの立体選択的合成法の開発. 第 55 回日本薬学会関東支部大会. 東京, 2011 年 10 月 8 日.

81) 相馬 晃, 野村 渉, 田中智博, 鳴海哲夫, 相川春夫, 玉村啓和. CXCR4 二量化状態解析のための 2 価結合型リガンドの合成. 第 55 回日本薬学会関東支部大会. 東京, 2011 年 10 月 8 日.

82) 森 あつみ, 野村 渉, 大橋 南美, 鳴海 哲夫, 玉村 啓和. 細胞内蛋白質のタグ - プローブシステムを利用した蛍光イメージングツールの創製. 第 55 回日本薬学会関東支部大会. 東京, 2011 年 10 月 8 日.

83) 鳴海哲夫, 野村 渉, 神戸千秋, 相川春夫, 古田寿昭, 玉村啓和. 光制御型 PKC リガンドの創製と新規光分解性保護基の開発研究.

第 37 回反応と合成の進歩シンポジウム. 徳島, 2011 年 11 月 7-8 日.

84) 相川春夫, 野村 渉, 鳴海哲夫, 田中智博, 玉村啓和. 蛍光ラベル化した二価結合型 CXCR4 リガンドの創製と応用. 第 37 回反応と合成の進歩シンポジウム. 徳島, 2011 年 11 月 7-8 日.

85) 鳴海哲夫, 新井啓之, 吉村和久, 原田恵嘉, 野村 渉, 松下修三, 玉村啓和. 低分子型 CD4 ミミック: HIV 外被タンパク質の構造変化を促す HIV 侵入阻害剤. 第 25 回日本エイズ学会学術集会・総会 日本エイズ学会. 東京, 2011 年 11 月 30 日-12 月 2 日.

86) 橋本知恵, 鳴海哲夫, 野村 渉, 村上 努, 山本直樹, 玉村啓和. HIV-1 第二受容体 CXCR4 の細胞外ドメインを基にしたエイズワクチンの開発研究. 第 25 回日本エイズ学会学術集会・総会 日本エイズ学会. 東京, 2011 年 11 月 30 日-12 月 2 日.

87) 鳴海哲夫, 新井啓之, 吉村和久, 原田恵嘉, 野村 渉, 松下修三, 玉村啓和. HIV 外被タンパク質 gp120 の構造変化誘起を指向した低分子 CD4 ミミックの構造活性相関研究. 第 25 回日本エイズ学会学術集会・総会 日本エイズ学会. 東京, 2011 年 11 月 30 日-12 月 2 日.

88) 尾崎太郎, 浦野恵美子, 鳴海哲夫, 野村渉, Kasthuraiah Maddali, Yves Pommier, 山本直樹, 駒野 淳, Vpr 由来インテグラーゼ阻害剤の構造活性相関. 第 25 回日本エイズ学会学術集会・総会 日本エイズ学会. 東京, 2011 年 11 月 30 日-12 月 2 日.

89) Narumi T, Tanaka T, Nomura W, Aikawa H, Tamamura H. HIV Inhibitors Targeting Entry, Fusion and Integrase. 244th American Chemical Society National Meeting & Exposition Fall 2012, Philadelphia,USA, Aug19-23, 2012.

90) Nomura W, Tanaka T, Aikawa H, Narumi T, Tamamura H. Bivalent Ligands for the Chemokine Receptor CXCR4 Dimer and Their Function. 32nd European Peptide Symposium 2012, Athens, Greek, Sep2-7, 2012.

91) Aikawa H, Matsumoto D, Nozue A, Urano E, Metifiot M, Maddai K, Nomura W, Narumi T, Komano JA, Murakami T, Pommier Y, Yamamoto N, Tamamura H. Structure-activity Relationship Studies of Peptidic HIV Integrase Inhibitors. The 13th Kumamoto AIDS Seminar・GCOE Joint International Symposium. Kumamoto, Japan, Oct 24-26, 2012.

92) 鳴海哲夫, 相川春夫, 玉村啓和. 高活性イミダゾールカルベンの創製研究:窒素原子上の置換基と触媒活性の相関. 日本化学会第 92 春季年会. 横浜, 2012 年 3 月 25-28 日.

93) 紺野 誠, 野村 渉, 鳴海哲夫, 相川春夫, 田中智博, 橋本知恵, 大橋南美, 尾崎太郎, 相馬 晃, 糸谷恭子, 村上 努, 山本直樹, 玉村啓和. ジピコリルアミンおよびアザマクロサイクル環を有する二核金属錯体型 CXCR4 アンタゴニストの構造活性相関研究. 日本化学会第 92 春季年会. 横浜, 2012 年 3 月 25-28 日.

94) 相川春夫, 野村 渉, 鳴海哲夫, 田中智博, 玉村啓和. 長さの調節が可能な二価結合型 CXCR4 リガンドの創製・蛍光ラベル化と応用. 日本化学会第 92 春季年会. 横浜, 2012 年 3 月 25-28 日.

95) 橋本知恵, 野村 渉, 鳴海哲夫, 相川春夫, 山本直樹, 玉村啓和. 宿主タンパク質を基にしたエイズワクチン候補の探索. 日本薬学会第 132 年会. 札幌, 2012 年 3 月 28-31 日.

96) 野村 渉, 湊 夏来, 奥田善章, 大橋南美, 鳴海哲夫, 相川春夫, 玉村啓和. 蛍光性

DAG lactone 誘導体の合成と PKC リガンドの新規スクリーニング法の開発. 日本薬学会第 132 年会. 札幌, 2012 年 3 月 28-31 日.

97) 野村 渉, 森 あつみ, 大橋南美, 相川春夫, 鳴海哲夫, 玉村啓和. プローブ導入型タグ付加標的タンパク質融合体による細胞内動態解析方法の開発. 日本ケミカルバイオロジー学会 第 7 回年会. 京都, 2012 年 6 月 7-9 日.

98) 橋本知恵, 野村 渉, 鈴木貴晴, 大矢亜紀, 浦野恵美子, 鳴海哲夫, 相川春夫, 駒野 淳. 山本直樹, 玉村啓和. HIV 侵入阻害剤としての外被タンパク質 gp41-C34 3 量体. 日本ケミカルバイオロジー学会 第 7 回年会. 京都, 2012 年 6 月 7-9 日.

99) 鳴海哲夫. 高活性イミダゾールカルベンの創製研究:窒素原子上の置換基と触媒活性の相関. 第一回全体班会議「有機分子触媒による未来型分子変換」. 京都, 2012 年 6 月 8-9 日.

100) 鳴海哲夫, 相川春夫, 玉村啓和. 高活性イミダゾールカルベンの創製を目指した構造活性相関研究. 第 56 回日本薬学会関東支部大会. 東京, 2012 年 10 月 13 日.

101) 紺野 誠, 鳴海哲夫, 野村 渉, 玉村啓和. 二核亜鉛錯体型 CXCR4 アンタゴニストの創製. 第 56 回日本薬学会関東支部大会. 東京, 2012 年 10 月 13 日.

102) 鳴海哲夫, 相川春夫, 玉村啓和. アゾリウム塩によるインドール類の Firedel-Crafts 型共役付加反応. 第 5 回有機触媒シンポジウム. 東京, 2012 年 10 月 26-27 日.

103) 鳴海哲夫, 相川春夫, 玉村啓和. アゾリウム塩を酸触媒とするインドール類の Friedel-Crafts 型共役付加反応の開発. 第 38 回反応と合成の進歩シンポジウム. 東京, 2012 年 11 月 5-6 日.

104) 小早川拓也, 鳴海哲夫, 相川春夫, 玉村啓和. 有機銅試薬によるアリル位アルキル化反応を基軸とする Z 型クロロアルケン骨格の立体選択的合成法の開発. 第 38 回反応と合成の進歩シンポジウム. 東京, 2012 年 11 月 5-6 日.

105) 野村 渉, 鳴海哲夫, 相川春夫, 玉村啓和. 細胞内でのタンパク質動態イメージングのための細胞膜透過型 ZIP タグ—プローブシステムの創製. 第 49 回ペプチド討論会. 鹿児島, 2012 年 11 月 7-9 日.

106) 大附寛幸, 一瀬裕太郎, 小林 剛, 原田恵嘉, 吉村和久, 鳴海哲夫, 玉村啓和, 松下修三, 五十嵐樹彦, 三浦智行. 中和感受性を増強する薬剤による抗 HIV-1 治療戦略に向けた新規 SHIV/アカゲザル評価モデルの開発. 第 60 回日本ウイルス学会学術集会. 大阪, 2012 年 11 月 13-15 日.

107) 村上 努, 高野 皓, 藤野真之, 鳴海哲夫, 相川春夫, 橋本知恵, 野村 渉, 山本直樹, 玉村啓和. HIV-1MA/CA 部分ペプチドの細胞内導入による新規 HIV-1 複製制御法の探索. 第 60 回日本ウイルス学会学術集会. 大阪, 2012 年 11 月 13-15 日.

108) 廣田雄樹, 鳴海哲夫, 橋本知恵, 吉村和久, 原田恵嘉, 大附寛幸, 三浦智行, 五十嵐樹彦, 相川春夫, 野村 渉, 松下修三, 玉村啓和. HIV 外被タンパク質 gp120 を標的とするインドール型低分子 CD4 ミミックの創製研究. 第 26 回日本エイズ学会学術集会・総会. 東京, 2012 年 11 月 24-26 日.

109) 相川春夫, 松本大地, 野末愛美, 浦野恵美子, Mathieu Metifiot, Kasthuraiah Maddali, 野村渉, 鳴海哲夫, 駒野 淳, 村上 努, Yves Pommier, 山本直樹, 玉村啓和. ペプチドミメティック型インテグラーゼ阻害剤の構造活性相関研究. 第 26 回日本エイズ学会学術集会・総会. 東京, 2012 年 11 月 24-26 日.

110) 高野 皓, 鳴海哲夫, 相川春夫, 橋本知恵, 藤野真之, 野村 渉, 村上 努, 山本直樹, 玉村啓和. HIV-1 MA, CA タンパク質を基にした新規抗 HIV-1 剤の創製研究. 第 26 回日本エイズ学会学術集会・総会. 東京, 2012 年 11 月 24-26 日.

111) 原田恵嘉, 鳴海哲夫, 玉村啓和,松下修三, 吉村和久. R5 臨床分離株を用いた CD4 類似低分子化合物誘導体に対する in vitro 耐性ウイルス誘導. 第 26 回日本エイズ学会学術集会・総会. 東京, 2012 年 11 月 24-26 日.

112) 鳴海哲夫, 廣田雄樹, 吉村和久, 原田恵嘉, 橋本知恵, 松下修三, 玉村啓和. 低分子型 CD4 ミミック:HIV 外被タンパク質の構造変化を促す HIV 侵入阻害剤. 第 30 回メディシナルケミストリーシンポジウム. 東京, 2012 年 11 月 28-30 日.

113) 高野 皓, 鳴海哲夫, 相川春夫, 野村 渉, 玉村啓和. 水性環境下で効率的に反応する 8-アザクマリニルメチル型光分解性保護基の開発. 日本薬学会第 133 年会. 横浜, 2013 年 3 月 27-30 日.

114) 小早川拓也, 鳴海哲夫, 相川春夫, 玉村啓和. *E* 型クロロアルケン骨格の立体選択的合成法の開発. 日本薬学会第 133 年会. 横浜, 2013 年 3 月 27-30 日.

115) 鳴海哲夫, 相川春夫, 玉村啓和. 新規アゾリウム塩によるインドール類の Friedel-Crafts 型共役付加反応. 日本薬学会第 133 年会. 横浜, 2013 年 3 月 27-30 日.

