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Structures of Four Methyltetrazole-Containing Antiviral Compounds in Human Rhinovirus Serotype 14

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Abstract

Four novel antiviral WIN compounds, that contain a methyl tetrazole ring as well as isoxazole, pyridazine or acetylfuran rings, have had their structures determined in human rhinovirus serotype 14 at 2.9 Å resolution. These compounds bind in the VP1 hydrophobic pocket, but are shifted significantly towards the pocket pore when compared to previously examined WIN compounds. A putative water network at the pocket pore is positioned to hydrogen bond with these four WIN compounds, and this network can account for potency differences seen in structurally similar WIN compounds.

Introduction

Human rhinoviruses (HRVs) are the major cause of the common cold in humans (Rueckert, 1990). Rhinoviruses belong to the family picornaviridae which are small icosahedral non-enveloped positive-sense-RNA viruses. Also included in the family picornaviridae are the enterovirus (*e.g.* polioviruses), aphthovirus (*e.g.* foot and mouth disease virus), heparnavirus (hepatitis A virus) and cardiovirus (*e.g.* mengovirus, EMC virus) (Melnick, 1992). There are 102 classified serotypes of HRVs. All but one of these serotypes bind to either of two receptors (Uncapher, DeWitt & Colonno, 1991). Most serotypes bind to ICAM-1 and belong in the major receptor group (Greve *et al.*, 1989). A subset of ten serotypes, the minor receptor group, binds to the low-density lipoprotein receptor family (Hofer *et al.*, 1994).

The X-ray crystal structures have been solved for a number of picornaviruses, and their structures share common features. Each picornavirus protein coat is comprised of 60 icosahedrally arranged copies of four distinct viral proteins (VP1 to VP4). VP1, VP2 and VP3 are eight-stranded β-barrels that extend through the capsid, and thus have both interior and exterior surfaces. VP4 is smaller than the other coat proteins and resides on the coat interior, in contact with the viral RNA. The HRVs and polioviruses have 15 Å deep exterior grooves, called canyons, which encircle each of the viral fivefold axes. In HRV16 this canyon has been shown to be the site of ICAM-1 binding (Chapman & Rossmann, 1993; Olson *et al.*, 1993). HRVs 14, 1A, 16 and 50 (HRV50 unpublished data) have had their structures elucidated crystallographically (Arnold & Rossmann, 1990; Badger *et al.*, 1988; Chapman, Minor, Rossmann, Diana & Andries, 1991; Kim *et al.*, 1993, 1989; Oliveira *et al.*, 1993; Smith *et al.*, 1986). HRVs 14, 16 and 50 belong to the major receptor group, and HRV1A belongs to the minor receptor group. Of these HRV sequences, HRV14 is the most divergent and is the most similar to the poliovirus sequences (Palmenberg, 1989).

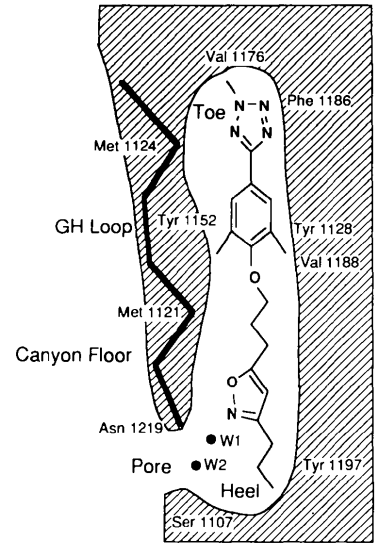

Fig. 1. A schematic representation of the hydrophobic pocket of VP1 occupied by compound (1). Residues in HRV14 are numbered according to the convention *xyyy*, where *x* indicates the viral protein number, VP1–4, and *yyy* indicates the sequence number in the VP*x*. The approximate locations of the pore water molecules are depicted as W1 and W2. The orientation of the schematic is similar to that of Fig. 4.

