DESIGN AND SYNTHESIS OF MONOCYCLIC β-LACTAMS AS MECHANISM-BASED INHIBITORS OF HUMAN CYTOMEGALOVIRUS PROTEASE.

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Abstract: Mechanism based inhibitors of HCMV protease have been designed based on the monocyclic β-lactam nucleus, which have been shown to acylate the viral enzyme in a time dependant manner. SAR in a series of monocyclic β-lactam N-ureas, has defined the size and relative stereochemisty of the C-3 substituent producing a low micromolar inhibitor **17b** with good aqueous stability and selectivity over the mammalian serine proteases. © 1998 Elsevier Science Ltd. All rights reserved.

Human herpes viruses cause a range of diseases HSV-1 (cold sores), HSV-2 (genital herpes), VZV (chicken pox, shingles) and HCMV (retinitis, pneumonitis). The current treatment of these diseases uses nucleoside (acyclovir, ganciclovir) and phosphonate (foscarnet) substrate analogues. Because of the toxicity associated with foscarnet and ganciclovir, together with the emergence of mutants resistant to acyclovir, there is a need for a new class of antiherpes compounds based on a novel mechanism. Mutation studies and reaction with protease inhibitors both for HCMV and HSV-1 indicate that the herpes virus protease, a member of the serine protease super family with little homology to the human (chymotrypsin) and bacterial (subtilisin) serine protease classes[1,2] is necessary for viral replication. The crystal structure of HCMV protease has recently been published [3,4,5,6] showing it to be a 7-stranded β-barrel stabilised by surrounding α-helices; it exists as a dimer of two of these units in the crystal structure. Moreover it has a novel catalytic strategy for peptide cleavage, where the serine nucleophile at position 132 is activated by two juxtapositioned histidine residues at positions 63 and 157. The substrate cleavage sites across all the herpes virus family are unique and highly conserved[1,2], the cleavage occurs between Ala/Ser and the consensus sequence for HCMV is Val-*Xxx*-Ala ↓ Ser. The β-lactam serine protease inhibitors developed as elastase[7] and thrombin inhibitors[8] are ideal starting points for a chemical

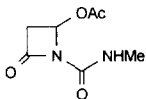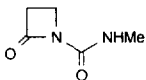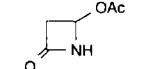Scheme 1   β-Lactam template and Natural Substrate for HCMV Protease
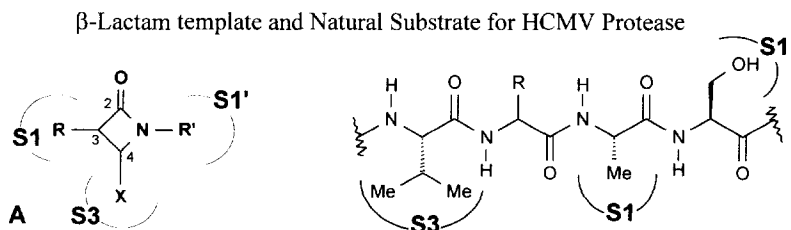

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programme as they have the potential to access all three specificity sites S1',S1 and S3. Our design of inhibitors was based on the β-lactam ring incorporating the natural substrate requirements of the consensus sequence. Thus template **A** with a methyl substituent alpha to the lactam carbonyl (for access of the S1 site) was our initial target, where variation of substituents on the the the ring at N-1 and C-4 would allow access to the S1' and S3 sites respectively (Scheme 1). We chose as our initial starting point the urea **1a** which was known[7] to give better hydrolytic stability than the corresponding amide or carbamate at this position. The requirement for a leaving group at the 4 position in this template, in conjunction with an electron withdrawing substituent on the lactam nitrogen, is seen from

