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Pyrrolidine-5,5-*trans*-lactams as Novel Mechanism-Based Inhibitors of Human Cytomegalovirus Protease. Part 3: Potency and Plasma Stability

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Abstract—Mechanism-based inhibitors of HCMV protease, which are stable to human plasma ($\geq$20 h) and have single-figure potency in the μM range against HCMV protease, have been developed based on the dansylproline α-methyl pyrrolidine-5,5-*trans*-lactam nucleus.

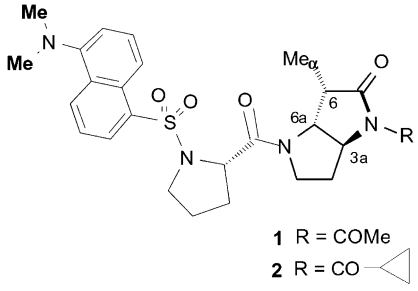
We recently reported the design and synthesis of a novel class of mechanism-based inhibitors (**1**, **2**) of the human cytomegalovirus protease (HCMV),[1,2] based on the α-methyl pyrrolidine-5,5-*trans*-lactam template (Fig. 1). These inhibitors are novel, potent in the nanomolar range and highly selective for the viral enzyme over the mammalian enzymes elastase, thrombin and acetylcholine esterase.[2] Mechanism of action studies showed that these compounds acylate the nucleophile Ser 132 present in the catalytic triad His, His, Ser at the active site of this viral protease.

These compounds have reactive functionality so they are potentially vulnerable to metabolism. The chemical reactivity of the *trans*-lactam ring will determine the rates of both the non-specific hydrolysis due to the hydrolytic plasma enzymes found in the blood as well as the acylation of HCMV protease. Thus, the goal was to find a compound that would react with the viral enzyme after binding to the active site, but would be sufficiently stable to hydrolysis by the plasma enzymes. Two ways were investigated to increase plasma stability and yet retain potency against the viral protease. One way was to sterically hinder the approach of the hydrolytic plasma enzymes to the lactam carbonyl, the other was to make the lactam carbonyl less reactive by making the substituent on the lactam nitrogen less electron withdrawing.

Initially a series of α-methyl pyrrolidine-5,5-*trans*-lactams with Cbz on the pyrrolidine nitrogen and a range of subsituents on the lactam nitrogen were tested for stability in human plasma (Table 1). The stability range was:

$$CONHMe >> COMe \geq CO_2Me > SO_2Me$$

The trend was similar to that seen with the mono-β-lactam[3] inhibitors of human leukocyte elastase. Increasing steric bulk adjacent to the lactam carbonyl at the C-6 position by introducing a second methyl group gave the gem-dimethyl derivative **3** which was >6 times more stable than the α-methyl *trans*-lactam **4**. Similarly



1 R = COMe
2 R = CO

Figure 1.

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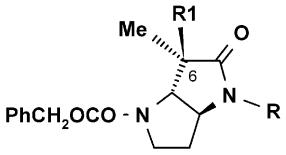
increasing the steric bulk of the acyl functionality on the lactam nitrogen of the α-methyl *trans*-lactam template with the *tert*-butyl acyl derivative **5** also increased stability to human plasma. However, both **3** and **5** lost in vitro activity against HCMV protease (Table 1). A similar situation has been observed in the mono-β-lactam series[4] of HCMV protease inhibitors. Interestingly the cyclopropyl carbonyl compound **6** is more potent and slightly more stable than the methyl carbonyl compound **4** (Table 1). The amide **9** was stable to human plasma as was the phosphonate **10** and the dinitrophenyl derivative **11** (Table 1), but all were inactive against HCMV protease.

To explore this plasma stability versus in vitro activity against HCMV protease, the more active and slightly more stable cyclopropyl acyl derivatives were investigated in both the Cbz and the more active dansylproline α-methyl pyrrolidine-5,5-*trans*-lactam templates.

Table 1.