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HRV and enterovirus infections have been inhibited in cell culture, animal models and humans by a number of hydrophobic compounds (Alarcon, Zerial, Dupiol & Carrasco, 1986; Andries, Dewindt, De Brabander, Stokbroekx & Janssen, 1988; Desideri *et al.*, 1992; Ishitsuka, Ninomiya, Ohsawa, Fujiu & Suhara, 1982; Kenny *et al.*, 1986; McKinlay & Steinberg, 1986). Prototypes of these compounds have been examined crystallographically (Badger *et al.*, 1988; Chapman *et al.*, 1991; Kim *et al.*, 1993, 1989; Oliveira *et al.*, 1993; Smith *et al.*, 1986; Zhang *et al.*, 1993). These compounds bind in a hydrophobic pocket inside the β-barrel of VP1. This pocket lies directly beneath the VP1 GH loop, which forms a portion of the canyon floor (Fig. 1). These compounds inhibit viral uncoating, and in some major receptor group viruses, also inhibit at the ICAM-1 binding step (Pevear *et al.*, 1989; Shepard, Heinz & Rueckert, 1993). *In vitro*, these compounds have been shown to stabilize the rhinoviruses to acid- and heat-induced loss of infectivity (Fox, Otto & McKinlay, 1986; Gruenberger, Pevear, Diana, Kuechler & Blaas, 1991). The prototypical WIN compound consists of an isoxazole ring, linked *via* an aliphatic chain, to an oxazolinophenoxy group [Fig. 2, compound (6)].

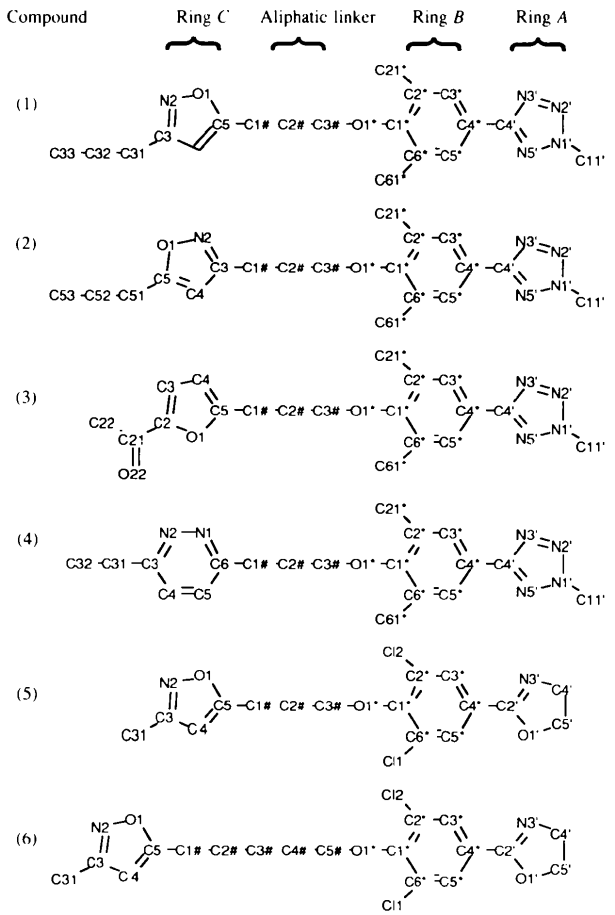


Fig. 2. WIN compound chemical structures (1) to (4) are presented here. Compound (1) contains an isoxazole ring at position *C*. Compound (2) also contains a *C* ring isoxazole, but the positions of the N and O atoms are reversed when compared with compound (1). Compounds (5) and (6) have been presented as WIN 56291 and WIN 54954, respectively (Kim *et al.*, 1993). Naming conventions for WIN compounds are consistent with previous publications.

Unlike polioviruses and encephalomyocarditis virus, HRVs require a pH-lowering step for productive infection of cells (Perez & Carrasco, 1993). The X-ray crystal structure of HRV14, exposed to acid, shows that three regions of the virion disorder (Giranda *et al.*, 1992). They are the GH loop, the putative fivefold Ca^{2+} ion, and VP3 and VP4 near the icosahedral fivefold axis. A series of mutants have been isolated that convey acid or antiviral resistance (without excluding antiviral compound binding inside VP1). These mutations predominate in the area surrounding the GH loop (Giranda *et al.*, 1992; Skern, Torgersen, Auer, Kuechler & Blaas, 1991). The acid-induced disorder of the GH loop and its proximity to mutations and antivirals, which effect virion stability, suggest that the uncoating blockade of WIN compounds is mediated through stabilization of the virion, especially at the GH loop.

Here we describe the structure of four novel WIN compounds bound to HRV14. These compounds contain a tetrazole ring and a three-carbon aliphatic linking region [Fig. 2, compounds (1)–(4)]. Their structures will be compared with other previously published compounds in HRV14, as well as in HRV1A. HRV16 and HRV50.

