Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure–activity relationship study of syringolin A as a potential anticancer agent

Takuya Chiba [a], Akira Matsuda [a,b], Satoshi Ichikawa [a,b,*]

[a] Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan
[b] Center for Research and Education on Drug Discovery, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

ARTICLE INFO

Article history:
Received 27 April 2015
Revised 29 May 2015
Accepted 2 June 2015
Available online xxxx

Keywords:
Proteasome inhibitor
Cancer
Structure–activity relationship

ABSTRACT

A detailed structure–activity relationship of syringolin A (**1**), which is a promising antitumor natural product, was described. We previously developed syringolin A analog **2** as a potent proteasome inhibitor by the structure-based drug design of syringolin A. In this Letter, we synthesized a range of analogs of **2**, having a different length of the lipophilic chain and substituted aryl group, and their cytotoxicity against human cancer cells was evaluated. It turned out that these modifications greatly affected the cytotoxicity. Further optimization would lead to develop a novel proteasome inhibitor.

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The eukaryotic 20S proteasome is an enzyme, which degrades ubiquitin-labeled proteins, and responsible for maintenance of an intracellular protein expression.[1] The barrel-shaped proteasome is composed of seven α and seven β subunits in a $\alpha_7\beta_7\beta_7\alpha_7$ arrangement, and β1, β2 and β5 subunits have a caspase-like, a trypsin-like and a chymotrypsin-like activities, respectively. After poly-ubiquitin-labeled proteins are taken up to a hole inside the proteasome, the proteins were hydrolyzed by proteasomal catalytic active sites. The inhibition of the proteasome results in accumulation of unnecessary proteins and ultimately causes cell death.[2] As a proteasome inhibitor, bortezomib (Velcade®)[3] and carfilzomib (Kyprolis®)[4] have been approved by U.S. Food and Drug Administration (FDA) for a treatment of multiple myeloma, and therefore, the proteasome is considered to be an attractive target for the development of anticancer agent. However, bortezomib and carfilzomib have several shortcomings such as side effects and their drug resistance,[5] and there is an urgent need for the next generation proteasome inhibitor. Syringolin A (**1**, Fig. 1) is a natural product isolated from a strain of the plant pathogen *Pseudomonas syringae* pv. *Syringae* in 1998.[6] Its structure consists of a highly strained 12-membered macrolactam, which contains β,γ-dehydrolysine, and a ureadipeptide side chain.

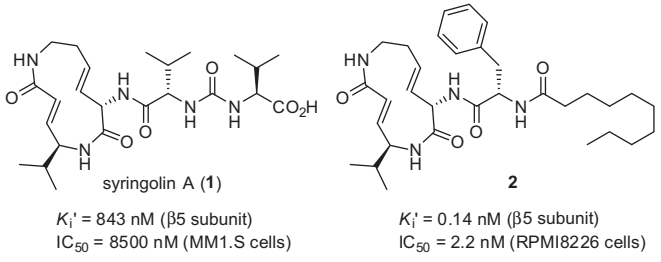

Figure 1. Structure and biological activities of syringolin A and its analog **2**.

In 2008, it was identified as a virulence factor by inhibiting the 20S proteasome.[7] Syringolin A reacts irreversibly with the *N*-terminal threonine (Thr) of the active site of the β5 subunit by a 1,4-addition of the hydroxyl group of the Thr to the α,β-unsaturated carboxamide moiety of syringolin A. Syringolin A exhibits a moderate β5 subunit inhibitory activity with the K_i' value of 0.8 μM and a weak β1/β2 subunits inhibitory activity. Its cytotoxicity is also weak with an IC_{50} value of 8.5 μM for human myeloma MM1.S cells in vitro. Several studies have been conducted by Kaiser's group in order to improve its biological activity.[8] Our group has accomplished the total synthesis of syringolin A, and its structure–activity relationship has also been investigated. We have found the compound **2**, where the ureadipeptide moiety was replaced with an *N*-decanoyl-L-phenylalanine, exhibited a strong β5 subunit inhibitory activity with a K_i' value of 0.14 nM as well as strong cytotoxicity with an IC_{50} value of 2.2 nM against

Abbreviations: Fmoc, 9-fluorenylmethyloxycarbonyl; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carboxamide; HOBt, 1-hydroxybenzotriazole; DMF, *N*,*N*-dimethylformamide.

