Enantiomeric Effects of Homologues of Disoxaril on the Inhibitory Activity against Human Rhinovirus-14

Guy D. Diana,*[†] Michael J. Otto,[†] Adi M. Treasurywala,[†] Mark A. McKinlay,[†] Richard C. Oglesby,[†] Edward G. Maliski,[†] Michael G. Rossmann,[‡] and Thomas J. Smith[‡]

Sterling-Winthrop Research Institute, Rensselaer, New York 12144, and Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907. *Received June 5, 1987*

X-ray crystallography studies of racemic 5-[7-[4-(4,5-dihydro-4-methyl-2-oxazolyl)phenoxy]heptyl]-3-methylisoxazole (**2**) bound to human rhinovirus-14 (HRV-14) indicate selective binding of the *S* isomer. This result correlates well with the 10-fold greater activity of the *S* isomer as compared to the *R* isomer. The enantiomeric effect on activity is explained by a hydrophobic interaction of the methyl group in the case of **2a**, with a pocket formed by Leu^{106} and Ser^{107}. The 4-ethyl, 4-propyl, and 4-butyloxazolinyl homologues were prepared and tested against HRV-14. All of these compounds exhibited a comparable stereochemical effect. In each case, the *S* isomer displayed greater levels of activity than the *R*. The results of energetic considerations of the oxazoline ring in an 8-Å pocket bound to the HRV-14 binding site suggest that the twist angle between the oxazoline and phenyl rings resulting from hydrophobic interactions of the alkyl substituents could be one of the determining factors for biological activity.

Disoxaril (**1**) (Table I) and the 4-methyloxazolinyl homologue **2** have been shown to be potent inhibitors of many human picornaviruses.[1-3] The *S* enantiomer of **2** was shown to exhibit greater activity than **1** against several rhinovirus serotypes and was 10 times more effective against human rhinovirus-14 (HRV-14)[2] (Table III).

These compounds and many structurally related analogues exert their antiviral effect by inhibiting the uncoating of the virion RNA without affecting adsorption or penetration of the virus.[4] The uncoating or disassembly of the virus takes place inside the infected cell within an endosome and appears to be mediated by a lowering of the endosomal pH.[5] Disoxaril prevents this from occurring by interacting directly with the virion capsid proteins. This direct interaction has been demonstrated by heat-inactivation studies in which disoxaril prevented the spontaneous uncoating of both HRV-2 and poliovirus-2 which occurs at 42 °C.[4]

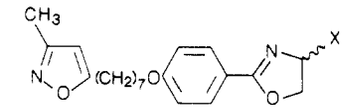
Recently, the three-dimensional crystal structure of HRV-14 was determined.[6] A major finding in these studies is the presence of a cleft or canyon 25 Å deep and 12–30 Å wide on the viral surface. At the base of this cleft is an opening or pore leading to the interior of the viral capsid. It was subsequently found by X-ray crystallography of the drug–virus complex that compound **2** binds reversibly to a hydrophobic pocket in viral protein 1 (VP1) located below the canyon.[7] Compound **2** is believed to insert into the pocket in VP1 with the isoxazole end inserted into the interior of the VP1 β-barrel. The methyloxazolinylphenyl moiety is located below the canyon floor with the nitrogen of the oxazoline ring within hydrogen-bonding distance to Asn^{219} (Figure 1). Compound **2** has a chiral center at the 4-position of the oxazoline ring, and although the studies were performed on the racemate, the results indicate preferential binding of the *S*-isomer **2a** to the binding site. This was consistent with the 10-fold difference in the antiviral activity observed between the two isomers (Table III).

In order to determine if the enantiomeric effect observed with the methyl compound could be extended to other homologues, the *R* and *S* 4-ethyl, 4-*n*-propyl, 4-isopropyl, and 4-*n*-butyl homologues were prepared and tested in vitro against HRV-14. In addition we have examined the X-ray coordinates of **2a** bound to HRV-14 using Chem-X.[8]

Chemistry

All of the 4-substituted oxazolines in Table II, with the exception of the propyl and butyl homologues, were prepared with the commercially available optically active amino alcohols according to the procedure previously re-

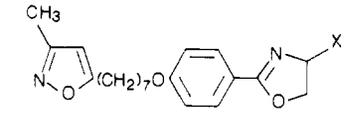
Table I. In Vitro Activity against Human Rhinovirus-14 (HRV-14)



compd[a]	X	MIC,[b] μg/mL
1	H	0.14 ± 0.04
2	CH_3 (racemic)	0.03 ± 0.007
3	$(CH_3)_2$	0.03 ± 0.02

[a] Reference 2. [b] Minimum inhibitory concentration.