Materials and methods

HRV14 crystals were prepared using established procedures (Arnold *et al.*, 1984). Crystals (cubic, $P2_13$, $a = 445.1$ Å) greater than 0.25 mm in at least one dimension were selected for data collection. These crystals were placed in 990 µl of 4%(*w/v*) PEG 8000 in 10 m*M* Tris buffer, pH = 7.2, with 20 m*M* $CaCl_2$ and 0.1%(*w/v*) NaN_3. To each well was added 10 µl of a 1 mg ml^{-1} WIN compound solution in 100% dimethyl sulfoxide (DMSO) for each of the compounds 1–4 (Fig. 2). For each compound, seven to 11 crystals were used in data collection. The crystals were soaked in inhibitor solutions for four to eight days prior to data collection.

Data were collected at the Cornell High Energy Synchrotron Source using the F1 station with an X-ray wavelength = 0.917 Å. Oscillation photographs (0.3°) were taken using Kodak DEF-5 film. Typical exposure times were between 60 and 90 s and were gauged using 1 to 2 s still photographs using Polaroid-type 57 film. The crystals continued to diffract X-rays for approximately eight exposures with the best data collected in the first four exposures. Approximately 20 films were used for each data set.

Data were processed using programs developed at Purdue University (Rossmann, 1985). The data were scaled to HRV14 native data (Arnold & Rossmann, 1988, 1990; Rossmann, 1985). Electron-density maps

Table 1. *Data-collection summary*

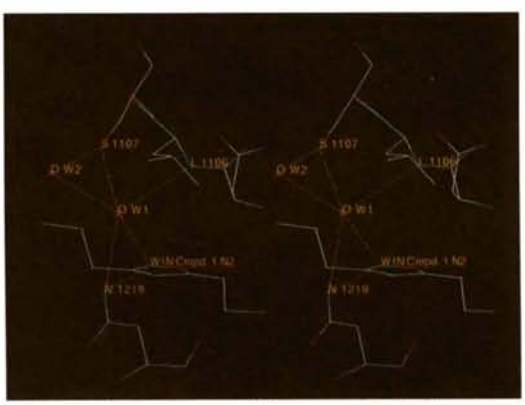
Summarized are all the data for which the partiality is >0.5, and are between 40 and 2.9 Å resolution.

WIN compound	Total accepted observations	Total accepted reflections	Data completeness (%)	R_{sym}* All data/3.0–2.9 Å data	$I/\sigma(I)$ 3.0–2.9 Å data
(1)	258318	202871	32	13.6/18.6	3.6
(2)	291396	227316	36	11.3/17.8	4.4
(3)	209978	177246	28	12.8/17.9	3.3
(4)	237328	188213	30	11.8/16.9	3.9

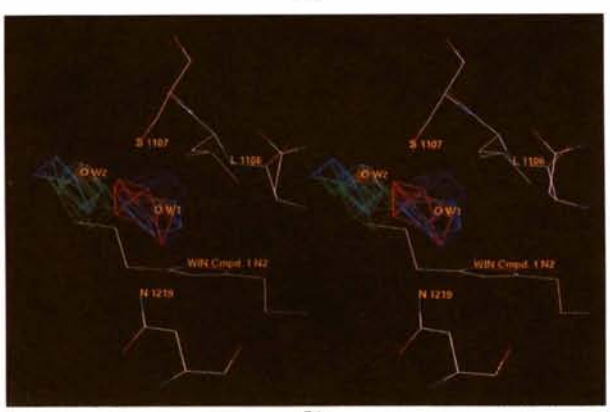
* $R_{sym} = (\sum_h \sum_i |F_h^2 - F_{hi}^2|)/(\sum_h \sum_i F_h^2) \times 100$ where F_h^2 is the mean of i observations F_{hi}^2 for reflection h.

were calculated according to $[F_{WIN} - (1 - k)F_N] \times \exp i\alpha_{MR}(N)$, where F_{WIN} and F_N are the structure-factor amplitudes for the WIN compound bound and native structures, respectively (Smith *et al.*, 1986). The phase terms, $\alpha_{MR}(N)$, are the native data molecular-replacement phases. The constant k, is empirically determined to allow the electron density of the compound to be approximately the same as that of the virus. In all cases reported here, $k = 0.65$ yielded the best result. The maps are dramatically improved by real-space averaging over the 20-fold non-crystallographic symmetry.

