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Structural modification of resveratrol leads to increased anti-tumor activity, but causes profound changes in the mode of action

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ABSTRACT

(*Z*)-3,5,4′-Trimethoxystilbene (Z-TMS) is a resveratrol analog with increased antiproliferative activity towards a number of cancer cell lines compared to resveratrol, which has been shown to inhibit tubulin polymerization *in vitro*. The purpose of this study was to investigate if Z-TMS still shows potential for the prevention of metabolic diseases as known for resveratrol.

Cell growth inhibition was determined with IC_{50} values for Z-TMS between 0.115 μM and 0.473 μM (resveratrol: 110.7 μM to 190.2 μM). Flow cytometric analysis revealed a G_2/M arrest after Z-TMS treatment, whereas resveratrol caused S phase arrest. Furthermore, Z-TMS was shown to impair microtubule polymerization. Beneficial effects on lipid accumulation were observed for resveratrol, but not for Z-TMS in an *in vitro* steatosis model. (*E*)-Resveratrol was confirmed to elevate cAMP levels, and knockdown of AMPK attenuated the antiproliferative activity, while Z-TMS did not show significant effects in these experiments. SIRT1 and AMPK activities were further measured indirectly *via* induction of the target gene small heterodimer partner (SHP). Thereby, (*E*)-resveratrol, but not Z-TMS, showed potent induction of SHP mRNA levels in an AMPK- and SIRT1-dependent manner, as confirmed by knockdown experiments.

We provide evidence that Z-TMS does not show beneficial metabolic effects, probably due to loss of activity towards resveratrol target genes. Moreover, our data support previous findings that Z-TMS acts as an inhibitor of tubulin polymerization. These findings confirm that the methylation of resveratrol leads to profound changes in the mode of action, which should be taken into consideration when conducting lead structure optimization approaches.

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Introduction

(*E*)-Resveratrol (3,5,4′-trihydroxy-*E*-stilbene, E-Res, Fig. 1A) is a phytoalexin found in the skin of red grapes and other food, *e.g.* peanuts and berries (Langcake and Pryce, 1976; Sanders et al., 2000; Siemann and Creasy, 1992). The thoroughly analyzed biochemical actions of (*E*)-resveratrol include potent anticarcinogenic and chemopreventive activities in a number of tumor cell lines (Jang et al., 1997; Ulrich et al., 2005; Kaminski et al., 2014). Furthermore, (*E*)-resveratrol is discussed as a preventive agent against type 2 diabetes, obesity, and other metabolic disorders (Lagouge et al., 2006), *e.g.* nonalcoholic fatty liver disease (NAFLD) (Heebøll et al., 2014).

NAFLD is recognized as the hepatic manifestation of the metabolic syndrome, initially characterized by accumulation of triglycerides in the liver. This benign steatosis can progress to an inflammatory state (nonalcoholic steatohepatitis, NASH), ultimately leading to fibrosis, cirrhosis, and liver cancer (Farrell and Larter, 2006). (*E*)-Resveratrol was shown to positively influence hepatic steatosis in mice fed a high fat diet. Baur et al. (2006) and Bujanda et al. (2008) obtained similar results from a steatosis model in rats. Investigations of an *in vitro* steatosis model showed beneficial effects for (*E*)-resveratrol on lipid accumulation in HepG2 cells *via* a signaling pathway involving the AMP-activated protein kinase (AMPK) and human silent mating type information regulation 2 homolog 1 (SIRT1) (Hou et al., 2008).

AMPK, known as the major cellular metabolic switch, has been shown to increase NAD^+ levels leading to activation of SIRT1 (Cantó et al., 2010), a NAD-dependent deacetylase also involved in the metabolic regulation of cells (Yu and Auwerx, 2010). (*E*)-Resveratrol has been shown to activate both metabolic target genes in a great number of cell lines and animal models (reviewed recently by Kulkarni and Canto, 2014). However, the molecular mechanism by which (*E*)-resveratrol mediates AMPK and SIRT1 activation remained unclear until 2012 when Park et al. (2012) proposed phosphodiesterases (PDEs) as direct