116) 廣田雄樹, 鳴海哲夫, 橋本知恵, 吉村和久, 原田恵嘉, 大附寛幸, 三浦智行, 五十嵐樹彦, 相川春夫, 野村 渉, 松下修三, 玉村啓和. HIV 外被タンパク質 gp120 を標的としたインドール型低分子 CD4 ミミックの創製. 日本薬学会第 133 年会. 横浜, 2013 年 3 月 27-30 日.

H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

1) HIV立体構造認識抗体誘導ペプチド：特願 2011-082813
2) HIV 立体構造認識抗体誘導ペプチド抗原、及びその合成方法：特願 2011-51432、
3) HIV 立体構造認識抗体誘導ペプチド抗原、及びその合成方法：米国出願番号：13/319,813
4) HIV 立体構造認識抗体誘導ペプチド抗原、及びその合成方法： EP 出願番号：10777543.9

I. 健康危惧情報

該当事項なし

別添４

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
鳴海哲夫、玉村啓和	ペプチドミメティックによる創薬研究	日本生化学会	「生化学」特集号「ペプチド科学と生化学の接点」	日本生化学会	東京	2010	第82巻6号頁515－523
Tanaka T, Nomura W ,Narumi T, Yamamoto N, Tamamura H, et al.	From Reverse to Forward Chemical Genomics: Development of Anti-HIV Agent	Kouji Okamoto (Eds.)	Peptide Science 2009	The Japanese peptide Society	Osaka	2010	105-106
Ohya A, Narumi T, Murakami T, Yamamoto N, Tamamura H, et al.	Synthesis and Evaluation of Artificial Antigen Peptide Based on the Trimeric Form of HIV Fusion Protein	Kouji Okamoto (Ed.)	Peptide Science 2009	The Japanese peptide Society	Osaka	2010	29-32
Nomura W Narumi T, Furuta T, Tamamura H, et al.	Caged DAG-Lactones for Study of Cellular Signaling in a Spatial-and Temporal Specific Manner	Kouji Okamoto (Eds.)	Peptide Science 2009	The Japanese peptide Society	Osaka	2010	347-348
Ohashi N, Nomura W ,Narumi T, Lewin NE, Blumberg PM, Tamamura H, et al.	Fluorescnt-Based Orthogonal Sensing Methods for Double Ealuation in PKC Ligands Screening	Kouji Okamoto (Ed.)	Peptide Science 2009	The Japanese peptide Society	Osaka	2010	353-354
Nomura W, Nakahara T, Hashimoto C, Ohba K , Narumi T, Yamamoto N, Tamamura T.	Synthesis of HIV Gp41 Trimer Mimics Inducing Neutralizing Antibodies Based on Remodeling of Dynamic Structures of HIV-1 Envelope Proteins.	Michal Lebl (Eds.)	Proceedings of the Twenty-Second American Peptide Symposium	American Peptide Society	San Diego	2011	132-133
Nomura W, Tanaka T, Masuda A, Narumi T, Tamamura H.	Development of Bivalent Ligands for CXCR4 with Rigid Linkers and Application to Detection of Cancer Cells.	Michal Lebl (Eds.)	Proceedings of the Twenty-Second American Peptide Symposium	American Peptide Society	San Diego	2011	134-135

Nomura W, Ohashi N, Mori A, Narumi T, Tanaka T, Masuda A, Tsutsumi H, Tamamura H.	Novel Tag-probe Pairs for Fluorescent Imaging of Proteins in Living Cells.	Nobutaka Fujii and Yoshiaki Kiso (Eds.)	Proceedings of the 5th International Peptide Symposium in conjunction with the 47th Japanese Peptide Symposium	The Japanese Peptide Society	Kyoto, Japan	2011	40
Nomura W, Tanaka T, Masuda A, Narumi T, Tamamura H.	Development of a Bivalent Ligand for a Chemokine Receptor CXCR4 by Utilizing Polyproline Helix as a Linker.	Nobutaka Fujii and Yoshiaki Kiso (Eds.)	Proceedings of the 5th International Peptide Symposium in conjunction with the 47th Japanese Peptide Symposium	The Japanese Peptide Society	Kyoto, Japan	2011	220
Narumi T, Bode JW.	α,α-Dicholoroisoxazolidinones for The Synthesis and Chemoselective Peptide Ligation of α-Peptide α-Ketoacids.	Nobutaka Fujii and Yoshiaki Kiso (Eds.)	Proceedings of the 5th International Peptide Symposium in conjunction with the 47th Japanese Peptide Symposium	The Japanese Peptide Society	Kyoto, Japan	2011	178
Hashimoto C, Narumi T, Nomura W, Yamamoto N, Tamamura H.	Synthesis and Evaluation of CXCR4-derived Peptides Targeting the Development of AIDS Vaccines.	Nobutaka Fujii and Yoshiaki Kiso (Eds.)	Proceedings of the 5th International Peptide Symposium in conjunction with the 47th Japanese Peptide Symposium	The Japanese Peptide Society	Kyoto, Japan	2011	103
Nomura W, Tanaka T, Narumi T, Tamamura H, et al.	Development of Designed Bivalent Ligands for CXCR4 and their Function on Receptor Binding	Sakaguchi, K., (Eds.)	Peptide Science 2011	The Japanese Peptide Society	Sapporo	2012	79
Nomura W, Tsutsumi H, Narumi T, Tamamura H, et al.	Intense Blue Fluorescence of Tag-probe Systems Based on a Leucine Zipper Assembly	Sakaguchi, K., (Eds.)	Peptide Science 2011	The Japanese Peptide Society	Sapporo	2012	317
鳴海哲夫, Jeffrey W. Bode	α-ケト酸とヒドロキシアミンの化学選択的な縮合反応によるペプチド合成	木曽良明	最新ペプチド合成技術とその創薬研究への応用	株式会社メディカルドゥ	大阪	2012	68-75

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yamada Y, Narumi T, Nomura W, Matsushita S, Tamamura H, et al.	CD4 mimics targeting the mechanism of HIV	*Bioorg. Med. Chem. Lett.*	20	354-358	2010
Nakahara T, Nomura W, Ohba K, Ohya A, Tanaka T, Hashimoto C, Narumi T, Murakami T, Yamamoto N, Tamamura H, et al.	Remodeling of dynamic structures of HIV-1 envelope proteins leads to synthetic antigen molecules inducing neutralizing antibodies	*Bioconjugate Chem*	21(4)	709-714	2010
Suzuki S, Narumi T, Yamamoto N, Pommier Y, Komano JA, Tamamura H, et al.	Peptidic HIV integrase inhibitors derived from HIV gene products: Structure-activity relationship studies	*Bioorg. Med. Chem.*	18	6771-6775	2010
Narumi T, Matsushita S, Tamamura H, et al.	CD4 mimics targeting the HIV entry mechanism and their hybrid molecules with a CXCR4 antagonist	*Bioorg. Med. Chem. Lett.*	20	5853–5858	2010
Tanaka T, Nomura W, Narumi T, Masuda A, Tamamura H, et al.	Bivalent ligands of CXCR4 with rigid linkers for elucidation of dimerization state in cells	*J. Am. Chem. Soc.*	132 (45)	15899-15901	2010
Nomura W, Narumi T, Tsutsumi H, Tamamura H, et al.	Development of Crosslink-Type Tag-Probe Pairs for Fluorescent Imaging of Proteins	*Biopolymers: Peptide Science*	94	843-852	2010
Narum T, Hayashi R, Oishi S, Fujii N, et al.	Synthesis and Biological Evaluation of Selective CXCR4 Antagonists Containing Alkene Dipeptide Isosteres	Org Biomol Chem	8	616-621	2010
Narumi T, Arai H, Yoshimura K, Harada S, Nomura W, Matsushita S, Tamamura H, et al.	Small Molecular CD4 Mimics as HIV Entry Inhibitors	Bioorg Med Chem	19	6735-6742	2011
Narumi T, Bode JW	α,α-Dichloroisoxazolidinones for The Synthesis and Chemoselective Peptide Ligation of α-Peptide α-Ketoacids.	Heterocycles	82	1515-1525	2011
Nomura W, Ohash N, Okuda Y, Narumi T, Ikura T, Ito N, Tamamura H, et al.	Fluorescence-Quenching Screening of Protein Kinase C Ligands with an Environmentally Sensitive Fluorophore	*Bioconjugate Chem*	22	923–930	2011
Tanaka T, Narumi T, Yamamoto N, Tamamura H et al.	Azamacrocyclic-metal Complexes as CXCR4 Antagonists	*ChemMedChem*	6	834-839	2011

Narumi T, Ohashi N, Serizawa Y, Lewin NE, Blumberg PM, Furuta T, Tamamura H, et al.	Synthetic Caged DAG-lactones for Photochemically-controlled Activation of Protein Kinase C	*ChemBioChem*	12	535–539	2011
Ohashi N, Nomura W, Narumi T, Lewin NE, Itotani K, Blumberg PM, Tamamura H, et al.	Fluorescent-responsive Synthetic C1b Domains of Protein Kinase Cδ as Reporters of Specific High Affinity Ligand Binding	*Bioconjugate Chem.*	22	82-87	2011
Hashimoto C, Tanaka T, Narumi T, Nomura W, Tamamura H, et al.	The Success and Failures of HIV Drug Discovery.	Expert Opin Drug Discovery	6	1067-1090	2011
Nomura W, Komano J, Narumi T, Yamamoto N, Tamamura H , et al.	Synthetic C34 Trimer of HIV-1 gp41 Shows Significant Increase of Inhibition Potency	ChemMedChem	7	205–208	2012
Narumi T, Yamamoto N, Murakami T, Tamamura H, et al.	Conjugation of Cell-penetrating Peptides Leads to Identification of Anti-HIV Peptides from Matrix Proteins	Bioorg Med Chem	20	1468-1474	2012
Hashimoto C, Nomura W, Ohya A, Urano E, Miyauchi K. Narumi T, Aikawa H, Komano AJ, Yamamoto, N, Tamamura H, et al.	Evaluation of a synthetic C34 trimer of HIV-1 gp41 as AIDS vaccines	Bioorg Med Chem	20	3287-3291	2012
Hashimoto C, Nomura W, Narumi T, Yamamoto N, Tamamura H, et al.	Evaluation of a Synthetic C34 Trimer of HIV-1 gp41 as AIDS Vaccines	*Bioorg. Med. Chem.*	20	3287–3291	2012
Narumi T, Tanaka T, Yamamoto N, Tamamura H, et al.	Pharmacophore-based Small Molecule CXCR4 Ligands	*Bioorg. Med. Chem. Lett.*	22	4169-4172	2012
Narumi T, Kobayakawa T, Aikawa H, Seike S, and Tamamura H	Stereoselective Formation of Trisubstituted (Z)-Chloroalkenes Adjacent to a Tertiary Carbon Stereogenic Center by Organocuprate-Mediated Reduction/Alkylation	*Org. Lett.*	14	4490-4493	2012
Narumi T, Arai H, Yoshimura K, Harada S, Matsushita S, and Tamamura H, et al.	CD4 Mimics as HIV Entry Inhibitors: Lead Optimization Studies of the Aromatic Substituents.	*Bioorg. Med. Chem.*	21	2518-2526	2013
Narumi T, Aikawa H, Kobayakawa T, Takano H, Hirota Y, Murakami T, Yamamoto N, Tamamura H, et al.	Low Molecular Weight CXCR4 Ligands with Variable Spacers	*ChemMedChem*	8	118-124	2013

研究成果の刊行物・別刷（抜粋）

Bioorganic & Medicinal Chemistry Letters 20 (2010) 354–358

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

CD4 mimics targeting the mechanism of HIV entry

Yuko Yamada [a], Chihiro Ochiai [a], Kazuhisa Yoshimura [b], Tomohiro Tanaka [a], Nami Ohashi [a], Tetsuo Narumi [a], Wataru Nomura [a], Shigeyoshi Harada [b], Shuzo Matsushita [b], Hirokazu Tamamura [a,*]

[a] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan
[b] Center for AIDS Research, Kumamoto University, Kumamoto 860-0811, Japan

ARTICLE INFO

Article history:
Received 19 September 2009
Revised 20 October 2009
Accepted 22 October 2009
Available online 4 November 2009

Keywords:
CD4 mimic
HIV entry
Synergistic effect
CXCR4

ABSTRACT

A structure–activity relationship study was conducted of several CD4 mimicking small molecules which block the interaction between HIV-1 gp120 and CD4. These CD4 mimics induce a conformational change in gp120, exposing its co-receptor-binding site. This induces a highly synergistic interaction in the use in combination with a co-receptor CXCR4 antagonist and reveals a pronounced effect on the dynamic supramolecular mechanism of HIV-1 entry.

