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NMR AND TOPOCHEMICAL STUDIES OF PEPTIDOMIMETIC HIV-I PROTEASE INHIBITORS CONTAINING A *CIS*-EPOXIDE AMIDE ISOSTERE

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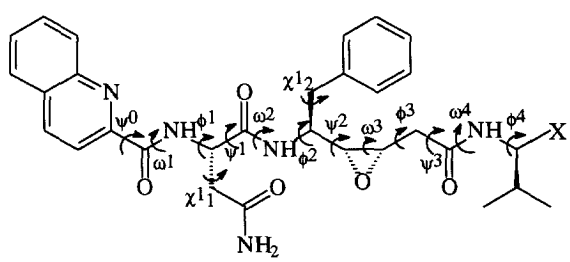
Abstracts: NMR and topochemical studies of irreversible HIV-1 protease inhibitors containing a cis-epoxide as amide isostere have been carried out to identify conformational preference of the inhibitors in solution. The inhibitors prefer to adopt extended conformations similar to the β-strand in solution.

We have designed and synthesized irreversible HIV-I protease inhibitors containing a *cis*-epoxide as a peptidomimetic modification[1] for the scissile peptide bond. These inhibitors strongly inhibited HIV-1 protease with time-dependent irreversible pattern.[2] Namely, when the inhibitors bind to the enzyme, a nucleophilic oxygen of Asp^{25} sidechain added to a carbon of the epoxide ring to form an ester bond and simultaneously, the epoxide ring is opened.[2a]

We believe the recognition of inhibitors by the enzyme in solution is one of the crucial factors governing the activity of inhibitors. Therefore, we have carried out NMR and topochemical studies[3] on our inhibitors, Qc-Asn-Pheψ[(1S,2R)-*cis*-epoxide]Gly-NH-CH(isopropyl) (X) where Qc stands for quinaldic acid; X = C(O)NH(OMe), LB71097; benzyl, LB71112; phenyl, LB71119 (see Figure 1). To our knowledge, this represents the first conformational study of peptidomimetics containing *cis*-epoxide as an amide isostere.

Details of the inhibitor synthesis have been previously described.[2] The second order rate constants (k_{ina}/K_i) and 50% inhibition concentration (IC_{50}) of these inhibitors are in the ranges of 10^8-10^{10} $M^{-1}min^{-1}$ and 10 - 50 nM, respectively.

Figure 1.



Samples for NMR spectroscopy were prepared in DMSO-d_6 solvent. All the spectra were recorded at 500 MHz on a JEOL GSX-500 spectrometer at temperature 303K. The ^1H chemical shifts are referred to the TMS signal. The phase sensitive ROESY experiments[4] were performed using pulse sequence $\pi/2$ - t_2 - spin lock - ACQ(t_1) - delay. A limited r.f. field strength[5] for the mixing period was used to suppress COSY and Hartmann-Hahn type[6] transfers. Mixing times were varied from 50 to 600 msec. In COSY and DQF-COSY experiments the FID's with 256 t_1 increments were acquired with 16 scans, and 4 and 2 dummy scans for t2 and t_1 domain. The spectral widths were 6060.6 Hz both in F2 and F1 for all the spectra. The Felix 2.30 program[7] was used for processing and analyzing the data on SGI Indigo II workstation.

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The proton resonances of the inhibitors were assigned employing COSY and DQF-COSY. The Vicinal coupling constants measured are summarized in Table 1. The NOEs were obtained from the ROESY experiments. The ROESY experiments with the maximum mixing time that maintains linearity of cross peak intensities were chosen to measure the volume of NOEs. These measured volumes of cross peaks are classified into 5 different categories: s, strong; sm, strong-medium; m, medium; mw, medium-weak; w, weak (Table 2).