Table II. Physicochemical Properties



compd	X	mp, °C	config[a]	$[\alpha]^D_{25}$,[b] deg	formula[c]
2a[d]	CH_3	71	*S*	−31.7	$C_{21}H_{28}N_2O_3$
2b	CH_3	71–72	*R*	+32.9	$C_{21}H_{28}N_2O_3$
4a	C_2H_5	74–75	*S*	−26.8	$C_{22}H_{30}N_2O_3$
4b	C_2H_5	72–73	*R*	+21.4	$C_{22}H_{30}N_2O_3$
5a	*n*-C_3H_7	80–81	*S*	−29.7	$C_{23}H_{32}N_2O_3$
5b	*n*-C_3H_7	79–80	*R*	+30.8	$C_{23}H_{32}N_2O_3$
6a	*i*-C_3H_7	67–68	*S*	−23.3	$C_{23}H_{32}N_2O_3$
6b	*i*-C_3H_7	66–68	*R*	+24.7	$C_{23}H_{32}N_2O_3$
7a	*n*-C_4H_9	83–84	*S*	−27.7	$C_{24}H_{34}N_2O_3$
7b	*n*-C_4H_9	83–84	*R*	+27.8	$C_{24}H_{34}N_2O_3$

[a] The absolute configurations are based on commercially available optically pure amino alcohols or amino acids. [b] Rotations were determined in ethanol (c1). [c] The elemental analyses (C, H, and N) for all new compounds were within ±0.4% of the theoretical values. [d] Reference 2.

[†] Sterling-Winthrop Research Institute.
[‡] Purdue University.

(1) Otto, M. J.; Fox, M. P.; Fancher, M. J.; Kuhrt, M. F.; Diana, G. D.; McKinlay, M. A. *Antimicrob. Agents Chemother.* **1985**, *27*, 883–886.
(2) Diana, G. D.; McKinlay, M. A.; Otto, M. J.; Akullian, V.; Oglesby, C. *J. Med. Chem.* **1985**, *28*, 1906.
(3) Diana, G. D.; Otto, M. J.; McKinlay, M. A. *Pharmacol. Ther.* **1985**, *24*, 287.
(4) Fox, M. P.; Otto, M. J.; Shave, W. J.; McKinlay, M. A. *Antimicrob. Agents Chemother.* **1986**, *30*, 110.
(5) Zeichhart, H.; Wetz, K.; Willingmann, P.; Habermehl, K. O. *J. Gen. Virol.* **1985**, *66*, 483.
(6) Smith, T. J.; Kremer, M. J.; Luo, M.; Vriend, G.; Arnold, E.; Kramer, G.; Rossmann, M. G.; McKinlay, M. A.; Diana, G. D.; Otto, M. J. *Science (Washington, D.C.)* **1986**, *233*, 1286.