Recently, remarkable success has attended the clinical treatment of HIV-infected and AIDS patients, with 'highly active antiretroviral therapy (HAART)'. This approach involves a combination of two or three agents from two categories: reverse transcriptase inhibitors and protease inhibitors.[1] In addition, the molecular mechanism involved in HIV-entry and -fusion into host cells has been described in detail.[2] The complex interactions of surface proteins on cellular and viral membranes, which are designated as a dynamic supramolecular mechanism of HIV entry, are reported to be crucial to the viral infection. In a first step, an HIV envelope protein, gp120 interacts with a cell surface protein, CD4, leading to a conformational change in gp120 followed by subsequent binding of gp120 to a co-receptor CCR5[3] or CXCR4.[4] CCR5 and CXCR4 are the major co-receptors for the entry of macrophage-tropic (R5-) and T cell line-tropic (X4-) HIV-1, respectively. The interaction of gp120 with CCR5 or CXCR4 triggers entry of another envelope protein, gp41 to the cell membrane and formation of a gp41 trimer-of-hairpins structure, which causes fusion of HIV/cell-membranes and completes the infection.

Informed by this mechanism, a fusion inhibitor, enfuvirtide (fuzeon, Trimers & Roche)[5] and a CCR5 antagonist, maraviroc (Pfizer)[6] in addition to an integrase inhibitor, raltegravir (Merck)[7] have been used clinically. However, serious problems with chemotherapy still persist, including the emergence of viral strains with multi-drug resistance (MDR), considerable adverse effects and high costs. Consequently, development of novel drugs possessing mechanisms of action different from those of the above inhibitors is currently required. We have previously developed selective CXCR4 antagonists[8] and fusion inhibitors.[9] Furthermore, *N*-(4-Bromophenyl)-*N'*-(2,2,6,6-tetramethylpiperidin-4-yl)-oxalamide (**1**) and *N*-(4-chlorophenyl)-*N'*-(2,2,6,6-tetramethylpiperidin-4-yl)-oxalamide (**2**) were previously found using chemical library screening to inhibit syncytium formation by other researchers.[10] **1** and **2** bind to gp120 with binding affinities of K_d = 2.2 μM and 3.7 μM, respectively, blocking the interaction of gp120 with CD4 in the first step of an HIV-1 entry. Thus, in the present study we focus on the development of CD4 mimics that can block the interaction between gp120 and CD4. We have investigated the effect of CD4 mimics on conformational changes of gp120 and on their use in combination use with a CXCR4 antagonist.

Initially, molecular modeling of compound **2** docked into gp120 was carried out using docking simulations performed by the FlexSIS module of SYBYL 7.1 (Tripos, St. Louis) (Fig. 1).[11] The atomic coordinates of the crystal structure of gp120 with soluble CD4 (sCD4) were retrieved from Protein Data Bank (PDB) (entry 1RZJ) (Fig. 1a) and it was observed that Phe^{43} and Arg^{59} of the CD4 have multiple contacts with Asp^{368}, Glu^{370} and Trp^{427} of gp120, which are all conserved residues. An inspection of the environment of compound **2** docked in gp120 revealed the presence of a large cavity around the *p*-position of the phenyl ring of compound **2**, which could interact with the viral surface protein gp120 (Fig. 1b and c). Several analogs of **2** with substituents on the phenyl ring were therefore synthesized.

All compounds except **12** were synthesized by previously published methods (Scheme 1).[10b,12,13] Aniline derivatives (**3**) were coupled with ethyl oxalyl chloride to yield the corresponding ethyl oxalamates **4**. Saponification of the above oxalamates to the corresponding free acids and the subsequent coupling with 4-ami-

* Corresponding author.
E-mail address: tamamura.mr@tmd.ac.jp (H. Tamamura).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.098

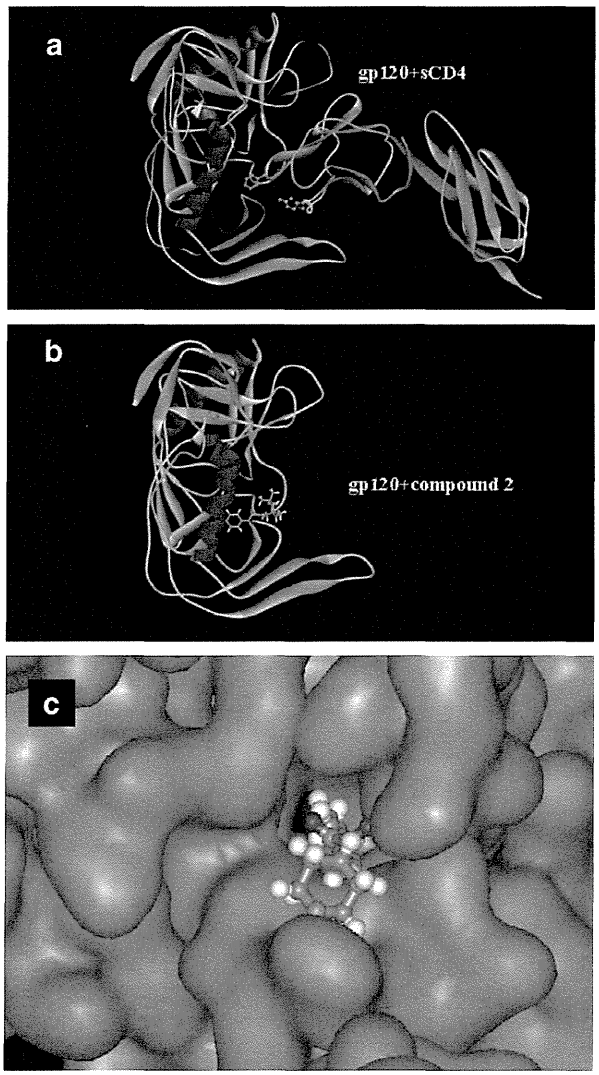
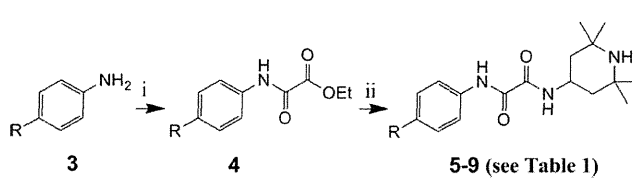
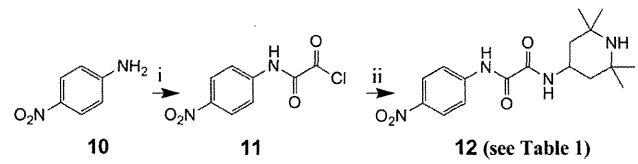


Figure 1. (a) The crystal structure of gp120 with soluble CD4 (sCD4) retrieved from the PDB (entry 1RZJ); (b) docking structure of compound **2** and gp120; (c) a focused figure of (b) shown by space-filling model.



Scheme 1. Reagents and conditions: (i) ethyl oxalyl chloride, Et_3N; (ii) 1 M NaOH; 4-amino-2,2,6,6-tetramethylpiperidine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole, Et_3N.

no-2,2,6,6-tetramethylpiperidine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) yielded compounds **5–9**. In the case of compound **12**, whose amide bond is not stable during the reaction of the saponification of the corresponding oxalamates, an alternative synthetic scheme was used (Scheme 2).[14] The reaction of *p*-nitroaniline (**10**) with oxalyl chloride gave the corresponding oxoacetamide **11**, which was subsequently coupled with 4-amino-2,2,6,6-tetramethylpiperidine to yield the desired compound **12**.



Scheme 2. Reagents and conditions: (i) oxalyl chloride, Et_3N; (ii) 4-amino-2,2,6,6-tetramethylpiperidine, Et_3N.

The anti-HIV activity of the synthetic compounds was evaluated against various viral strains including both laboratory and primary isolates (Table 1). IC_{50} values were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[15] as the concentrations of the compounds which conferred 50% protection against HIV-1-induced cytopathogenicity in PM1/CCR5 cells. Cytotoxicity of the compounds based on the viability of mock-infected PM1/CCR5 cells was also evaluated using the MTT method. CC_{50} values were determined as the concentrations achieving 50% reduction of the viability of mock-infected cells. Compounds **1** and **2** showed potent anti-HIV activity against laboratory isolates, IIIB (X4, Sub B) and 89.6 (dual, Sub B) strains, and compound **2** also possessed potent activity against a primary isolate, an fTOI strain (R5, Sub B). All of the IC_{50} values were between 4 μM and 10 μM. Compound **1** was not tested against primary isolates. The potencies of compounds **1** and **2** are comparable to the reported binding affinities for gp120 (K_d = 2.2 and 3.7 μM, respectively).[10] Several of the new analogs of compounds **1** and **2** showed significant anti-HIV activity. Compound **5**, which has a phenyl group in place of the *p*-chlorophenyl group of compound **2**, did not show significant anti-HIV activity at concentrations below 100 μM against all strains tested except for an fTOI strain (R5, Sub B). This result suggests that a substituent at the *p*-position of the phenyl ring is critical for potent activity. Compound **6**, which has a fluorine atom at the *p*-position of the phenyl ring, showed moderate anti-HIV activity against laboratory isolates, IIIB (X4, Sub B) and 89.6 (dual, Sub B) strains (IC_{50} = 61 and 81 μM, respectively), but, at concentrations below 100 μM, failed to show significant anti-HIV activity against a primary isolate, a KYAG strain (R5, Sub B). Among halogen atoms, fluorine is less suitable than bromine or chlorine as a substituent at the *p*-position of the phenyl ring, as evidenced by compound **6**, which is 8–15-fold less potent than compounds **1** and **2** against IIIB (X4, Sub B) and 89.6 (dual, Sub B) strains. Compound **7**, which has a methyl group at the *p*-position of the phenyl ring, showed relatively more potent activity against IIIB (X4, Sub B) and 89.6 (dual, Sub B) strains (IC_{50} = 23 and 41 μM, respectively) than compound **6**. Compound **7** also showed significant anti-HIV activity against primary isolates, fTOI (R5, Sub B) and KYAG (R5, Sub B) strains (IC_{50} = 16 and 51 μM, respectively). Compound **8**, with a methoxy group at the *p*-position of the phenyl ring, did not show significant anti-HIV activity against all strains tested until a concentration of 100 μM was reached. In the biological assays, derivatives having electron-withdrawing substituents such as bromine, chlorine and fluorine at the *p*-position of the phenyl ring are relatively potent, whereas derivatives having electron-donating groups such as methoxy at this position are not potent. Furthermore, the steric effect of a substituent at the *p*-position of the phenyl ring appears to be critical to anti-HIV activity. The sum of Hammett constants (σ) of benzoic acid substituents[16] shown in Table 1 can be used to evaluate the electron-withdrawing or -donating effect of the substituents on the aromatic ring. The Taft E_s values[16a,17] were used as steric parameters for substituents at the *p*-position of the phenyl ring. The order of potency found for the halogen-containing derivatives in anti-HIV activity against laboratory isolates, IIIB (X4, Sub B) and 89.6 (dual, Sub B), is: compound **1** (R = Br) (σ = 0.23, E_s = −1.16), **2**

Table 1
Hammett constants (σ) and steric effects (E_s) of substituted aromatic rings and anti-HIV activity and cytotoxicity of synthetic compounds

Compd	R[a]	σ[b]	E_s[c]	IC_{50}[e] (μM)				CC_{50}[e] (μM)
				Lab. isolates		Primary isolates		
				IIIB (X4)	89.6 (dual)	fTOI (R5)	KYAG (R5)	
1	Br	0.23	−1.16	4	9	ND	ND	150
2	Cl	0.23	−0.97	8	10	5	>30	170
5	H	0	0	>100	>100	81	>100	350
6	F	0.06	−0.46	61	81	ND	>100	320
7	CH_3	−0.17	−1.24	23	41	16	51	210
8	OCH_3	−0.27	−0.55	>100	>100	ND	>100	340
9	CF_3	0.54	−2.40	ND	27	ND	ND	72
12	NO_2	0.78	−1.77[d]	ND	42	ND	ND	230
sCD4				0.010	0.021	0.0044	ND	ND

[a] See Schemes 1 and 2.
[b] σ = Hammett constant of a substitutent on a benzoic acid derivative.[16]
[c] E_s = steric effect of a substituent at the *para* position on the aromatic ring.[16a,17]
[d] The average value of −1.01 and −2.52, which are E_s values of the NO_2 group, −1.77, was used.
[e] Values are means of at least three experiments (ND = not determined).

