Conformationally Restricted Analogues of Disoxaril: A Comparison of the Activity against Human Rhinovirus Types 14 and 1A

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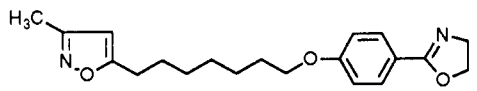
A series of conformationally restricted analogs of disoxaril has been synthesized and evaluated against human rhinovirus types (HRV) 14 and 1A. The sensitivity of these serotypes to this series varied and was dependent upon the length of the molecule as well as upon the flexibility of the aliphatic chain. Minimum energy conformations of these compounds were overlaid with the X-ray structure of a closely related analog **9** bound to the capsid protein of both HRV-14 and -1A and then modeled in the compound-binding site of both serotypes. A comparative sweep volume of these compounds about the isoxazole ring revealed an inaccessible region of space for the *cis*-olefin **8b**, which is not the case for either the *trans*-olefin **8a** or the acetylene **5**. This region may be important to the binding of the compounds to the HRV-14 site particularly during entry into the pocket.

The rhinovirus group of picornaviruses, which are responsible for 40–50% of common cold infections, are comprised of over 100 serotypes.[1] The effectiveness of a chemotherapeutic agent for the common cold would depend on the spectrum of activity and potency, in addition to the bioavailability of the drug. Several factors affect the sensitivity of the various serotypes to an antirhinovirus agent, among which is the ability of the compound to bind to the capsid protein, in the case of uncoating and adsorption inhibitors. The fact that the amino acid sequences among serotypes are not entirely conserved would suggest that the sensitivity of each serotype to this group of antipicornavirus agents would vary.

The determination of the three-dimensional structure of HRV-14[2] and HRV-1A[3] and the subsequent determination of the binding site of disoxaril **1**[4] and of related



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compounds[5] have assisted in better understanding the nature of interactions of these compounds with the capsid protein. This information has also provided some knowledge with regard to binding constants,[6] which have been shown to correlate well with antiviral activity.

The binding sites in both HRV-14 and 1A have been shown to consist of a hydrophobic pocket on the surface of the capsid protein in the vicinity of the cell receptor binding site. Conformational changes in the pocket occur to the extent of 3–5 Å in the case of HRV-14[4] while the HRV-1A pocket undergoes very minimal change as a result of drug binding.[3] The differences in these conformational changes may be responsible for the differences in the mechanism of inhibition of viral replication of the two serotypes by these compounds. Disoxaril has been shown to block adsorption of HRV-14, a member of the major group, while inhibiting uncoating of HRV-1A (minor group).[7] It has been demonstrated that critical amino acid residues in the canyon floor are displaced to an appreciable degree, which may prevent attachment of the HRV-14 to ICAM-1, which has been identified as the major binding site.[8]

We have examined the compound-binding sites of HRV-14 and -1A in an effort to better understand the differences in the sensitivities of these serotypes to compounds related to disoxaril. It has been shown that several amino acid residues are not conserved in the drug binding site, the most critical being Tyr^{128}, Cys^{199}, and Pro^{174} of HRV-14 (Figure 1), which are replaced by Ile, Met, and Met, respectively.[3] The result of these structural differences is that the HRV-1A pocket is shorter and wider.

The chain connecting the isoxazole and phenyl rings in the compounds studied adopts a bowed conformation when

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(1) Hamparian, V. V.; Colonno, R. J.; Cooney, M. K.; Dick, E. C.; Gwaltney, R. M., Jr.; Hughes, J. H.; Jordan, W. S., Jr.; Kapikian, A. Z.; Mogabgab, W. J.; Monto, A.; Phillips, C. A.; Reuckert, R. R.; Schieble, J. H.; Stott, E. J.; Tyrrell, D. A. J. A Collaborative Report: Rhinoviruses-Extension of the Numbering System from 89-100. *Virology* **1987**, *159*, 191–192.

(2) Rossmann, M. G.; Arnold, E.; Erickson, J. W.; Frankenberger, E. A.; Griffith, J. P.; Hecht, J. H.; Johnson, J. E.; Kamer, G.; Luo, M.; Mosser, A. G.; Reuckert, R. R.; Sherry, B.; Vriend, G. Structure of a Common Cold Virus and Functional Relationship to other Picornaviruses. *Nature* **1985**, *317*, 145–152.

(3) Kim, S.; Smith, T. J.; Chapman, M. S.; Rossmann, M. G.; Pevear, D. C.; Dutko, F. J.; Felock, P. J.; Diana, G. D.; McKinlay, M. A. Crystal Structure of Human Rhinovirus Serotype 1A (HRV 1A). *J. Biol. Chem.* **1989**, *210*, 91-111.

