Brief Articles

Synthesis and Antiviral Activity of New Anti-HIV Amprenavir Bioisosteres

Luc Rocheblave,[†,‡] Frédéric Bihel,[†,‡] Céline De Michelis,[†,‡] Ghislaine Priem,[†,‡] Jérôme Courcambeck,[†,‡] Brice Bonnet,[§] Jean-Claude Chermann,[‡] and Jean-Louis Kraus*[,†,‡]

Laboratoire de Chimie Biomoléculaire, Faculté des Sciences de Luminy, case 901, Université de la Méditerranée, 70 route Léon Lachamp, 13288 Marseille Cedex 9, France, INSERM U322, Campus Universitaire de Luminy, BP33, 13273 Marseille Cedex 9, France, and ENSSPICAM, Université Aix-Marseille III, 13397 Marseille Cedex 20, France

Received January 23, 2002

Starting from the chemical structure of the recent FDA-approved anti-HIV drug Amprenavir (Agenerase), a potent HIV-protease inhibitor, we have designed new series of Amprenavir bioisoteres in which the methylene group of the benzyl group was replaced by a sulfur atom. This structural modification has required an original multistep synthesis. Unfortunately, introduction of the sulfur atom abolished or drastically decreased both inhibitory activity on recombinant HIV protease and HIV infection protection on MT4 cell cultures.

Introduction

Inhibition of the virally encoded aspartyl protease[1−5] of the human immunodeficiency virus (HIV) results in the production of immature noninfectious virions.[6,7] This discovery has led to new strategies for the treatment of AIDS, based on the inhibition of this essential viral protein. Most successful to date in this area is the use of structures[8] that mimic the transition state[9] from the cleavage of the enzyme's natural substrate. Among the recently approved anti-HIV protease drugs, Amprenavir (Agenerase), or VX-478, is of particular interest because of its reduced size compared to that of Indinavir, Ritonavir, or Saquinavir.[10] Recently Amprenavir monotherapy was terminated because of moderate results in clinical trials.[11] Part of this failure was due to the induction of mutations and associated resistances. Indeed, most clinically used antiprotease drugs[12] have induced multiple HIV mutations,[13−15] bringing to light the necessity of finding new anti-HIV drugs.

Taking into account these structural and clinical observations of Amprenavir, we investigated the possibility of modifying the structure of Amprenavir, using the concept of bioisosterism.[16] Our approach was to use this bioisosterism concept by replacing the methylene group of the benzyl substituent in the Amprenavir derivative by a sulfur atom (Figure 1).

This bioisosteric replacement could induce several modifications in terms of size, shape, electronic distribution, lipophilicity, chemical reactivity, and hydrogen bonding capacity. Among these physicochemical modifications, chemical and biochemical aspects could be of major interest. We describe herein the original synthesis of Amprenavir bioisostere mixture **10** and the corresponding pure diastereomeric forms (**10a$_1$**, **10a$_2$**, **10b$_1$**, **10b$_2$**). These compounds were evaluated for their ability to inhibit recombinant HIV-1 protease and for their anti-HIV activity on infected MT4 cells.

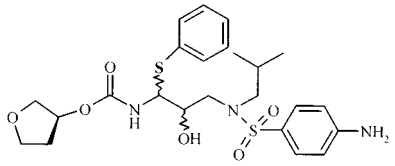


Figure 1. Structure of the Amprenavir bioisostere **10**.