The limit of diffraction for a typical crystal was 2.8 Å. Typically, 10–15% of collected reflections between 40 and 2.9 Å resolution had $I/\sigma_I < 2.5$ and were rejected as unobserved. A 0.3° oscillation photograph yields approximately 10 000 whole and 9000 partial reflections (Table 1). The resulting electron-density maps when contoured at 1.5σ are continuous in both WIN compound and virus structures. Residue side chains are clearly visible and carbonyl bulges of the peptide backbone are visible for many residues.



(a)



(b)

Fig. 3. A representative hydrogen-bond network formed between WIN compound (1), two water molecules, and the pore of the hydrophobic pocket of HRV14 showing possible hydrogen bonds (*a*). The electron densities for these waters (*b*) where red corresponds to WIN compound (1); blue, (2); green, (3) and cyan, (4).

The models of the four WIN compounds were built *in vacuo* using the program *QUANTA*'s molecular editor module (MSI, Waltham MA). The initial structures were minimized using the method of steepest descents for 50 cycles in the *CHARMm* module in *QUANTA*. The WIN compounds were fit manually into their electron densities using the graphics program *CHAIN* (Sack, 1988). The model was manually adjusted to minimize close contacts (Table 2). Where bonds needed to be broken during fitting, bond lengths and angles were regularized using *CHAIN*. Waters in the pore of the pocket were placed manually after visual inspection of the map (Fig. 3*b*). Waters in the pore of the WIN compound were placed within 0.5σ electron-density baskets, within hydrogen-bonding distance of at least one other hydrogen-bonding group. The electron-density peaks for waters 1 (W1) for compounds (1), (2) and (4) were 0.9, 0.9 and 0.8σ respectively. The peaks for waters 2 (W2) for compounds (1)–(4) were 0.7, 0.5, 0.8 and 0.8σ respectively.

Results and discussion

The structures of the four methyltetrazole compounds reported here are similar to other described WIN compounds bound in HRV14. All four compounds bind with their tetrazole ring towards the toe of the HRV14 hydrophobic pocket. The isoxazole [compounds (1) and (2)], furan [compound (3)] and pyridizine [compound (4)] rings are all twisted approximately 70° about the long axis of the molecule with respect to the phenoxy ring (Fig. 4). The changes induced in the native conformation of HRV14 are also similar to those previously reported, with r.m.s. deviations of Cα positions between native and WIN-bound VP1 of 0.7 Å. (Badger *et al.*, 1988; Kim *et al.*, 1993; Smith *et al.*, 1986). The largest deviations, 4–5 Å, occur in the GH loop between residues 1116 and 1122.

Despite the overall similarities between the four structures reported here and those reported previously,

Table 2. *WIN compound contacts less than* 4 Å *with HRV*14 (Å)