(4) Smith, T. J.; Kremer, M. J.; Luo, M.; Vriend, G.; Arnold, E.; Kamer, G.; Rossmann, M. G.; McKinlay, M. A.; Diana, G. D.; Otto, M. J. The Site of Attachment in Human Rhinovirus 14 for Antiviral Agents that Inhibit Uncoating. *Science* **1986**, *233*, 1286–1293.

(5) Badger, J.; Minor, I.; Kremer, M. J.; Oliviera, M. A.; Smith, T. J.; Griffith, J. P.; Guerin, D. M. A.; Krishnaswamy, S.; Luo, M.; Reuckert, R. R.; Heinz, B. A. Structural Analysis of a Series of Antiviral Agents Complexed with Human Rhinovirus 14. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3304–3308.

(6) Fox, M. P.; McKinlay, M. A.; Diana, G. D.; Dutko, F. J. Affinities of Structurally Related Human Rhinovirus Capsid Binding Compounds are Related to Their Activities Against Human Rhinovirus Type 14. *Antimicrob. Agents Chemother.* **1991**, *35*, 1040–1047.

(7) Pevear, D. C.; Fancher, M. J.; Felock, P. J.; Rossmann, M. G.; Miller, M. S.; Diana, G. D.; Treasurywala, A. M.; McKinlay, M. A.; Dutko, F. J. Conformational Change in the Floor of the Human Rhinovirus Canyon Blocks Adsorption to Hela Cell Receptors. *Virology* **1989**, *63*, 2002–2007.

(8) Staunton, D. E.; Merluzzi, V. J.; Rothlein, R.; Barton, R.; Marlin, S. D.; Springer, T. A. ICAM-1 is the Major Surface Receptor for Rhinovirus. *Cell* **1989**, *56*, 849–853.

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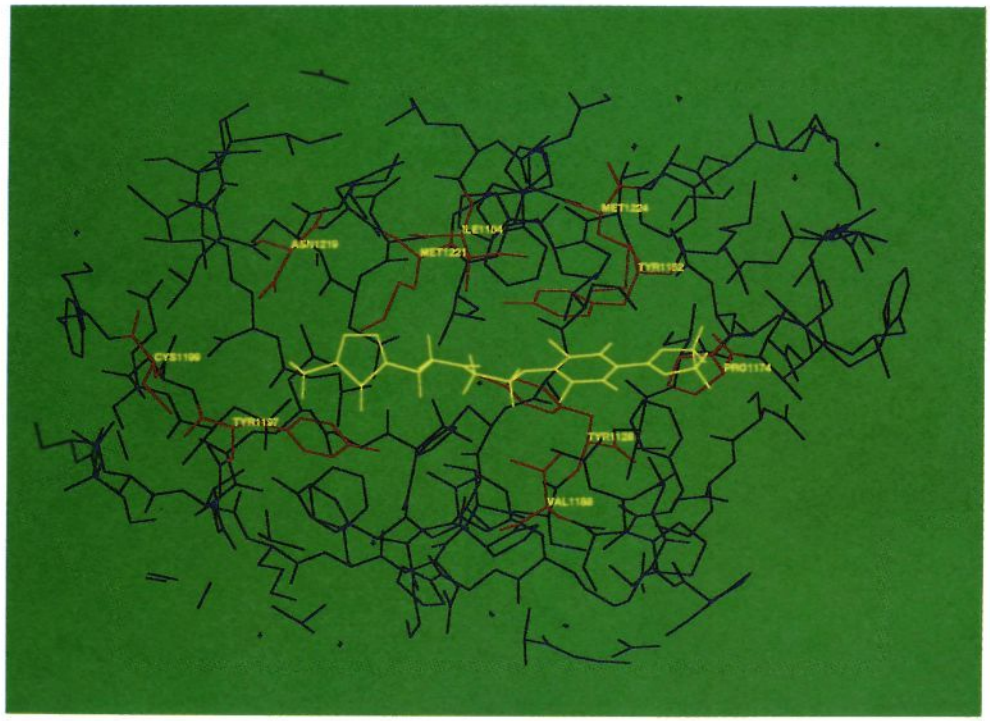
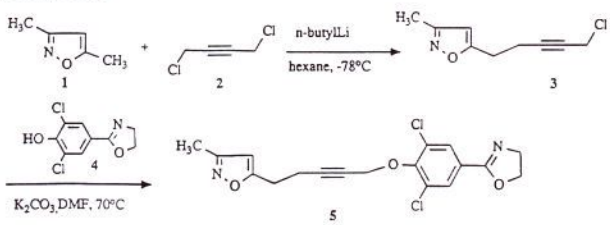
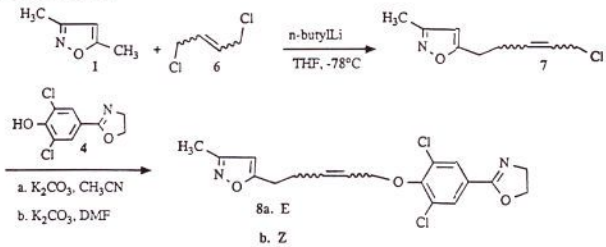


Figure 1. Compound **9** (in yellow) bound to HRV-14 with several of the closest residues (in red). These residues are within 4 Å of the compound.