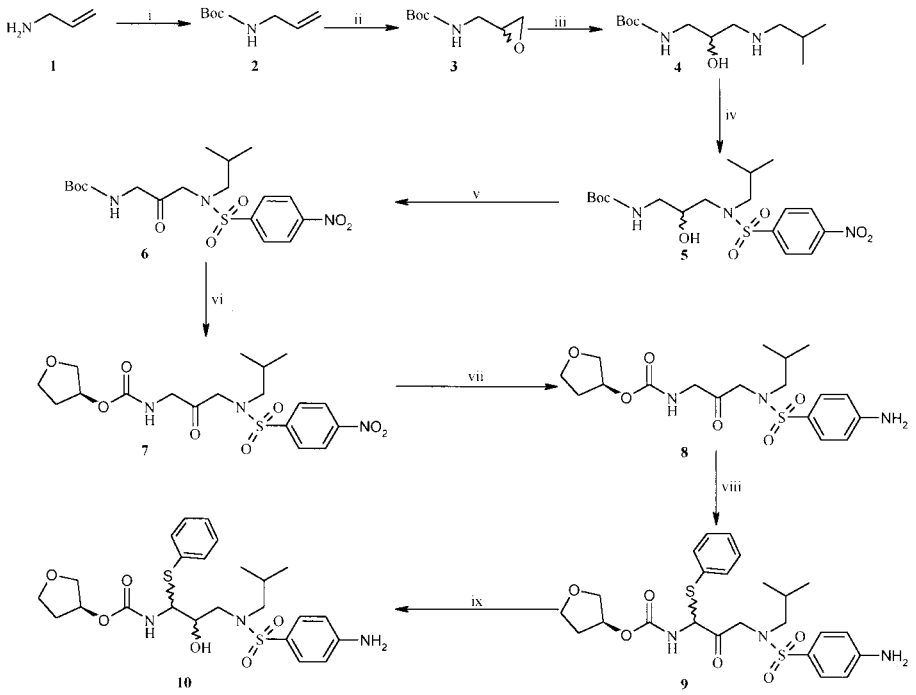
Chemistry

The synthesis of this new series of Amprenavir analogues required the original synthetic route shown in Scheme 1. Allylamine **1** treated with Boc_2O in CH_2Cl_2 at 0 °C led to the corresponding *N*-allylcarbamate **2**, which after oxidation[17] with *m*CPBA gave the corresponding epoxide **3**. Selective opening of these epoxides using isobutylamine in methanol, followed by N-sulfonylation[18] using 4-nitrobenzenesulfonyl chloride in acetonitrile, resulted in the formation of the corresponding N-substituted sulfonamidohydroxypropylene derivative **5**. Oxidation of this intermediate in Swern conditions[19] led to the corresponding ketone **6** in 88% yield. After Boc deprotection of compound **6**, the resulting free amine derivative coupled with *S*(+)-3-hydroxytetrahydrofuran using DSC (*N*,*N*′-disuccinimidyl carbonate[20]) led to carbamate **7**, which gave the desired 4-aminobenzenesulfonamide ketone **8** after reduction with activated palladium. By introduction of only one thiophenoxy group, regioselectively at the α position of the ketone intermediate was overcome using *S*-phenylbenzene thiosulfonate[21] as a reagent in the presence of lithium diisopropylamide (LDA) in dry dichloroethane. The use of LDA[22] appeared to be suitable to allow specifically the monocarbanion formation at the α position of the ketone. In contrast, as we have previously reported,[23] the use of sodium hydride followed by *n*-butyllithium

* To whom correspondence should be addressed. Phone: +33-0-4-91-82-91-41. Fax: +33-0-4-91-82-94-16. E-mail: kraus@luminy.univ-mrs.fr.
† Université de la Méditerranée.
‡ Campus Universitaire de Luminy.
§ Université Aix-Marseille III.

10.1021/jm0208323 CCC: $22.00
Published on Web 06/20/2002

Scheme 1[a]



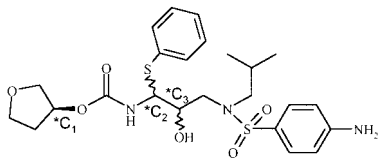
[a] Reagents: (i) Boc_2O, CH_2Cl_2; (ii) *m*CPBA, CH_2Cl_2; (iii) $^tBuNH_2$, MeOH; (iv) 4-nitrobenzenesulfonyl chloride, K_2CO_3, CH_3CN; (v) TFAA, DMSO, Et_3N, CH_2Cl_2; (vi) (1) TFA, CH_2Cl_2, (2) TEA, DSC, (*S*)-3-hydroxytetrahydrofuran, CH_2Cl_2; (vii) H_2, Pd/C, EtOH; (viii) $PhSSO_2Ph$, LDA, $ClCH_2CH_2Cl$; (ix) $NaBH_4$, MeOH.

allowed the formation of the 1,3-dithiophenoxy ketone intermediate. The last step of the synthesis required reduction of the obtained α-sulfanyl ketone intermediate **9**. Compound **10** was obtained from **9** by reduction using $NaBH_4$ as reducing reagent. Compound **10** was a racemic mixture of four diastereoisomers (**10a$_1$**, **10a$_2$**, **10b$_1$**, **10b$_2$**). Since separation by HPLC could not be achieved directly, separation of diastereoisomers was achieved as follows. First, the mixture of the α-thiophenoxy ketone diastereoisomers **9** was separated on a chiralpack AS column into isomers **9a** and **9b**. It should be emphasized that the different fractions containing **9a** and **9b** had to be kept cold (−30 °C) in order to avoid racemization through the enolization process. These fractions were directly reduced into their corresponding α-thiophenoxy alcohols using $NaBH_4$ as reagent. Reduction of ketones **9a** and **9b** led respectively to diastereoisomeric mixtures (**10a$_1$** + **10a$_2$**) and (**10b$_1$** + **10b$_2$**). Separation of the diastereoisomeric mixtures (**10a$_1$** + **10a$_2$**) and (**10b$_1$** + **10b$_2$**) was achieved using the same HPLC protocole (α-thiophenoxy alcohols were less sensitive to racemization than their corresponding ketone precursors). The four diastereoisomers (**10a$_1$**, **10a$_2$**, **10b$_1$**, **10b$_2$**) were isolated, characterized (^1H and ^{13}C NMR, mass spectroscopy, elemental analyses), and screened for antiviral evaluation.