(R = Cl) (σ = 0.23, E_s = −0.97), **6** (R = F) (σ = 0.06, E_s = −0.46) and **5** (R = H) (σ = 0, E_s = 0). This is the order of substituents' electron-withdrawing ability and also of their size. Methyl (σ = −0.17, E_s = −1.24) is an electron-donating group, but is almost as bulky as a bromine atom. Thus, the *p*-methyl derivative **7** has relatively potent anti-HIV activity against laboratory isolates, IIIB (X4, Sub B) and 89.6 (dual, Sub B), higher than that of compound **6** (R = F) but lower than that of compound **1** (R = Br) or **2** (R = Cl). The electron-donating ability of a methoxy group is stronger (σ = −0.27), but the bulk size is smaller (E_s = −0.55), than that of a methyl group. Thus, the *p*-methoxy derivative **8** has no significant anti-HIV activity against all strains tested at concentrations below 100 μM. Two derivatives containing bulkier and more potent electron-withdrawing substituents such as trifluoromethyl (R = CF_3) (σ = 0.54, E_s = −2.40) and nitro (R = NO_2) (σ = 0.78, E_s = −1.77) at the *p*-position of the phenyl ring were evaluated. Compounds **9** (R = CF_3) and **12** (R = NO_2) showed significant anti-HIV activity against an 89.6 (dual, Sub B) strain. These are less potent than compounds **1** and **2** and this is perhaps due to the excessive size of the substituents at the *p*-position. This suggests that a certain level of the bulk size and a potent electron-withdrawing ability of the substituents are preferable for anti-HIV activity. It is estimated that a cavity around the *p*-position of the phenyl ring of CD4 mimicking compounds would be optimally filled by bromine (E_s = −1.16) or a methyl group (E_s = −1.24) at *p*-position, and that an electron-deficient aromatic ring might interact tightly with a negatively charged group such as carboxy of Glu^{370}. In isothermal titration calorimetry (ITC) experiments reported elsewhere,[10c] compound **5** (R = H) does not have significant affinity for gp120, and compound **6** (R = F) has less potent affinity for gp120 than compound **2**, consistent with the present data. In all but one of the compounds, no significant cytotoxicity was detected (CC_{50} >150 μM, Table 1), the exception being compound **9** (R = CF_3) (CC_{50} = 72 μM). Compounds **7** and **12** have relatively low cytotoxicities, compared to compounds **1** and **2**.

Fluorescence activated cell sorting (FACS) analysis was performed[15] to investigate whether these synthetic compounds interact with gp120 inducing the conformational change necessary for the approach of an anti-envelope antibody or a co-receptor to the gp120. The profile of binding of an anti-envelope CD4-induced monoclonal antibody, 4C11, to the Env-expressing cell surface (an R5-HIV-1 strain, JR-FL,-infected PM1 cells) pretreated with the above CD4 mimic analogs was examined. Comparison of the binding of 4C11 to the cell surface was measured in terms of the mean fluorescence intensity (MFI), and is shown in Figure 2. Pretreatment of the Env-expressing cells with compound **2** (MFI = 38.42) produced a remarkable increase in binding affinity for 4C11, similar to that observed in pretreatment with sCD4 (MFI = 37.90). This is consistent with the results in the previous paper[10] where it was reported that compound **2** enhances the binding of gp120 to the 17b monoclonal antibody which recognizes the co-receptor binding site of gp120. Env-expressing cells, which were not pretreated with sCD4 or a CD4 mimic compound, did not show significant binding affinity for 4C11 (Fig. 2, blank). The increase in binding affinity for monoclonal antibodies may be due to conformational changes in gp120, which were caused by the interaction of sCD4 or a CD4 mimic with gp120. It is hypothesized that such conformational changes involve the exposure of the co-receptor binding site of gp120 (the V3 loop), which is hidden internally, since the binding of gp120 to 17b is enhanced. Compound **5**, which failed to show significant anti-HIV activity, and compounds **7**, **9** and **12**, which had significant anti-HIV activity, were assessed in the FACS analysis. The profile of the binding of 4C11 to the Env-expressing cell surface pretreated with compound **5** (MFI = 14.34) was similar to that of the blank (MFI = 11.24), suggesting that compound **5** offers no significant enhancement of binding affinity for 4C11. This result is compatible with the anti-HIV activity of compound **5**. The profile of the binding of 4C11 to the Env-expressing cell surface pretreated with compound **7** (MFI = 38.33) was entirely similar to that of compound **2** used as a pretreatment. Pretreatment of the cell surface with compounds **9** and **12** (MFI = 29.09 and 30.01, respectively) produced a slightly lower enhancement of binding affinity for 4C11, compared to those of compounds **2** and **7** as pretreatments. However, in the ITC experiments reported elsewhere,[10c] compound **9** (R = CF_3) has a high affinity for gp120, comparable to that of compound **2**, but compound **12** (R = NO_2) does not have significant affinity for gp120, indicating that these are not consistent with the current FACS studies, possibly due to the difference in the assay systems. Although the anti-HIV activity of **7** is weaker than that of compound **2**, the level of compound **7** inducing an enhancement of binding affinity of gp120 for 4C11 is comparable to that of compound **2**. The concentration of compounds used in the FACS analysis was 100 μM, much beyond the IC_{50} values of compounds **2** and **7**. A concentration of 100 μM would be also sufficient for the expression of anti-HIV activity caused by compounds **2** and **7**.

An effect on the use of compound **2** combined with another entry inhibitor was investigated. Analysis of the synergistic effects of anti-HIV agents was performed according to the median effect principle using the CalcuSyn version 2 computer program[18] to estimate IC_{50} values of compounds in different combinations. Combination indices (CI) were estimated from the data evaluated using the MTT assay

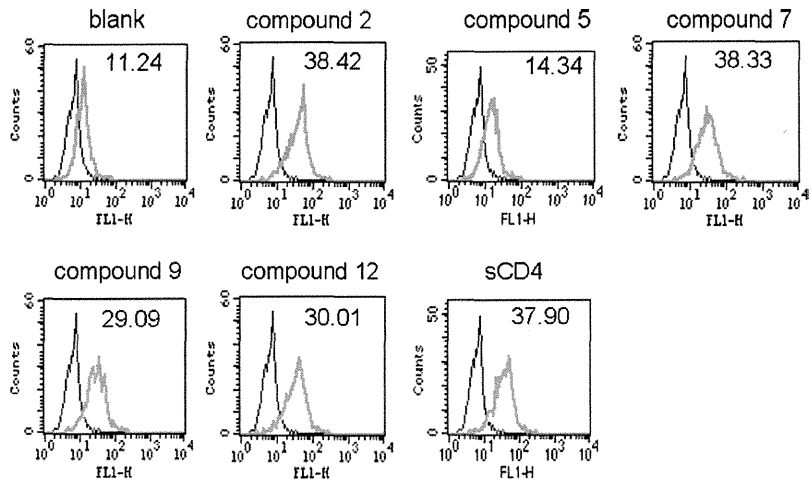


Figure 2. JR-FL (R5, Sub B) chronically infected PM1 cells were preincubated with 100 μM of a CD4 mimic or sCD4 (11 nM) for 15 min, and then incubated with an anti-HIV-1 mAb, 4C11, at 4 °C for 15 min. The cells were washed with PBS, and fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG antibody was used for antibody-staining. Flow cytometry data for the binding of 4C11 (green lines) to the Env-expressing cell surface in the presence of sCD4 or a CD4 mimic are shown among gated PM1 cells along with a control antibody (anti-human CD19: black lines). Data are representative of the results from a minimum of two independent experiments. The number at the top of each graph shows the mean fluorescence intensity (MFI) of the antibody 4C11.

Table 2
Combination indices (CI) for compound **2** or sCD4 and a CXCR4 antagonist, T140, against an HIV IIIB strain

Combination	HIV strain	CI values at different IC[a]		
		IC_{50}	IC_{75}	IC_{90}
2 + T140	IIIB	0.786	0.713	0.655
sCD4 + T140	IIIB	0.705	0.528	0.400

[a] The multiple-drug effect analysis reported by Chou et al. was used to analyze *the effects of combinational uses of compounds.*[18] CI <0.9: *synergy*, 0.9 < CI < 1.1: additivity, CI >1.1: antagonism.

(Table 2).[15] Compound **2** showed a highly remarkable synergistic anti-HIV activity with a co-receptor CXCR4 antagonist, T140,[8a] against an X4-HIV-1 strain, IIIB at various IC values (IC_{50}, IC_{75} and IC_{90}). However, sCD4 exhibited a higher synergistic effect (lower CI values) with T140 (Table 2). The interaction of sCD4 or a CD4 mimic with gp120 would expose the co-receptor-binding site of gp120, and the co-receptor CXCR4 could then easily approach gp120. Thus, an inhibitory effect of a CXCR4 antagonist would be meaningful, and a significant synergistic effect might also be brought about by a combination of sCD4 or a CD4 mimic and T140.

In summary, a series of CD4 mimic compounds were synthesized and evaluated for their anti-HIV activity. Several compounds showed significant anti-HIV activity with relatively low cytotoxicity. SAR studies showed that a certain level of size and electron-withdrawing ability of the substituents at the *p*-position of the phenyl ring are suitable for potent anti-HIV activity. In addition, the treatment of Env-expressing cells with several CD4 mimicking compounds causes a conformational change, exposing the co-receptor-binding site of gp120 externally. Thus, a CD4 mimic exhibited a remarkable synergistic effect with a co-receptor antagonist. These compounds are essential probes directed to the dynamic supramolecular mechanism of HIV entry, and important leads for the cocktail therapy of AIDS.

Acknowledgments

This work was supported by Mitsui Life Social Welfare Foundation, Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and Health and Labour Sciences Research Grants from Japanese Ministry of Health, Labor, and Welfare.