Scheme I



Scheme II



bound to both HRV-1A and -14. It has been assumed that the flexibility of the hydrocarbon chain is critical for binding and biological activity since the molecules could adjust to the size of the binding site and consequently exhibit activity against both HRV-14 and -1A. Since there are over 100 rhinovirus serotypes whose binding sites probably differ in size, the flexibility of the hydrocarbon chain may be a necessity for broad-spectrum activity. To test this hypothesis, several compounds with rigidity incorporated into the chain have been synthesized, their activity against HRV-14 and -1A has been examined, and the compounds have been modeled in the binding site of these serotypes, using the X-ray structures of the virus–drug complex of compound **9** as a starting point.

Chemistry

Both triple and double bonds were incorporated into the hydrocarbon bridge. The synthesis of the acetylene analog is shown in Scheme I. Treatment of 3,5-dimethylisoxazole with *n*-butyllithium at -78 °C followed by the dropwise addition to 1,4-dichloro-2-butyne in THF provided 3-methyl-5-(5-chloro-3-pentynyl)isoxazole (**3**) in 80% yield. The reaction of **3** with 2,6-dichloro-4-(4,5-dihydro-2-oxazolyl)phenol[9] (**4**) and potassium carbonate in DMF gave **5** in 90% yield.

The (*E*)- and (*Z*)-pentene analogs **8a** and **8b**, respectively, were prepared in a similar fashion from the appropriate dichlorobutenes (Scheme II). Compound **8a** was prepared using potassium carbonate in refluxing acetonitrile; however, conditions using DMF at 100 °C were required for the preparation of the *Z*-isomer **8b**, resulting in a 61% yield. The saturated propyl analog **10** was prepared by the alkylation of 3,5-dimethylisoxazole with 1-bromo-2-chloroethane to give an 83% yield of the chloropropylisoxazole, which was then treated with **4**.

Results

The compounds were tested against HRV-14 and -1A in a plaque-reduction assay[10] and the results are shown in Table I. Compound **9**, Win 54954, which has been

(9) Diana, G. D.; Cutcliffe, D.; Oglesby, R. C.; Otto, M. J.; Mallamo, J. P.; Akullian, V.; McKinlay, M. A. Synthesis and Structure-Activity Studies of Some Disubstituted Phenylisoxazoles against Human Rhinovirus. *J. Med. Chem.* **1989**, *32*, 450–455.

(10) Diana, G. D.; McKinlay, M. A.; Brisson, C. J.; Zalay, E. S.; Miralles, J. V.; Salvador, U. J. Isoxazoles with Antipicornavirus Activity. *J. Med. Chem.* **1985**, *28*, 748–752.

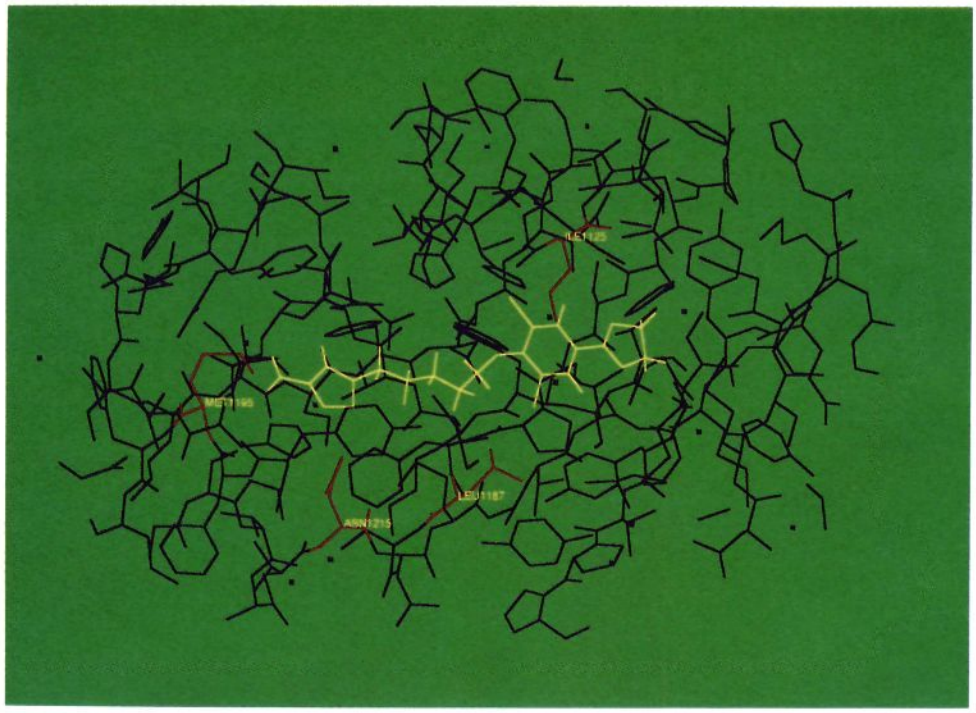
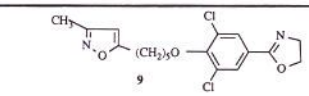
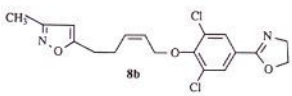
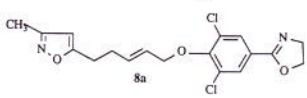
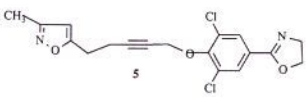
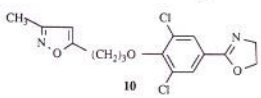


