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PEPTIDOMIMETIC INHIBITORS OF HERPES VIRUS RIBONUCLEOTIDE REDUCTASE. CORRELATION BETWEEN HERPES SIMPLEX AND VARICELLA ZOSTER VIRUS.

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Abstract: Peptidomimetic inhibitors based on the C-terminal sequence of herpes simplex virus (HSV) ribonucleotide reductase (RR) small subunit (R2) also inhibit varicella zoster virus (VZV) RR. There is generally good correlation between the potencies against both HSV and VZV RR. There are, however, two important differences. A urea moiety at the N-terminus improves potency against HSV RR but offers no advantages against VZV RR. γ-Methylleucine at the C-terminus is also beneficial for HSV RR but decreases potency considerably for VZV RR.

Several herpes viruses such as herpes simplex (HSV) and varicella zoster (VZV) encode a distinct ribonucleotide reductase (RR).[1] This enzyme catalyses the conversion of ribonucleoside diphosphates into their corresponding 2'deoxy analogs that are essential for DNA biosynthesis.[2] Active ribonucleotide reductase is formed by the reversible association of two distinct homodimeric subunits.[3-7] It has been shown that a nonapeptide corresponding to the C-terminal amino acid sequence of the small subunit (R2) of HSV RR inhibits enzymatic activity by preventing subunit association.[4,5] We have previously reported on peptidomimetic inhibitors of HSV RR enzyme based on the C-terminal sequence of the HSV R2[8-10] and showed that these compounds bind more tightly to the large subunit (R1) than does the natural ligand (R2).[11]

The C-terminal sequence of VZV R2 (YAGTVINDL) is quite similar to that of HSV R2 (YAGAVVNDL). Therefore, we argued that peptidomimetic inhibitors of HSV RR based on the C-terminal sequence of HSV R2 should also inhibit VZV RR. In this paper we correlate the binding potencies of

previously described inhibitors of HSV RR subunit association with the potencies these same inhibitors show against the VZV RR.

Materials

Compounds **1** and **2** were prepared by automated solid-phase N-Boc chemistry as published.[11] The syntheses of compounds **3** to **9**[9] and **10** to **13**[10] have been described.

Binding Assays

Inhibitor potency against HSV RR was assessed using a competitive binding assay described previously.[11] To assess the potency of the inhibitors against VZV RR, a novel binding assay was developed using purified recombinant VZV R1.[12]

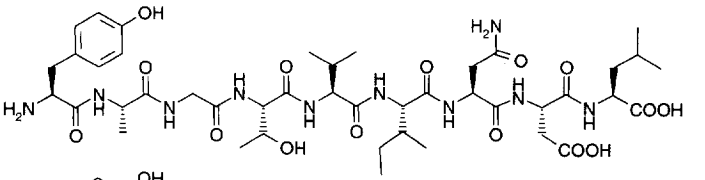
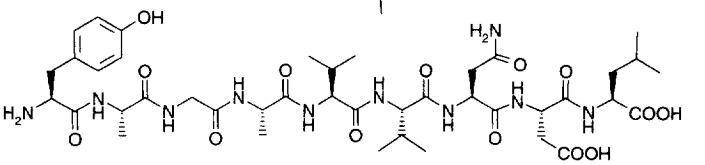
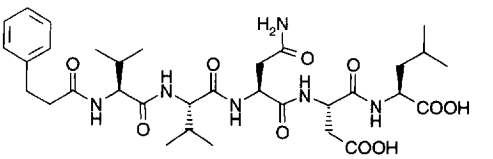
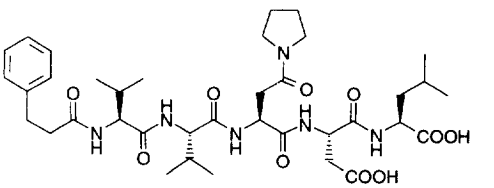
The standard reaction mixture, in a final volume of 200 μL, contained 50 mM HEPES pH 7.6, 0.15 M NaCl, 0.1% casein, 0.02% NaN_3, 3% DMSO, 1 μM aprotinin, 1 μM leupeptin, 0.1 μM pepstatin A, 0.37 μg recombinant VZV R1 and 0.015 pmol (~50,000 cpm) ^{125}I-labelled tracer described previously.[11] After incubating at room temperature for 2 h to reach equilibrium, 100 μL of sample were transferred to the upper sample reservoir of Ultrafree-MC filter units with regenerated cellulose membranes, molecular weight cutoff 30,000 (Millipore Corporation). After centrifugation at 7,000 rpm for 15 min in a Eppendorf centrifuge, the upper sample reservoirs were transferred to a gamma counter to measure bound radioactivity. Under these conditions the total binding of tracer was about 20%. Non specific binding was less than 4%. For inhibition experiments, compounds were tested in quadruplicate in serial 4-fold dilutions. IC_{50} values were determined from plots of inhibition of binding as a function of compound concentration using non linear regression analysis as described previously.[11]