Table 1. ^1H-^1H Coupling constants of HIV protease inhibitors.

protons	coupling constants 097 (Hz)	112 (Hz)	119 (Hz)	protons	coupling constants 097 (Hz)	112 (Hz)	119 (Hz)
Qc C^5H, Qc C^6H	8.3	8.3	8.1	Phe(cep) NH, Phe(cep) $C^{\beta}H_h$	6.3	6.7	6.3
Qc C^5H, Qc C^7H	1.5	1.5	1.3	Phe(cep) NH, Phe(cep) $C^{\beta}H_l$	5.9	4.8	5.9
Qc C^6H, Qc C^7H	7.8	6.8	7.6	Phe(cep) $C^{\alpha}H$, CEP C^1H	9.3	9.1	9.1
Qc C^6H, Qc C^8H	1.5	1.1	1.1	Phe(cep) $\phi_{2,6}H$, Phe(cep) $\phi_{3,5}H$	8.3	7.4	7.2
Qc C^7H, Qc C^8H	8.3	8.3	8.3	Phe(cep) $\phi_{3,5}H$, Phe(cep) ϕ_4H	7.8	7.2	7.0
Asn NH, Asn $C^{\alpha}H$	8.3	8.5	8.7	CEP C^1H, CEP C^2H	4.4	4.3	4.4
Asn $C^{\alpha}H$, Asn $C^{\beta}H_h$	7.8	7.7	7.7	CEP C^2H, (cep)Gly $C^{\alpha}H_h$	7.3	6.6	7.4
Asn $C^{\alpha}H$, Asn $C^{\beta}H_l$	4.9	5.2	5.2	CEP C^2H, (cep)Gly $C^{\alpha}H_l$	4.9	5.5	4.6
Asn $C^{\beta}H_h$, Asn $C^{\beta}H_l$	15.1	15.3	15.5	(cep)Gly $C^{\alpha}H_h$, (cep)Gly $C^{\alpha}H_l$	15.6	15.2	15.4
Phe(cep) NH, Phe(cep) $C^{\alpha}H$	8.3	8.5	8.7				

Phe(cep) and (cep)Gly: residues generated by the replacement of the Phe-Gly peptide bond with *cis*-epoxide;
CEP: *cis*-epoxide.

Table 2. ^1H-^1H ROE intensity of HIV protease inhibitors.

NOE	intensity 097	112	119	NOE	intensity 097	112	119
Asn NH - Asn $C^{\alpha}H$	mw	mw	mw	Phe(cep) $C^{\alpha}H$ - Phe(cep) $C^{\beta}H_l$	sm	sm	sm
Asn NH - Asn $C^{\beta}H_h$	w	w	mw	Phe(cep) $C^{\alpha}H$ - (cep)Gly $C^{\alpha}H_h$	s	s	s
Asn NH - Asn $C^{\beta}H_l$	w	w	w	Phe(cep) $C^{\alpha}H$ - (cep)Gly $C^{\alpha}H_l$	sm	sm	sm
Asn $C^{\alpha}H$ - Asn $C^{\beta}H_h$	m	mw	m	CEP C^1H - CEP C^2H	s	-	s
Asn $C^{\alpha}H$ - Asn $C^{\beta}H_l$	sm	sm	sm	CEP C^2H - (cep)Gly $C^{\alpha}H_h$	mw	m	m
Asn $C^{\alpha}H$ - Phe(cep) NH	s	s	s	CEP C^2H - (cep)Gly $C^{\alpha}H_l$	mw	m	m
Asn $C^{\beta}H_h$ - Asn $C^{\beta}H_l$	s	s	s	(cep)Gly $C^{\alpha}H_h$ - (cep)Gly $C^{\alpha}H_l$	s	s	s
Phe(cep) NH - Phe(cep) $C^{\alpha}H$	-	mw	m	(cep)Gly $C^{\alpha}H_h$ - NH-X NH	sm	sm	sm
Phe(cep) NH - CEP C^1H	sm	sm	sm	(cep)Gly $C^{\alpha}H_l$ - NH-X NH	mw	mw	mw
Phe(cep) $C^{\alpha}H$ - Phe(cep) $C^{\beta}H_h$	mw	w	w	NH-X NH - NH-X C^1H	-	w	mw

Conformation of peptides or peptidomimetics can be presented by combination of torsion angles, which show the rotational status around the single bonds of the molecule. The torsion angles defining conformations of our inhibitors are demonstrated in Figure 1. The preferences of these torsion angles in solution are analyzed on the basis of the above NMR data.

