Biochemical and Biophysical Research Communications 496 (2018) 961–966

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Piperlongumine and some of its analogs inhibit selectively the human immunoproteasome over the constitutive proteasome

Elodie Bosc [a, 1], Jhennifer Nastri [b, 1], Valérie Lefort [a], Marilia Valli [b], Fernando Contiguiba [c], Renan Pioli [d], Maysa Furlan [b], Vanderlan da Silva Bolzani [b], Chahrazade El Amri [a, **], Michèle Reboud-Ravaux [a, *]

[a] *Sorbonne Université, UPMC Univ Paris 06-CNRS, IBPS, UMR 8256, Inserm ERL1164, B2A, 7 Quai Saint Bernard, F75005 Paris, France*
[b] *Nuclei of Bioassays, Biosynthesis and Ecophysiology of Natural Products (NuBBE), Department of Organic Chemistry, Institute of Chemistry, Sao Paulo State University - UNESP, 14800-060, Araraquara, SP, Brazil*
[c] *Institute for Natural Products Research Walter Mors, Health Sciences Center — HSC, Federal University of Rio de Janeiro - UFRJ, 21941902, Rio de Janeiro, RJ, Brazil*
[d] *Institute of Chemistry, Department of Organic Chemistry, University of São Paulo — USP, 05508-000, São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 8 January 2018
Accepted 15 January 2018

Keywords:
Piperlogumine
Piperlongumine analogs
Proteasome
Immunoproteasome

ABSTRACT

The natural small molecule piperlongumine A is toxic selectively to cancer cells *in vitro* and *in vivo*. This toxicity has been correlated with cancer cell ROS, DNA damage and apoptotic cell death increases. We demonstrate here a new mechanistic property of piperlongumine: it inhibits selectively human immunoproteasome with no noticeable inhibition of human constitutive proteasome. This result suggests that immunoproteasome inhibition, a mechanism independent of ROS elevation, may also partly play a role in the anticancer effects observed with piperlongumine. Structure-activity relationships of piperlongumine analogs suggest that the lactam (piperidonic) ring of piperlongumine A may be replaced by the linear olefin $-NHCO\text{-}CH_2{=}CH_2$ to improve both *in vitro* inhibitory efficiency against immunoproteasome and cellular toxicity.

1. Introduction

Piperlongumine A (PL) is an alkaloid isolated from some vegetal species of *Piper* (Piperaceae) (Fig. 1A). Several PL biological effects have been reported such as cytotoxic, genotoxic, anti-angiogenic, anti-metastatic and anti-tumor activities [1]. The natural PL molecule induces apoptosis in osteosarcoma, breast, bladder and lung cancer cells, but importantly not in normal cells [2]. A potent inhibition of breast cancer cell line migration was observed with PL analogs [3]. PL increases reactive oxygen species (ROS) and apoptotic cell death in both cancer cells and normal cells engineered to have a cancer genotype, irrespective of p53 status, with little effect in primary normal cells [4]. Using a panel of PL analogs, Adams et al. analyzed the mechanism of action of PL and suggested that ROS-independent mechanisms, including cross-linking events, may also contribute to PL's induction of apoptosis [5]. An inhibition of the ubiquitin-proteasome system by PL was then reported by Jarvius et al. [6] but no inhibition of the 20S proteasome itself, or of 19S deubiquitinating activity was observed at concentrations inducing cytotoxicity. These observations motivated us to investigate the potential interaction between PL and proteasome. Indeed, the proteasome is now a valuable anticancer drug target [7]. This highly complex protease is formed of a 20S catalytic core particle (CP) complexed with regulatory particles such as 19S for constitutive proteasome or 11S for inducible immunoproteasome [8,9]. The CPs of both proteasomes are composed of four stacked heptameric rings with two outer rings (α1-7) formed by the α subunits and two inner rings formed of β subunits (β1-7). The two β1c, two β2c and two β5c catalytic units of the constitutive cCP are each replaced by the β1i, β2i and β3i subunits in inducible iCP. They bear caspase-like or post-acid activity (PA) for β1, trypsin-like activity (T-L) for β2 and chymotrypsin-like activity (ChT-L) for β5 subunits. Considerable efforts to develop proteasome inhibitors [7,8] have been made leading to noncovalent inhibitors [10–13] or covalent ones such as

---

* Corresponding author.
** Corresponding author.
E-mail addresses: chahrazade.el_amri@upmc.fr (C. El Amri), michele.reboud@upmc.fr (M. Reboud-Ravaux).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.01.100
0006-291X/