Results and Discussion

Compounds **10** obtained as mixture of diastereoisomers were first screened for their inhibitory effects on HIV replication in MT4 cell cultures. Anti-HIV activities were determined according to a procedure described by Rey et al.[24,25] by the observation of the fusogenic effect (*syncytium* formation assay). Antiviral potencies (EC_{50}) and cytotoxicities (CC_{50}) are reported in Table 1.

Table 1. Amprenavir Analogues and Associated Biological Activities



compd	configuration *C1	*C2	*C3	$[\alpha]_D$[a]	IC_{50}[b] (μM)	EC_{50}[c] (μM)	CC_{50}[d] (μM)	SI[e]
10	racemic mixture			0°	nd	50	>50	>1
10a1	*S*	*S*	*R*	+6.8° *c* = 2.92	1.4	>50	>50	nd
10a2	*S*	*S*	*S*	−0.5° *c* = 1.46	11.6	>50	>50	nd
10b1	*S*	*R*	*S*	−24.8° *c* = 3.23	12.5	>50	>50	nd
10b2	*S*	*R*	*R*	−3.6° *c* = 1.92	16.7	>50	>50	nd
Amprenavir	*S*	*S*	*R*	nd	0.001	0.01	>50	>5000

[a] $[\alpha]_D$: optical rotation was measured at 20 °C in *c* (g/100 mL) concentration. [b] IC_{50}: concentration in μM required to inhibit HIV-1 protease cleavage by 50%. [c] EC_{50}: concentration in μM required to inhibit *syncytia* formation by 50% on MT4 cells. [d] CC_{50}: concentration in μM required to cause 50% death of uninfected MT4 cells. [e] SI: selective index = CC_{50}/EC_{50} (nd= not determinated).

Under our assay conditions, the racemic mixture tested elicited anti-HIV activity with an EC_{50} value up to 50 μM. Under the same conditions, Amprenavir inhibited *syncytium* formation with $EC_{50} = 0.01$ μM. The results obtained led us to separate the four constitutive isomers (**10a$_1$**, **10a$_2$**, **10b$_1$**, **10b$_2$**) from the diastereoisomeric mixture (compound **10**) and to screen each of them under the same assay conditions.The results show that none of the four separated isomers were found active at concentrations below 50 μM. Compound **10a$_1$** (*S*, *S*, *R*), in which the configurations of the three asymmetric

carbons are identical to those of Amprenavir (*S, S, R*), was inactive. The four diastereoisomers (**10a$_1$**, **10a$_2$**, **10b$_1$**, **10b$_2$**) were then tested as recombinant HIV protease inhibitors according to a classical procedure.[26] IC_{50} values are given in Table 1. It can be seen that the four diastereoisomeric resolved Amprenavir bioisosteres (**10a$_1$**, **10a$_2$**, **10b$_1$**, **10b$_2$**) were only weak inhibitors of recombinant HIV protease (IC_{50} values were 1.4, 11.6, 12.5, and 16.7 μM, respectively). One explanation for the lack of HIV-protease inhibitory properties of these thiophenoxy bioisosteres could be their high sensitivity to hydrolysis. We have seen through the recombinant anti-HIV-1-protease inhibition studies that the new bioisosteres are not protease substrates. The HPLC profile showed that the peak corresponding to the bioisostere remained unchanged. Since the bioisosteres were not degraded by the recombinant HIV-1 protease, we have also studied their stabilities in normal human serum. The half-lives ($t_{1/2}$) of some representative compounds were determined at 37 °C by the HPLC method. The half-life value found for compound **10** was 10 min, while Amprenavir was recovered unchanged after 1440 min.

These results were inconsistent with molecular modeling studies (data not shown) that supported the hypothesis that the replacement of the methylene group in the phenylalanine moiety of Amprenavir did not affect drastically the binding energy of the resulting analogue within the active site of the HIV-1 protease. Introduction of a thiophenoxy moiety abolished both protease inhibition activities and viral cytopathogenicity protection in HIV-infected MT4 cells, indicating that the presence of a sulfur atom disturbed the capabilities of the thiophenoxy drug to form a complex within the HIV-protease active site.

