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Research Brief

Trypanocidal activity of peptidyl vinyl ester derivatives selective for inhibition of mammalian proteasome trypsin-like activity

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ABSTRACT

Nine vinyl ester tripeptides selective for inhibition of mammalian proteasome trypsin-like activity were tested for *in vitro* activity against *Trypanosoma brucei*. Interestingly, two compounds showed trypanocidal activity in the low micromolar range without displaying cytotoxicity against human cells. However, the compounds did not inhibit the trypsin-like activity of the trypanosome proteasome although their effect correlates with inactivation of the chymotrypsin-like activity. This finding shows that the inhibitor sensitivities between mammalian and trypanosome proteasome are distinct. This difference may be exploited for rational anti-trypanosomal drug development.

1. Introduction

The protozoan parasite *Trypanosoma brucei* is the causative agent of sleeping sickness in sub-Saharan Africa. Since the 1970s, sleeping sickness has re-emerged and currently over 60 million people living in 36 sub-Saharan countries are at risk of contracting the disease (Steverding, 2008; Brun et al., 2010). Due to reinforced surveillance the number of new cases reported has fallen and at present the estimated number of infected patients is thought to be between 50,000 and 70,000 (Steverding, 2008; Brun et al., 2010). If left untreated, sleeping sickness is a fatal disease. For chemotherapy of human African trypanosomiasis only four drugs, which were developed decades ago, are available (Steverding, 2010). Current therapies of the disease are unsatisfactory because of serious side effects and poor efficacy of these drugs (Fairlamb, 2003). In addition, the occurrence of drug-resistant trypanosome strains is an increasing problem (Matovu et al., 2001; Delespaux and de Koning, 2007). Therefore, the identification of novel trypanocidal lead compounds is required if new treatment of sleeping sickness are to be developed.

Research in recent years has shown that the proteasome is a valid drug target for sleeping sickness (Li et al., 2002; Nkemgu-Njinkeng et al., 2002; Glenn et al., 2004; Steverding et al., 2005, 2006; Steverding, 2007). Although the structure of the trypanosome proteasome resembles that of the mammalian counterpart, the enzyme complexes differ from each other with respect to peptidase activity, substrate specificity and inhibitor sensitivity (Hua et al., 1996; Wang et al., 2003; Glenn et al., 2004; Steverding et al., 2006). In addition, enzymatic analyses have demonstrated that the trypanosome and mammalian proteasome functions are particularly sensitive to inhibition of the trypsin-like and chymotrypsin-like activities, respectively (Genn et al., 2004, Steverding et al., 2005, 2006). Thus, compounds specifically targeting the trypsin-like activity of the trypanosome proteasome may be a basis for rational anti-trypanosomal drug development. We therefore investigated the trypanocidal activity of tripeptidic-based vinyl ester derivative proteasome inhibitors selective for the trypsin-like activity.

2. Materials and methods

2.1. Reagents

N-succinyl-leucyl-leucyl-valyl-tyrosyl-7-amido-4-methylcoumarin (Suc–Leu–Leu–Val–Tyr–AMC) and *N*-t-butoxycarbonyl-leucyl-seryl-threonyl-arginyl-7-amido-methylcoumarin (Boc–Leu–Ser–Thr–Arg–AMC) were purchased from Bachem (Weil am Rhein, Germany). Benzyloxycarbonyl–leucyl–leucyl–leucinal (Z–Leu–Leu–Leu–CHO, MG-132) and 7-amino-4-methylcoumarin (AMC) were from Sigma–Aldrich (Gillingham, UK).

2.2. Cell cultures

Bloodstream forms of *T. brucei* clone 427-221a and human myeloid leukaemia HL-60 cells (LGC Promochem, Middlesex, UK) were grown in Baltz medium and RPMI medium, respectively (Genn

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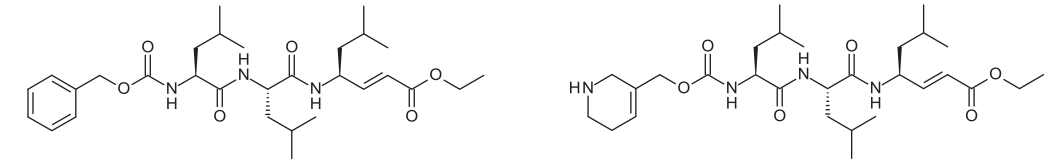
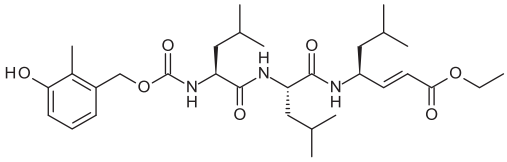
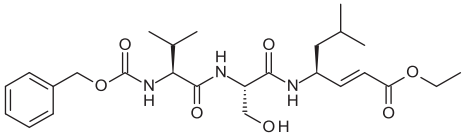


