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Synthesis and anti-HCMV activity of 1-acyl-β-lactams and 1-acylazetidines derived from phenylalanine

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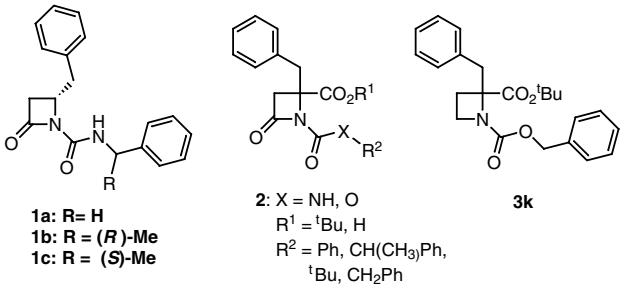
Abstract—Different Phe-derived 1-acyl-β-lactams, analogous to a series of 2-azetidinones acting as HCMV serine protease inhibitors, were synthesized. Some of these compounds were modest inhibitors of the HCMV replication. Interestingly, removal of the carbonyl group of the β-lactam ring, most likely acting as the serine trap, resulted in an azetidine derivative with anti-HCMV activity comparable to that of the reference compound ganciclovir.

Human cytomegalovirus (HCMV) is a ubiquitous member of the herpes virus family. Although most infections are asymptomatic, severe manifestations of HCMV can be seen in individuals whose immune system has been weakened by a disease, such as late-stage cancers and AIDS, or by immunosuppressive therapy following organ transplantation.[1–3] At present there are four drugs that have been approved for the treatment of HCMV infections, namely the viral DNA polymerase inhibitors ganciclovir (DHPG), cidofovir [(*S*)-HPMPC] and foscarnet, and the antisense oligonucleotide fomivirsen.[1–3] However, because of toxicity and bioavailability problems, together with the emergence of resistance to these compounds, the need for effective, and safe anti-HCMV drugs based on other mechanisms continues.

Due to its critical role in capsid assembly and viral maturation, HCMV serine protease has become an attractive target for the development of anti-HMCV drugs.[2–4] The three dimensional structure of HCMV protease shows that it has a unique catalytic triad composed of Ser132, His63, and His157.[5,6] The structure also suggests that two conserved arginine residues at positions 165 and 166 form the oxyanion hole that is thought to stabilize the tetrahedral intermediate during catalysis.[5,7] Since the active site of this enzyme has been identified, the search for protease inhibitors has been a high priority. Most of these inhibitors contain classical serine protease inhibitor motifs based on an activated carbonyl group such as peptidyl ketoamides,[8,9] as well as mechanism-based inhibitors such as oxazazinones[10,11] and pyrrolidine-5,5-*trans*-lactams.[12–14] Among these latter inhibitors, a series of monocyclic β-lactams **1** (compound **1a** being the prototype) has resulted in highly potent derivatives in the isolated enzyme assay, but their efficacy in cell culture was quite limited, as for all described inhibitors of this enzyme.[15–19]



This paper deals with the synthesis and evaluation of a series of new azetidinones **2**, derived from phenylalanine, which were designed on the basis of the structure of the reported β-lactam inhibitors[16] and of the residues

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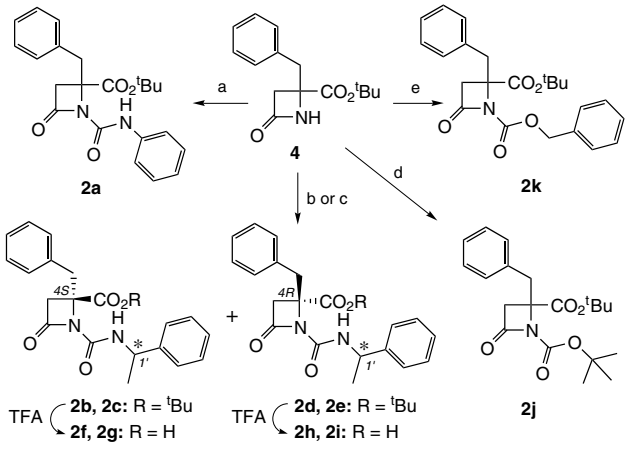
implicated in the active site of the HCMV protease.[5] In comparison with model compounds **1**, these new β-lactams have an additional 4-carboxylate group that could be suitable for extra interactions with the guanidine group of the Arg165 or Arg166 residue of the viral protease. In addition, we have also synthesized and evaluated the azetidine derivative **3k**, analogous to the resulting most active β-lactam within this series.