Rhinovirus 14		Compound number			
		(1)	(2)	(3)	(4)
Ile1104	CD1	3.54 C61*	3.54 C61*	3.52 C61*	3.50 C61*
	CG2	3.67 C1#	3.67 C1#	3.76 C1#	3.59 C1#
		3.90 C61*	3.90 C61*	3.93 C61*	3.92 C61*
		3.77 O1*	3.77 O1*	3.85 O1*	3.70 O1*
Leu1106	CB			3.94 O22	
	CD1	3.69 C1#	3.69 C1#		3.86 C1#
		3.87 C4	3.87 C4	3.51 O1	3.96 C5
		3.46 C5	3.48 C3	3.68 C5	3.55 C6
		3.66 O1	3.72 N2		3.72 N1
Val1122	CG1	3.61 C33	3.61 C53		
Tyr1128	CE1	3.84 C61*	3.84 C61*	3.89 C61*	3.89 C61*
	CE2	3.80 C61*	3.80 C61*	3.86 C61*	3.84 C61*
	CZ	3.43 C61*	3.43 C61*	3.50 C61*	3.49 C61*
	OH			3.97 C3#	
		3.40 C61*	3.40 C61*	3.49 C61*	3.48 C61*
Tyr1152	CB	3.91 C4′	3.91 C4′	3.79 C4′	3.91 C4′
				3.94 N2′	
		3.81 N3′	3.81 N3′	3.70 N3′	3.82 N3′
				3.99 N5′	
Pro1174	CB	3.74 N2′	3.74 N2′	3.65 N2′	3.71 N2′
	O			3.83 C11′	
		3.82 N2′	3.82 N2′	3.64 N2′	3.80 N2′
Phe1186	CE1	3.99 C11′	3.99 C11′		
	CE2	3.90 N1′	3.90 N1′		3.89 N1′
		3.53 N5′	3.53 N5′	3.70 N5′	3.50 N5′
	CZ	3.78 C11′	3.78 C11′		3.82 C11′
		3.92 N1′	3.92 N1′		3.91 N1′
		3.66 N5′	3.66 N5′	3.80 N5′	3.63 N5′
Val1188	CG1	3.96 C3*	3.96 C3*	3.97 C3*	
		3.99 C4*	3.99 C4*		
	CG2	3.74 C2*	3.74 C2*	3.68 C2*	3.83 C2*
		3.64 C21*	3.64 C21*	3.51 C21*	3.74 C21*
		3.94 C3*	3.94 C3*	3.95 C3*	
Val1191	CB			3.98 C21*	
	CG1	3.40 C21*	3.40 C21*	3.32 C21*	3.47 C21*
	CG2	3.39 C21*	3.39 C21*	3.40 C21*	3.44 C21*
Tyr1197	CB	3.73 C31	3.73 C51		3.52 C31
				3.79 C22	
	CD1				3.94 C3
					3.89 C31
					3.74 C4
	CD2	3.59 C31	3.59 C51		
				3.38 C3	
				3.92 C4	
					3.62 C4
	CE1			3.91 C4	
					3.67 C4
					3.98 C5
	CE2				
		3.99 C4	3.99 C4		3.55 C4
				3.41 C3	
				3.43 C4	
	CG			3.69 C3	
					3.97 C3
					3.72 C4
		3.52 C31	3.52 C51		3.58 C31
	CZ			3.76 C3	
				3.42 C4	
					3.57 C4
					3.75 C5
	OH			3.72 C4	
					3.95 C5
Asn1219	ND2	3.45 N2	3.38 O1		3.43 N2
				3.29 C21	
				3.62 C22	
				2.75 O22	
Met1221	SD		3.99 N2		
Met1224	SD	3.94 N5′	3.94 N5′	3.87 N5′	3.97 N5′
WaterW1	O	3.3 N2			3.4 N2
			3.3 O1		
					4.0 C31
WaterW2	O			3.4 C21	
		3.9 C32		3.3 C22	3.6 C32
				2.9 O22	
		3.7 C33			

important differences were observed. This is the first report of the pyridizine [compound (4)], acetylfuran [compound (3)] and 'reversed isoxazole' [compound (2)] heterocycles at ring position *C*. The three-methylene linker is shorter than that of other reported WIN compound structures, with the exception of compound (5) (WIN 56291).

All four compounds have similar electron density for their methyl tetrazole rings (Fig. 4). The methyl group, on the tetrazole ring, fits into a pocket bounded by the peptide backbones at residues Ser1175 and Met1151, as well as the side chains of residues Phe1186 and Val1176. The effect of the methyl group and the tetrazole ring, combined with a shorter three-methylene linker is to shift the *A* (tetrazole) and *B* (phenoxy) rings further towards the pocket pore than any previously reported WIN compound (Fig. 5). The ability of the hydrophobic pocket to accommodate the *A* and *B* rings in a variety of positions supports the hypothesis that the binding energy for compounds in the pocket toe derives from hydrophobic interactions (Diana *et al.*, 1992; Diana, Nitz, Mallamo & Treasurywala, 1993). If more energetic, more directional interactions (*e.g.* hydrogen bonding between the virus and the phenoxy oxygen O1*) predominated, a more discrete distribution of atomic positions would be expected.

The shift in the position of these WIN compounds require that the C3# atom resides on the opposite side of the phenoxy ring than reported for other WIN compounds. The C3# atom in the aliphatic linker of WIN 56291 resides on the Met1221 side of the phenoxy-ring plane (Kim *et al.*, 1993). The electron-density maps of both compounds (1) and (2) clearly show that their C3# atoms reside on the opposite side of the phenoxy-ring plane from Met1221 (Fig. 4). The electron-density maps of compounds (3) and (4) while not as clear, are still consistent with the C3# atoms on the side of the phenoxy ring plane opposite Met1221. This shift in position of the phenoxy ring, and C3# atoms allows the C1# atom of compound (5) and the C3# atoms of compounds (1), (2) and (4) to be nearly coincident. The shift of the phenoxy ring towards the heel of the pocket requires less than 1 Å movement of the Tyr1128, Val1191 and Ile1104 side chains relative to their positions in the compound (5) (WIN 56291) structure in order to relieve close contacts.

