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INHIBITION OF HERPES PROTEASES AND ANTIVIRAL ACTIVITY OF 2-SUBSTITUTED THIENO[2,3-d]OXAZINONES

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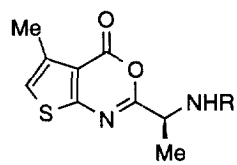
Abstract: Cinnamyl derivatives of thieno[2,3-d]oxazinones are mechanism-based inhibitors of the HSV-2, VZV and CMV herpes proteases which demonstrate nanomolar potency. Compounds **5** and **28** inhibit protease processing in HSV-2 infected cells with a selectivity index of at least 30.

The recent discovery of a new family of herpes proteases encoded by the UL26 gene in herpes simplex type 1 (HSV-1)[1] and by the homologous UL80 gene of cytomegalovirus (CMV),[2] has afforded a new potential target for therapy of herpesvirus infections. This protease plays an essential role in virus capsid maturation, cleaving a scaffold protein which is encoded in-frame with the C-terminal part of the gene product.[3] The protease, which is self processing, shows a varying degree of sequence homology across the herpesvirus family with a highly conserved P_4-P_1' cleavage motif, in which proteolysis occurs between alanine and serine residues. These proteases do not show homology with any known proteases outside of the herpes virus family and the recent determination of the crystal structure of CMV protease indicates that they belong to an entirely new family of serine proteases with a novel Ser-His-His catalytic triad.[4]



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Several mechanism based approaches to the inhibition of the herpes proteases have now been reported including spiro-oxazolones,[5] imidazolones,[5] oxazinones[6,7] and β-lactams.[8] We now describe further details of a series of 5-methylthieno[2,3-d]oxazinone[9] based inhibitors **1** which was identified as conferring enhanced biological stability relative to other oxazinone herpes protease inhibitors. A parallel synthesis approach was utilised to prepare a range of N-acyl analogues as described in Scheme 1. Coupling of 2-aminothiophene **2** and Boc-alanine followed by triphenylphosphine/carbon tetrachloride mediated cyclisation yielded thieno[2,3-d]oxazinone **3**. Previous work had already identified the 2-(S)-methyl group as being optimal for activity in the thieno[3,2-d]oxazinone analogues[7] and this group was retained in the current series. Deprotection of the

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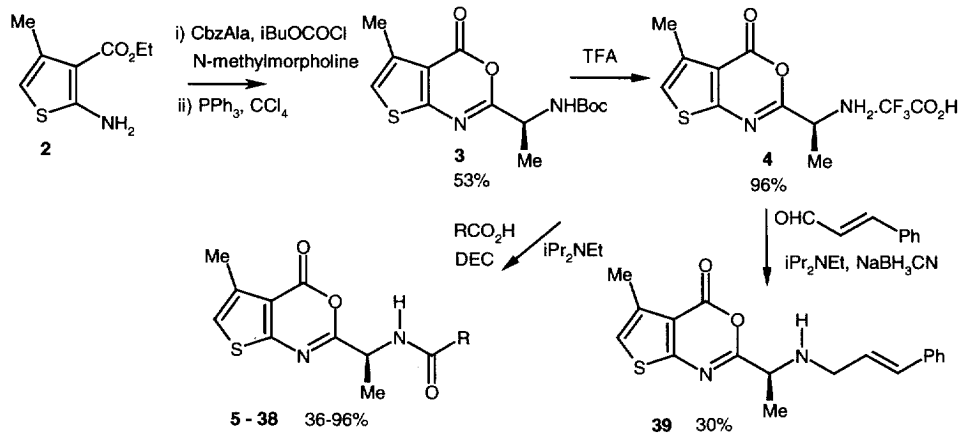
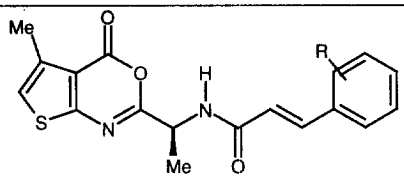
amino group with trifluoroacetic acid provided the key intermediate **4** that was acylated with the requisite acids using DEC in the presence of Hunig's base while reductive alkylation of the amine **4** with cinnamaldehyde provided **39**. The thiophene oxazinones were evaluated in quenched fluorescence assays of the peptidolytic activity of the HSV-2, VZV and CMV proteases[9]. The kinetics of selected compounds were also studied in more detail through measurement of their inactivation rates and acylation-enzyme half-lives.



