Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Medicinal Chemistry 41 (2006) 978–984
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Original article

Synthesis and biological evaluation of new vinyl ester pseudotripeptide proteasome inhibitors

M. Marastoni [a,*], A. Baldisserotto [a], C. Trapella [a], R. Gavioli [b], R. Tomatis [a]

[a] Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, I-44100 Ferrara, Italy
[b] Department of Biochemistry and Molecular Biology, University of Ferrara, I-44100 Ferrara, Italy

Received in revised form 23 March 2006; accepted 3 April 2006
Available online 18 May 2006

Abstract

Here we report the synthesis and biological activities of new tripeptidic-based vinyl ester derivative proteasome inhibitors. Starting from Hmb-Val-Ser-Leu-VE prototype, we investigated P2 position and N-terminal substitution. The more effective inhibitors of the series showed remarkable inhibition and selectivity for the trypsin-like (β2) subunit and were revealed to be specific for the proteasome. In vitro metabolic stability studies of the new vinyl ester analogues are also reported here.

Keywords: Proteasome inhibitors; Vinyl ester pseudopeptides; Synthesis; Trypsin-like activity

1. Introduction

The 26S proteasome (2.4 MDa) is a multicatalytic protease complex which plays an essential role in cells able to recognize and degrade ubiquitinated proteins. The proteasome is made up of multiple subunits and, in mammalian cells, its modular structure consists of a 20S proteolytic chamber capped at both ends by 19S regulatory particles. The 20S portion is formed by four stacked rings, and each of the two inner rings is composed of seven different β subunits [1–3]. It has numerous physiological functions including most non-lysosomal proteolysis in prokaryotes and eukaryotes [4], and its principal activities, located respectively in the β1, β2 and β5 subunits of each β-ring, are referred to as post-acidic-like (PGPH), trypsin-like (T-L) and chymotrypsin-like (ChT-L) [5,6]. The proteolytic sites utilize the γ-hydroxyl group of the N-terminal threonine residue of β subunits as nucleophile [7–12].

The proteasome is fundamental for innumerable cellular functions such as cell cycle regulation, stress response, hydrolysis of abnormal proteins and production of antigenic peptides presented by class I-MHC [13–16], and the ubiquitin-proteasome pathway represents a promising target for the development of bioactive molecules potentially applicable in treatment of pathologies such as cancer, inflammation, immune diseases and others [17–22]. Thus far, the study of proteasome inhibitors, which are normally C-terminally functionalized oligopeptides with a pharmacophore able to interact with catalytic threonine of the proteasome, has revealed useful information about enzyme structure, catalytic mechanism and biology. Classes of these substances are being used to study the role of the proteolytic complex in various cellular processes and dipeptidyl boronic acid PS-341 has been approved by the FDA for the treatment of multiple myeloma [23–34].

We previously studied a number of tripeptidic sequences derivatized at the C-terminal with arecoline derivatives and found that these analogues showed an interesting inhibition against tryptic and chymotryptic active sites with an IC_{50} < 1 μM in in vitro enzyme assays [35,36], and we recently reported a series of peptide-based inhibitors bearing a C-terminal leucine vinyl ester (Leu-VE) as pharmacophore able to function as substrate of the N-terminal catalytic threonine [37]. The most promising derivative, Hmb-Val-Ser-Leu-VE (Fig. 1), displayed potent and selective inhibition for trypsin-like activity and good pharmacokinetic properties Fig. 2.

On the basis of these features, we describe here the synthesis and biological activities of a new series of vinyl ester pseu-

* Corresponding author. Dipartimento di Scienze Farmaceutiche, Via Fossato di Mortara 17-19, 44100, Ferrara, Italy.
E-mail address: mru@dns.unife.it (M. Marastoni).