Figure 2. Compound **9** in yellow bound to HRV-1A. Residues Met1195, Leu1187, and Ile1125 are nonconserved in HRV-14.

Table I. Comparative in Vitro Antirhinovirus Activity

	MIC (μmol)	
	1A	14
 **9**	2.5	1.2
 **8b**	4.72	3.9
 **8a**	2.25	0.44
 **5**	>8.2	0.26
 **10**	0.46	8.14

evaluated in the clinic,[11] was used as a comparator. The *Z*-olefin **8b** demonstrated a 2–3-fold reduction in activity against both serotypes when compared to **9**, while the corresponding *E*-isomer was comparable in activity to **9** against HRV-1A but showed a 3-fold enhancement in activity against HRV-14. The butyne analog **5** was inactive against HRV-1A but was greater than 4-fold more potent than **9** against HRV-14 and comparable in activity to the *E*-isomer against this serotype.

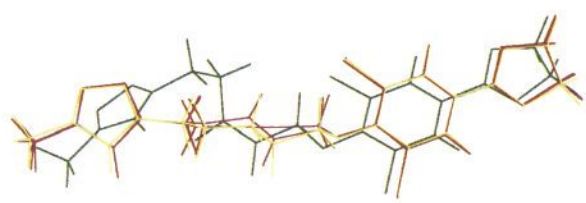


Figure 3. An overlay of the energy-minimized structures of compounds **5** (red), **8a** (magenta), **8b** (green) and **9** (yellow).

Molecular Modeling

The X-ray conformations of compound **9** bound to HRV-1A and HRV-14[12] were determined and the small molecule conformations were used in subsequent modeling experiments. The compound is bound in the same orientation in both serotypes. In HRV-14 (Figure 1), the phenyl ring of compound **9** is in a stacking conformation between Tyr^{128} and Tyr^{152}, in a herring bone pattern. Val^{188} is in close contact with one of the chlorine atoms on the phenyl ring while the other chlorine is in van der Waals contact with Tyr^{128}, Ile^{104}, and Met^{224}. The isoxazole ring lies in the "pore" area of the binding site in close proximity to Tyr^{197}, Met^{221}, and Asn^{219}. This area of the binding site is considerably more hydrophyllic. In the case of HRV-1A, Val^{191} in HRV-14 is replaced by Leu^{187} and Tyr^{128} by Ile^{125}, resulting in a 45° rotation of the phenoxy and oxazoline rings from their relative position in HRV-14, in order to avoid steric interactions with these residues (Figure 2). The replacement of Cys^{199} with Met^{195} in HRV-1A causes the position of the isoxazole ring to shift while its relative position near Asn^{215} and Met^{195} is maintained as in HRV-14.

(11) Turner, R. B.; Hayden, F. G.; Efficacy of Oral Win 54954 for the Prophylaxis of Experimental Rhinovirus Infection. *Antiviral Res., Suppl. 1*, **1992**, *17*, 92. Schiff, G. M.; Sherwood, E. C.; Young, L. J.; Mason, J. N. Prophylactic Efficacy of Win 54954 in Prevention of Experimental Human Coxsackievirus A21 Infection and Illness. *Antiviral Res., Suppl. 1*, **1992**, *17*, 92.

(12) Kim, K. H.; Willingmann, P.; Gong, Z. X.; Kremer, M. J.; Chapman, M. S.; Minor, I.; Oliveira, M. A.; Rossmann, M. G.; Andries, K.; Diana, G. D.; Dutko, F. J.; McKinlay, M. A.; Pevear, D. C. A Comparison of the Antirhinoviral Drug Binding Pocket in Hrv14 and Hrv1A. Submitted to *J. Mol. Biol.* **1992**.