The shift in the methyl tetrazole and phenoxy rings towards the pocket heel allows for the heterocycle ring *C* to be in a hydrogen-bonding position with Asn1219 in spite of the short three-methylene linker. This is in contrast to the other published three-methylene structure, compound (5) (WIN 56291), where ring *C* is not within hydrogen-bonding distance of Asn1219 (Kim *et al.*, 1993).

In the compound (4) structure a single rotomer for Asn1219 is preferred. In the other three structures [compounds (1)–(3)], the Asn1219 side chain appears to exist as two rotamers. Either of these rotomers could form hydrogen bonds with the heterocyclic N atom N2, of compound (1), or with the carbonyl O atom O32, of compound (3). The electron density, while suggesting two rotomers at Asn1219, is not sufficient to define either rotomer well enough to unequivocally demonstrate the existence of a hydrogen bond to the WIN compounds.

Compounds (1), (2) and (4) all contain two densities consistent with water (W1, W2) near the pore of the hydrophobic pocket (Figs. 3 and 4, Table 3). Compound (3)'s carbonyl O atom excludes one of these waters, W1 [Figs. 4(*c*) and 3(*b*) (green), Table 2]. These waters are in position to form hydrogen-bond bridges between the compounds and HRV14 (Fig. 3). A similar network of water molecules surrounding the entrance to the hydrophobic pocket has been seen in other HRV14 structures (Badger *et al.*, 1988; Chapman *et al.*, 1991; Kim *et al.*, 1993).

In HRV1A, HRV16 and polioviruses similar networks of hydrogen bonds to putative fatty acids have been observed (Filman *et al.*, 1989; Oliveira *et al.*, 1993). In HRV50 bound to compound (10) (Fig. 6) a water analogous to W1 exists hydrogen bonded to compound (10) and the backbone N atom of L1103 (equivalent to L1106 in HRV14). In HRV50 the water analogous to W2, which is hydrogen bonded to Ser1107 in HRV14, is replaced by the amide side chain of Gln1104. The electron density surrounding Asn1215 in HRV50 (Asn1219 in HRV14) clearly supports that the Asn1215 is not in a position to hydrogen bond to either the WIN compound or the water W1.

These data suggest that the hydrogen bonding of the WIN compounds to a conserved pore Asn (1219 in HRV14) is not critical for potency. Rather, some hydrogen-bonding network, possibly mediated through solvent, is important in WIN-compound binding. The existence of a hydrogen-bonding network which does not require the pore Asn could explain the observation that compound potency is unchanged when Asn1219 of HRV14 is mutated to Ala (unpublished data).

Compounds that can participate in this hydrogen-bond network require some hydrogen-bonding groups to be facing into the pore region of the pocket. Compounds which contain such a group would be expected to be more potent than those which do not. The importance of participation in the pore hydrogen-bonding network is most clearly illustrated by the comparison of the structures of compounds (1) and (2). The only difference between these two compounds is the reversal of the N and O-atom positions in the isoxazole ring. The electron densities of these two structures suggest that they bind in identical conformations, within experimental error (Figs. 4*a* and 4*b*). Thus, the difference in inhibition constants for compounds (1) and (2) is likely to be indicative of the different hydrogen-bonding potential of the isoxazole and reversed isoxazole rings.

In compound (1), the isoxazole N atom is in a position to hydrogen bond with solvent or Asn1219. In compound (2) the isoxazole O atom is in this position. Heterocyclic O atoms, if π-donors, tend to be poor hydrogen-bond acceptors (Abraham, Grellier, Prior, Morris & Taylor, 1990; Abraham *et al.*, 1989; Edwards *et al.*, 1992). Because the O atom of the reversed ixoazole is a poor hydrogen-bond acceptor unlike the N atom of the isoxazole, it is reasonable to expect that the reversed isoxazole would be a poorer inhibitor of viral replication (Fig. 6).

The existence of this pore hydrogen-bond network, can also explain antirhinoviral activity differences between other closely related structural analogs (Bailey *et al.*, 1994). Compounds with good hydrogen-bond acceptors (*e.g.* heterocyclic N or acetyl O atoms) are more potent inhibitors than those with poor hydrogen-bond acceptors (*e.g.* heterocyclic O atoms). A hydrophobic pocket pore hydrogen-bond network has been observed in all four solved HRV structures. Thus, it is not surprising that the preference for compounds which can interact with such a network appears to be generalized among many HRV serotypes (Fig. 6).