Table 1

Compound No.	Structure	%Inhibition 500μM
1a		68.5
2		7.7
3		0

Table 1. Compounds were evaluated in an HPLC assay measuring the activity against a 14mer peptide substrate based on the release site[13]. To investigate the mechanism of action of **1a**, the interaction of δAla HCMV protease[11] and inhibitor **1a** has been studied by electrospray ion / mass spectroscopy (ESI/MS) after 3 mins, 0.5, 3 and 21hrs incubation (Scheme 2). These studies indicate the formation of a covalent δAla HCMV protease/**1a** complex after a period of 30 mins. The average complex molecular weight (M_{av}) is 27,951, a difference of + 127 on the native protease (M_{av} = 27,824). This indicates the formation of an enzyme complex with **1a** less a molecular weight of 59, which is equivalent to the loss of OAc. A mechanism which would support this evidence is shown in Scheme 3; the serine hydroxyl moiety attacks the lactam carbonyl with loss of the OAc group.
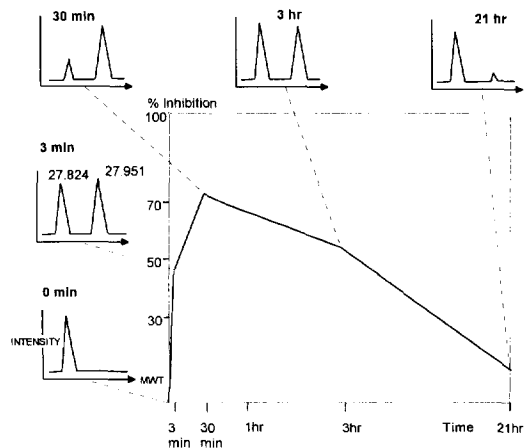
Scheme 2   Scheme 3
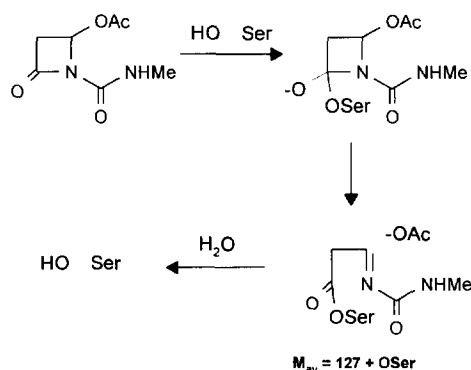
The enzyme gradually turns over the compound within a 21 h period, which suggests the His has not reacted with the imine. Thus **1a** has been shown by ESI/MS studies to acylate δAla HCMV protease in a reversible and covalent fashion. Analysis of peptide fragments from a tryptic digest of the covalently modified enzyme by

A. D. Borthwick et al. / Bioorg. Med. Chem. Lett. 8 (1998) 365–370

367

tandem mass spectrometry showed[15] that Ser 132 was the covalently modified amino-acid and hence is the active site serine of the catalytic triad seen in the X-ray crystallographic structure [3,4,5,6] of HCMV protease.