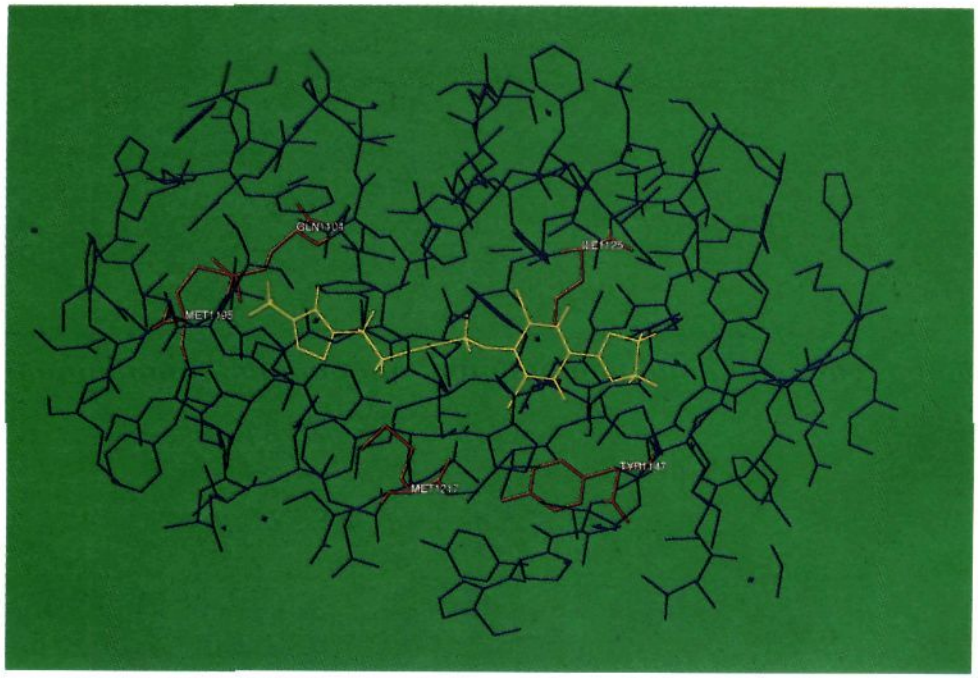
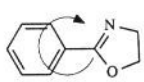


Figure 4. Compound **5** modeled in the HRV-1A pocket showing steric interaction between the chlorine atom on the aromatic ring and Ile125.

Table II. Torsion Angle between the Oxazoline and Phenyl Rings



compd	torsion angle (deg)	
	HRV-14	HRV-1A
9[a]	3.2	3.2
8a[b]	2.8	3.3
8b[b]	0.9	4.0
5[b]	1.4	9.4

[a] Torsion angle obtained from *X*-ray structure. [b] Torsion angle obtained from minimized structure fitted to X-ray structure of compound **9**.

Compound **9** was removed in the X-ray-determined conformation from the virus binding site (1A and 14) and converted to **5**, **8a**, and **8b** by modification of the appropriate atom types. The resulting structures were subjected to the Tripos force field (Maximin 2), using Sybyl version 5.41, with default settings. Rotatable bonds in the alkyl ether chain were defined and the structures flexibly fitted to compound **9**, in virus-bound conformation, for insertion into HRV-1A and -14 binding sites. An overlay of these structures with compound **9** in an HRV-14-bound configuration is depicted in Figure 3. The optimized and fitted structures **5**, **8a**, and **8b** were inserted into each serotype by replacement of virus-bound **9**. The drug-bound conformation of the virus binding site with several of the compounds has been determined[5] revealing only minor variation in both the protein side chain and backbone conformation despite the variation in compound structure. Consequently, insertion of the modeled compounds into the binding site configuration derived from compound **9** is supported by these observations. The torsion angle between the oxazoline and phenyl rings for each compound after energy minimization and flexible fitting is shown in Table II.

Discussion

Two of the most striking results in Table I are the inactivity of the acetylene **5** against HRV-1A and the difference in activity between the *Z* (**8b**) and *E* (**8a**) olefins against HRV-14. The relative sizes of the HRV-1A and -14 pockets was discussed in the introduction. From previous work we have found that in this series, the HRV-1A binding site accommodates shorter molecules. This is evident from the increase in activity between the three and five-carbon-chained homologs **10** and **9**, respectively. Conversely, reducing the length of the chain causes a concomitant decrease in activity against HRV-14. These results support the premise that the activity is dependent upon the length of the molecule and also confirm the conclusions obtained from volume map studies which demonstrated that optimum activity is associated with a high degree of occupancy of the pocket, particularly in the pore area.[13] It is reasonable to assume that these conclusions also apply to HRV-1A.