Compd	R	R1	Human[8] plasma stab $t_{1/2}$ min	HCMV protease[2] *p*NA assay IC_{50} (μM)
3	COMe	Me	6.5 h	> 500
4	COMe	H	54	40
5	$COCMe_3$	H	3.7 h	> 500
6	CO-cyclopropyl	H	1.4 h	9
7	CO_2Me	H	50	148
8	SO_2Me	H	< 5	~500
9	CONHMe	H	> 24 h	> 500
10	$PO(OMe)_2$	H	> 24 h	> 500
11	Ph2,4-$(NO_2)_2$	H	> 24 h	~500

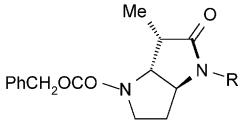
Steric hindrance was achieved by substitution of the cyclopropyl ring with methyl groups. The stability of these derivatives to plasma increased cyclopropyl < methylcyclopropyl < *cis*-dimethyl *cis*-cyclopropyl < tetramethylcyclopropyl. Methylation in the cyclopropyl ring gave **12** and **13** with increased stability to plasma, but the dimethyl compound lost some potency, and the tetramethyl analogue **14** although stable to plasma lost all activity (Table 1). A similar increase in stability to human plasma and decrease in potency was seen in the dansylproline series: cyclopropyl > methylcyclopropyl > *cis*-dimethyl *cis*-cyclopropyl > tetramethylcyclopropyl with the dimethyl analogue **19** in the dansylproline series having the best profile (K_i 1.1 μM and $t_{1/2} = 20$ h in plasma).

In contrast, replacing the cyclopropyl carbonyl substituent in the Cbz series with an amide, an electron-withdrawing aryl or heterocyclic ring produced stable compounds but they were inactive against HCMV protease. However, the increase in potency in going from the Cbz to the dansylproline series seen with the cyclopropyl carbonyl derivatives was developed for these plasma stable compounds (Table 2). Thus the *N*-methylamide **9** and *N*-benzylamide **15** were inactive (IC_{50} > 500 μM) in the Cbz series, but in the dansylproline series both the *N*-methylamide **21** and *N*-benzylamide **22** had low μM activity against HCMV protease in the order CH_2Ph > Me.

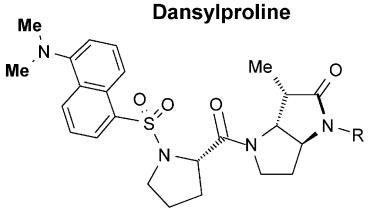
Electronegative aromatic heterocycles have been used[5] to activate peptidyl ketones towards nucleophilic addition by the active-site serine hydroxyl of human neutrophile elastase and prolyl endopeptidase. The potency of peptidyl α-keto heterocyclic inhibitors of the mammalian serine protease HLE have been correlated[5a,c] with the electon-withdrawing character σ_I of the heterocyclic group. Although the plasma stable *para*-nitrophenyl analogue **16** in the Cbz series was inactive the corresponding derivative **23** in the dansylproline series was weakly active (IC_{50} 41 μM). The stable thiazole was

Table 2.

Cbz



Dansylproline



Compd	R	Human[8] plasma stab $t_{1/2}$ min	HCMV[2] Protease *p*NA assay IC_{50} (μM)	Compd	R	Human[8] plasma stab $t_{1/2}$ min	HCMV protease[2] *p*NA assay (μM)	
							IC_{50}	K_i
6	CO-cyclopropyl	1.4 h	9	**2**	CO-cyclopropyl	5 h	0.34	
12	CO-cyclopropyl-Me	2.6 h	16	**18**	CO-cyclopropyl-Me	2.5 h	1.4	
13	CO-(Z) diMecyclopropyl	9.8 h	72	**19**	CO-(*Z*) diMecyclopropyl	20 h	5.1	1.1
14	CO-Me_4cyclopropyl	33 h	> 500	**20**	CO-Me_4cyclopropyl	> 24 h	> 100	
9	CONHMe	> 24 h	> 500	**21**	CONHMe	12 h	22	
15	$CONHCH_2Ph$	12 h	250	**22**	$CONHCH_2Ph$	> 33 h	6.6	2.6
16	Ph-4-NO_2	40 h	~500	**23**	Ph-4-NO_2		41	
17	2-Thiazole	44 h	~500	**24**	2-Thiazole	> 50 h	2.1	0.4