0022-2623/88/1831-0540$01.50/0

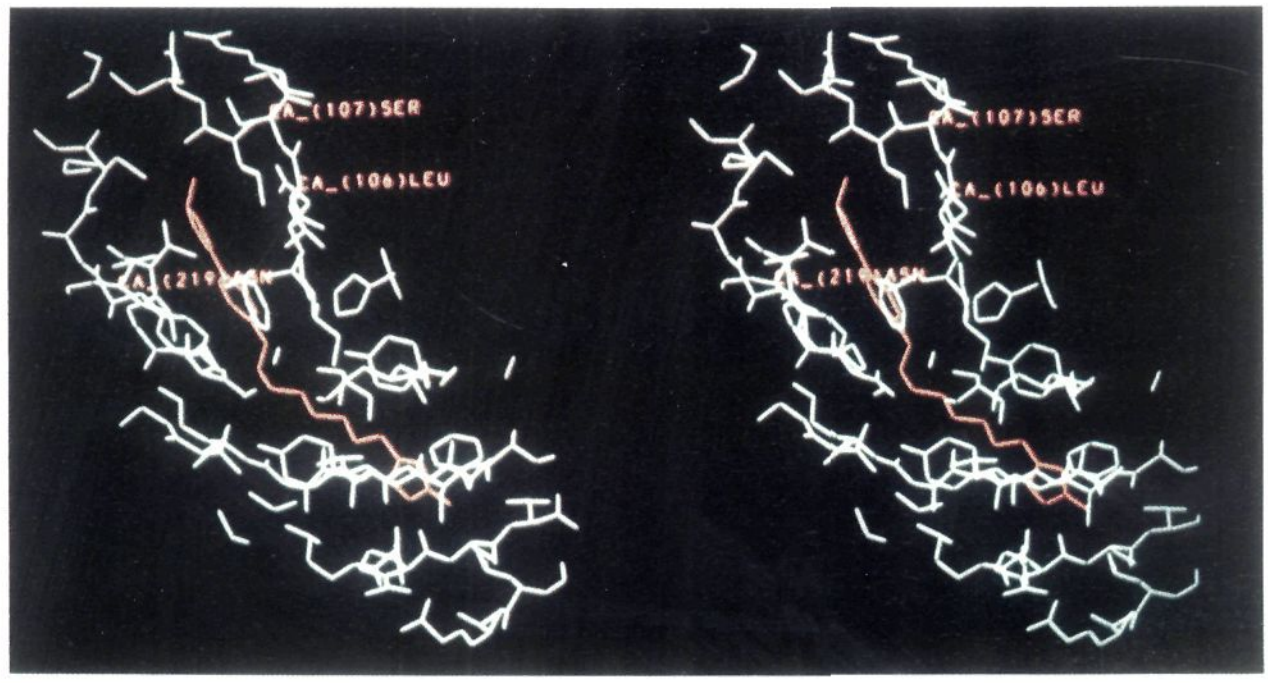


Figure 1. Compound **2a** bound to VPI of HRV-14. The X-ray data was obtained at CHESS and visualized on an Evans and Sutherland computer using the program Chem-X.

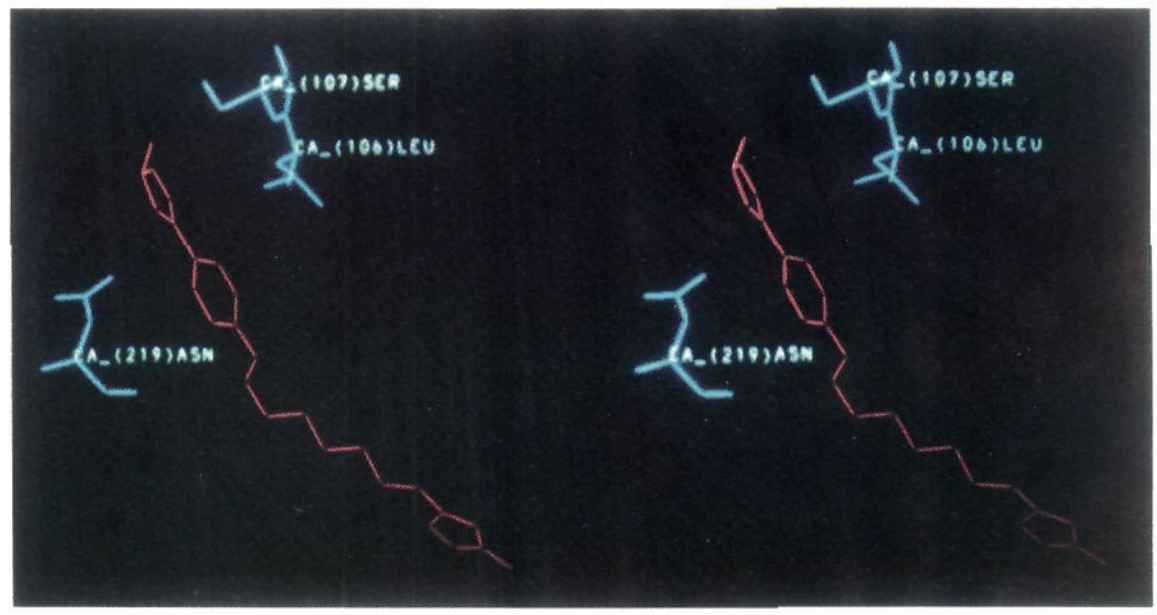


Figure 2. The interaction of the methyl group of **2a** with Leu^{106} and Ser^{107} and the interaction of Asn^{219} with the nitrogen of the oxazoline group.

ported.[2] The (*R*)- and (*S*)-aminobutanol and -aminopentanol were prepared by the reduction of norvaline and norleucine, respectively (see the Experimental Section).

Activity against HRV-14

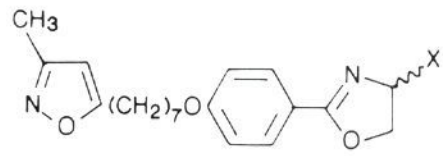
Compounds were screened against HRV-14 by a plaque reduction assay method[2] and the results are shown in Table III. In every case, the *S* isomer was more active than the corresponding *R* isomer. Enhanced activity was observed when the methyl group was replaced with ethyl (**4**) and propyl (**5**). An increase in the chain length to butyl (**7**) resulted in a reduction in activity.