The data presented here and elsewhere suggest two principles that direct binding of WIN compounds in the VP1 hydrophobic pocket. First, the toe region binding is dominated by hydrophobic interactions, and can accommodate considerable structural diversity. Second, com-

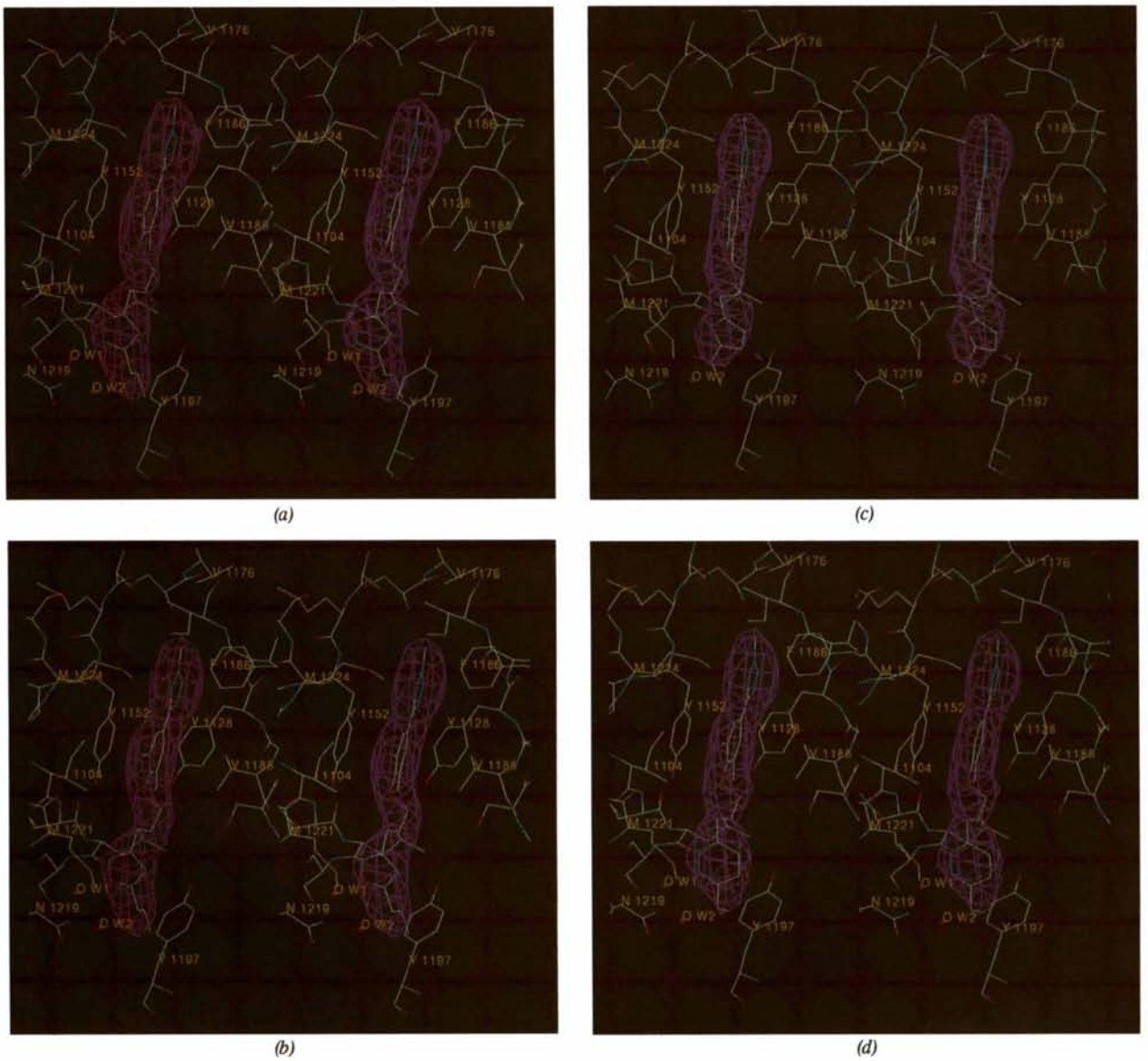


Fig. 4. WIN compounds (1)–(4) [(*a*)–(*d*) respectively] in the hydrophobic pocket of HRV14. The WIN compounds are depicted in electron-density baskets, that are contoured at 1.5σ with respect to the entire map.