Two ψ^0 angles (~ 0 and 180°) are possible since the quinoline ring and the attached amide are almost in the same plane. No NOE is observed between the proton on the carbon 3 of the quinoline ring and Asn NH for the all three compounds. These results indicate that the proton on the carbon 3 of the quinoline and Asn NH are in the opposite side of the bond between quinoline and carbonyl group. Thus, the ψ^0 angles of these inhibitors are about 0° in solution.

The coupling constants between NH and $C^{\alpha}H$ ($J_{NH\text{-}C^{\alpha}H}$) of Asn are between 8.3 and 8.7 Hz (Table 2). These constants are closely related to the rotation of NH - $C^{\alpha}H$ (ϕ^1). If one consider the Karplus-type equation,[8] the ϕ^1 angles can be estimated as about 60, -60, -90 and -145°. However, since medium-weak NOEs are observed between NH and $C^{\alpha}H$ of Asn, only -90 and -145° are possible. The ψ of Asn is estimated to be in the range of approximately 60 - 180° since strong NOEs were observed between Asn $C^{\alpha}H$ and Phe(cep) NH.

The coupling constants between NH and $C^{\alpha}H$ ($J_{NH\text{-}C^{\alpha}H}$) of Phe(cep) are 8.3 to 8.7 Hz (Table 2) and medium-weak NOEs are observed between NH and $C^{\alpha}H$ of Phe(cep). Thus, the ϕ^2 angles are -90 or -145° as in the case of ϕ^1 estimation. Strong-medium or medium NOEs are observed between Phe(cep) NH and the C^1H of the epoxide, but there is no NOE between Phe(cep) $C^{\alpha}H$ and the C^1H of the epoxide. Thus, the Ψ angles of Phe(cep) must be larger than 130° in solution.

The ϕ^3 angles can be estimated from the NOEs between Phe(cep) $C^{\alpha}H$ and (cep)Gly $C^{\alpha}H_2$. The intensities of the both NOEs are close to strong with one being somewhat smaller. Thus, the ϕ^3 angles are approximately -150 or 150°. The ψ^3 angles are estimated around -90 or 90° from the strong and medium NOEs between (cep)Gly $C^{\alpha}H_2$ and NH of the terminal residue. The ϕ angles of the terminal residues are estimated as about 120 or -120° because there is no NOE between NH and C^1H of NH-X.

None of the three amide bonds of the inhibitors are alkylated. Thus, the torsion angles ω^1, ω^2 and ω^4 must be close to 180° (*trans* configuration). The observed coupling constant between the two protons of the epoxide is 4.3 to 4.4 Hz. This is similar to the coupling constant between *cis* protons of ethyl epoxide (4.5 Hz).[9] In addition, strong and strong-medium NOEs are observed between Phe(cep) $C^{\alpha}H$ and (cep)Gly $C^{\alpha}H_2$. These results indicate that the epoxide unit of these compounds is in a *cis* configuration with the ω^3 angles being 0°.

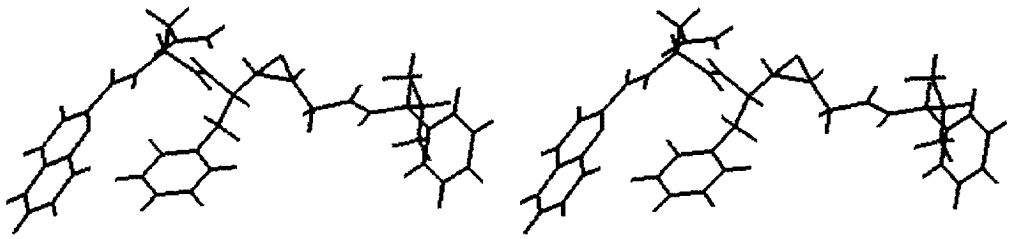
The side chains of Asn and Phe(cep) are known to be flexible adopting all three possible conformations (g-, t, g+). However, since weak NOEs between Asn NH and $C^{\beta}Hs$ and strong and medium NOE between Asn $C^{\alpha}H$ and $C^{\beta}H$'s are observed, the side chain of Asn may prefer g- conformation. The conformational preference of Phe(cep) side chain can not be determined because the two β-proton signals are in complete overlap of each other. All the estimated torsion angles are summarized in Table 3.