References and notes

1. Mitsuya, H.; Erickson, J. In *Textbook of AIDS Medicine*; Merigan, T. C., Bartlett, J. G., Bolognesi, D., Eds.; Williams & Wilkins: Baltimore, 1999; pp 751–780.
2. Chan, D. C.; Kim, P. S. *Cell* **1998**, *93*, 681.
3. (a) Alkhatib, G.; Combadiere, C.; Broder, C. C.; Feng, Y.; Kennedy, P. E.; Murphy, P. M.; Berger, E. A. *Science* **1996**, *272*, 1955; (b) Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P. D.; Wu, L.; Mackay, C. R.; LaRosa, G.; Newman, W.; Gerard, N.; Gerard, C.; Sodroski, J. *Cell* **1996**, *85*, 1135; (c) Deng, H. K.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Marzio, P. D.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. *Nature* **1996**, *381*, 661; (d) Doranz, B. J.; Rucker, J.; Yi, Y. J.; Smyth, R. J.; Samson, M.; Peiper, S. C.; Parmentier, M.; Collman, R. G.; Doms, R. W. *Cell* **1996**, *85*, 1149; (e) Dragic, T.; Litwin, V.; Allaway, G. P.; Martin, S. R.; Huang, Y.; Nagashima, K. A.; Cayanan, C.; Maddon, P. J.; Koup, R. A.; Moore, J. P.; Paxton, W. A. *Nature* **1996**, *381*, 667.
4. Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. *Science* **1996**, *272*, 872.
5. Wild, C. T.; Greenwell, T. K.; Matthews, T. J. *AIDS Res. Hum. Retroviruses* **1993**, *9*, 1051.
6. (a) 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; (b) Price, D. A.; Armour, D.; De Groot, M.; Leishman, D.; Napier, C.; Perros, M.; Stammen, B. L.; Wood, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4633.
7. (a) Cahn, P.; Sued, O. *Lancet* **2007**, *369*, 1235; (b) Grinsztejn, B.; Nguyen, B.-Y.; Katlama, C.; Gatell, J. M.; Lazzarin, A.; Vittecoq, D.; Gonzalez, C. J.; Chen, J.; Harvey, C. M.; Isaacs, R. D. *Lancet* **2007**, *369*, 1261.
8. (a) Tamamura, H.; Xu, Y.; Hattori, T.; Zhang, X.; Arakaki, R.; Kanbara, K.; Omagari, A.; Otaka, A.; Ibuka, T.; Yamamoto, N.; Nakashima, H.; Fujii, N. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 877; (b) Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z.; Peiper, S. C. *Angew. Chem., Int. Ed.* **2003**, *42*, 3251; (c) Tanaka, T.; Nomura, W.; Narumi, T.; Esaka, A.; Oishi, S.; Ohashi, N.; Itotani, K.; Evans, B. J.; Wang, Z.; Peiper, S. C.; Fujii, N.; Tamamura, H. *Org. Biomol. Chem.* **2009**, *7*, 3805.
9. Otaka, A.; Nakamura, M.; Nameki, D.; Kodama, E.; Uchiyama, S.; Nakamura, S.; Nakano, H.; Tamamura, H.; Kobayashi, Y.; Matsuoka, M.; Fujii, N. *Angew. Chem., Int. Ed.* **2002**, *41*, 2937.
10. (a) Zhao, Q.; Ma, L.; Jiang, S.; Lu, H.; Liu, S.; He, Y.; Strick, N.; Neamati, N.; Debnath, A. K. *Virology* **2005**, *339*, 213; (b) Schön, A.; Madani, N.; Klein, J. C.; Hubicki, A.; Ng, D.; Yang, X.; Smith, A. B., III; Sodroski, J.; Freire, E. *Biochemistry* **2006**, *45*, 10973; (c) Madani, N.; Schön, A.; Princiotto, A. M.; LaLonde, J. M.; Courter, J. R.; Soeta, T.; Ng, D.; Wang, L.; Brower, E. T.; Xiang, S.-H.; Do Kwon, Y.; Huang, C.-C.; Wyatt, R.; Kwong, P. D.; Freire, E.; Smith, A. B., III; Sodroski, J. *Structure* **2008**, *16*, 1689; (d) Haim, H.; Si, Z.; Madani, N.; Wang, L.; Courter, J. R.; Princiotto, A.; Kassa, A.; DeGrace, M.; McGee-Estrada, K.; Mefford, M.; Gabuzda, D.; Smith, A. B., III; Sodroski, J. *ProS Pathogens* **2009**, *5*, 1.
11. The structure of compound **2** was built in Sybyl and minimized with the MMFF94 force field and partial charges. (see: Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.) Docking was then performed using FlexSIS through its SYBYL

module, into the crystal structure of gp120 (PDB, entry 1RZJ). The binding site was defined as residues Val^{255}, Asp^{368}, Glu^{370}, Ser^{375}, Ile^{424}, Trp^{427}, Val^{430} and Val^{475}, and included residues located within a radius 4.4 Å. The ligand was considered to be flexible, and all other options were set to their default values. Figures were generated with ViewerLite version 5.0 (Accelrys Inc., San Diego, CA).

12. *For example, the synthesis of compound* **7**: To a solution of ethyl oxalyl chloride (0.400 mL, 3.48 mmol) in THF (20 mL) were added triethylamine (Et_3N) (0.480 mL, 3.48 mmol) and *p*-toluidine (373 mg, 3.48 mmol) with stirring at 0 °C. The reaction mixture was allowed to warm to room temperature, and then stirred for 6 h. After removal by filtration of the resulting salts, the filtrate was concentrated under reduced pressure. The residue was extracted with EtOAc (50 mL), and the extract was washed successively with brine (20 mL), 1 M HCl (20 mL × 2), brine (20 mL), saturated $NaHCO_3$ (20 mL × 2) and brine (20 mL × 3), then dried over $MgSO_4$. Concentration under reduced pressure gave the crude ethyl oxalamate, which was used without further purification. To a solution of the crude ethyl oxalamate (640 mg, 3.09 mmol) in THF (30 mL) were added aqueous 1 M NaOH (3.40 mL, 3.40 mmol), water (50 mL) and MeOH (20 mL) with stirring at 0 °C. The reaction mixture was allowed to warm to room temperature, and then stirred for 20 h. After the addition of aqueous 1 M HCl (5 mL), MeOH and THF were evaporated under reduced pressure. The residue was acidified to pH 2 with 1 M HCl, and extracted with EtOAc (50 mL × 2). The combined organic layer was washed with brine (20 mL × 3), and dried over $MgSO_4$. Concentration under reduced pressure gave the crude acid, which was used for the next reaction without further purification. To a solution of the above crude acid (514 mg, 2.87 mmol) in THF (10 mL) were added 1-hydoxybenzotriazole (484 mg, 3.16 mmol), 4-amino-2,2,6,6-tetramethylpiperidine (446 μL, 2.58 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (606 mg, 3.16 mmol) and Et_3N (0.439 mL, 3.16 mmol) with stirring at 0 °C. The reaction mixture was allowed to warm to room temperature, and then stirred for 20 h. After evaporation of THF, the residue was dissolved in $CHCl_3$ (50 mL). The mixture was washed with saturated $NaHCO_3$ (20 mL × 2) and brine (20 mL × 3), and dried over $MgSO_4$. Concentration under reduced pressure gave the crude crystalline mass. The usual work-up followed by recrystallization from EtOAc–*n*-hexane gave the title compound **7** (363 mg, 1.14 mmol, 39.8%) as colorless crystals, mp = 176 °C; δ_H (400 MHz; $CDCl_3$) 1.07 (1H, m, NH), 1.16 (6H, s, CH_3), 1.29 (6H, s, CH_3), 1.44 (2H, m, CH_2), 1.91 (1H, d, *J* 3.7, C*H*H), 1.94 (1H, d, *J* 3.7, CH*H*), 2.34 (3H, s, CH_3), 4.25 (1H, m, CH), 7.17 (2H, d, *J* 8.3, ArH), 7.33 (1H, m, NH), 7.50 (2H, d, *J* 8.4, ArH), 9.18 (1H, s, NH); HRMS (FAB), *m*/*z* calcd for $C_{18}H_{28}N_3O_2$ $(MH)^+$ 318.2182, found 318.2173.
13. McFarland, C.; Vicic, D. A.; Debnath, A. K. *Synthesis* **2006**, 807.
14. *The synthesis of compound* **12**: To a solution of Et_3N (417 μL, 3.00 mmol) and 4-nitroaniline (138 mg, 1.00 mmol) in THF (1.3 mL) was added oxalyl dichloride (85.8 μL, 1.00 mmol) with stirring at 0 °C. After being stirred for 30 min at 0 °C, Et_3N (167 μL, 1.20 mmol) and 4-amino-2,2,6,6-tetramethylpiperidine (156 μL, 0.90 mmol) were added. The reaction mixture was stirred for 6 h at 0 °C. After removal by filtration of the resulting salts, the filtrate was concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (20 mL), and the mixture was washed successively with brine (10 mL), saturated $NaHCO_3$ (10 mL × 2) and brine (10 mL × 3), and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with $CHCl_3$–MeOH (9:1) gave 42.4 mg (0.122 mmol, 13.5%) of the title compound **12** as colorless crystals, mp = 190 °C; δ_H (400 MHz; $CDCl_3$) 1.09 (1H, m, NH), 1.17 (6H, s, CH_3), 1.29 (6H, s, CH_3), 1.43 (2H, m, CH_2), 1.92 (1H, d, *J* 3.8, C*H*H), 1.95 (1H, d, *J* 3.8, CH*H*), 4.28 (1H, m, CH), 7.29 (1H, m, NH), 7.82 (2H, d, *J* 9.1, ArH), 8.28 (2H, d, *J* 9.1, ArH), 9.55 (1H, s, NH); HRMS (FAB), *m*/*z* calcd for $C_{17}H_{25}N_4O_4$ $(MH)^+$ 349.1876, found 349.1871.
15. Yoshimura, K.; Shibata, J.; Kimura, T.; Honda, A.; Maeda, Y.; Koito, A.; Murakami, T.; Mitsuya, H.; Matsushita, S. *AIDS* **2006**, *20*, 2065.
16. (a) Chapman, N. B.; Shorter, J. *Advances in Linear Free Energy Relationship*; Plenum Press: London, 1972; (b) Chapman, N. B.; Shorter, J. *Correlation Analysis in Chemistry*; Plenum Press: London, 1978; (c) Hansch, C.; Leo, A. J.; Hoekman, D. *Exploring QSAR, Hydrophobic, Electronic, and Steric Constants*; American Chemical Society: Washington, DC, 1995.
17. Taft, R. W. In *Steric Effects in Organic Chemistry*; Newman, M. S., Ed.; John Wiley: New York, 1956; p 556.
18. (a) Chou, T. C.; Talalay, P. *J. Biol. Chem.* **1977**, *252*, 6438; (b) Chou, T. C.; Hayball, M. P. *CalcuSyn*, 2nd ed.; Biosoft: Cambridge, UK, 1996.

Bioorganic & Medicinal Chemistry 18 (2010) 6771–6775

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Peptidic HIV integrase inhibitors derived from HIV gene products: Structure–activity relationship studies

Shintaro Suzuki [a], Kasthuraiah Maddali [b], Chie Hashimoto [a], Emiko Urano [c], Nami Ohashi [a], Tomohiro Tanaka [a], Taro Ozaki [a], Hiroshi Arai [a], Hiroshi Tsutsumi [a], Tetsuo Narumi [a], Wataru Nomura [a], Naoki Yamamoto [c,d], Yves Pommier [b], Jun A. Komano [c], Hirokazu Tamamura [a,*]

[a] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan
[b] Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255, USA
[c] AIDS Research Center, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo 162-8640, Japan
[d] Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore

ARTICLE INFO

Article history:
Received 28 June 2010
Revised 17 July 2010
Accepted 20 July 2010
Available online 25 July 2010

Keywords:
HIV integrase
Inhibitory peptide
Glu-Lys pairs
Ala-scan

ABSTRACT

Structure–activity relationship studies were conducted on HIV integrase (IN) inhibitory peptides which were found by the screening of an overlapping peptide library derived from HIV-1 gene products. Since these peptides located in the second helix of Vpr are considered to have an α-helical conformation, Glu-Lys pairs were introduced into the *i* and *i* + 4 positions to increase the helicity of the lead compound possessing an octa-arginyl group. Ala-scan was also performed on the lead compound for the identification of the amino acid residues responsible for the inhibitory activity. The results indicated the importance of an α-helical structure for the expression of inhibitory activity, and presented a binding model of integrase and the lead compound.