Conclusion

The simple replacement of the methylene group in the benzyl moiety of Amprenavir by a sulfur atom abolished drastically antiviral activity on HIV replication in infected MT4 cells, as well as inhibitory properties on recombinant HIV-1 protease. Our finding is that the hydrophobic binding of the aromatic moiety of the Phe residue and its orientation within the protease binding site are critical to enzyme inhibition. In addition to the drastically observed decrease of Amprenavir bioisostere stability in human serum, the presence of the sulfur atom induces a significant entropic disadvantage to the resulting bioisostere for coincident binding of the thiophenoxy moiety in the protease active site.

Acknowledgment. This research was financially supported by the Institut National de la Recherche Médicale (INSERM). We are indebted to U-322 members for technical assistance in antiviral activity evaluation and to Dr. T. Williamson for help with the manuscript. We thank M. Meyer and Dr. G. Pepe for molecular modeling studies. Also, we give a special thanks to Professor Roussel from Université Aix-Marseille III ENSSPICAM (Marseille) for his precious help in chiral separation.

Supporting Information Available: Experimental procedure for the syntheses of compounds **2**−**10**, along with some analytical data, and procedures for diastereoisomer separation, antiviral activity measurements, and HIV-1 protease inhibition assay. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pearl, L. H.; Taylor, W. R. A Structural Model for the Retroviral Proteases. *Nature* **1987**, *329*, 351−354.
(2) Power, M. D.; Marx, P. A.; Bryant, M. L.; Gardner, M. B.; Barr, P. J.; Luciw, P. A. Nucleotide Sequence of SRV-1, a Type D Simian Acquired Immune Deficiency Syndrome. *Science* **1986**, *231*, 1567−1572.
(3) Ratner, L.; Haseltine, W.; Patarca, R.; Livak, K. J.; Starcich, B.; Josephs, S. F.; Doran, E. R.; Rafalski, J. A.; Whitehorn, E. A.; Baumeister, K.; Ivanoif, L.; Petteway, S. R., Jr.; Pearson, M. L.; Lautenberger, J. A.; Papas, T. S.; Ghrayeb, J.; Chang, N. T.; Gallo, R. C.; Wong-Staal, F. Complete Nucleotide Sequence of the AIDS Virus, HTLV III. *Nature* **1985**, *313*, 277−284.
(4) Wain-Hobson, S.; Sonigo, P.; Danos, O.; Cole, S.; Alizon, M. Nucleoside Sequence of the AidsVirus, LAV. *Cell* **1985**, *40*, 9−10.
(5) Muesing, M. A.; Smith, D. H.; Cabradilla, C. D.; Benton, C. V.; Lasky, L. A.; Capon, D. J. Nucleic Acid Structure and Expression of the Human AIDS/Lymphadonopathy Retrovirus. *Nature* **1985**, *313*, 450−458.
(6) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. Active HIV-1 Protease Is Required for Viral Infectivity. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686−4690.
(7) Kramer, R. A.; Ganguly, K.; Reddy, E. P.; Shaber, M. D.; Skalka, A. M.; Wongstaal, F. HTLV III GAG Protein Is Processed in Yeast-Cells by the Virus Pol-Protease. *Science* **1986**, *231*, 1580−1584.
(8) Dorsey, B. D.; McDonough, C.; McDaniel, S. L.; Levin, R. B.; Newton, C. L.; Hoffman, J. M.; Darke, P. L.; Zugay-Murphy, J. A.; Emini, E. A.; Schleif, W. A.; Olsen, D. B.; Stahlhut, M. W.; Rutkowski, C. A.; Kuo, L. C.; Lin, J. H.; Chen, I. W.; Michelson, S. R.; Holloway, M. K.; Huff, J. R.; Vacca, J. P. Identification of MK-944a: A Second Clinical Candidate from the Hydroxylaminepentanamide Isostere Series of HIV Protease Inhibitors. *J. Med. Chem.* **2000**, *43*, 3386-3399.
(9) Kempf, D. J.; Sham, H. L. HIV Protease Inhibitors. *Curr. Pharm. Des.* **1996**, *2*, 225−246.
(10) Swanstrom, R.; Erona, J. Human Immunodeficiency Virus Type-1 Protease Inhibitors: Therapeutic Successes and Failures, Suppression and Resistance. *Pharmacol. Ther.* **2000**, *86*, 145−170.
(11) Murphy, R. L.; Gulick, R. M.; DeGruttola, V.; D'Aquila, R. T.; Eron, J. J.; Sommadossi, J. P.; Currier, J. S.; Smeaton, L.; Frank, I.; Caliendo, A. M.; Gerber, J. G.; Tung, R.; Kuritzkes, D. R. Treatment with Amprenavir Alone or Amprenavir with Zidovudine and Lamivudine in Adults with Human Immunodeficiency Virus Infection. *J. Infect. Dis.* **1999**, *109*, 808−816.
(12) Molla, A.; Granneman, G. R.; Sun, D.; Kempf, E. Recent Development in HIV Protease Therapy. *Antiviral Res.* **1998**, *39*, 1−23.
(13) Shirasaka, T.; Yarchoan, R.; O'Brien, M. C.; Husson, R. N.; Anderson, B. D.; Kojima, E.; Shimada, T.; Broder, S.; Mitsuya, H. Changes in Drug Sensitivity of Human Immunodeficiency Virus Type 1 during Therapy with Azidothymidine, Dideoxycytidine, and Dideoxyinosine: An In Vitro Comparative Study. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 562−566.
(14) Arts, E. J.; Wainberg, M. A. Mechanisms of Nucleoside Analog, Antiviral Activity and Resistance during HIV Reverse Transcription. *Antimicrob. Agents Chemother.* **1996**, *40*, 527−540.
(15) De Clercq, E. Antiviral Therapy for HIV Infections. *Clin. Microbiol. Rev.* **1995**, *8*, 200−239.
(16) Patani, G. A.; LaVoie, E. J. Bioisosterism: A Rational Appoach in Drug Design. *Chem. Rev.* **1996**, *96*, 3147−3106.
(17) Romeo, S.; Rich, D. H. Diastereoselective Epoxidation of Dipeptides Olefins. *Tetrahedron Lett.* **1993**, *34*, 7187−7190.
(18) Freskos, J.; Bertenshaw, D.; Getman, D.; Heintz, R.; Mischke, B.; Blystone, L.; Bryant, M.; Funckes-Shippy, C.; Houseman, K.; Kishore, N.; Kocan, G.; Mehta, P. (Hydroxyethyl) Sulfonamide HIV-1 Protease Inhibitors: Identification of the 2-Methylbenzoyl Moiety at P-2. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 445−450.
(19) Mancuso, A. J.; Swern, D. Activated Dimethyl Sulfoxyde: Useful Reagents for Synthesis. *Synthesis* **1981**, 165−185.
(20) Ghosh, A. K.; Duong, T. T.; McKee, S. P.; Thompson, W. J. *N,N′*-Disuccinimidyl Carbonate: A Useful Reagent for Alkoxycarbonylation of Amines. *Tetrahedron Lett.* **1992**, *33*, 2781−2784.
(21) Semmelhack, M.; Chou, C.; Cortes, D. Nitroxyl-Mediated Electooxidation of Alcohols to Aldehydes and Ketones. *J. Am. Chem. Soc.* **1983**, *105*, 4492−4494.
(22) Seebach, D.; Bossler, H.; Grunder, H.; Shoda, S.; Wenger, R. C-Alkylation of Peptides through Polylithiated and LiCl-Solvated Derivatives Containing Sarcosine Li-Enolate Units. *Helv. Chim. Acta* **1991**, *74*, 197−224.