VE1 Z-Leu-Leu-Leu-VE

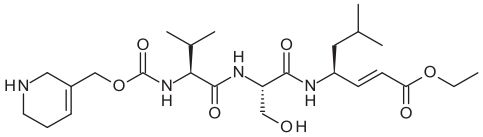
VE3 Guv-Leu-Leu-Leu-VE



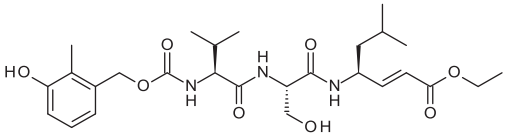
VE4 Hmb-Leu-Leu-Leu-VE



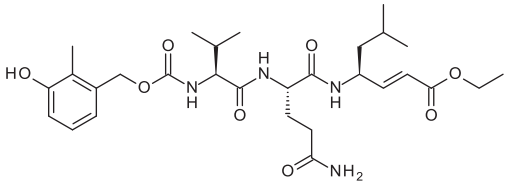
VE5 Z-Val-Ser-Leu-VE



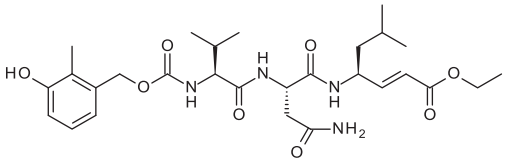
VE7 Guv-Val-Ser-Leu-VE



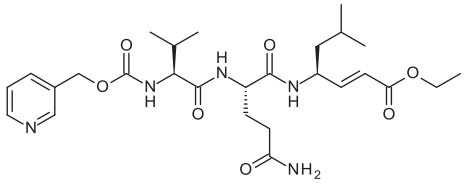
VE8 Hmb-Val-Ser-Leu-VE



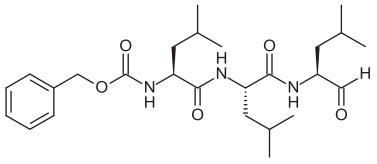
VE14 Hmb-Val-Gln-Leu-VE



VE15 Hmb-Val-Asn-Leu-VE



VE21 Nic-Val-Gln-Leu-VE



MG-132 Z-Leu-Leu-Leu-CHO

Fig. 1. Structures of vinyl ester tripeptides and MG-132. Z, benzyloxycarbonyl; Guv, guvacine; Hbm, 3-hydroxy-2-methylbenzoyl; Nic, pyridine-3-carbonyl; VE, vinly ester.

Table 1
MICs and GI_{50}s of peptidyl vinyl ester derivatives for *T. brucei* bloodstream forms and HL-60 cells.

Compound		IC_{50}[a]		*T. brucei*		HL-60	
		T-L (μM)	ChT-L (μM)	MIC (μM)	GI_{50} (μM)	MIC (μM)	GI_{50} (μM)
VE1	Z–Leu–Leu–Leu–VE	0.28	2.45	>100	3.16 ± 0.54	>100	>100
VE3	Guv–Leu–Leu–Leu–VE	0.21	0.86	100	2.03 ± 0.38	>100	>100
VE4	Hmb–Leu–Leu–Leu–VE	0.041	4.21	100	2.11 ± 0.31	>100	3.20 ± 0.67
VE5	Z–Val–Ser–Leu–VE	0.071	>10	>100	30.0 ± 3.2	>100	34.5 ± 12.2
VE7	Guv–Val–Ser–Leu–VE	0.38	6.54	>100	>100	>100	>100
VE8	Hmb–Val–Ser–Leu–VE	0.033	>10	>100	31.6 ± 1.3	>100	>100
VE14	Hmb–Val–Gln–Leu–VE	0.018	8.95	>100	32.5 ± 6.5	>100	>100
VE15	Hmb–Val–Asn–Leu–VE	0.041	>10	>100	>100	>100	>100
VE21	Nic–Val–Gln–Leu–VE	0.035	0.87	>100	29.5 ± 3.6	>100	26.1 ± 1.6

Data are mean values ± SD of three experiments. T-L, trypsin-like activity; ChT-L, chymotrypsin-like activity.
[a] IC_{50}s were determined with partially purified proteasomes obtained from lymphoblastoid cells pretreated with varying concentrations of test compounds for 12 h at 37 °C. Data were taken from Marastoni et al. 2006 and 2007.

et al., 2004; Steverding et al., 2005, 2006). Both media were supplemented with 16.7% (v/v) heat-inactivated foetal calf serum. All cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C.