1. Introduction

Highly active anti-retroviral therapy (HAART), which involves a combination of two or three agents from two categories, reverse transcriptase inhibitors and protease inhibitors, has brought us remarkable success in the clinical treatment of HIV-infected and AIDS patients.[1] However, it has been accompanied by serious clinical problems including the emergence of viral strains with multidrug resistance (MDR), considerable adverse effects and nonetheless high costs. As a result, new categories of anti-HIV agents operating with mechanisms of action different from those of the above inhibitors are sought. HIV-1 integrase (IN) is a critical enzyme for the stable infection of host cells since it catalyzes the insertion of viral DNA into the genome of host cells, by means of strand transfer and 3′-end processing reactions and thus it is an attractive target for the development of anti-HIV agents. Recently, the first IN inhibitor, raltegravir (Merck),[2] has appeared in a clinical setting. It is assumed that the activity of IN must be negatively regulated during the translocation of the viral DNA from the cytoplasm to the nucleus to prevent auto-integration. The virus, as well as the host cells, must encode mechanism(s) to prevent auto-integration since the regulation of IN activity is critical for the virus to infect cells.[3] By screening a library of overlapping peptides derived from HIV-1 SF2 gene products we have found three Vpr-derived peptides, **1**, **2** and **3**, which possess significant IN inhibitory activity, indicating that IN inhibitors exist in the viral pre-integration complex (PIC).[4] The above inhibitory peptides, **1**, **2** and **3**, are consecutive overlapping peptides (Fig. 1). Compounds **4** and **5** are 12- and 18-mers from the original Vpr-sequence with the addition of an octa-arginyl group[5] into the C-terminus for cell membrane permeability, respectively. Compounds **4** and **5** have IN inhibitory activity and anti-HIV activity. Here, we report structure–activity relationship studies on these lead compounds for the development of more potent IN inhibitors.

2. Results and discussion

To determine which lead compound is most suitable for further experiments, five peptides **6–10**, which were elongated by one amino acid starting with compound **4** and extended ultimately to **5**, were synthesized (Fig. 2). Judging by the 3′-end processing and strand transfer reactions in vitro,[6] these peptides **4–10** had similar inhibitory potencies (Table 1). As a result, we concluded that 12 amino acid residues derived from the original Vpr-sequence are of sufficient for IN inhibitory activity, and any peptide among **4–10** is a suitable lead.

* Corresponding author.
E-mail address: tamamura.mr@tmd.ac.jp (H. Tamamura).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.07.050

1 AGVEAIIRILQQLLF
2 IIRILQQLLFIHFRI
3 LQQLLFIHFRIGCQH
4 Ac-LQQLLFIHFRIG-RRRRRRRR-NH_2
5 Ac-EAIIRILQQLLFIHFRIG-RRRRRRRR-NH_2

Figure 1. Amino acid sequences of compounds **1–5**. Compounds **1–3** are consecutive overlapping peptides with free N-/C-terminus. These were found by the IN inhibitory screening of a peptide library derived from HIV-1 gene products. Compounds **4** and **5** are cell penetrative leads of IN inhibitors.

4 Ac-LQQLLFIHFRIG-RRRRRRRR-NH_2
6 Ac-ILQQLLFIHFRIG-RRRRRRRR-NH_2
7 Ac-RILQQLLFIHFRIG-RRRRRRRR-NH_2
8 Ac-IRILQQLLFIHFRIG-RRRRRRRR-NH_2
9 Ac-IIRILQQLLFIHFRIG-RRRRRRRR-NH_2
10 Ac-AIIRILQQLLFIHFRIG-RRRRRRRR-NH_2
5 Ac-EAIIRILQQLLFIHFRIG-RRRRRRRR-NH_2

Figure 2. Amino acid sequences of compounds **6–10**, which are elongated by one amino acid from compound **4** to **5**.

Table 1
IC_{50} values of compounds **4–10** toward the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN

Compound	IC_{50} (μM)	
	3′-End processing	Strand transfer
4	0.13 ± 0.02	0.06 ± 0.01
5	0.09 ± 0.01	0.04 ± 0.01
6	0.10 ± 0.01	0.07 ± 0.01
7	0.13 ± 0.02	0.11 ± 0.01
8	0.26 ± 0.04	0.11 ± 0.03
9	0.11 ± 0.01	0.07 ± 0.01
10	0.08 ± 0.01	0.05 ± 0.01

Structural analysis showed that the Vpr-derived peptides, **1**, **2** and **3**, are located in the second helix of Vpr and were thus considered to have an α-helical conformation.[7] Compound **5** was adopted as a lead for the development of compounds with an increase in α-helicity since a longer peptide is likely to form a more stable α-helical structure than a shorter one. Initially, Glu (E) and Lys (K) were introduced in pairs into compound **5** at the i and $i+4$ positions. In general, such disposition of Glu-Lys pairs at i and $i+4$ positions is considered to cause an increase in α-helicity due to formation of an ionic interaction of a β-carboxy group of Glu and an ε-amino group of Lys. Several analogs of **5** with Glu-Lys pairs were synthesized by Fmoc-solid phase peptide synthesis (Fig. 3). In the inhibitory assay against the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN in vitro, compounds **11** and **15** showed more potent inhibitory activities than **5** (Table 2). Substitution of Glu-Lys for His^{14}-Gly^{18} or Ile^{3}-Leu^{7} caused no decrease in IN inhibitory activity but a significant increase in activity, suggesting that Ile^3, Leu^7, His^{14} and Gly^{18} are not indispensable for activity. Substitution of Glu-Lys for Ala^2-Ile^6 or Gln^9-Ile^{13} caused a slight decrease in IN inhibitory activity against the 3′-end processing and strand transfer reactions (compounds **12** and **13**), indicating that Ala^2 and/or Ile^6, and Gln^9 and/or Ile^{13} are partly required for activity. Substitution of Glu-Lys for Ile^4-Gln^8 caused a 2–4-fold decrease in IN inhibitory activity against the 3′-end processing and strand transfer reactions (compound **14**), showing that Ile^4 and/or Gln^8 are essential for activity. Substitution of Glu-Lys for Leu^{11}-Phe^{15} caused an eightfold decrease in IN inhibitory activity against the 3′-end processing reaction and a 1.5-fold decrease in IN inhibitory activity against the strand transfer reaction (compound **16**), indicating that Leu^{11} and/or Phe^{15} are indispensable for activity, especially for inhibition against 3′-end processing. Compound **17** has two substitutions of Glu-Lys for His^{14}-Gly^{18} and for Ala^2-Ile^6, which are common to compounds **11** and **12**, respectively. A twofold decrease in both IN inhibitory activities of compound **17** is mostly due to the substitution for Ala^2-Ile^6 common to **12**, although **17** is slightly less active than **12** in both IN inhibitory assays.

1 5 10 15
5 Ac-EAIIRILQQLLFIHFRIG-RRRRRRRR-NH_2
11 Ac-EAIIRILQQLLFI*E*FRI*K*-RRRRRRRR-NH_2
12 Ac-E*E*IIR*K*LQQLLFIHFRIG-RRRRRRRR-NH_2
13 Ac-EAIIRILQ*E*LLF*K*HFRIG-RRRRRRRR-NH_2
14 Ac-EAI*E*RIL*K*QLLFIHFRIG-RRRRRRRR-NH_2
15 Ac-EA*E*IRI*K*QQLLFIHFRIG-RRRRRRRR-NH_2
16 Ac-EAIIRILQQL*E*FIH*K*RIG-RRRRRRRR-NH_2
17 Ac-E*E*IIR*K*LQQLLFI*E*FRI*K*-RRRRRRRR-NH_2

Figure 3. Amino acid sequences of compounds **11–17**, into which Glu-Lys pairs have been introduced.

Table 2
IC_{50} values of compounds **5** and **11–17** toward the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN

Compound	IC_{50} (μM)	
	3′-End processing	Strand transfer
5	0.09 ± 0.01	0.04 ± 0.01
11	0.05 ± 0.01	0.01 ± 0.001
12	0.12 ± 0.01	0.047 ± 0.01
13	0.14 ± 0.02	0.065 ± 0.01
14	0.23 ± 0.03	0.15 ± 0.002
15	0.04 ± 0.01	0.031 ± 0.01
16	0.71 ± 0.21	0.06 ± 0.004
17	0.18 ± 0.06	0.08 ± 0.02

Anti-HIV activity of these compounds was assessed by an MT-4 Luc system, in which MT-4 cells were stably transduced with the firefly luciferase expression cassette by a murine leukemia viral vector. MT-4 Luc cells constitutively express high levels of luciferase. HIV-1 infection significantly reduces luciferase expression due to the high susceptibility of MT-4 cells to HIV-1 infection. Protection of MT-4 Luc cells from HIV-1-induced cell death maintains the luciferase signals at high levels. In addition, the cytotoxicity of test compounds can be evaluated by a decrease of luciferase signals in these MT-4 Luc systems. The parent compound **5** showed significant anti-HIV activity at concentrations above 1.25 μM, as reported previously (Fig. 4).[4] Compound **15** showed a significant inhibitory effect against HIV-1 replication, and is thus comparable to compound **5**. Compounds **11**, **14** and **16** also displayed weak antiviral effects at concentrations of 2.5 and 5.0 μM and compounds **12**, **13** and **17** failed to show any significant anti-HIV activity. These results suggest that there is a positive correlation between IN inhibitory activity and anti-HIV activity of the compounds. None of these compounds showed significant cytotoxic effects at concentrations below 5.0 μM.

The structures of compounds **5** and **11–17** were assessed by CD spectroscopy. Because the aqueous solubility of these peptides is not high the peptides were dissolved in 0.1 M phosphate buffer, containing 50% MeOH at pH 5.6. The CD spectra suggest that the parent compound **5**, which has no Glu-Lys pair, forms a typical α-helical structure, and the other compounds, with the exception of **11** and **15**, form α-helical structures similarly (Fig. 5). The order of strength of α-helicity is **12**, **16** > **14** > **17** > **5** > **13**. Compounds **11** and **15** have no characteristic pattern, although IN inhibitory activities of both compounds are superior to that of the parent

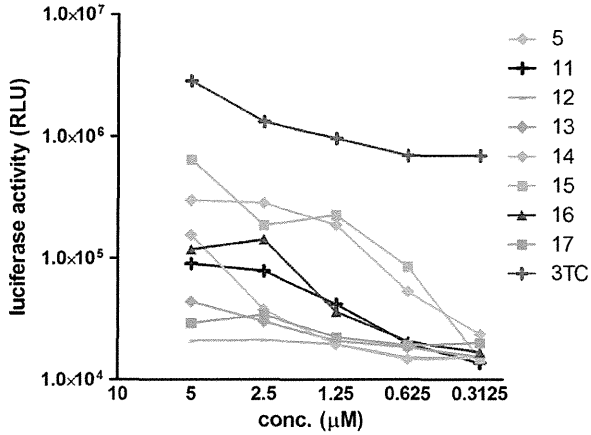


Figure 4. Luciferase signals in MT-4 Luc cells infected with HIV-1 in the presence of different concentrations of compounds **11–17**. Luciferase activity is expressed as relative luciferase units (RLU). 3TC is an HIV reverse transcriptase inhibitor, which was used as a positive control.