Synthesis: The starting β-lactams **4-7** [7,9,10] were treated with isocyanates to afford the C-4 acetoxy N-1 urea derivatives **1a-e, 9, 10, 11a,b,** and **12a,b** (Scheme 4). Displacement of the C-4 acetoxy group in **4,7,8** with the Na salt of 4-*tert*butoxycarbonyl phenol gave **13,** and **16a-d.** In the case of β-lactams **7** and **8,** which consisted of a separable mixture of *cis* and *trans* isomers, displacement of the acetoxy group by the Na salt of
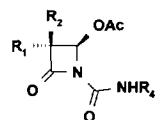
Scheme 4
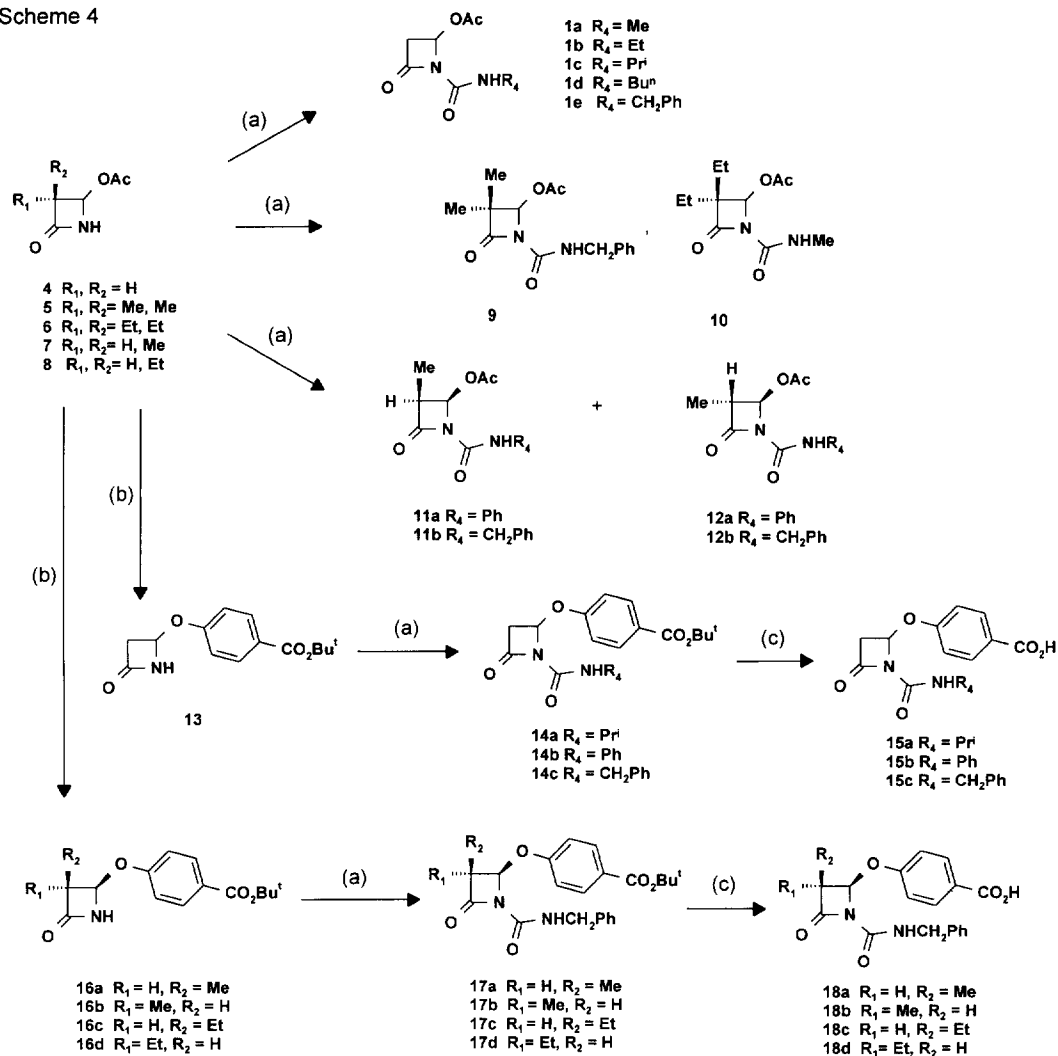
Reagents and conditions: (a) R_4NCO, Et_3N, DMAP, CH_2Cl_2, RT. (b) pHOPhCO$_2$Bu^t, 1M NaOH/Acetone, RT. (c) TFA, anisole.
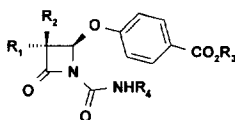
4-*tert*butoxycarbonyl phenol inverted the *cis* to *trans* isomer ratio, in accordance with the published findings of Clauss.[9] For example, **7** (*cis:trans* 3:1) gave **16a:16b** (*cis:trans* 1:2), and **8** (*cis:trans* 2:1) afforded **16c:16d** (*cis:trans* 1:3). The *cis* and *trans* isomers were assigned based on the coupling constant, $J_{3,4}$ in the ^{1}H NMR[12]. The *cis* isomers show a coupling constant $J_{3,4}$ of 4.0-4.5 Hz, whereas in the *trans* isomers $J_{3,4}$ is 0-1.0 Hz. The β-lactams **13, 16a-d** were converted in a straightforward manner to urea derivatives **14a-c, 17a-d** by reaction with isocyanates. The *tert*butyl group was removed with TFA/anisole to afford **15a-c, 18a-d**.

Results and Discussion: Modifications to the urea substituent showed that the order of potency was Me ≈ Et ≈ iPr < Bu^n < PhCH$_2$, (**1a-1e**) in the C-3 unsubstituted β-lactams (Table 2). The *para*-alkoxybenzoic acid moiety was introduced at C-4 because it is known to increase stability and solubility[7], however these analogues were less active than the 4-acetoxy analogues, **15a** < **1c** and **15c** < **1e**; also the benzyl urea and the phenyl urea were equipotent **15c** ≡ **15b** in this series. Methyl substitution at C-3 to access the S1 pocket showed (Table 2) that the *trans*-isomer **12b** was more potent than the *cis*-isomer **11b** in the benzyl ureas, and the *trans*-isomer **12a** and the *cis*-isomer **11a** in the phenyl ureas were less active but showed the same trend. Addition of another methyl to give the gem-dimethyl analogue **9** caused a loss of activity, and the gem-diethyl analogue **10**, was similarly less active than **1a**. Similarly, a consistent pattern was observed when the *para*-alkoxybenzoic acid moiety was introduced into the 3-methyl series (Table 2), the *trans*-isomer **18b** was more potent than the *des*-methyl analogue **15c** which was more potent than the *cis*-isomer **18a**, the *trans-tert*butyl ester **17b** was more potent than the *cis-tert*butyl ester **17a**, and in the 3-ethyl analogues the *trans*-isomer **18d** was more potent than the *cis*-isomer **18c**.