Scheme 1

The most potent class of compounds identified from the initial arrays were cinnamide derivatives with the parent compound **5** demonstrating good pan-herpetic activity (Table 1). The amide carbonyl was required for activity as the amine **39** was inactive against all three enzymes up to 20μM. Docking of the inhibitors into the active site[4] suggested a possible H-bond interaction of the amide carbonyl with Arg165. The absolute IC_{50} values for HSV-2 were limited by enzyme concentration (0.5μM) but in general the trend was similar to that observed with VZV, consistent with the good sequence homology between the two enzymes. For **5** the fact that the differences in IC_{50} are merely an artifact of the higher HSV-2 protease enzyme concentration was confirmed by similar rates of inactivation and acyl-enzyme half-lives for both enzymes. The HSV-2 and VZV proteases afforded inactivation rates of $k_{obs}/[I]$ = 65,000 and 44,000 $M^{-1}s^{-1}$ respectively and the acyl enzyme half-lives were 2.4 and 1.9h respectively. Relative to the previous reported Cbz analogue of **5**, the cinnamyl side chain has afforded an order of magnitude increase in HSV-2 protease inactivation rate with only a small increase in acyl-enzyme half-life.[9]

Functionalisation of the aryl ring had little effect on the VZV protease IC_{50} values, with the exception of the increased potency of the 2,6-dichloro analogue **14.** However, the VZV acyl-enzyme half-life of 2-halo substituted analogues was significantly increased, up to a maximum for **14**. Thus the analogues **6,7**, **13** and **14** afforded adducts with half-lives of 12, 8.2, 14 and 25 hrs respectively. The increased potency of **14** is accounted for entirely by the increased half-life as the inactivation rate, 33,000 $M^{-1}s^{-1}$, is very similar to that of

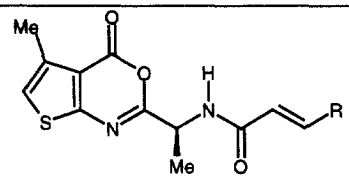


No.	R	IC_{50} (μM) HSV-2	VZV	'CMV
5	H	0.3	0.038	0.5
6	2-Cl	0.61	0.035	0.38
7	2-Br	0.48	0.039	0.38
8	2-Me	1.5	0.036	20
9	2-NO_2	0.72	0.076	2.2
10	2-EtO	0.56	0.015	0.51
11	2-Cl,4-OH	0.23	0.015	0.19
12	2,6-di-F	0.63	0.06	1.7
13	2-Cl, 6-F	0.52	0.036	2.0
14	2,6-di-Cl	1.97	0.01	>34
15	2,6-di-MeO	1.3	0.18	21
16	3-Cl	0.98	0.042	0.96
17	3,5-di-MeO	0.35	0.021	8.5
18	4-CHO	0.53	0.094	2.4
19	4-NMe_2	0.57	0.038	1.8
20	4-NO_2	0.94	0.060	3.6

Table 1.

5. In contrast to the halogen substituted analogue, the 2-ethoxy derivative **10** had a more modest effect on off-rates ($t_{1/2}$ 2.5 and 5.1h for HSV-2 and VZV proteases respectively) and the 2,6-dimethoxy analogue **15** was the least potent derivative against VZV protease. A greater divergence in potency was observed with CMV protease. While a 2-halo or 2-ethoxy substituent was tolerated a 2-methyl as in **8** led to a substantial drop in CMV potency. 2,6 Di-substitution was also detrimental to potency as witnessed by **14** and **15** and to a less pronounced extent with the 2,6-difluoro and 2-chloro-6-fluoro derivatives **12** and **13**. However **11**, a 2-chloro-4-hydroxy di-substituted aromatic provided the optimal activity in this series.