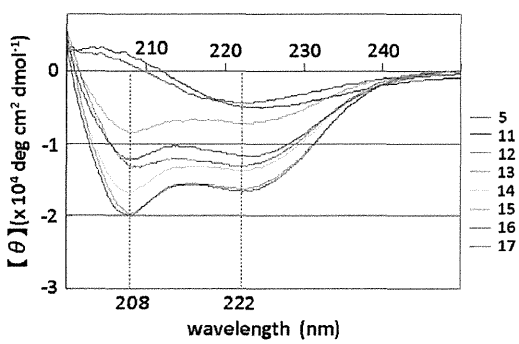


Figure 5. CD spectra of compounds **5** and **11–17** (5 μM) in 0.1 M phosphate buffer, pH 5.6 containing 50% MeOH at 25 °C.

compound **5**. Replacement of His^{14}-Gly^{18} and Ile^{3}-Leu^{7} by Glu-Lys in compounds **11** and **15**, respectively, caused a significant decrease in α-helicity, possibly due to formation of unfavorable salt bridges such as Glu^{14}-Arg^{16} and Glu^{3}-Arg^{5}. Introduction of a Glu-Lys pair into Gln^{9}-Ile^{13} in compound **13** caused a slight decrease in α-helicity, possibly due to interference in the formation of a salt bridge of Glu^{1}-Arg^{5} by that of Arg^{5}-Glu^{9}. In the other analogs, increases in α-helicity were observed to result from the introduction of Glu-Lys pairs as we had initially postulated. Overall, there is no positive correlation between IN inhibitory or anti-HIV activity and the degree of α-helicity of the compounds.

In order to identify the amino acid residues responsible for IN inhibitory and anti-HIV activities of these peptides, an Ala-scan of compound **4** was performed (Fig. 6). Compounds **18–22**, **25**, **27** and **29** showed IN inhibitory activities against the 3′-end processing and strand transfer reactions similar to those of **4** (Table 3). Ala-substitution for Leu^{7}, Gln^{8}, Gln^{9}, Leu^{10}, Leu^{11}, His^{14}, Arg^{16} or Gly^{18} did not cause any significant change in either of IN inhibitory activities, indicating that the replaced amino acids are not essential for IN inhibition. Ala-substitution for Phe^{12}, Ile^{13}, Phe^{15} or Ile^{17} gave compounds **23**, **24**, **26** and **28**, which were 2–4 times less active in both the IN inhibitory assays, suggesting that Phe^{12}, Ile^{13}, Phe^{15} and Ile^{17} are indispensable for IN inhibition. Assessment of anti-HIV activity in the MT-4 Luc system showed that all compounds **18–29** produced dose-dependent inhibition of HIV-1 replication, although they displayed cytotoxicity at 10 μM (**4**, **19–23**, **26** and **27**) or above 5 μM (**24** and **25**) (Fig. 7). Compounds **23** and **24**,

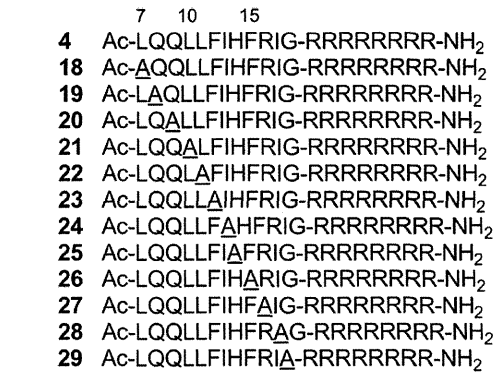


Figure 6. Amino acid sequences of compounds **18–29** based on an Ala-scan of compound **4**. Position numbers correspond to alignment with compound **5**.

Table 3
IC_{50} values of compounds **18–29** toward the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN

Compound	IC_{50} (μM)	
	3′-End processing	*Strand transfer*
4	0.11 ± 0.03	0.05 ± 0.01
18	0.12 ± 0.004	0.08 ± 0.01
19	0.13 ± 0.02	0.06 ± 0.01
20	0.10 ± 0.004	0.06 ± 0.01
21	0.12 ± 0.02	0.07 ± 0.01
22	0.13 ± 0.003	0.06 ± 0.01
23	0.34 ± 0.06	0.18 ± 0.03
24	0.33 ± 0.02	0.22 ± 0.01
25	0.13 ± 0.01	0.06 ± 0.01
26	0.25 ± 0.02	0.12 ± 0.01
27	0.11 ± 0.01	0.05 ± 0.01
28	0.20 ± 0.03	0.16 ± 0.02
29	0.09 ± 0.01	0.09 ± 0.01

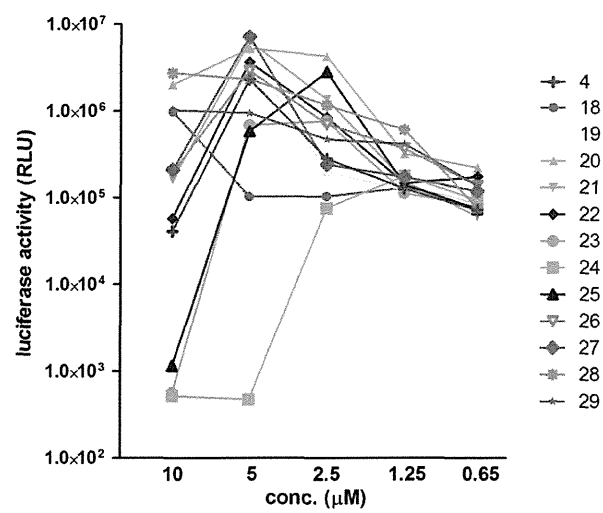


Figure 7. Luciferase signals in MT-4 Luc cells infected with HIV-1 in the presence of various concentrations of compounds **18–29**. Luciferase activity was valued as RLU.

with Ala-substitution for Phe^{12} and Ile^{13}, respectively, showed weaker inhibitory activity than **4** at 5 μM. Consequently, Phe^{12} and Ile^{13} were deemed to be critical for activity, which is consistent with the IN inhibitory activity results. A control peptide isomer of **5** (Ac-QIFEHLAGIIQILRFLRI-R_8-NH_2) did not show anti-HIV activity at

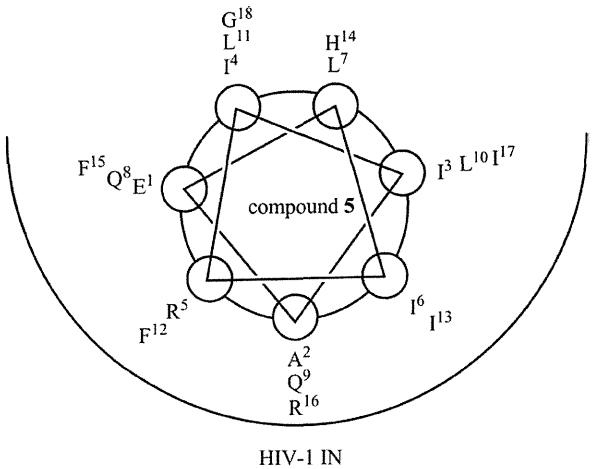


Figure 8. Brief presumed drawing of the binding model of HIV-1 IN and compound **5**.

concentrations below 10 μM, suggesting that the original Vpr-sequence, with the exceptions of Phe^{12}, Ile^{13}, Phe^{15} and Ile^{17}, is critical for activity.

The assumption that compound **5** forms an α-helical structure when binding to HIV-1 IN suggests the binding model of IN and **5** shown in Figure 8, as **5** forms an α-helical structure in 50% aqueous MeOH solution. In this model, Phe^{12}, Ile^{13}, Phe^{15} and Ile^{17}, which were identified by the Ala-scan experiment as critical residues, are located in the pocket of IN. His^{14} and Gly^{18}, which can be replaced by Glu-Lys with an increase of activity in compound **11**, are located outside of the pocket of IN. Ile^3 and Leu^7 can also be replaced by Glu-Lys while retaining activity in compound **15**, and Leu^7 is located outside of the pocket, whereas Ile^3 is located in the edge of the pocket. Compounds **11** and **15** might form α-helical structures when binding to IN, although **11** or **15** does not show α-helicity in the CD spectrum. Thus, these compounds might retain IN inhibitory activity. This binding model is compatible with the results of structure–activity relationship studies involving Glu-Lys substitution and Ala-scan. The reason for decreases in IN inhibitory and anti-HIV activity of compounds **12** and **17**, which show increases of α-helicity, are possibly due to substitution of Glu-Lys for Ala^2 and Ile^6, which are located in the pocket of IN. The reason for a decrease in activity of compounds **14** and **16**, which show increased α-helicity, might be due to substitution of Lys for Gln^8 and Phe^{15}, respectively, which are located in the pocket of IN. The reason for decreases in IN inhibitory and anti-HIV activity of compound **13**, which also shows a decrease of α-helicity, are possibly due to substitution of Glu-Lys for Gln^9 and Ile^{13}, which are located in the pocket of IN.

3. Conclusion

In the present study, structure–activity relationship studies were performed on Vpr-derived peptides **4** and **5**, which had been previously identified as HIV-1 IN inhibitors.[4] The Glu-Lys substitution experiments and Ala-scan data suggest that several amino acid residues of **4** and **5** are indispensable for IN inhibitory and anti-HIV activities, and a binding model of IN and **5** were proposed. Furthermore, two novel compounds **11** and **15**, which contained Glu-Lys pairs and showed more potent IN inhibitory activities than compound **5**, were found. These data including the binding model should be useful for the development of potent HIV-1 IN inhibitors based on Vpr-peptides.

4. Experimental

4.1. Chemistry

All peptides were synthesized by the Fmoc-based solid-phase method. The synthetic peptides were purified by RP-HPLC and identified by ESI-TOF-MS. Fmoc-protected amino acids and reagents for peptide synthesis were purchased from Novabiochem, Kokusan Chemical Co., Ltd and Watanabe Chemical Industries, Ltd. Protected peptide resins were constructed on NovaSyn TGR resins (0.26 meq/g, 0.025 and 0.0125 mmol scales for Glu-Lys substitution and Ala-scan peptides, respectively). All peptides were synthesized by stepwise elongation techniques. Each cycle involves (i) deprotection of an Fmoc group with 20% (v/v) piperidine/DMF (10 mL) for 15 min and (ii) coupling with 5.0 equiv of Fmoc-protected amino acid, 5.0 equiv of diiopropylcarbodiimide (DIPCI) and 5.0 equiv of 1-hydroxybenzotriazole monohydrate ($HOBt{\cdot}H_2O$) in DMF (3 mL) for 90 min. N-Terminal α-amino groups of Glu-Lys substitution and Ala-scan peptides were acetylated with 100 equiv of acetic anhydride in DMF (10 mL). Cleavage from the resin and side chain deprotection were carried out by stirring for 1.5 h with *m*-cresol (0.25 mL), thioanisole (0.75 mL), 1,2-ethanedithiol (0.75 mL) and TFA (8.25 mL). After removal of the resins by filtration, the filtrate was concentrated under reduced pressure, the crude peptides were precipitated in cooled diethyl ether and purified by preparative RP-HPLC on a Cosmosil 5C18-AR II column (10 × 250 mm, Nacalai Tesque, Inc.) with a LaChrom Elite HTA system (Hitachi). The HPLC solvents employed were water containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). All peptides were purified using a linear gradient of solvents A and B over 30 min at a flow rate of 3 $cm^3\ min^{-1}$. The purified peptides were identified by ESI-TOF-MS (Brucker Daltonics micrOTOF-2focus) (shown in Table S1 in Supplementary data). All peptides were obtained after lyophilization as fluffy white powders of the TFA salts. The purities of these peptides were checked by analytical HPLC on a Cosmosil 5C18-ARII column (4.6 × 250 mm, Nacalai Tesque, Inc.) eluted with a linear gradient of solvents A and B at a flow rate of 1 $cm^3\ min^{-1}$, and eluted products were detected by UV at 220 nm (shown in Figs. S1–S3 in Supplementary data).