Table 2a[14]

Compound	R$_1$	R$_2$	R$_4$	% Inhibition at 100μM	IC$_{50}$ (μM)	Ki (μM)
1a	H	H	Me	29	>100	-
1b	H	H	Et	36	>100	-
1c	H	H	Pr^i	30	>100	-
1d	H	H	nBu	-	68	26
1e	H	H	CH$_2$Ph	-	42	20
9	Me	Me	CH$_2$Ph	6	>100	-
10	Et	Et	Me	5	>100	-
11b	H	Me	CH$_2$Ph	-	27	-
12b	Me	H	CH$_2$Ph	-	6.5	-
11a	H	Me	Ph	-	>100	-
12a	Me	H	Ph	-	86	-

Table 2b[14]

Compound	R_1	R_2	R_3	R_4	% Inhibition at 100μM	IC_{50} (μM)	Ki (μM)
15a	H	H	H	Pr^i	8	>100	-
15b	H	H	H	Ph	43	>100	-
15c	H	H	H	CH_2Ph	48	>100	-
18a	H	Me	H	CH_2Ph	12	>100	-
18b	Me	H	H	CH_2Ph	-	20	5.6
18c	H	Et	H	CH_2Ph	25	>100	-
18d	Et	H	H	CH_2Ph	-	50	8.3
17a	H	Me	Bu^t	CH_2Ph	35	>100	-
17b	Me	H	Bu^t	CH_2Ph	-	17	5.7

This difference between the *cis* and *trans*-isomers may be because the 4-phenoxy substituent in the *cis*-isomers **18a** and **18c** interferes with the initial binding of the C-3 group with the S1 pocket of HCMV protease and prevents formation of a stable acyl-enzyme. The 3-ethyl compound **18d** was less active than the methyl one **18b** probably because in this template the larger alpha-ethyl substituent couldn't fit the S1 specificity pocket which permits only a methyl in the conserved substrate. Of the two most potent compounds **17b** and **18b** active

Table 3

Compound No.	δAla HCMV IC_{50} (μM)	Elastase IC_{50} (uM)	Chymotrypsin IC_{50} (uM)	Acetylcholine esterase IC_{50} (uM)
18b	20	3.9	1.6	>100
17b	17	>100	>100	>100

against HCMV protease in this series, the ester **17b** was shown to be selective for the viral enzyme relative to acetylcholine esterase and the mammalian proteases elastase and chymotrypsin (Table 3).

Table 4

Compound	IC_{50} (μM)	Ki (μM)	$t_{1/2}$ hrs
1e	42	20	0.53
15c	>100	-	1.4
18b	20	5.6	3.1
18d	50	8.3	7.6
17b	17	5.7	22.8

The stability of a number of these monocyclic β-lactams in aqueous solution (HEPES buffer, pH = 7.6) was assayed by HPLC. The compounds had a wide range of half-lives and there was no correlation of potency with reactivity (Table 4). Compound **17b** the most potent and selective analogue is of interest as a micromolar inhibitor with high aqueous stability.

Summary: We have developed mechanism based inhibitors of HCMV protease based on the monocylic β-lactam template that are stable to hydrolysis, have low μM activity against the viral enzyme and have selectivity

over acetylcholine esterase and the mammalian serine proteases elastase and chymotrypsin. ESI/MS studies have shown that these inhibitors can bind covalently and reversibly in a time dependant manner by a mechanism which involves acylation of δAla HCMV protease at the active site nucleophile Ser 132. We have defined the size Me >Et, relative sterochemistry trans > des > cis, and bulk of the substituent Me > diMe that accesses the S1 specificity pocket for this template. Further work to exploit the 1 and 4 position of this template should enable access to the conserved S1' and S3 pockets of the protease and give an increase in potency.