A comparable structure–activity relationship was observed with the *R* isomers, where optimum activity was obtained with the ethyl and propyl homologues. Both of these compounds showed a 3-fold increase in activity over the *R* methyl homologue.

Molecular Graphics

The coordinates of **2a** bound to HRV-14 were generated by X-ray crystallography[6] and displayed on an Evans and Sutherland PS 300 computer using the program Chem-X. The difference in activity between the *S* methyl and the *R* methyl homologues is consistent with a hydrophobic interaction of the (*S*)-methyl group of **2a** with a pocket formed by Leu^{106} and Ser^{107} (Figure 2). The hydrophilic hydroxyl group of Ser^{107} is oriented away from the methyl group. Such an interaction would favorably orient the nitrogen of the oxazoline ring to hydrogen bond with Asn^{219}. The proximity of the methyl group of **2a** to the hydrophobic region of the binding site is illustrated in Figure 3. The carbon atom of this methyl group approaches C_B of Ser^{107} (4.1 Å), C_B of Leu^{106} (4.7 Å), and C_A

Table III. Comparative Evaluation of Enantiomers against HRV-14



compd	*X*	MIC, μg/mL	
		S[a]	*R*[a]
2	CH_3	0.02 ± 0.002	0.20 ± 0.02
4	C_2H_5	0.01 ± 0.003	0.06 ± 0.01
5	n-C_3H_7	0.01 ± 0.001	0.07 ± 0.008
6	i-C_3H_7	0.03 ± 0.004	0.13 ± 0.01
7	n-C_4H_9	0.06 ± 0.03	0.52 ± 0.007

[a] Absolute configuration.

(7) Rossmann, M. G.; Arnold, E.; Erickson, J. W.; Frankenberger, E. A.; Griffith, J. P.; Hecht, H. J.; Johnson, J. E.; Kramer, G.; Luo, M.; Mosser, A. G.; Reukert, R. R.; Sherry, B.; Vriend, G. *Nature (London)* **1985**, *317*, 145.
(8) Chem-X, developed and distributed by Chemical Design Limited, Oxford, England. Geistiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219.