4.2. Expression and purification of F185K/C280S HIV-1 integrase from *Escherichia coli*

Plasmid encoding IN1–288/F185K/C280S was expressed in *Escherichia coli* strain C41. The solubility of the mutant protein was examined in a crude cell lysate, as follows. Cells were grown in 1 L of culture medium containing 100 μg/mL of ampicillin at 37 °C until the optical density of the culture at 600 nm was between 0.4 and 0.9. Protein expression was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.1 mM. After 2 h, the cells were collected by centrifugation at 6000 rpm for 30 min. After removal of the supernatant, the cells were resuspended in HED buffer (20 mM HEPES, pH 7.5, 1 mM EDTA, 1 mM DTT) with 0.5 mg/mL lysozyme and stored on ice for 30 min. The cells were sonicated until the solution exhibited minimal viscosity then it was centrifuged at 15,000 rpm for 30 min. After removal of the supernatant, the pellet was dissolved in TNM buffer (20 mM Tris/HCl, pH 8.0, 1 M NaCl, 2 mM 2-mercaptoethanol) with 5 mM imidazole and stored on ice for 30 min. The cells were then centrifuged at 15,000 rpm for 30 min and the supernatant was collected. The supernatant was then filtered through 0.45 μm filter cartridge and applied to a HisTrap column at 1 mL/min flow rate. After loading, the column was washed with 10 volume of TNM buffer with 5 mM imidazole. Protein was eluted with a linear gradient of 500 mM imidazole, containing TNM buf-

fer. Fractions containing IN were pooled and checked with SDS–PAGE.

4.3. CD spectroscopy of peptides with Glu-Lys substitution

CD measurements were performed on a JASCO J720 spectropolarimeter equipped with thermo-regulator (JASCO Corp., Ltd), using 5 μM of peptides dissolved in 0.1 M phosphate buffer, pH 5.6 containing 50% MeOH. UV spectra were recorded at 25 °C in a quartz cell 1.0 mm path length, a time constant of 1 s, and a 100 nm/min scanning speed with 0.1 nm resolution.

4.4. Integrase assays

Expression and purification of the recombinant IN in *E. coli* were performed as previously reported with addition of 10% glycerol to all buffers. Oigonucleotide substrates were prepared as described.[6] Integrase reactions were performed in 10 μL with 400 nM of recombinant IN, 20 nM of 5′-end [^{32}P]-labeled oligonucleotide substrate and inhibitors at various concentrations. Solutions of 10% DMSO without inhibitors were used as controls. Reaction mixtures were incubated at 37 °C (60 min) in buffer containing 50 mM MOPS, pH 7.2, 7.5 mM $MgCl_2$, and 14.3 mM 2-mercaptoethanol. Reactions were stopped by addition of 10 μL of loading dye (10 mM EDTA, 98% deionized formamide, 0.025% xylene cyanol and 0.025% bromophenol blue). Reactions were then subjected to electrophoresis in 20% polyacrylamide–7 M urea gels. Gels were dried and reaction products were visualized and quantitated with a Typhoon 8600 (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Densitometric analyses were performed using ImageQuant from Molecular Dynamics Inc. The concentrations at which enzyme activity was reduced by 50% (IC_{50}) were determined using 'Prism' software (GraphPad Software, San Diego, CA) for nonlinear regression to fit dose–response data to logistic curve models.

4.5. Replication assays (MT-4 luciferase assays)

MT-4 luciferase cells (1×10^3 cells) grown in 96-well plates were infected with HIV-1_{HXB2} in the presence of various concentrations of peptides. At 6–7 days post-infection, cells were lysed and the luciferase activities were measured using the Steady-Glo assay kit (Promega), according to the manufacturer's protocol. Chemiluminescence was detected with a Veritas luminometer (Promega).

Acknowledgments

N.O. and T.T. are supported by JSPS research fellowships for young scientists. This work was supported by Mitsui Life Social Welfare Foundation, Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Health and Labour Sciences Research Grants from Japanese Ministry of Health, Labor, and Welfare, and by the NIH Intramural Program, Center for Cancer Research, US National Cancer Institute.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.050.

References and notes

1. Mitsuya, H.; Erickson, J. In *Textbook of AIDS Medicine*; Merigan, T C., Bartlett, J. G., Bolognesi, D., Eds.; Williams & Wilkins: Baltimore, 1999; pp 751–780.
2. (a) Cahn, P.; Sued, O. *Lancet* **2007**, *369*, 1235; (b) Grinsztejn, B.; Nguyen, B.-Y.; Katlama, C.; Gatell, J. M.; Lazzarin, A.; Vittecoq, D.; Gonzalez, C. J.; Chen, J.; Harvey, C. M.; Isaacs, R. D. *Lancet* **2007**, *369*, 1261.
3. (a) Farnet, C. M.; Bushman, F. D. *Cell* **1997**, *88*, 483; (b) Chen, H.; Engelman, A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15270; (c) Gleenberg, I. O.; Herschhorn, A.; Hizi, A. *J. Mol. Biol.* **2007**, *369*, 1230; (d) Gleenberg, I. O.; Avidan, O.; Goldgur, Y.; Herschhorn, A.; Hizi, A. *J. Biol. Chem.* **2005**, *280*, 21987; (e) Hehl, E. A.; Joshi, P.; Kalpana, G. V.; Prasad, V. R. *J. Virol.* **2004**, *78*, 5056; (f) Tasara, T.; Maga, G.; Hottiger, M. O.; Hubscher, U. *FEBS Lett.* **2001**, *507*, 39; (g) Gleenberg, I. O.; Herschhorn, A.; Goldgur, Y.; Hizi, A. *Arch. Biochem. Biophys.* **2007**, *458*, 202.
4. Suzuki, S.; Urano, E.; Hashimoto, C.; Tsutsumi, H.; Nakahara, T.; Tanaka, T.; Nakanishi, Y.; Maddali, K.; Han, Y.; Hamatake, M.; Miyauchi, K.; Pommier, Y.; Beutler, J. A.; Sugiura, W.; Fuji, H.; Hoshino, T.; Itotani, K.; Nomura, W.; Narumi, T.; Yamamoto, N.; Komano, J. A.; Tamamura, H. *J. Med. Chem.* **2010**, *53*, 5356.
5. Suzuki, T.; Futaki, S.; Niwa, M.; Tanaka, S.; Ueda, K.; Sugiura, Y. *J. Biol. Chem.* **2002**, *277*, 2437.
6. (a) Yan, H.; Mizutani, T. C.; Nomura, N.; Tanaka, T.; Kitamura, Y.; Miura, H.; Nishizawa, M.; Tatsumi, M.; Yamamoto, N.; Sugiura, W. *Antivir. Chem. Chemother.* **2005**, *16*, 363; (b) Marchand, C.; Zhang, X.; Pais, G. C. G.; Cowansage, K.; Neamati, N.; Burke, T. R., Jr.; Pommier, Y. *J. Biol. Chem.* **2002**, *277*, 12596; (c) Semenova, E. A.; Johnson, A. A.; Marchand, C.; Davis, D. A.; Tarchoan, R.; Pommier, Y. *Mol. Pharmacol* **2006**, *69*, 1454; (d) Leh, H.; Brodin, P.; Bischerour, J.; Deprez, E.; Tauc, P.; Brochon, J. C.; LeCam, E.; Coulaud, D.; Auclair, C.; Mouscadet, J. F. *Biochemistry* **2000**, *39*, 9285; (e) Marchand, C.; Neamati, N.; Pommier, Y. In Vitro Human Immunodeficiency Virus Type 1 Integrase Assays. In *Methods in Enzymology (Drug-Nucleic Acid Interactions)*; Chaires, J. B., Waring, M. J., Eds.; Elsevier: Amsterdam, 2001; Vol. 340, pp 624–633.
7. Morellet, N.; Bouaziz, S.; Petitjean, P.; Roques, B. P. *J. Mol. Biol.* **2003**, *327*, 215.

Bioorganic & Medicinal Chemistry Letters 20 (2010) 5853–5858

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

CD4 mimics targeting the HIV entry mechanism and their hybrid molecules with a CXCR4 antagonist

Tetsuo Narumi [a], Chihiro Ochiai [a], Kazuhisa Yoshimura [b], Shigeyoshi Harada [b], Tomohiro Tanaka [a], Wataru Nomura [a], Hiroshi Arai [a], Taro Ozaki [a], Nami Ohashi [a], Shuzo Matsushita [b], Hirokazu Tamamura [a,*]

[a] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan
[b] Center for AIDS Research, Kumamoto University, Kumamoto 860-0811, Japan

ARTICLE INFO

Article history:
Received 14 June 2010
Revised 22 July 2010
Accepted 26 July 2010
Available online 3 August 2010

Keywords:
CD4
HIV entry
Hybrid molecule
gp120

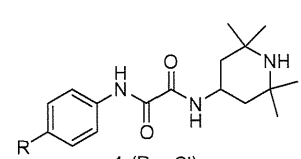
ABSTRACT

Small molecules behaving as CD4 mimics were previously reported as HIV-1 entry inhibitors that block the gp120–CD4 interaction and induce a conformational change in gp120, exposing its co-receptor-binding site. A structure–activity relationship (SAR) study of a series of CD4 mimic analogs was conducted to investigate the contribution from the piperidine moiety of CD4 mimic **1** to anti-HIV activity, cytotoxicity, and CD4 mimicry effects on conformational changes of gp120. In addition, several hybrid molecules based on conjugation of a CD4 mimic analog with a selective CXCR4 antagonist were also synthesized and their utility evaluated.

The infection of host cells by HIV-1 takes place in multiple steps via a dynamic supramolecular mechanism mediated by two viral envelope glycoproteins (gp41, gp120) and several cell surface proteins (CD4, CCR5/CXCR4).[1] Cell penetration begins with the interaction of gp120 with the primary receptor CD4. This induces conformational changes in gp120, leading to the exposure of its V3 loop allowing the subsequent binding of gp120 to a co-receptor, CCR5[2] or CXCR4.[3]

N-(4-Chlorophenyl)-*N'*-(2,2,6,6-tetramethyl-piperidin-4-yl)oxalamide (NBD-556: **1**) and the related compounds NBD-557 (**2**) and YYA-021 (**3**) have been identified as a novel class of HIV-1 entry inhibitors, which exert potent cell fusion and virus cell fusion inhibitory activity at low micromolar levels (Fig. 1).[4] Furthermore, compound **1** can also induce thermodynamically favored conformational changes in gp120 similar to those caused by CD4 binding. The X-ray crystal structure of gp120 complexed with CD4 revealed the presence of a hydrophobic cavity, the Phe43 cavity, which is penetrated by the aromatic ring of Phe^{43} of CD4.[5] Molecular modeling revealed that compound **1** is also inserted into the Phe43 cavity, the *para*-chlorophenyl group of **1** entering more deeply than the phenyl ring of Phe^{43} of CD4 and interacting with the conserved gp120 residues such as Trp^{427}, Phe^{382}, and Trp^{112}.[4c] The modeling also suggested that an oxalamide linker forms hydrogen bonds with carbonyl groups of the gp120 backbone peptide bonds. Our model of **1** docked into gp120 revealed that eight other gp120 residues, Val^{255}, Asp^{368}, Glu^{370}, Ser^{375}, Ile^{424}, Trp^{427}, Val^{430}, and Val^{475} are located within a 4.4 Å-radius of **1** and that a large cavity exists around the *p*-position of the aromatic ring of **1**.[4e] Based on these observations, we conducted a structure–activity relationship (SAR) study of a series of analogs of CD4 mimics with substituents at the *p*-position of the aromatic ring. This study revealed that a certain size and electron-withdrawing ability of the substituents are indispensable for potent anti-HIV activity.[4e]

Although several reported SAR studies of **1** have revealed the contributions of the phenyl ring and the oxalamide linker of **1** to the binding affinity with gp120, the anti-HIV activity and the CD4 mimicry on conformational changes of gp120,[4] there has been, to the best of our knowledge, no prior report describing SAR studies of the piperidine ring of **1**. In this paper, the contributions of the piperidine ring of **1** to the anti-HIV activity, CD4 mimicry and cytotoxicity were investigated through the SAR studies focused on the piperidine ring of **1**. Furthermore, to apply the utility of CD4 mimics to the development of potent anti-HIV agents, a series of the



1 (R = Cl)
2 (R = Br)
3 (R = Me)

Figure 1. NBD-556 (**1**) and related compounds.

* Corresponding author.
E-mail address: tamamura.mr@tmd.ac.jp (H. Tamamura).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.106