Structure-Activity Relationship Studies

Table **1** compares the binding affinities of the nonapeptides corresponding to the C-terminal sequence of the VZV and HSV R2 (compound **1** and **2,** respectively) for the R1 subunit of both enzymes. The potencies of the nonapeptides are very similar against both RR enzymes. It is interesting to note that compound **1** corresponding to the C-terminal sequence of VZV R2 binds more strongly to HSV R1 than does compound **2** corresponding to its natural sequence. It has been reported that the C-terminal pentapeptide sequence contains the minimum structural requirements for effective binding to the large subunit of the HSV enzyme.[13] We have previously shown that the four N-terminal amino acids in compound **2** can be replaced with a phenylpropionyl group to produce an inhibitor (compound **3**) that is 6 times more potent against HSV RR.[9] In the case of VZV RR, the potency of compound **3** is comparable to that of the nonapeptide corresponding to its R2 C-terminal sequence (compound **1**). We have also shown that, for HSV RR, the potency of these peptidic inhibitors can be increased by almost 200 times by replacing the NH_2 on the asparagine side chain with a pyrrolidino moiety (compound **3** *vs* compound **4**).[9,14] This modification also improves binding potency against VZV R1, however by a more modest 25-fold. It should be pointed out that although the nonapeptides

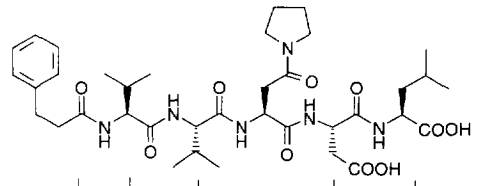
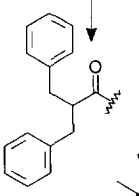
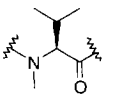
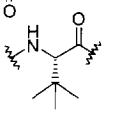
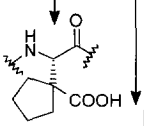
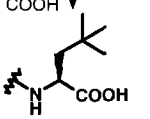
1 and **2** have similar binding affinities for either R1, the modified pentapeptide (compound **4**) binds 25 times better to HSV R1 than to VZV R1.

Table 1

Compound		Binding Assay IC_{50} (nM)	
		HSV	VZV
1		8,000	11,000
2		25,000	42,000
3		4,000	15,000
4		24	588

Compounds **5** to **9** in Table **2** illustrate the best substitutions found so far for all the residues of compound **4** for binding to the HSV R1.[9] The introduction of a second benzyl group at the N-terminus (compound **5**) is not only beneficial for binding to HSV R1 but also greatly increases the binding affinity of the inhibitor against VZV R1. Furthermore, modification of compound **4** with a N-methylvaline (compound **6**), *tert*-leucine (compound **7**) or cyclopentylaspartic acid (compound **8**) increases the binding potency of the inhibitors for R1 of both enzymes. In general, the increase in potency observed for each modification is very similar for both enzymes. However, we observe a distinct difference in the structural requirements at the inhibitor C-terminus.

