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Cephalostatin 1 analogues activate apoptosis via the endoplasmic reticulum stress signaling pathway

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ABSTRACT

The current study was conducted to compare the cytotoxicity of two stereospecific cephalostatin 1 analogues (CAs) against several human normal cell types and cancer cell lines and to determine their cytotoxic mechanism. Both CA analogues induced apoptosis and were cytotoxic with 50% growth inhibition (GI_{50}) at ~1 μM or less in six human cancer cell lines but neither analogue at 10 μM killed more than 14% of any of three types of normal human cells suggesting their cytotoxicity is cancer-specific. CA treatment inhibited clonogenic tumor growth and activated caspase 3 and 9 but not caspase 8. CA-induced apoptosis was inhibited by the pan caspase inhibitor indicating the importance of caspase activation. CA treatment released smac/DIABLO but not cytochrome c from mitochondria and induced phosphorylation of eIF-2 and the activation of procaspase 4 in cancer cells, similar to cell treatment with thapsigargin, a known endoplasmic reticulum (ER) stress inducer. Finally, cells pretreated with a caspase 4 inhibitor were resistant to CA-induced apoptosis. In conclusion, both CAs induced apoptosis by triggering ER stress. Because of their ease of synthesis and low GI_{50}, these cephalostatin analogues represent promising anticancer drugs.

1. Introduction

Cancer is a leading cause of death worldwide and the number of deaths is projected to rise in the coming years (Siegel et al., 2016). Surgery, chemotherapy and radiotherapy are the most common treatment options against cancer (Abdullah and Chow, 2013). However, the response to treatment varies substantially in different types of cancer, or even among patients with the same type of cancer (Gatti and Zunino, 2005). The variations in responses might indicate that intrinsic or acquired therapeutic resistance exists in cancer patients (Hammond et al., 2016). Nowadays, there are great efforts focusing on understanding the molecular mechanisms responsible for drug resistance and identifying new drugs which might work through signaling pathways that differ from what regular drugs use to overcome drug resistance (Housman et al., 2014).

Genetic and biochemical studies have identified two major pathways of apoptosis, the death receptor-mediated pathway and the mitochondria-dependent pathway (Elmore, 2007). Recently, many studies have implicated a regulatory role for the endoplasmic reticulum (ER) in apoptosis (Bravo-Sagua et al., 2013). The ER under stress induces the overexpression of chaperones and phosphorylation of the eukaryotic initiation factor-2 (eIF-2), which attenuates translation initiation and protein synthesis (Bravo-Sagua et al., 2013).

In addition and under severe stress, the ER can initiate its own apoptotic signals that independently activate caspase 4 by localizing it to the ER membrane. Caspase 4 in turn activates caspase 9 independently of cytochrome c release from the mitochondria (Breckenridge et al., 2003; Iurlaro and Muñoz-Pinedo, 2016). Caspase 9 then activates one or more of the effector caspases (caspase 3, 6 and 7) (Groenendyk and Michalak, 2005). In addition, ER stress causes the release of second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low isoelectric point (smac/DIABLO) from the mitochondria which inhibits the inhibitors of apoptosis proteins (IAPs) contributing to the activation of caspases (Parrish et al., 2013).

Cephalostatin 1 (Fig. 1) is a member of strongly-related bis-steroidal compounds (Pettit et al., 1988, 2011). Cephalostatin 1 has proved to be one of the most powerful experimental anticancer agents tested with a