* Corresponding author. Tel.: +81 11 706 3228; fax: +81 11 706 4980.
E-mail address: ichikawa@pharm.hokudai.ac.jp (S. Ichikawa).

http://dx.doi.org/10.1016/j.bmcl.2015.06.015
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human myeloma RPMI8226 cells.[9] In this paper, we describe the structure–activity relationship of **2** aiming at further improvement of its cytotoxicity.

The X-ray crystal structure of the yeast 20S proteasome bound to syringolin A (PDB code: 2ZCY)[7] revealed several aspects for molecular interaction in addition to its characteristic covalent binding. Namely, syringolin A is stabilized by hydrogen bond formation with Asp114 and Gly47 residues (Fig. 2a). The isopropyl group located on the 12-membered ring is recognized by a small S1 pocket, and the isopropyl group located on the internal Val side chain is recognized by a large S3 pocket, which consists of hydrophobic residues. The S3 pocket could accept a larger substituent than the isopropyl group, and we have examined the effect of the size of the substituent and found that **2** possessing a benzyl group exhibited a stronger proteasome inhibitory activity than syringolin A. The decanoyl group of **2** was considered to be necessary both for cell-membrane permeability and proteasome inhibitory activity (Fig. 2b). In order to optimize the structure in more detail, we planned to change the length of the lipophilic chain and the phenylalanine moiety to other amino acids (Fig. 2c).

We planned to synthesize syringolin A analogs by condensation of the amine unit containing the 12-membered ring **8** with various carboxylic acid units. The carboxylic acid units **7a**–**mm** were prepared by a solid phase synthesis in order to synthesize various analogs efficiently (Scheme 1). Loading amino acids **3a**–**mm** to a 2-chlorotrityl (2-CT) resin gave solid-supported Fmoc amino acids **4a**–**mm**. After deprotection of the Fmoc group, acylation with the carboxylic acids and cleavage from the 2-CT resin afforded cleanly the *N*-acyl carboxylic acid units **7a**–**mm**. Finally, the amine **8** prepared by the previously reported procedure[10] was condensed with **7a**–**mm** in the presence of EDCI, HOBt and $^{i}Pr_2NEt$ in DMF to yield the corresponding syringolin A analogs (**9a**–**mm**).

Cytotoxicity of **9a**–**mm** against several human cancer cells (human epidermoid carcinoma A431, human colon carcinoma HCT-116 and RKO, human ovarian carcinoma TOV21G, human lymphoblastic leukemia CCRF-CEM, human myeloma RPMI8226) was evaluated.[11] Bortezomib and carfilzomib as controls exhibited cytotoxicity with IC_{50} values of 11.0–28.2 nM and 6.1–27.1 nM, respectively (Table 1). Under these assay conditions, compound **2** exhibited a strong cytotoxicity with IC_{50} values between 4.3 and