Isosteric replacement of the phenyl ring of the cinnamide **5** with a 3-thiophene as in **21** was investigated next and found to provide inhibitors of the three proteases with similar potencies (Table 2). Substitution of the 3-thienyl analogue as with the bromide **22** maintained potency while the introduction of a dimethyl substitution in **23** enhanced the VZV potency in particular providing a 7nM inhibitor. The VZV protease IC_{50} value for **23** is at the limit of what can be determined in the VZV protease assay as the enzyme concentration is 20nM. Determination of the rate of acylation showed that it had the highest VZV inactivation rate in this series, $k_{obs}/[I]$ = 76,000 $M^{-1}s^{-1}$. The 2-thienyl series **25** also provided potent inhibitors though moving the methyl substituent from the 5-position as in **25** to the 3-position as in **24** caused a close to ten fold drop in potency for VZV. Introduction of a bromine substituent in the 2-thienyl group also provided good HSV-2 and VZV



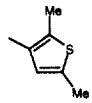
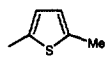
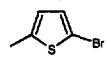
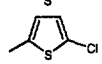
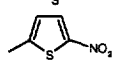
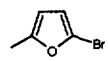
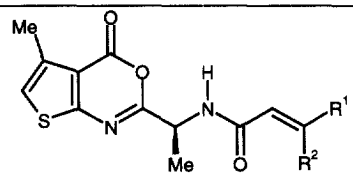
No.	R	IC_{50} (μM) HSV-2	VZV	CMV
21		0.33	0.089	0.38
22		0.2	0.051	0.62
23		0.19	0.007	0.23
24		0.33	0.29	0.67
25		0.65	0.033	0.53
26		0.25	0.058	1.11
27		0.19	0.033	1.12
28		0.27	0.008	0.21
29		0.21	0.017	0.28
30		0.57	0.054	0.9
31		0.49	0.041	0.18
32		1	0.029	0.22
33		0.5	0.17	1.24
34		0.25	0.019	1.1

Table 2

inhibitors though in the case of **26** and **27** there was a slight drop in CMV potency. The 5-bromo substituted compound **28,** however proved optimal with excellent activity against VZV (8nM) in particular. A nitro group at the 5-position as with **30** is also tolerated though it is not as favourable as the bromide . A furan analogue **31** of the 5-bromo 2-thiophene **28** was also prepared and shown to have good CMV and HSV-2 activity though a slight drop in VZV activity was observed. The phenyl ring of **5** could also be replaced with an N-methyl

pyrrole **32** and with a 2-pyridyl ring **33**, although the quinoline **34** proved more potent than the pyridine. Several of the heterocyclic analogues afforded both HSV-2 and VZV protease IC_{50} values below the enzyme concentrations and these were characterised in more detail: HSV-2 $k_{obs}/[I]$ **23**, **28**, **29**, **34** = 53,000, 12,000, 58,000, 76,000 $M^{-1}s^{-1}$; VZV $k_{obs}/[I]$ **23**, **28**, **29**, **34** = 76,000, 63,000, 60,000, 45,000 $M^{-1}s^{-1}$; HSV-2 $t_{1/2}$ **23**, **28**, **34** = 2.9, 4.1, 3.2h ; VZV $t_{1/2}$ **23**, **28**, **34** = 5, 12, 9h. With the exception of **28**, the HSV-2 data tracks well with that observed for VZV again showing the similarity of the interactions with these enzymes and that the IC_{50} differences for potent compounds are a result of the assay limitations. The increased VZV potency of these compounds is also seen to be a result of subtle effects on both acylation rate and adduct half-life.

Introduction of a second aryl ring onto the cinnamide double bond in the terminal position was investigated next. The di-phenyl compound **35** was again similar in potency to the parent **5** though replacement of the *trans* phenyl ring with a 2-thienyl group improved activity against all three enzymes providing good pan-herpetic activity (Table 3). Replacement of both phenyls with thiophene as in **37** led to a significant improvement in HSV-2 and CMV activity while the VZV inhibition was still in the 40nM range. Compound **37** had the highest HSV-2 inactivation rate observed in the thieno[2,3-d]oxazinone series, kobs/[I] = 120,000 $M^{-1}s^{-1}$. Replacement of one phenyl ring with a thiophenyl group as in **38** resulted in a reduction in potency against all enzymes.