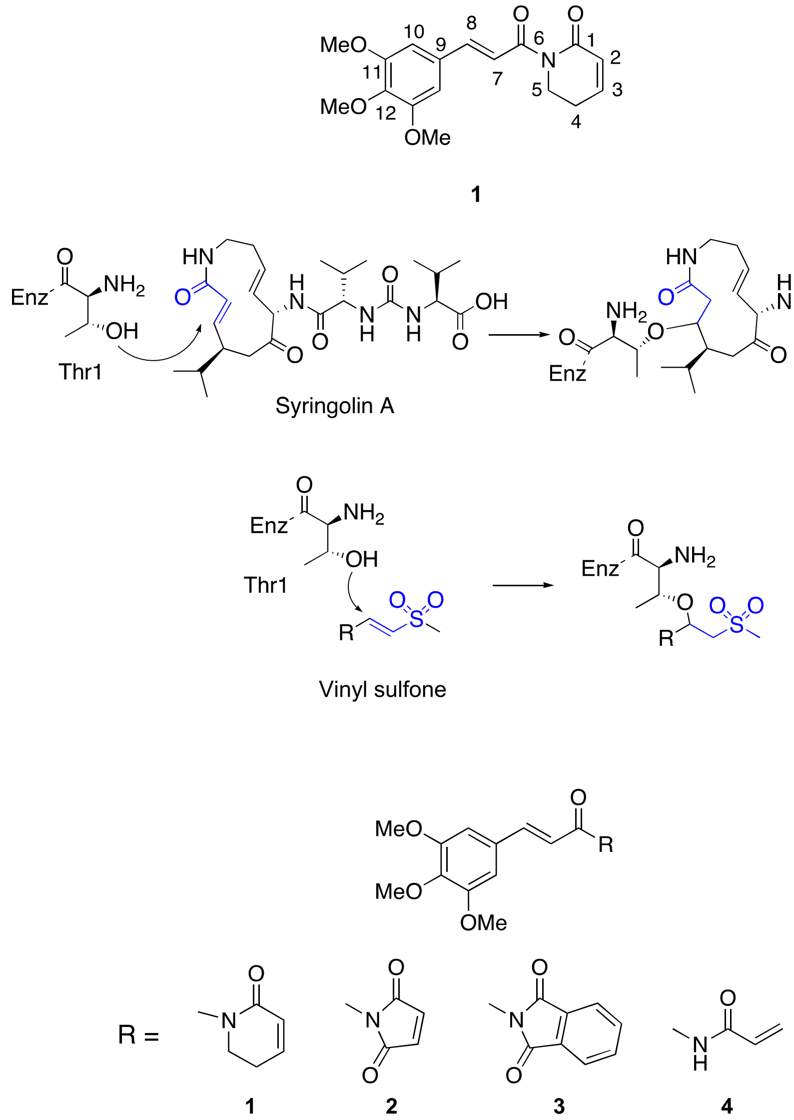

Fig. 1. Structure of piperlogumine (PL, **1**) showing the two Michael acceptor units (olefins C2-C3 and C7-C8), and reactions of syringolin and vinyl ketone derivatives with Thr1O$^{\gamma}$ 1 residues of cCP (A). Newly synthesized analogs of **1** (B).

the three approved drugs used in the treatment of hematologic malignancies, bortezomib (Velcade®) [14], carfilzomib (Kyprolis®) [15] and the orally administered ixazomib (Ninlaro®) [16]. These drugs inhibit mainly the β5 activity of the catalytic core of the cCP but also that of the iCP by creating covalent bonds with the catalytic Thr1. When considering the chemical structure of PL (**1**, Fig. 1A), it appears that its non-symmetric di-vinyl imide functions implicating the double bonds C2-C3 and C7-C8 (Fig. 1A) may lead to two possibilities of Michael addition with the N-terminal nucleophilic residue Thr1O$^{\gamma}$ present in all catalytic subunits of CPs. Inhibitors displaying the parent amide vinyl group such as the natural syringolin A are known to react with cCP Thr1O$^{\gamma}$ to give Michael adduct (Fig. 1A) [17]. The same mechanism was observed with synthetic vinyl ketones [18]. Nevertheless, Jarvius et al. found that PL did not inhibit 20S proteasome (presumably constitutive proteasome) [6]. In the other hand, the interference of *N*-acetyl-L-cysteine with PL suggested that PL displays proteasome inhibitory properties [19]. In this paper, we re-examine the interaction of PL with proteasome by analyzing the inhibitory effect of PL and three of its newly synthesized analogs [3] (Fig. 1B) not only on human cCP but also human iCP. Immunoproteasome has been associated to progression of certain types of cancer, autoimmune disorders and inflammation [20]. Previous studies indicated that the 3,4,5-trimethoxy cinnamic moiety of **1** does not affect its antitumor property and the C7-C8 site has a lesser electrophiliciy than the C2-C3 one [5]. We thus examined the effect of ring isomerism by replacing the PL δ-valerolactam cyclic ring by the known nucleophile trap succinimide (compound **2**) or by a non-reactive more expanded bicycle (compound **3**) whereas the pharmacophore involving the C7-C8 olefin was conserved. Compound **4** showed a molecular simplification with the conserved Michael acceptor C7-C8 and double bond C2-C3, but the lactam (piperidonic) ring was removed and replaced by a linear vinyl keto group [3]. We demonstrated that the human cCP is poorly inhibited by the tested compounds whereas iCP is efficiently inhibited by **1** and two of its analogs. The mechanism by which the iCP was inhibited by the tested compounds was determined and their potential as cytotoxic tumor reagents was evaluated.