References and Notes:

1.   Gibson, W.; Welch, A. R.; Hall, M. R. T. *Perspect. Drug Discov. Design* **1994** 2, 413-426.
2.   Holwerda, B. C. *Antiviral Res.* **1997**, 35, 1-21.
3.   Chen, P.; Tsuge, H.; Almassy, R. J.; Gribskov, C. L.; Katoh, S.; Vanderpool, D. L.; Marbosiak, S. A.; Pinko, C.; Matthews, D. A.; Kan, C.C. *Cell.* **1996**, 86, 835-843.
4.   Tong, L.; Qian C. G.; Massariol, M. J.; Bonneau, P. R.; Cordingley, M. G.; LaGace, L. A. *Nature* **1996**, 383(N6597), 272-275.
5.   Qiu, X. Y.; Culp, J. S.; Dilella, A. G.; Hellmig, B.; Hoog, S. S.; Janson, C. A.; Smith, W. W.; Abdelmeguid, S.S. *Nature* **1996**, 383(N6597), 275-279.
6.   Shieh, H. S.; 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(N6597), 279-282.
7.   Shah, K. S.; Dorn, C. P.; Finke, P. E.; Hale, J. J.; Hagmann, W. K.; Brause, K. A.; Chandler, G. O.; Kissenger, A. L.; Ashe, B. M.; Weston, H.; Knight, W. B.; Maycock, A. L.; Dellea, P. S.; Flechter, D. S.; Hand, K. M.; Mumford, R.A.; Underwood, D. J.; Doherty, J.B. *J.Med.Chem.***1992**, 35, 3745-3754.
8.   Han, T. W.; Trehan, A. K.; Wright, J.J.K.; Federici, M. E.; Seiler, S. M.; Meanwell, N. A. *Bioorganic Med. Chem.* **1995**, 3, 1123-1143.
9.   Clauss, K.; Grimm, D.; Prossel, G. *Liebigs Ann. Chem.* **1974**, 539-560.
10.   Firestone, R.A.; Barker, P.L.; Pisano, J.M.; Ashe, B.M.; Dahlgren, M.E. *Tetrahedron* **1990**, 46, 2255 2262.
11.   δAla HCMV is a mutant prepared by deleting residues Ala 143 and Ala 144 to decrease the self cleavage seen in the wild-type HCMV protease.
12.   van der Steen, F.H.; Kleijn, H.; Britovesek, G.J.P.; Jastrzebski, J.T.B.H.; van Koten, G. *J.Org.Chem.***1992**, 57, 3906-3916. Yamashita, H.; Minami, N.; Sakakibara, K.; Kobayashi, S.; Ohno, M. *Chem. Pharm. Bull.* **1988**, 36, 469-480.
13.   Compounds are dissolved in DMSO and added at a concentration of 500μM (2% DMSO carry over) to a reaction mixture containing 6.65μM HCMV δAla protease with a final buffer composition of 85mM HEPES pH7.5, 170mM EDTA, 8.5mM NaCl, 1.7mM DTT and 25.5% glycerol. The reaction mixture is then pre-incubated at 37 °C for 15min, prior to addition of 1mM substrate (RESYVKASVSPEAA), and then incubated for a further 15 minutes. The reaction is stopped by the addition of 1% TFA and the extent of inhibition of peptide substrate cleavage is analysed by reverse phase HPLC. The results obtained provide a % inhibition for each compound.
14.   Compounds are dissolved in DMSO, serially diluted and added at a range of concentrations (from 100μM - 0.195μM) to a reaction mixture containing 0.5μM HCMV δAla protease, 100mM HEPES pH7.5, 0.2mM EDTA, 10mM NaCl, 1mM DTT, 2%DMSO and 30% glycerol. The reaction mixture is pre-incubated at 32°C for 15 minutes prior to addition of 4mM substrate (RESYVKA-pNA), and then incubated for a further 30 minutes at 32°C in a BIO-TEK Bio Kinetics Reader EL340i. The plate reader monitors production of pNA and calculates the reaction rates over 30 minutes. The rates are plotted against inhibitor concentration and IC_{50} results determined.
15.   Full mass spectral and sequencing analysis will be presented elsewhere.