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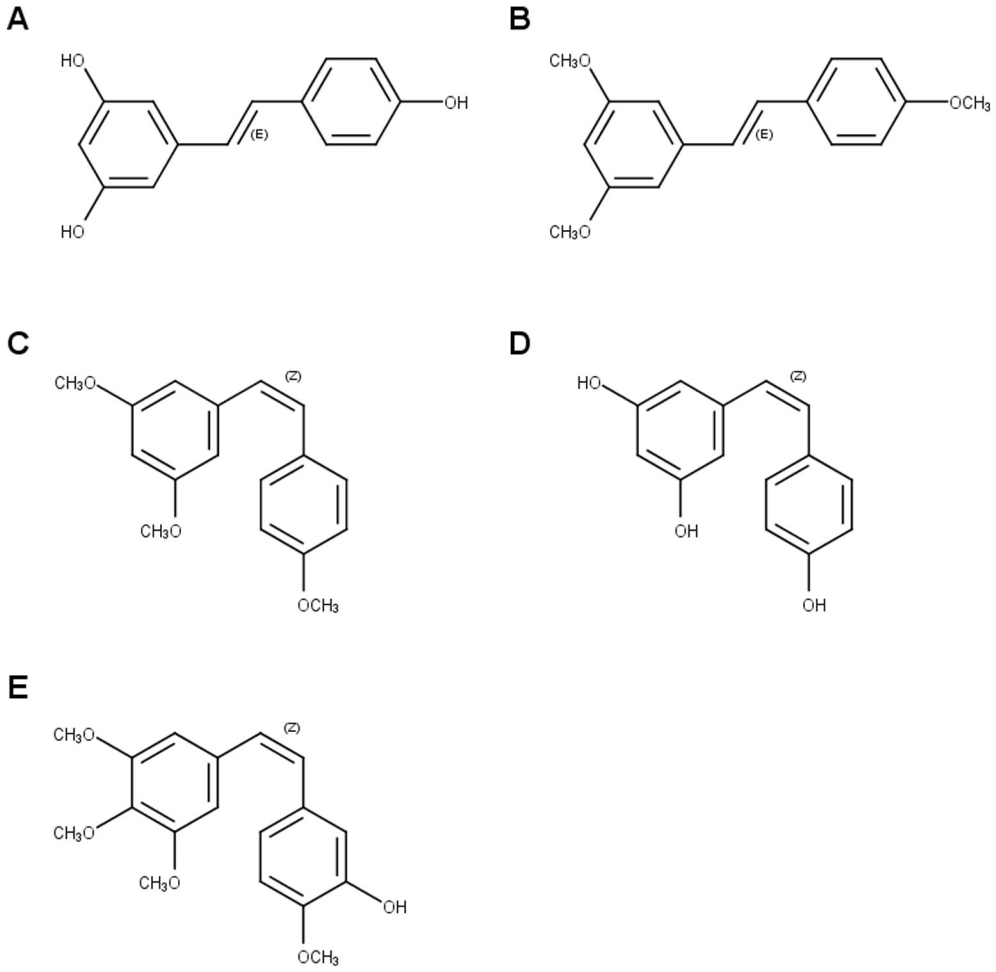

Fig. 1. Chemical structures of (*E*)-resveratrol (A), E-TMS (B), Z-TMS (C), (*Z*)-resveratrol (D), and combretastatin A-4 (E).

targets of (*E*)-resveratrol. Inhibition of PDEs by (*E*)-resveratrol leads to elevation of intracellular cAMP levels, subsequent elevation of NAD^+ levels and thus downstream activation of AMPK and SIRT1.

A molecular target which has been shown to interfere with both AMPK and SIRT1 is the nuclear receptor small heterodimer partner (SHP) (Yuk et al., 2011; Wei et al., 2011). SHP belongs to the orphan nuclear receptors without a known endogenous ligand and lacks a DNA-binding domain (Seol et al., 1996). An *in vivo* global gene expression profiling revealed that SHP is capable of regulating a great number of target genes involved in bile acid synthesis and cholesterol, glucose, and lipid metabolism (Boulias et al., 2005).

Despite its potent *in vitro* activities described above, (*E*)-resveratrol lacks important biophysical properties for drug candidates like bioavailability, metabolic stability, and target specificity (Walle et al., 2004). Common concentrations of (*E*)-resveratrol used in *in vitro* experiments are in the range of 10–200 μM. Significant effects are often observed in concentrations above 50 μM, while pharmacodynamic studies using a repeated dosing scheme showed that the highest serum concentrations reached were below 5 μM after oral administration of up to 5.0 g of (*E*)-resveratrol daily (Brown et al., 2010).

There are two main approaches to increase the bioavailability of (*E*)-resveratrol: chemical modification of the core structure or the development of formulations with improved pharmacokinetic properties. The latter involve liposomal formulations (Mukherjee et al., 2011) and nanoemulsions (Zhou et al., 2015), while lead structure optimization focused on higher hydroxylated (Murias et al., 2004), halogenated (Lee et al., 2003), or methylated (Cardile et al., 2007) analogs of resveratrol.

Methylation of the free hydroxyl groups seems a promising approach to enhance metabolic stability of (*E*)-resveratrol by preventing metabolic phase II steps (Zhang and Go, 2007). Lin et al. conducted two studies addressing the determination of a pharmacokinetic profile of (*E*)-3,5,4′-trimethoxystilbene (E-TMS, Fig. 1B) and its isomer (*Z*)-3,5,4′-trimethoxystilbene (Z-TMS, Fig. 1C) compared with (*E*)-resveratrol (Lin et al., 2010; Lin and Ho, 2009). Both methylated analogs showed a longer elimination half-life and lower clearance after intravenous application, suggesting a lesser extent of metabolism. Methylated resveratrol analogs are intensively studied and recognized as more potent regarding cell growth inhibition of various cancer cell lines compared to (*E*)-resveratrol (Horvath et al., 2007; Zhang and Go, 2007). Additionally, methylated (*Z*)-analogs show superior antiproliferative activity in contrast to their (*E*)-isomers (Cushman et al., 1991), whereas (*E*)-resveratrol is more potent towards colorectal cancer cell lines than the corresponding (*Z*)-isomer (Mazué et al., 2010, Fig. 1D).

Z-TMS shows distinct antiproliferative activity towards a number of cancer cell lines (Paul et al., 2010; Cardile et al., 2007), and is widely recognized as a successful lead structure optimization of (*E*)-resveratrol. However, recent findings suggest a mode of action different from that of (*E*)-resveratrol (Chabert et al., 2006).

The purpose of this study was to examine the antiproliferative activity of Z-TMS towards colon and liver cancer cells lines. In addition, we wanted to investigate if the structural modifications from (*E*)-resveratrol to Z-TMS are able to maintain the ability to elevate intracellular cAMP levels, or the activity towards enzymes involved in metabolic regulation, *e.g.* SIRT1 and AMPK. For a more extensive understanding of the

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