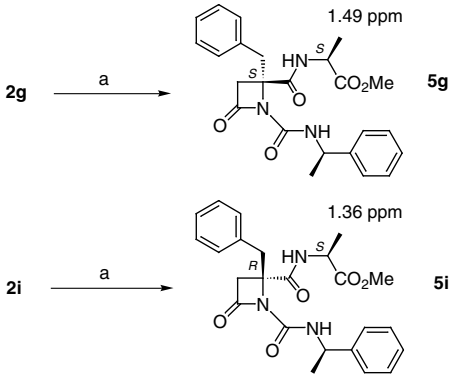
The reaction of the Phe-derived 2-azetidinone **4**[20] with phenyl isocyanate under different conditions was first investigated, since acylation of related β-lactams with isocyanates generally proceeded in low yield (Scheme 1).[16] Among TEA/DMAP, DBU, and Cs_2CO_3, the latter base was found to be the most effective for the synthesis of compound **2a** (82%). Reaction of compound **4** with (*S*)-1-phenylethyl isocyanate under these conditions afforded a mixture of the 1-acyl-β-lactams **2b** and **2d** in an approximately 1.5:1 ratio (74% total yield),[21] which were separated by column chromatography. In a similar way, compounds **2c** (50%) and **2e** (34%) were prepared by treatment of **4** with (*R*)-1-phenylethyl isocyanate, followed by chromatographic resolution.

Compounds **2b–e** were hydrolyzed to the corresponding free carboxylic acids **2f–i**, respectively, by treatment with (1:4) TFA/CH_2Cl_2. Compounds **2j** and **2k** were prepared by treatment of the 2-azetidinone derivative **4** with *tert*-butyldicarbonate and benzyl chloroformate, respectively, as previously described.[22]

The assignment of the absolute configuration at C_4 position in compounds **2g** and **2i**, was based on the chemical shifts of the β-H protons of the aliphatic residue of dipeptide derivatives **5g** and **5i** (Scheme 2),[23,24] and on their respective retention times in HPLC.[25] The knowledge of the configuration in compounds **2g** and **2i** allowed the subsequent configurational assignment of their enantiomers **2h** and **2f**, respectively, and hence of their respective precursors **2b–e**.



Scheme 1. Reagents and conditions: (a) PhNCO/Cs_2CO_3/THF; (b) (*S*)-PhCH(CH_3)NCO/Cs_2CO_3/THF; (c) (*R*)-PhCH(CH_3)NCO/Cs_2CO_3/THF; (d) (^{t}BuO)$_2$CO/TEA/DMAP/CH_2Cl_2; (e) BzlOCOCl/DBU/CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) H-L-Ala-OMe/BOP/TEA/THF.

Compounds **2** were evaluated against HCMV in human embryonic lung (HEL) cells,[26] and the results compared to those obtained for the reference compounds DHPG (ganciclovir) and HMPC (cidofovir) (Table 1). For comparative purposes, the reported anti-HCMV activity of the model β-lactam **1a** was also included in the table.