0223-5234/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.04.001

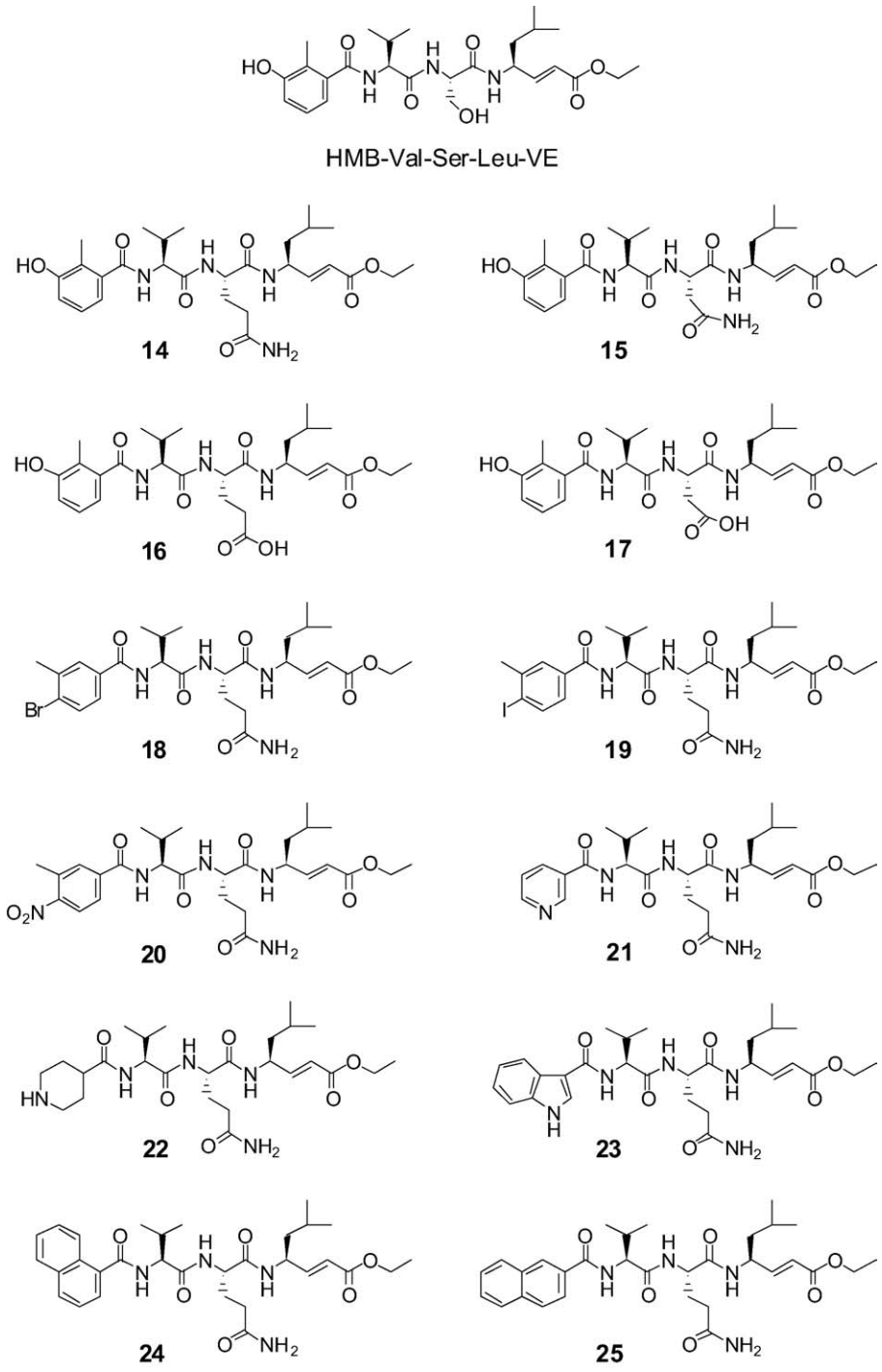

Fig. 1. Structure of the prototype and new vinyl ester pseudotripeptides inhibitors **14**–**25**.

dotripeptides (Fig. 1) possessing an ethyl acrylate group which can function as a substrate of the γ-hydroxy threonine side chain in Michael addition in a similar way to that suggested for the well-know peptide vinyl sulfone inhibitors [38]. Compared to the prototype, compound **14**–**17** bear an acidic or amidic residue in place of serine in P2 position with the aim of increasing the selectivity for the β2 subsite, analogous to previous studies on vinyl sulfone inhibitors [39]. Cyclic N-terminal substituents with different physicochemical properties replaced the 3-hydroxy-2-methylbenzoyl (Hmb) moiety at the P4 position in analogues **18**–**25**.

2. Chemistry

Vinyl ester pseudotripeptides were synthesized by the classical solution method using C-terminal stepwise elongation, as reported in Scheme 1 and the N_{α}–Boc-protected leucine vinyl ester was prepared from the corresponding aldheyde [40] by