When **5** was modeled in the HRV-1A pocket there were some steric interactions between the chlorine atom and Ile^{125} which is within 3 Å of the compound (Figure 4). These interactions appear to be no more severe than those shown by **9**, **8a**, and **8b**, which suggest that **5** should exhibit activity comparable to these compounds. The fact that this compound is inactive against HRV-1A is, at this point, unexplainable. The enhancement in activity against HRV-14 shown by compound **5** may be due to two factors. First, the rigidity of the triple bond forces the chain to adopt a linear conformation, thus maximizing the distance between the isoxazole and oxazoline rings and consequently filling space within the pocket. In addition, the planarity of the triple bond and adjacent carbon atoms minimizes any steric interactions within the pocket requiring less conformational changes within the binding site.

(13) Diana, G. D.; Treasurywala, A. M.; Bailey, T. R.; Oglesby, R. C.; Pevear, D. C.; Dutko, F. T. A Model for Compounds Active Against Human Rhinovirus-14 Based on X-ray Crystallography Data. *J. Med. Chem.* **1990**, *33*, 1306–1311.

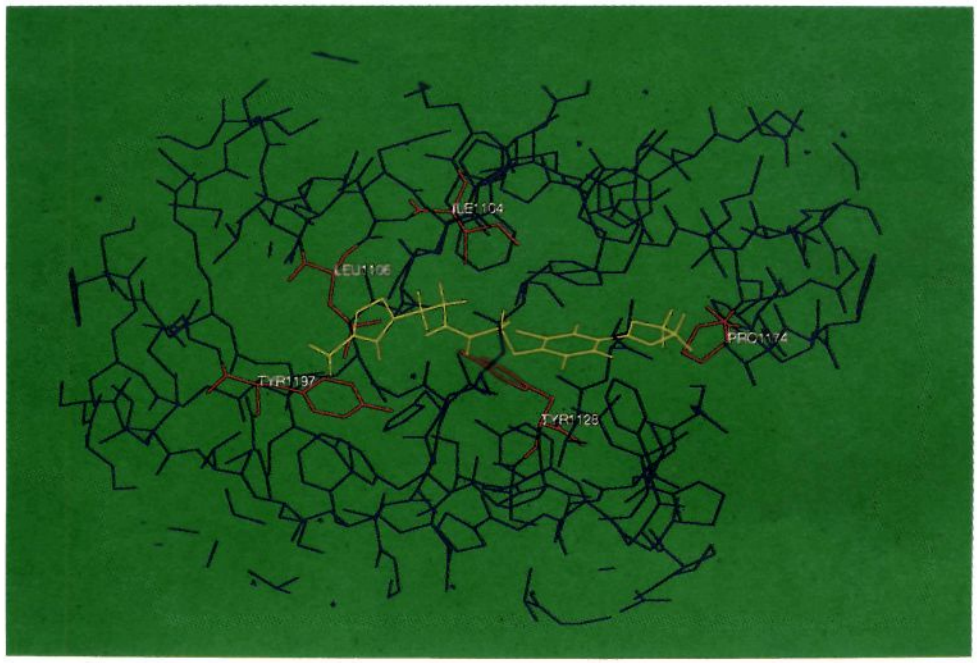


Figure 5. Compound **8b** modeled in HRV-14 showing negative interactions with Ile104 and Tyr128. Residues in red are within 2.8 Å.

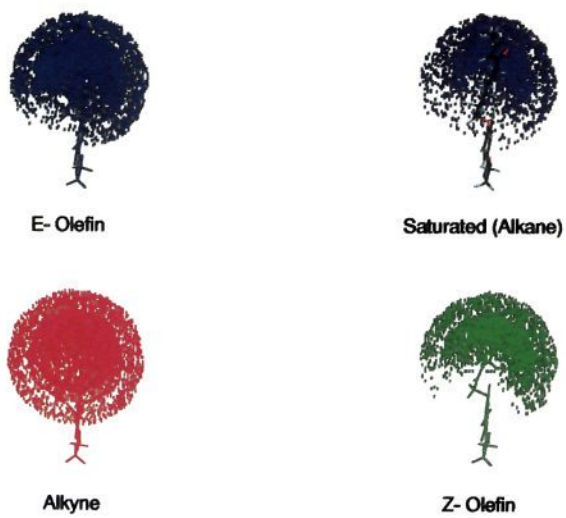


Figure 6. Conformational sweep graphs illustrating the conformational space accessible to the isoxazolylethyl chain of compounds **5** (red), **8a** (purple), **8b** (green), and **9** (blue). The volumes are obtained by rotating about the adjacent carbon atoms in the connecting chain.