As shown in Table 1, trisubstituted β-lactams **2a–e** were found to exhibit some antiviral activity, slightly higher than that reported for the prototype 2-azetidinone **1a**. No appreciable influence of the absolute configuration, either at C_4 or at the 1-phenylethyl substituent, on the inhibition of viral replication was observed. However, the lack of activity of the free carboxylic acid derivatives **2f–i** suggests a role for the *tert*-butyl ester moiety. In a similar way, the presence of an aromatic group at the 1-acyl substituent seems important for the antiviral activity, as deduced from the lack of activity of the *tert*-

Table 1. Activity of compounds **2** and **3** against human cytomegalovirus in HEL cell cultures[a]

Compounds	Configurations	AD-169 strain EC_{50} (μM)[b]	Davies strain EC_{50} (μM)[b]	CC_{50} (μM)[c]
2a	4*R*,*S*	50	50	118
2b	4*S*,1′*S*	35	35	184
2c	4*S*,1′*R*	32	33	70
2d	4*R*,1′*S*	32	33	164
2e	4*R*,1′*R*	100	107	>200
2f	4*S*,1′*S*	>50	>50	187
2g	4*S*,1′*R*	>200	>200	>200
2h	4*R*,1′*S*	>200	>200	>200
2i	4*R*,1′*R*	>50	>50	>200
2j	4*R*,*S*	>200	>200	>200
2k	4*R*,*S*	11	13	36
3k	2*R*,*S*	0.74	0.62	34
1a[d]	4*S*	53	—	>750
DHPG	—	0.9	0.8	>150
(*S*)-HPMPC	—	0.16	0.5	>150

[a] The anti-HCMV assay was based on scoring the virus-induced cytopathicity in HEL cell cultures at 7 days post infection as described in Ref. 26.

[b] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).

[c] Cytotoxic concentration required to reduce cell growth by 50%.

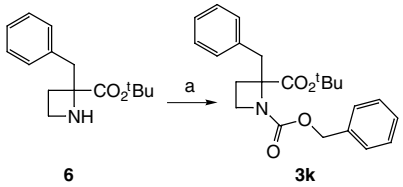
[d] From Ref. 16.

butoxycarbonyl derivative **2j** and the presence of activity of the benzyloxycarbonyl analogue **2k**. The latter compound showed the highest anti-HCMV activity within the β-lactam series, but it had a very narrow therapeutic window, with a cell toxicity value close to the IC_{50} data for viral inhibition.

Considering the structural analogy between β-lactams **2** and model compounds **1**, it is expected that compounds **2** could inhibit HCMV replication in an irreversible manner, through acylation of the virus serine protease. It is well known that irreversible enzyme inhibitors could bind to many nucleophiles en route to the intended target, most likely resulting in toxic side effects.[27] Taking into account that we have recently reported that compound **2k** is highly reactive toward *O*- and *N*-nucleophiles,[22] this reactivity could be masking, at least to some extent, its real antiviral effect. With this in mind, we thought that the elimination of the electrophilic carbonyl lactam group, while maintaining enough hydrogen bonds, ionic, and/or van der Waals interactions with the enzyme, could led to more active and safe HCMV inhibitors. A similar approach has successfully guided the design of *noncovalent* inhibitors of some mammalian serine proteases.[27,28] Following this idea, we deleted the carbonyl group in the supposedly covalent inhibitor **2k** to obtain the corresponding azetidine derivative **3k** (Scheme 3).[29,30]

Interestingly, compound **3k** showed a considerable improvement in the antiviral activity with respect to **2k** and all other β-lactamic inhibitors, although certain toxicity persisted (Table 1). Its antiviral selectivity was at least 10-fold increased. The EC_{50} value obtained for this azetidine derivative was similar to that of the standard reference compound ganciclovir (DHPG) and slightly higher than that of cidofovir (HPMPC). In addition, compound **3k** did not show appreciably activity in viral cytopathicity reduction assays for a broad panel of viruses, pointing to its HCMV selectivity. Compound **3k** could represent the first noncovalent inhibitor for HCMV protease reported to date, although its exact mechanism of action remains to be determined.

In conclusion, starting from different β-lactam derivatives, analogous to a series of covalent inhibitors of the HCMV serine protease, and following a deletion strategy involving the reduction of the carbonyl group of the β-lactam ring, we have obtained an effective inhibitor of HCMV replication. The possible modulation of the antiviral activity/toxicity profile in the azetidine series, through suitable modifications of the substituents in compound **3k**, is one of our current endeavors.



Scheme 3. Reagents and conditions: (a) BzlOCOCl/propylene oxide/CH_2Cl_2.

Acknowledgements

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