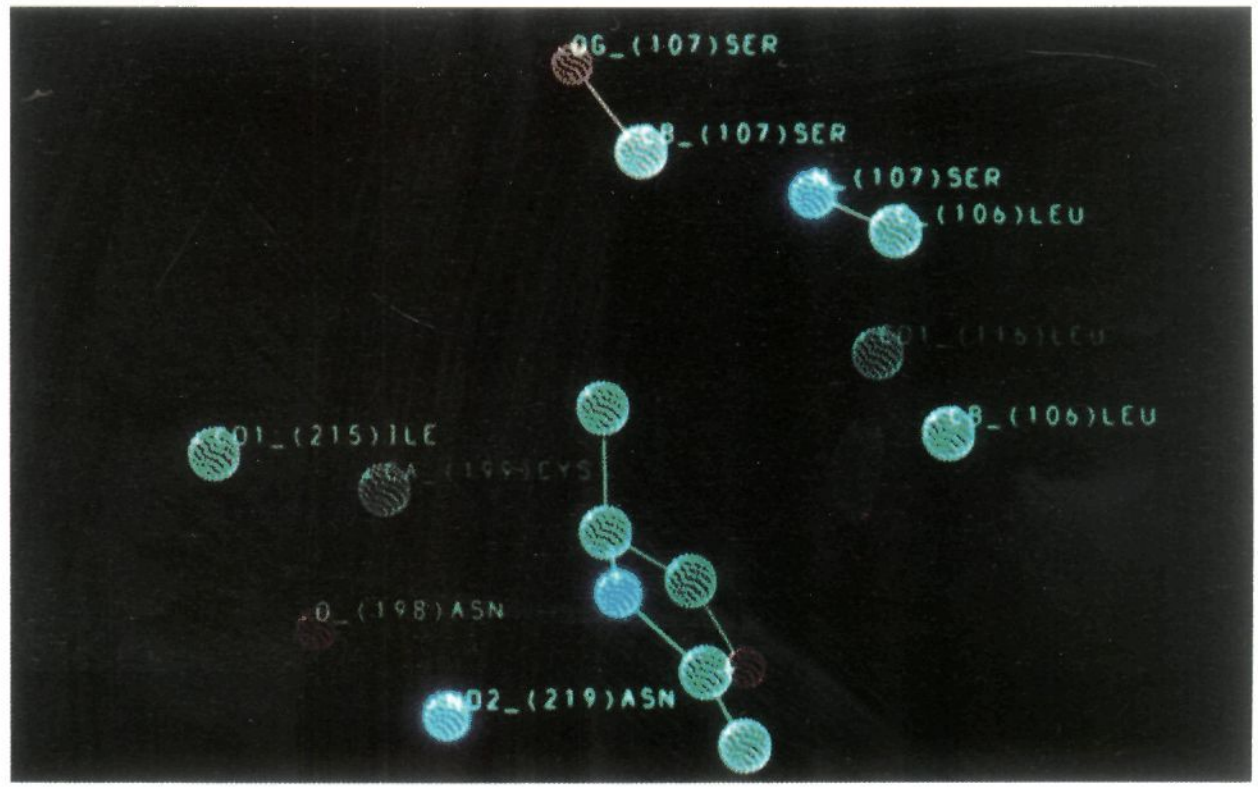


Figure 3. The interatomic distances between the methyl group of **2a**, C_B of Ser^{107} (4.1 Å), C_B of Leu^{106} (4.7 Å), and C_A of Cys^{199} (4.95 Å).