Table 3. Preferred torsion angles of HIV protease inhibitors (LB71 series) in solution.

Torsion angles	preferred values	Torsion angles	preferred values
ψ^0	0°	ω^3	0°
ω^1	180°	ϕ^3	-150 or 150°
ϕ^1	-90 or -145°	ψ^3	-90 or 90°
ψ^1	60 - 180°	ω^4	180°
ω^2	180°	ϕ^4	-120 or 120°
ϕ^2	-90 or -145°	$\chi_1{}^1$	-60°
ψ^2	> -130°	$\chi_1{}^2$	-

Based on these NMR results, we have carried out molecular mechanical calculations using DISCOVER[10] program, and Figure 2 shows one of the resulting preferred conformations in the frame of LB71119. When this is compared with the results from the X-ray studies of our inhibitor / HIV-I protease complex.[2] They are similar to each other. These results indicate that the conformation of the free inhibitors in solution is almost identical to that of bound to HIV-1 protease.

Figure 2.



In conclusion, our HIV-1 protease inhibitors in solution adopt extended conformations similar to the β-strand. The epoxide seems to play a role in stabilizing such conformations since the absolute values of torsion angles about the bonds directly attached to the epoxide are bigger than 130°.

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References and Notes

1. Goodman, M. and Ro, S. in "Burger's Medicinal Chemistry and Drug Discovery (5th Ed.), Vol. I: Principles and Practice" **1995**, M.E.Wolff Eds, John Wiley and Sons, Inc, 803-861.
2. a) Lee, C. S.; Choy, N.; Park, C.; Choi, H.; Son, Y. C.; Kim, S.; Ok, J. H.; Kim, S. C.; Yoon, H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 589. b) Park, C.; Koh, J. S.; Son, Y.C.; Choi, H.; Lee, C. S.; Choy, N.; Moon, K. Y.; Jung, W. H.; Kim, S. C.; Yoon, H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1843. c) Choy, N.; Choi, H.; Jung, W. H.; Kim, C. R.; Yoon, H.; Kim, S. C.; Lee, T. G.; Koh, J.S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2635.
3. a) Yamazaki, T.; Ro, S.; Goodman, M. Chung, N. N.; Schiller, P.W. *J. Med. Chem.* **1993**, *36*, 708. b) Yamazaki, T.; Ro, S.; Goodman, M. *Biochem. Biophys. Res. Commun.*, **1991**, *181*, 664.
4. Bothner-By, A. A.; Stephens, R. L.; Lee, J. M.; Warren C. D.; Jeanloz, R. W. *J. Am. Chem. Soc*, **1984**, *106*, 811.
5. Bax, A. and Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207.
6. Hartmann, S. R. and Hahn, E. L. *Phys. Rev.* **1962**, *128*, 2042.
7. Felix V-2.30 Biosym, San Diego, CA, 1993.
8. a) Karplus, M.; *J. Chem. Phys.* **1959**, *30*, 11. b) Karplus, M. *J. Am. Chem. Soc*, **1963**, *85*, 2870.
9. "Tables of Spectral Data for Structure Determination of Organic Compounds: 13C-NMR, 1H-NMR, IR, MS, UV/VIS (2nd edition)" **1989**, W. Fresenius, J. F. K. Huber, E. Pungor, G. A. Rechnitz, W. Simon, Th. S. West Eds, Springer-Verlag, Berlin Heidelberg.
10. DISCOVER – V 2.9.5, Biosym/MSI, San Diego, CA, 1994.