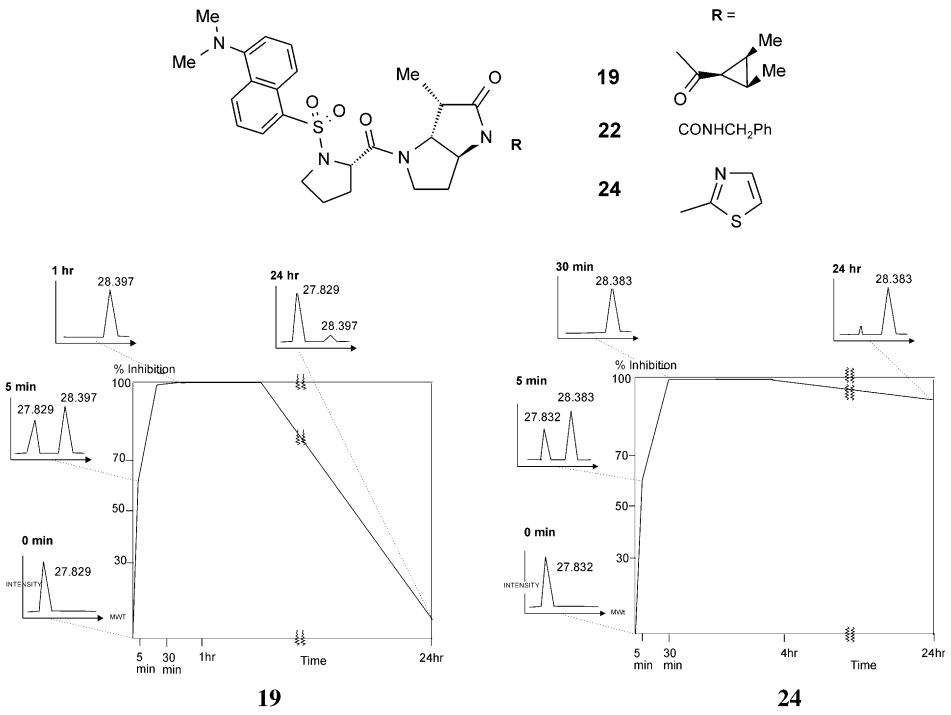
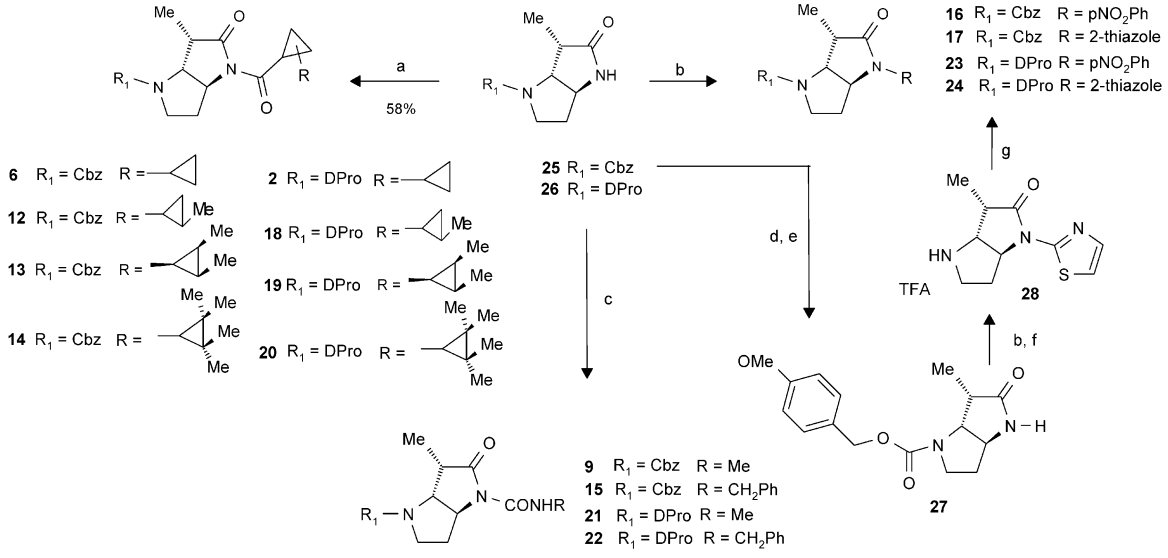