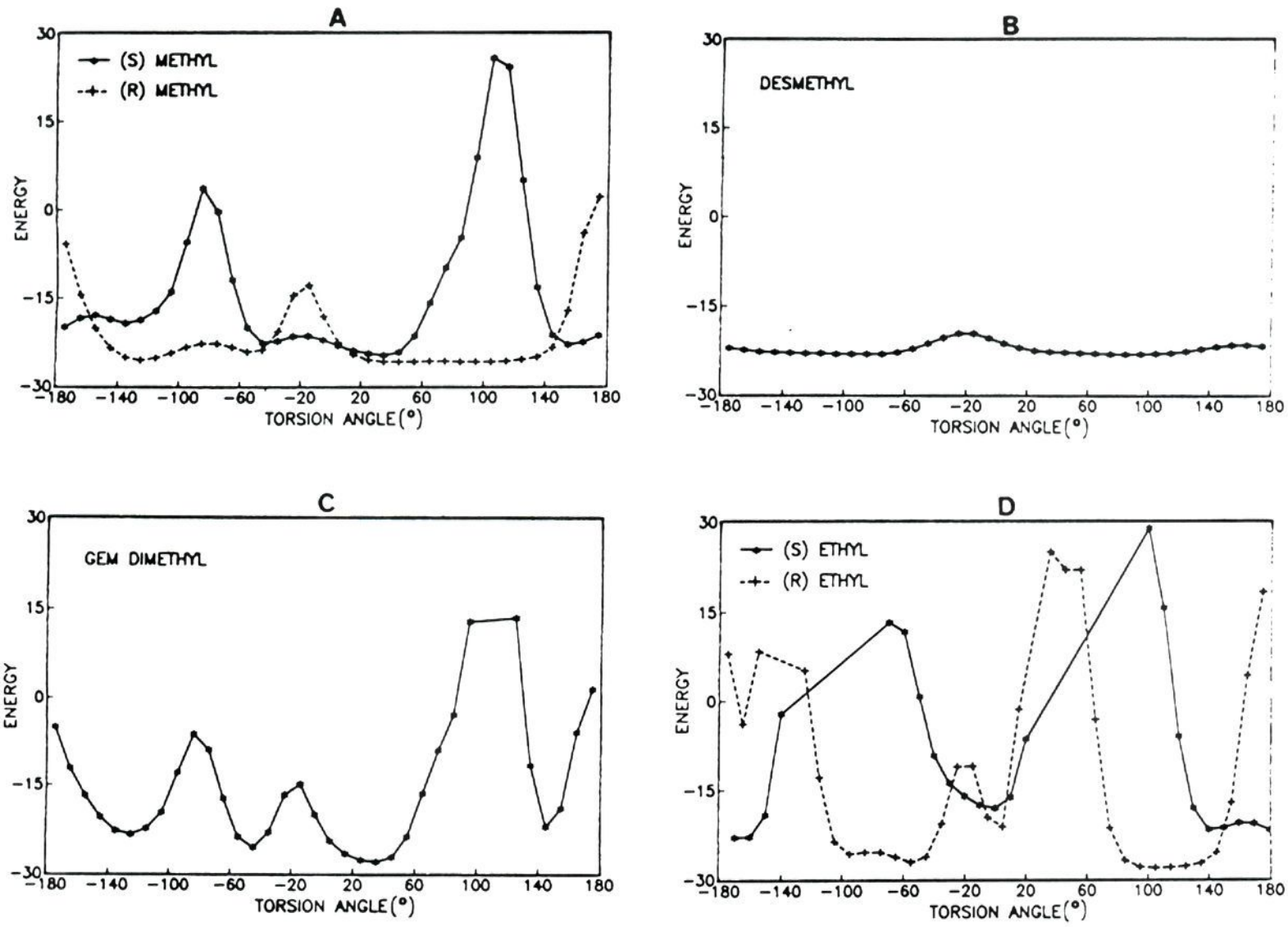


Figure 4. A plot of van der Waals energy versus the torsion angle between the oxazoline and phenyl rings for compounds 1–4, bound in the HRV-14 virus pocket. The pocket consists of all residues within 8 Å of any atom of the appropriate compound. All calculations were performed on a VAX 11785 using Chem-X. The intramolecular van der Waals energy was calculated with use of a 6–12 function.

of Cys^{198} (4.95 Å). These distances are beyond the overlap of the van der Waals radii.

To further analyze the interactions of the *R* and *S* conformers in the binding site, an energy-profiling study was performed. The X-ray crystal structure of **2a**, found in the HRV-14 virus pocket, was used as the starting point for this work. A pocket, consisting of all residues within 8 Å of any atom of the drug molecule, was cut out of this starting structure. All calculations were performed on a VAX 11/785.

Charges were set on the atoms of the resulting pocket as well as the drug according to a method defined within Chem-X.[8] The hydrogen atoms on both the virus pocket as well as the drug were removed in order to avoid excessively high values of energy during the calculations. In our experience, this has not changed the position of the peaks and valleys but only the amplitude. The intermolecular van der Waals energy was calculated via a 6–12 function for the conformations resulting from a rotation about the bond between the phenyl ring and the oxazoline

ring in steps of 10° (36 points in all). A plot was finally obtained of the function of this van der Waals energy versus the rotation angle about this bond. The results of this calculation are shown in Figure 4A. They show two peaks at −90° and roughly 100° with a number of smaller peaks.

The argument can be made that the valley point at roughly 10–30°, visible in the *S* methyl analogue, was important in providing a conformer that was biologically relevant. A repeat of this calculation on the *R* methyl homologue reproduced roughly the pattern found with the desmethyl compound 1 (Figure 4B), showing a large flat valley between 30° and roughly 120°.

Further studies with the *gem*-dimethyl analogue, embodying both the methyl group of the *S* and of the *R* compounds shown in Figure 4C reproduced a pattern that may be labeled as characteristic of the *S* methyl analogue. It is interesting to note that the *S* methyl and the *gem*-dimethyl analogues have similar levels of biological activity whereas the *R* methyl and the desmethyl compounds have lower activity.

Extension of this methodology to similar calculations performed for the *R* and *S* ethyl homologues **4a** and **4b** provided the results in Figure 4D. This case was particularly important because the *R* ethyl compound showed considerable improvement of activity over the *R* methyl compound and was almost as active as the *S* ethyl compound. As the figures demonstrate, the *S* ethyl compound has a relatively low lying valley at around 10°. The *R* ethyl compound, on the other hand, has a valley in this area which is not relatively low lying, although it does exist. Clearly the correlation with the presence of valley points around 10° for this torsion angle with improved biological activity in the range of 0.01–0.06 μg/mL MIC against HRV-14 is a tenuous one, depending on the type of calculation performed. Nonetheless, it seems to suggest that the twist angle between the two ring systems could be an important factor in determining biological activity. It seems that activity may exist in those cases where a conformer in which the two rings are only slightly tilted is stabilized over other conformations inside the virus pocket. Whether this stabilization of a coplanar conformation is imposed by the pocket in which the molecule resides or is preferred by the molecule a priori is almost irrelevant to this discussion, although it may have tremendous repercussions in the design of bioactive analogues.

Discussion

The enantiomeric effect of the substituents in the 4-position of the oxazoline ring on antiviral activity is similar to that seen with the binding of D- and L-*N*-acyl-α-amino amides[9] to chymotrypsin. It has been proposed that the active site of chymotrypsin includes, in addition to the hydrolytic site occupied by the amide moiety, a hydrophobic area to which the L isomer is bound.[10] In this case, the L isomers are substrates while the D isomers are inhibitors of the enzyme. Other examples of this type have been reported[11,12] for a number of enzymes.