Table 3. *Possible hydrogen bonds at the hydrophobic pocket pore between HRV14 and WIN compounds*

Interacting atoms		Distance between atoms (Å) Compound number			
Atom 1	Atom 2	(1)	(2)	(3)	(4)
Asn1219	WIN N2	3.5			3.4
	WIN O1		3.4		
	WIN O32			2.8	
	W1	3.2	3.3		3.2
W1	W2	3.1	3.7		2.8
	WIN N2	3.3			3.4
	WIN O1		3.3		
	Leu1106	3.5	3.3		3.5
	Ser1107	3.3	3.2		3.3
W2	Ser1107	3.4	3.1	3.3	3.4
	WIN O32			2.9	

pounds should be capable of forming a hydrogen bond at the pocket pore. The flexibility of the pore hydrogen-bonding network also allows for compound structural diversity in this region. It has been proposed that a naturally occurring molecule or 'pocket factor' occupies this hydrophobic pocket and thus may stabilize the virion in transition between hosts. Data from antiviral-compound binding, presented here and elsewhere, suggest that this pocket could accommodate a structurally diverse group of amphiphilic pocket factors.

We would like to thank the staff at CHESS for their help in collecting data, Michael Rossmann and Greg Kamer for providing and helping to transport programs for data processing and Marcia Kramer for guidance in production of HRV14 for crystallization.

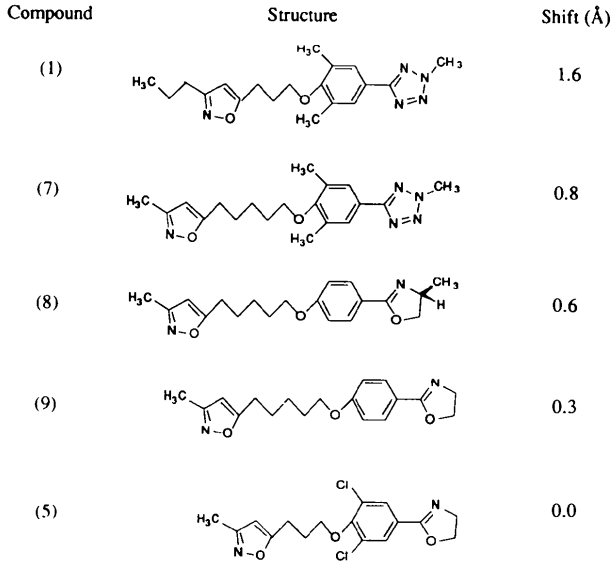
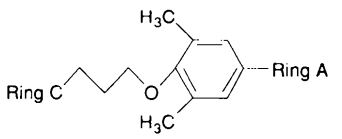


Fig. 5. Shift towards hydrophobic pocket pore of methyl tetrazole compounds. Measurements have been made between the C1* atoms of the compounds, using C1* of compound (5) (WIN 56291) as the origin. Compounds (8) and (9) are listed elsewhere as compounds V(S) and IV, respectively (Badger *et al.*, 1988).



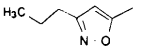
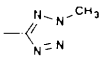
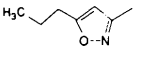
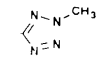
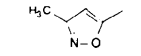
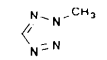
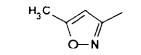
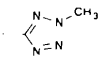
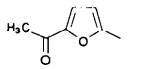
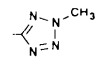
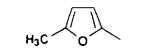
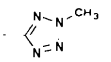
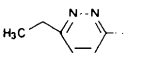
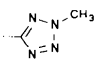
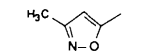
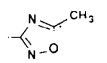
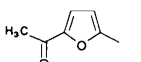
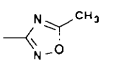
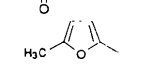
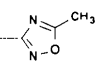
Compound	Ring C	Ring A	*In vitro* activity (m*M*) HRV14	MIC_{80}
(1)			0.11	0.25
(2)			1.24	>2.25
(10)			2.32	0.71
(11)			7.24	>4.9
(3)			0.51	0.51
(12)			>38.	>2.45
(4)			0.24	0.45
(13)			0.20	0.30
(14)			0.53	0.42
(15)			8.0	>2.45

Fig. 6. The effect of the *C* ring hydrogen-bonding potential on rhinovirus inactivation by WIN compounds. The MIC_{80} is the concentration of compound at which 80% of the virus serotypes tested are inhibited in replication by 50% (Oliveira *et al.*, 1993). Also shown is the compound concentration which inhibits HRV14 replication by 50%.

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