No.	R^1	R^2	IC_{50} (μM) HSV-2	VZV	CMV
35	Ph	Ph	0.50	0.037	0.91
36	2-thienyl	Ph	0.26	0.016	0.30
37	2-thienyl	2-thienyl	0.11	0.042	0.25
38	Ph	SPh	3.1	0.14	1.9

Table 3

In a MRC-5 cell culture assay (XTT) for cytotoxicity the most potent compounds from the series described above demonstrated low to moderate cytotoxicity (Table 4). These compounds were further evaluated for their effect on protease processing in HSV-2 virus infected cells by pulse chase (PC) assay[9] and cytotoxicity data under the assay conditions was also obtained. Compounds **5**, **2**, **28** and **29** demonstrated micromolar inhibition of viral protease processing with **5** and **28** demonstrating a >30 fold separation from cytotoxicity under the assay conditions (Table 4). Selectivity, where tested, against other serine proteases such as elastase and trypsin demonstrated no significant inhibition up to 100μM.

No.	TC_{50} (μM) XTT assay	TC_{50} (μM) under PC conditions HSV-2	PC_{50} (μM) HSV-2
5	56	>250	7.5
11	100	56	NI
23	180	130	12
28	83	>250	8
29	130	>250	18
37	95	21	NI

Table 4

In summary we have described a series of thieno[2,3-d]oxazinones which are potent inhibitors of HSV-2, VZV and CMV herpes proteases with good rates of inactivation and prolonged rates of reactivation. Selected compounds on further evaluation in HSV-2 pulse chase assays demonstrated micromolar inhibition of protease processing in virus infected cells with good to excellent separation from cytotoxicity under the assay conditions.

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References and footnotes:

1. Liu, F.; Roizman B. *J. Virol.*, **1991**, *65*, 5149.
2. Welch, A.R.; Woods, A.S.; McNally, L.M.; Cotter, R.J.; Gibson, W. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 10792.
3. Gao, M.; Matusick-Kumar, L.; Hurlburt, W.; DiTusa S.F.; Newcomb, W.W.; Brown, J.C.; McCann, P.J. III; Deckman I.; Colonno, R.J. *J. Virol.*, **1994**, *68*, 3702.
4. **a.** Tong, L.; Qian, C.; Massariol, M.-J.; Bonneau, P.R.; Cordingley, M.G.; Lagacé, L. *Nature*, **1996**, 383, 272. **b.** Qiu, X.; Culp, J.S.; DiLella, A.G.; Hellmig, B.; Hoog, S.S.; Janson, C.A.; Smith, W.W.; Abdel-Meguid, S.S. *Nature*, **1996**, 383, 275. **c.** Shieh H.-J.; Kurumbail, R.G.; Stevens, A.M.; Stegeman, R.A.; Sturman, E.J.; Pak, J.Y.; Wittwer, A.J.; Palmier, M.O.; Wiegand, R.C.; Holwerda, B.C.; Stallings, W.C. *Nature*, **1996**, 383, 279. **d.** Chen P.; Tsuge, H,; Almassy, R.J.; Gribskov, C.L.; Katoh, S.; Vanderpool, D.L.; Margosiak, S.A.; Pinko, C.; Matthews, D.A.; Kan, C.-C. *Cell*, **1996**, *86*, 835.
5. Pinto, I.P.; West, A.; Debouck, C.M.; DiLella, A.G.; Gorniak J.G.; O'Donnell, K.C.; O'Shannessy, D.J.; Patel, A.; Jarvest, R.L. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2467.
6. Jarvest, R.L.; Parratt, M.J.; Debouck, C.M.: Gorniak J.G.; Jennings, L.J.; Serafinowska, H.T.; Strickler, J.E. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2463.
7. Jarvest, R.L.; Connor, S.C.; Gorniak, J.G.; Jennings, L.J.; Serafinowska, H.T.; West, A. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1733.
8. **a.** Deziel, R.; Malenfant, E., *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 1437. **b.** Borthwick, A.D.; Weingarten, H.M; Haley, T.M.; Tomaszewski, M.; Wang, W.; Hu, Z.; Bedard, J.; Jin, H; Yuen, L.; Mansour, T.S. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 365.
9. Dabrowski, C.E.; Ashman, S.M.; Fernandez, A.V.; Gorczyca, M.; Lavery, P.; Parratt, M.J.; Serafinowska, H.T.; Sternberg, E.J.; Tew, D.G.; West,A.; Jarvest, R.L., submitted for publication