The importance of hydrophobic bonding in protein structure has been well established.[13–15] Although hydrogen bonding represents a major factor in the organization of polypeptides, hydrophobic bonds that occur intramolecularly contribute to the stability of the secondary and tertiary structure. Likewise, in this homologous series of compounds, hydrophobic interactions may play an important role in determining the extent of binding to the HRV-14. The hydrophobicity of the substituents is proportional to the surface area of the alkyl chain,[16,17] which is consistent with the increase in activity with successive increase in the size of the substituent. Reduced activity of the butyl homologue may be attributed to either a saturation of the binding site[18] or steric hindrance due to space constraints in the region surrounding the oxazoline ring.

Hydrophobic interactions of the substituents were taken into account in the energy study. The results show a similarity between each of the *S* conformers, consisting of a narrow valley at a twist angle between the phenyl and oxazoline rings of 10–30°. This result indicates that the range of the stable conformations of the oxazoline ring is rigidly confined. This contrasts with the wide energy minimum displayed by the *R* conformers, which occurs between 30° and 120°. The *gem*-dimethyl homologue **3** was comparable in activity to the *S* methyl conformer **2a** and produced an energy pattern similar to the latter.

The analogous study with the ethyl homologues **4a** and **4b** further support the apparent correlation between the twist angle and antiviral activity. Both isomers display low areas around 10° but of different magnitudes. Furthermore, **4b** is considerably more active than the *R* methyl homologue **2b** and this is consistent with the broad valley shown by the latter.

The effect of the hydrophobic interactions of the 4-alkyl substituents with amino acid residues of VP1 on activity against HRV-14 becomes clearer if one considers the mode of action of these compounds. In the process of insertion into the binding site in VP1, two residues, Met^{221} and Tyr^{152}, are displaced from 3 to 5 Å, creating a hydrophobic pocket into which the drug fits. The displacement of these two residues causes a concomitant shift of other amino acids in VP1 to a more stable conformation of the polypeptide. Therefore these compounds may prevent uncoating by altering the conformation of the entire viral capsid and stabilizing the modified conformation. In fact, the spontaneous uncoating of poliovirus that occurs at 42 °C is prevented by disoxaril. This indicates that the intramolecular forces that maintain the structural integrity of the capsid in the poliovirus are stronger in the presence of disoxaril than in the native virus. The stability of this conformation is dependent upon the interaction of these compounds with residues within the binding site, which are similar for poliovirus and RV-14. The hydrogen bonding of Asn^{219} to the nitrogen of the oxazoline ring and the hydrophobic interaction with the alkyl substituents on the oxazoline ring are two of these important interactions. Hydrogen bonding contributes between 3 and 10 kcal/mol[18] and hydrophobic bonding has a maximum en-

(9) Yoshimoto, B.; Hansch, C. *J. Org. Chem.* **1976**, *41*, 2269. Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*; Wiley-Intersciences: New York, 1973.
(10) Hein, G. E.; Niemann, C. J. *J. Am. Chem. Soc.* **1962**, *84*, 4495.
(11) Martin, Y. C. *Drug Design*; Academic: New York, 1979; Vol. 8, pp 1–72.
(12) Belleau, B.; LoCassi, G. *J. Med. Chem.* **1964**, *7*, 768.
(13) Scheraga, H. A. *Protein Structure*; Academic: New York, 1961.
(14) Kauzman, W. *Adv. Protein Chem.* **1959**, *14*, 1.
(15) Klotz, I. M. *Science (Washington, D.C.)* **1956**, *128*, 815.
(16) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*; Wiley-Intersciences: New York, 1973.
(17) Davis, S. S.; Higuchi, T.; Rytting, J. H. *Advances in Pharmaceutical Science*; Academic: London, 1974; Vol. 4, pp 73–201.
(18) Franke, R. *Biological Activity and Chemical Structures*; Elsevier: Amsterdam, 1977.

ergy value of 0.7 kcal/mol for a CH_2 to CH_2 interaction.[20,21]

The enantiomeric effect produced by 4-alkyl substituents on the activity against HRV-14 clearly demonstrates the specificity with which these compounds bind to the viral capsid. It is hoped that studies such as these that examine the drug–HRV-14 complexes may serve as models for the design of more potent antipicornavirus agents.

Experimental Section

Melting points were determined according to the USP procedure and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results are within 0.4% of the theoretical values. Analyses were performed by Galbraith Laboratories, Knoxville, TN. NMR spectra were determined on a JEOL FX-270 spectrophotometer and the mass spectra on a JEOLCO double-focusing high-resolution mass spectrophotometer.