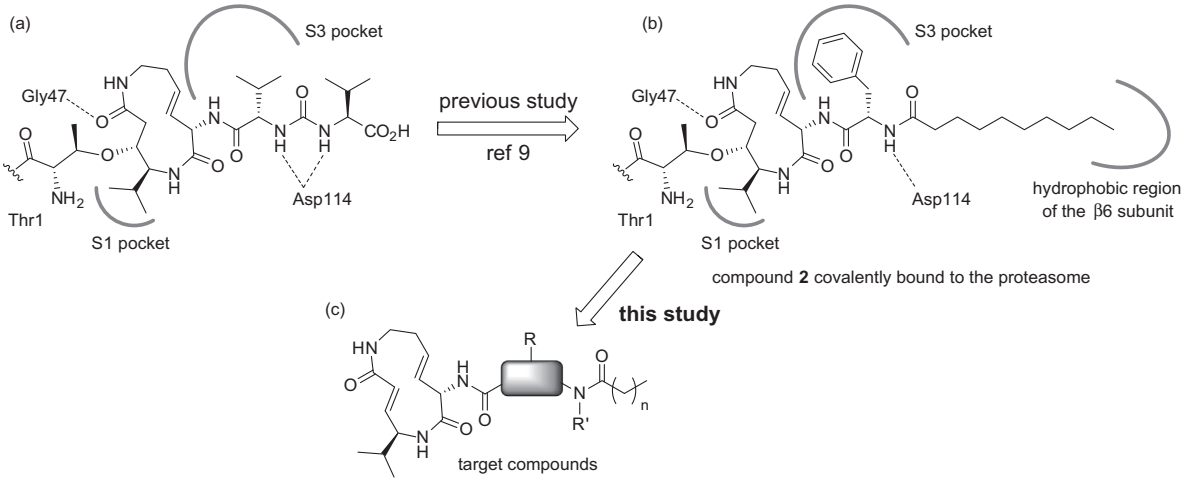
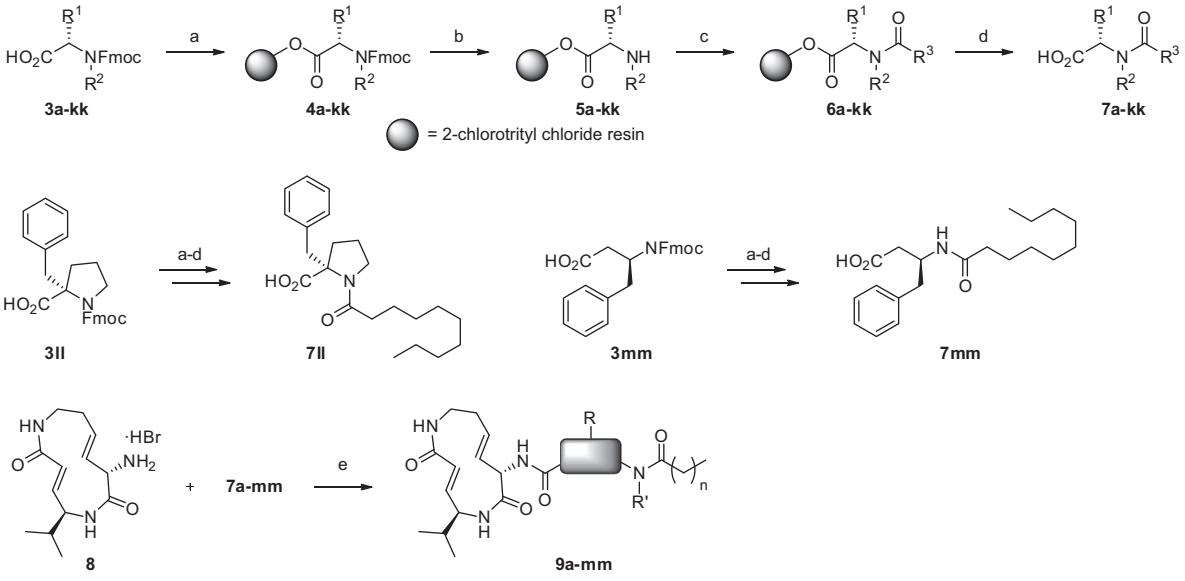


Figure 2. (a) Binding mode of syringolin A with proteasome. (b) Expected binding mode of previously synthesized compound **2** with proteasome. (c) The structure of the target compounds in this structure–activity relationship study.



Scheme 1. Synthesis of syringolin A analogs (**9a**–**mm**). Reagents and conditions: (a) 2-chlorotrityl chloride resin, $^{i}Pr_2NEt$, CH_2Cl_2, rt; (b) 20% piperidine/DMF, rt; (c) acyl chloride, Et_3N, CH_2Cl_2, rt; (d) 5% TFA/CH_2Cl_2, rt; (e) EDCI, HOBt, $^{i}Pr_2NEt$, DMF, rt.

Please cite this article in press as: Chiba, T.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.06.015

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