Table 2

Compound	Binding Assay IC_{50} (nM) HSV	VZV
4 	24	588
5 	0.7	22
6 	7.5	210
7 	4	123
8 	1	37
9 	5	9,800

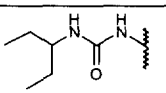
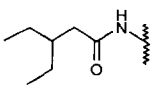
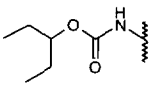
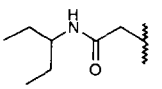
Previous SAR indicates that the side chain of the C-terminal leucine is involved in a strong hydrophobic interaction with the HSV R1.[14] The same appears to be true for the VZV enzyme as replacement of the C-terminal Leu by an Ala in a related inhibitor series dramatically decreases binding affinity (from 1 μM to >1000 μM). Interestingly, introduction of an extra methyl group to the hydrophobic side chain of the leucine, as in compound **9**, improves potency four times against HSV RR but *decreases potency 16-fold against VZV RR*. This result suggests that the size and/or shape of the hydrophobic pocket in the R1 subunit of each enzyme may be significally different.

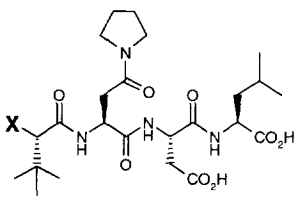
In addition to the inhibitor series shown in Table **2**, we have also described a shorter series of peptidomimetic inhibitors of HSV RR. This inhibitor series is characterized by the presence of a ureido functionality at the N-terminus (see compound **10** in Table **3**).[10] Replacement of the (phenylpropionyl)valine moiety in compound **7** with an [(ethylpropyl)amino]carbonyl group (compound **10**) provides an equipotent

inhibitor. We were surprised to discover, however, that compound **10** was over 300-fold less potent against VZV RR.

The compounds shown in Table **3** provide evidence that for HSV RR, the N-terminal NH of this new ureido based inhibitor series partakes in a hydrogen bonding interaction with R1. Replacement of this NH (compound **10**) by a methylene group (compound **11**) or by an oxygen (compound **12**) considerably lowers binding potency (>40 and 200 times, respectively). The similar IC_{50} values of compounds **10** and **13** against the HSV enzyme indicate that the *tert*-leucine NH does not interact with R1. In contrast, compounds **10** to **13** all show relatively similar binding potencies towards the VZV RR. This suggest that any hydrogen bonding interaction of the N-terminal NH with VZV R1 is at best weak. The differences between VZV and HSV RRs are evident when the potencies of compounds **10** and **12** are compared. Compound **10** is over 200 times more potent than compound **12** against HSV RR, but only 7 times more potent against VZV RR. Similar IC_{50} values for compounds **11** and **13** against VZV RR indicate that neither NH of the urea moiety are strongly involved in a hydrogen bonding interaction with VZV R1.

Table 3

Compound	X	Binding Assay IC_{50} (nM) HSV	VZV
10		3	925
11		140	2,200
12		635	7,000
13		5	3,500



Conclusions

We have shown that peptidomimetic compounds based on the C-terminal sequence of the small subunit (R2) of HSV RR are able to bind to the large subunit (R1) of VZV RR. Consistent with the similarities in the C-terminal sequences of each R2, there is generally good correlation between the potencies of inhibitors against both HSV and VZV RR. There are however two important differences. Replacement of the C-terminal leucine with a γ-methylleucine improves inhibitor potency 4-fold against HSV RR, but decreases

potency 16-fold against VZV RR. The second difference is found in the ureido-based inhibitor series. While the ureido functionality in compound **10** improves binding potency over 45-fold in comparison to the corresponding amide analog (compound **11**) against HSV R1, it offers little advantage against VZV R1. The inability of VZV R1 to interact as strongly with our best peptidomimetic HSV RR inhibitors may lessen their importance as effective anti-VZV agents.

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