General Methods of Synthesis. Ethyl 4-[[7-(3-Methyl-5-isoxazolyl)heptyl]oxy]benzenecarboximidate Hydrochloride. A slurry of 8.95 g (0.03 mol) of 4-[[7-(3-methyl-5-isoxazolyl)-heptyl]oxy]benzonitrile[2] in 50 mL of absolute ethanol was saturated at 0–5 °C with dry HCl gas. After sitting several days at 0 °C, the solution was concentrated and the residual solid triturated with Et_2O and collected. Recrystallization from EtOH–Et_2O gave 10.1 g (89%) of the imidate: mp 105–106 °C; NMR (DMSO-d_6) δ 8.15 (2 H, d, arom), 7.15 (2 H, d, arom), 6.1 (1 H, s, CH), 4.6 (2 H, q, OCH_2Me), 4.1 (2 H, t, $ArOCH_2$), 3.4 (1 H, br s, NH), 2.7 (2 H, t, $ArCH_2$), 2.2 (3 H, s, $ArCH_3$), 1.5 (3 H, t, OCH_2Me), 1.4–1.7 (10 H, m, $CH_2 \times 5$). Anal. ($C_{20}H_{28}N_2O_3$·HCl) C, H, N.

(*S*)-2-Amino-1-pentanol. A mechanically stirred slurry of 10.4 g (0.09 mol) of L-norvaline and 5.1 g (0.13 mol) of LAH in 200 mL of dry tetrahydrofuran was heated to reflux under nitrogen for 20 h. After cooling, the thick gray mass was diluted with ether and carefully quenched at −5 °C by sequential dropwise addition of 5 mL of H_2O, 5 mL of 15% NaOH, and 15 mL of H_2O. The resulting suspension was separated by filtration and the filtrate concentrated to dryness. The oily residue was distilled to give 6.6 g of the amino alcohol, bp 39 °C (0.1 mm) (mp 45–47 °C); $[\alpha]_D$ +5.2° (*c* 1, MeOH) (lit.[20] $[\alpha]_D$ 5.5°).

(*S*)-5-[7-[4-(4,5-Dihydro-4-propyl-2-oxazolyl)phenoxy]-heptyl]-3-methylisoxazole (5a). A mixture of 7.7 g (0.02 mol) of ethyl 4-[[7-(3-methyl-5-isoxazolyl)heptyl]oxy]benzenecarboximidate hydrochloride and 2.6 g (0.025 mol) of (*S*)-2-amino-1-pentanol was heated with stirring to 100 °C for 2 h. After cooling, the solid was treated with EtOAc and the resulting solid collected by filtration. Recrystallization from MeOH yielded 5.4 g (70%) of the desired product: mp 80–81 °C; NMR ($CDCl_3$) δ 7.9 (2 H, d, aromatic), 6.9 (2 H, d, aromatic), 5.8 (1 H, s, CH), 4.5 (1 H, dd, OCH), 4.25 (1 H, m, NCH), 4.0 (3 H, m, OCH_2, OCH), 2.7 (2 H, t, $ArCH_2$), 1.3–1.9 (14 H, m, $(CH_2)_5$, $(CH_2)_2$), 1.0 (3 H, t, CH_3); $[\alpha]_D$ − 29.7° (*c* 1, EtOH). Anal. ($C_{23}H_{32}N_2O_3$) C, H, N.

Registry No. **2**, 98034-32-3; **2a**, 98102-61-5; **2b**, 98524-87-9; **4a**, 112270-39-0; **4b**, 112270-40-3; **5a**, 112270-41-4; **5b**, 112270-42-5; **6a**, 112270-43-6; **6b**, 112270-44-7; **7a**, 112270-45-8; **7b**, 112270-46-9; ethyl 4-[[7-(3-methyl-5-isoxazolyl)heptyl]oxy]benzenecarboximidate hydrochloride, 98360-44-2; 4-[[7-(3-methyl-5-isoxazolyl)heptyl]oxy]benzonitrile, 91944-98-8; (*S*)-2-amino-1-pentanol, 22724-81-8; (*R*)-2-amino-1-pentanol, 80696-30-6; L-norvaline, 6000-40-4.

(19) Dixon, M.; Webb, E. *Enzymes*; Academic: New York, 1964; Chapter 6.
(20) Stryer, L. *Biochemistry*; W. H. Freeman: San Francisco, 1981; Chapter 6.
(21) Cohen, E. J.; Edsall, J. T. *Proteins, Amino Acids and Peptides*; Reingold: New York, 1943; Chapter 9.
(22) Nemely, G.; Scheraga, H. A. *J. Phys. Chem.* **1963**, *66*, 1173.