Figure 2.

inactive in the Cbz series **17** but in the dansylproline series **24** had a K_i of 0.4 μM (IC_{50} 2.1 μM). In the dansylproline series the greater potency of the thiazole over the *para*-nitrophenyl derivative is reflected in the greater electon-withdrawing character of the thiazole group $\sigma_I = 3.4$ over the *para*-nitrophenyl group $\sigma_I = 2.3$.[6]

Examples of the three classes of compounds [the dimethylcyclopropyl carbonyl compound **19** (K_i 1.1 μM), the amide **22** (K_i 2.6 μM) and the thiazole **24** (K_i 0.4 μM)], which were stable to human plasma (≥20 h) and had single figure potency in the μM range against HCMV protease were investigated by ESI-MS to study their mechanism of action and to see how quickly they were turned over by the enzyme.

The ESI-MS study of these three compounds, incubated with δ-Ala protease, has shown three modes of action. The *N*-benzylamide **22** inhibits without acylation, the dimethylcyclopropyl carbonyl compound **19** acylates within 30 min and the enzyme is restored after 24 h, and the thiazole **24** also acylates within 30 min but the enzyme remains almost completely acylated for 24 h (Fig. 2). The thiazole **24** is thus the most potent



Scheme 1. (a) LiHMDS (1.5 equiv)/THF, −78° then RCOOCOCMe$_3$ (2 equiv) −78 to 0 °C, 2 h; (b) *p*-NO$_2$-bromobenzene or 2-bromothiazole, CuCl, K_2CO_3, TDA-1, xylene; (c) NaH (0.2–0.5 equiv)/THF, then RN=C=O (1.3–2.5 equiv), 2 h; (d) H_2, 10% Pd/C, IPA: HCl/Et_2O; (e) 2-(4-methoxybenzyloxycarbonyloxyimino)-2-phenylacetonitrile, Et_3N, aq dioxan; (f) TFA/rt; (g) dansylproline, TBTU, HOBT, *i*Pr$_2$Net, MeCN, DMF.

(K_i 0.4 μM) and stable ($t_{1/2}$ > 50 h in plasma) acylating inhibitor of HCMV protease and is essentially stable to turnover by the viral enzyme during 24 h.

Chemistry

The cyclopropyl acyl derivatives **2**, **6**, **12**–**14**, **18**–**20** were prepared by acylating the anion of the required lactam **25** or **26**, with the corresponding mixed anhydride or acid chloride,[1,2] while the amides **9**, **15**, **21**, **22** were prepared by reacting the lactam nitrogen with the corresponding isocyanate under base-catalysed conditions (Scheme 1). The aryl **16**, **23**, or heterocyclic ring **17**, **24**, derivatives were prepared by reacting the lactam nitrogen with the corresponding aryl or heterocyclic bromide under Cu catalysis using modified Goldberg conditions.[7] The plasma stable thiazole derivative **17** was prepared in 67% yield, however when the modified Goldberg reaction was carried out on the dansylproline translactam **26** only a 3% yield of thiazole **24** could be obtained, possibly due to co-ordination of Cu by the dansylproline moiety. The Cbz group could not be removed by hydrogenolysis from the more readily prepared Cbz analogue **17**, hence a more labile protecting group was used. The Cbz group was removed from the lactam **25** and the pyrrolidine nitrogen protected by a *p*-methoxybenzyloxy group to give **27**. The thiazole was then added in the usual way,[7] in 53% yield, and the protecting group removed with TFA to give the proline **28**. Coupling with dansylproline gave **24** (Scheme 1).

References and Notes

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8. Each compound was incubated in fresh human plasma at a concentration of 10 mM and aliquots deproteinated with acetonitile at the following times 0, 15, 30, 60, 120, 240 min, and 22 h. The samples were then assayed individually by LC–MS on an API-300 using an APCI source and single-ion monitoring.