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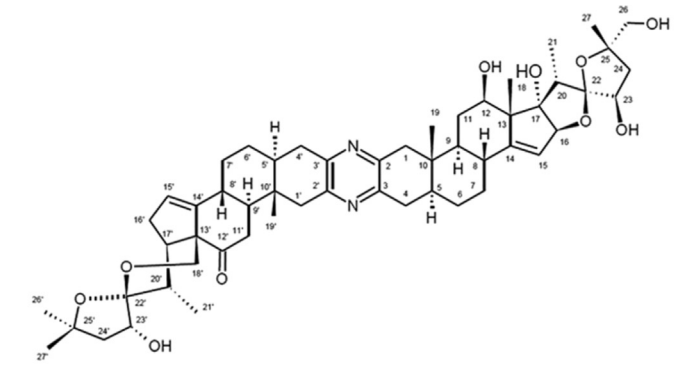
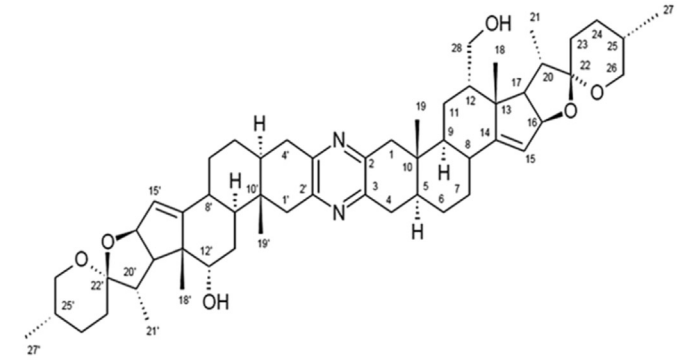


Cephalostatin 1



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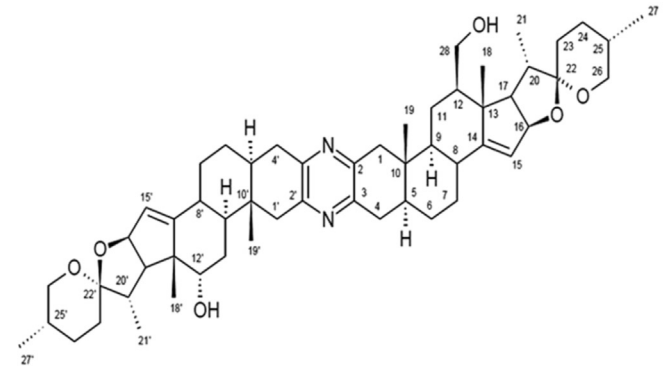

12'α-hydroxy,12β-hydroxymethyl bis-steroidal pyrazine (CAB)

Fig. 1. Structures of molecules important to this study. Chemical structure of cephalostatin 1, cephalostatin analogue A (CAA) and CAB.

GI_{50} in the nano to pico molar range (Lee et al., 2009). In addition, cephalostatin 1 induces apoptosis through the ER-mediated pathway and selectively uses smac/DIABLO as a mitochondrial signaling molecule (Dirsch et al., 2003; Muller et al., 2005; Rudy et al., 2008).

However, the availability of cephalostatin 1 from its natural sources is extremely limited and its synthesis is very complicated (Gryszkiewicz et al., 2003; Li and Dias, 1997; Poza et al., 2010). Difficulties in obtaining quantities of cephalostatin 1 led to approaches synthesizing cephalostatin 1 analogues (CAs) looking for utilizable alternatives (Guo et al., 1996; Iglesias-Arteaga and Morzycki, 2013; Li et al., 2002; Li and Fuchs, 2003; Nawasreh, 2007).

Two 12'α-derivatives of cephalostatin 1, 12'-α hydroxy-12α-hydroxymethyl-bis-steroidal pyrazine (CAA) and 12'α-hydroxy-12β-hydroxymethyl-bis-steroidal pyrazine (CAB) (Fig. 1), were synthesized previously in small amounts and when tested as a mixture with the 12'-β-hydroxy derivatives, showed cytotoxicity (Nawasreh, 2007). However, the cytotoxicity of the isolated 12'-β-hydroxy derivatives was diminished. Thus, we have scaled up synthesis of these compounds to obtain sufficient amounts of the purified 12'α-hydroxy derivatives to characterize their biological activity in cell culture and to deduce their mechanism of cell killing. The amounts of these compounds should be sufficient for their eventual use in vivo to analyze their pharmacological efficacy against cancers.

2. Materials and methods

2.1. Compounds

Broad spectrum pan-caspase inhibitor (benzyloxycarbonyl-Val-Ala-Asp(OMe)-fluoromethylketone; zVAD-fmk) was purchased from Bachem Americas, Inc. (CA, USA), thapsigargin (TG) was purchased from LC Laboratories (MA, USA). Caspase 4 inhibitor (Ac-LEVD-CHO)