The difference in activity against HRV-14 between the *E*- and *Z*-olefins can be explained by examining the relative low-energy virus bound conformations of the two compounds. An overlay of compound **9** (based on X-ray crystallography data) and minimized structures for **5**, **8a**, and **8b** shown in Figure 3 indicates that compound **8a** shows a reasonable fit with respect to **9** while **8b** does not. Furthermore, when compound **8b** is inserted into HRV-14 (Figure 5), unfavorable interactions occur between the molecule and several residues in the pocket including Ile^{104} and Tyr^{128}. Reorganization of the HRV-14 pocket to accommodate **8b** may cost free energy, which is reflected by the higher MIC value.

The very high MIC (Table I) for **8b** against HRV-14 and -1A may also reflect a slow k_{on} in both cases. The conformational space accessible to the isoxazole of compounds **5**, **8a**, **8b**, and **9** is shown in Figure 6. The conformational sweep graph for the *Z*-olefin **8b** discloses a significant inaccessible region of space; however alkyne **5**, *E*-olefin **8a**, and alkane **9** do not show this deficit. Along with the steric interactions which result when **8b** is modeled into the static HRV-14 pocket, binding to this site may be dependent on conformational permissibility in this region which is required during entry into the pocket. The result is that **8b** is 2–8 times less active against HRV-14 than the other five-carbon-chain analogs, **5**, **8a**, and **9**. However, **8b** has weak but comparable activity to these analogs against HRV-1A. Although entry into the HRV-1A pocket (k_{on}) may be slow, once the compound enters the binding site it is able to attain the required conformation.

The results of this study have shown that the activity of this series of compounds against HRV-14 and -1A is strongly dependent upon the flexibility of the hydrocarbon chain and of the ability of the molecule to fit into the conformational space of both of the pockets of these serotypes.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 20SX FTIR. NMR spectra were acquired in the indicated solvent on a JEOL-FX270, General Electric QE-300 or Bruker-AC200 FTNMR. HETCOR (^{1}H–^{13}C correlation) and DEPT experiments were utilized to assist in peak assignments. Mass spectra were recorded on a Nermag R10/10 coupled to a Varian 3400 gas chromatograph or on a JEOL JMS-01SC spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on E. Merck 5 × 20, Kieselgel 60 F-254 plates. Preparative chromatography was performed using a Büchi B680 MPLC system coupled to an ISCO UV detector and fraction collector. Columns were packed with Kieselgel 60, 230–400 mesh. High-boiling solvents (DMF) were stage-dried over molecular

sieve,[14] and anhydrous THF was distilled from sodium benzophenone ketyl. Alkyllithium reagents were titrated with diphenylacetic acid.[15]

5-[5-[2,6-Dichloro-4-(4,5-dihydro-2-oxazolyl)phenoxy]-3-pentynyl-3-methylisoxazole (5). To a stirred solution of 3,5-dimethylisoxazole (10 g, 0.10 mol) in 210 mL of THF at −78 °C under an atmosphere of N_2 was added by dropwise addition 11.9 mL of 9.5 M *n*-BuLi in hexanes (0.11 mol). The resulting mixture was stirred at −78 °C for 30 min. In a separate 1-L flask, a solution of 1,4-dichloro-2-butyne (38 g, 0.31 mol) in 300 mL of THF under N_2 was cooled to −78 °C. The resulting anion was transferred to the flask containing 1,4-dichloro-2-butyne with a 12-gauge cannula under N_2 pressure. The resulting mixture was stirred at −78 °C for 60 min, warmed to ca. 10 °C, and quenched with 200 mL of 20% NaH_2PO_4. The volatiles were removed in vacuo, and the resulting aqueous mixture was extracted with Et_2O. The combined ethereal extracts were washed three times with saturated NaCl, dried over anhydrous K_2CO_3, and concentrated to give 41 g of a mobile liquid. Flash chromatography on 600 g of silica gel eluted with hexane recovered unreacted dichloride. Subsequent elution with Et_2O and evaporation gave 15.2 (80%) of 3-methyl-5-(5-chloro-3-pentynyl)isoxazole (**3**). A solution of **3** (15.0 g, 82 mmol) and 2,6-dichloro-4-(4,5-dihydro-2-oxazolyl)-phenol hydrochloride[8] (15 g, 56 mmol) in 300 mL of DMF containing 75 g of anhydrous K_2CO_3 was heated at 70 °C for 12 h, after which time the reaction was filtered, and the volatiles were removed in vacuo. The resulting semisolid was triturated with 500 mL of 1:1 Et_2O/EtOAc aided by sonication and was filtered. The organic solution was washed with 2 × 100 mL of H_2O and 2 × 100 mL of saturated NaCl and dried over K_2CO_3 containing a small amount of Darco G-60. Filtration and rotary evaporation gave 19.3 g (90%) of an off white solid, mp 65–69 °C. The desired product (R_f = 0.4, Et_2O) was isolated by MPLC on a 50 × 600 mm column eluting with 9:1 Et_2O/hexane to give 9.6 g (45%) of a white solid. Recrystallization from hexane/Et_2O gave 8.25 g of **5**: mp 70–72 °C; ^1H NMR ($CDCl_3$) δ 7.90 (2 H, s), 5.81 (1 H, s), 4.84 (2 H, t, J = 1.5 Hz), 4.45 (2 H, t, J = 10.3 Hz), 4.08 (2 H, t, J = 8.6 Hz), 2.90 (2 H, t, J = 10.3 Hz), 2.6 (2 H, m), 2.3 (3 H, s). Anal. ($C_{18}H_{16}Cl_2N_2O_3$) C, H, N.