(23) Medou, M.; Priem, G.; Rocheblave, L.; Pepe, G.; Meyer, M.; Chermann, J. C.; Kraus, J. L. Synthesis and Anti-HIV Activity of α-Thiophenoxy-hydroxyethylamide Derivative. *Eur. J. Med. Chem*. **1999**, *34*, 625−638.
(24) Rey, F.; Barré-Sinoussi, F.; Schmidtmayerova, H.; Chermann, J. C. Detection and Titration of Neutralizing Antibodies to HIV Using an Inhibition of the Cytopathic Effect of the Virus on MT4 Cells. *J. Virol. Methods* **1987**, *16*, 239−249.
(25) Rey, F.; Donker, G.; Hirsch, I.; Chermann, J. C. Productive Infection of CD4+ Cells by Selected HIV Strains Is Not Inhibited by Anti-CD4 Monoclonal Antibodies. *Virology* **1991**, *181*, 165−101.
(26) Betageri, R.; Hopkins, J. L.; Thibeault, D.; Emmanuel, M. J.; Chow, G. C.; Skoog, M. J.; Debreu, P.; Cohen, K. A. Rapid, Sensitive and Efficient HPLC Assays for HIV-1 Proteinase. *J. Biochem. Biophys. Methods* **1993**, *27*, 191−197.

JM0208323