(*E*)-5-[5-[2,6-Dichloro-4-(4,5-dihydro-2-oxazolyl)phenoxy]-3-pentenyl]-3-methylisoxazole (8a). (*E*)-5-(5-Chloro-3-pentenyl)-5-methylisoxazole (**7**) was prepared as an oil, according to the procedure described in the previous experiment. The reaction of **7** with 2,6-dichloro-4-(4,5-dihydro-2-oxazolyl)phenol hydrochloride as previously described followed by purification by MPLC (elution with 35:5 Et_2O/hexane) gave 8.39 g of **8a** in 56% yield: mp 59–61 °C; ^1H NMR ($CDCl_3$) δ 7.89 (1 H, s), 5.7–6.05 (3 H, m), 4.5–4.7 (2 H, m), 4.45 (2 H, t), 2.8 (2 H, t), 2.46 (2 H, m), 2.26 (3 H, s). Anal. ($C_{18}H_{18}Cl_2N_2O_3$) C, H, N.

(*Z*)-5-[[2,6-Dichloro-4-(4,5-dihydro-2-oxazolyl)phenoxy]-3-pentenyl]-3-methylisoxazole (8b). (*Z*)-3-Methyl-5-(5-chloro-3-pentenyl)isoxazole was prepared in 90% purity according to the procedure previously described. The reaction of the above crude isolate (21.1 g) and 2,6-dichloro-4-(4,5-dihydro-2-oxazolyl)-phenol hydrochloride (8.0 g, 30 mmol) in 500 mL of DMF containing 90 g of anhydrous K_2CO_3, as described above, gave 6.9 g (61%) of an off-white solid. Recrystallization from *tert*-butyl methyl ether/pentane gave 4.34 g of **8b**: mp 52–54 °C; ^1H NMR ($CDCl_3$) δ 7.91 (2 H, S), 6.0–5.84 (1 H, m), 5.82 (1 H, s), 5.76–5.62 (1 H, m), 2.27 (3 H, s). Anal. ($C_{18}H_{18}Cl_2N_2O_3$) C, H, N.

3-[3-[2,6-Dichloro-4-(4,5-dihydro-2-oxazolyl)phenoxy]propyl]-3-methylisoxazole (10). 5-(3-Chloropropyl)-3-methylisoxazole was prepared in 92% yield as a yellow oil, of ca. 95% purity, by the method described above: ^1H NMR ($CDCl_3$) δ 5.71 (1 H, s), 3.45 (2 H, t), 2.8 (2 H, t), 2.4–2.0 (3 H, s, 2 H, m).

Reaction of 20 g of the above crude chloride, 2,6-dichloro-4-(4,5-dihydro-2-oxazolyl)phenol hydrochloride (11.2 g, 42 mmol), 2 g of NaI, and 60 g of anhydrous K_2CO_3 in 800 mL of CH_3CN, as described above, gave 14 g (81%) of **10** as an off-white solid after chromatography on 700 g of SiO_2, eluted with 100% Et_2O. Recrystallization from Et_2O gave 12.5 g (73%) of **10** as fine white crystals: mp 67–68 °C; ^1H NMR ($CDCl_3$) δ 7.63 (2 H, s), 5.7 (1 H, s), 4.32 (2 H, t), 4.25–3.9 (4 H, m), 2.95 (2 H, t), 2.20 (3 H, s), 2.1 (2 H, q). Anal. ($C_{16}H_{16}Cl_2N_2O_3$) C, H, N.

(14) Burfield, D. R.; Smithers, R. H. Desicant Efficiency in Solvent and Reagent Drying. 7. Alcohols. *J. Org. Chem.* **1983**, *48*, 2420–2422.

(15) Kofron, W. G.; Baclawski, L. M. A Convenient Method for Estimation of Alkyllithium Concentrations. *J. Org. Chem.* **1976**, *41*, 1879–1880.