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Anti-inflammatory and antioxidant properties of a novel resveratrol–salicylate hybrid analog

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ABSTRACT

Resveratrol is a natural compound with a plethora of activities as well as limitations. We recently reported a series of resveratrol–salicylate analogs with potential chemopreventive activity. Herein, we report the anti-inflammatory and antioxidant properties of these resveratrol derivatives. Using an in vitro COX inhibition assay, and two in vivo protocols (carrageenan-induced peritonitis and paw edema), we identified a novel compound (**C10**) as a potent anti-inflammatory agent. The enhanced potency of **C10** was associated with the ability of **C10** to decrease the activity of myeloperoxidase (MPO) enzyme at 10 mg/kg, whereas resveratrol and it's natural analog (TMS) did not exert the same effect. Additionally, **C10** significantly reduced the concentration of intracellular reactive oxygen species. Because of the proven association between cancer, inflammation, and oxidative stress, we believe that **C10** is a promising chemopreventive molecule.

Chronic inflammation and oxidative stress are two commonly associated conditions involved in the pathophysiology of cancers, atherosclerosis, diabetes, Alzheimer's disease, pulmonary diseases and others.[1] Additionally, prolonged exposure to reactive oxygen species (ROS) can trigger the overexpression of numerous transcription factors that can in turn activate many genes involved in cell cycle regulation, inflammation, and chemotaxis.[1] Polyphenols and other natural agents are capable of counteracting stress-induced tissue injury by acting as antioxidant and anti-inflammatory agents.[2]

Resveratrol (3,4′,5-*trans*-trihydroxystilbene, Fig. 1) is a natural phytoalexin that is produced by many plant species as a defensive agent. It regulates many biological processes including (but not limited to) hemostasis,[3] glucose metabolism,[4] cell division,[5] neuroprotection,[6] free radical scavenging[7] and inflammation,[2] and it is considered in the literature as a 'multi-target'[8] molecule. However, despite the considerable number of studies published over the last two decades, the therapeutic potential of resveratrol remains elusive, at least due to its low oral bioavailability[9] and its efficacy.[10] Consequently, most of the medicinal chemistry projects in this field have concentrated on developing resveratrol derivatives with better pharmacokinetic profiles and enhanced potencies.

Our group has recently designed a new class of resveratrol hybrids based on the addition of a carboxylic acid group adjacent to one of the phenol groups present in resveratrol structure. This series of molecules were designed as multi-target chemopreventive agents according to literature[8,11] which emphasize that modulation of several pathways underlying a disease state is better than targeting a single receptor or enzyme. Some of these molecules exert a potential chemopreventive activity by inhibiting the activity and expression of CYP1A1[12] (a carcinogen-activating CYP450 protein), and inhibiting the DNA methyltransferase (DNMT)[13] enzymes which are responsible for the inactivation of tumor suppressor proteins. We herein, report the anti-inflammatory profile of these resveratrol–salicylate hybrids, as well as their ability to decrease intracellular oxidative stress,[1] as these processes are implicated in the different stages of carcinogenesis.

DPPH is relatively stable nitrogen radical that is frequently used in free radical scavenging assays, to determine the ability of test drugs to exert a potential antioxidant activity.[14] We used this in vitro assay to investigate whether resveratrol–salicylate analogs could quench free radicals using a previously reported procedure.[14]

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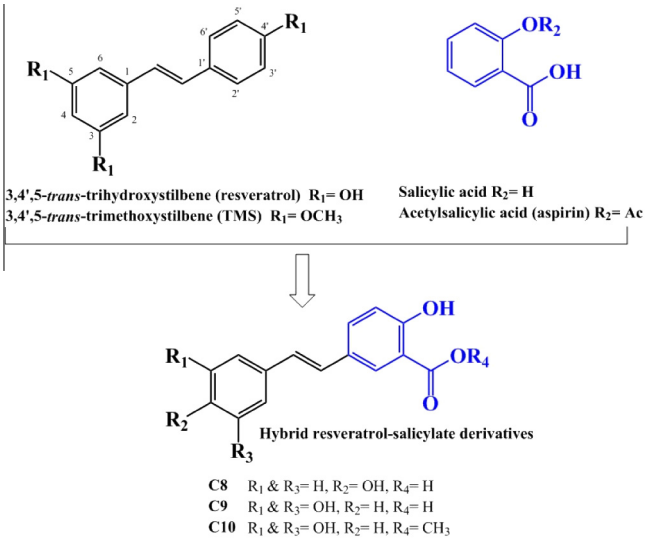

Figure 1. Chemical structures of resveratrol, TMS, salicylates and resveratrol–salicylate derivatives described in this study.

Figure 2 shows the results of the DPPH assay for the active compounds in the series of resveratrol–salicylate derivatives.

Compound **8** (**C8**) demonstrated a free radical-scavenging activity higher than that of resveratrol only at the maximum tested compound concentration (200 μM). From structural perspective, **C8** is a resveratrol–salicylate hybrid that has 4,4′-dihydroxy substitution pattern, as opposed to the parent compound resveratrol, which has a 3,4′,5-trihydroxy substitution pattern (Fig. 1). This observation is consistent with a previous report by Fan et al., in which they demonstrated that 4,4′-dihydroxystilbene scavenged galvinoxyl radicals to a greater extent than resveratrol.[7]

Previous data showed that the hydroxyl group at position 4′-in resveratrol structure participates in radical scavenging to a higher extent than hydroxyls at 3-, and 5-positions.[7] Furthermore, recent studies showed that acetylated resveratrol derivatives (some of which retained the 4′-hydroxyl group) exhibited higher antioxidant and anti-thrombotic activities than resveratrol.[15] Our results showed that compound **9** (**C9**) and compound **10** (**C10**) showed moderate free radical-scavenging properties, a bit lower than those obtained for resveratrol. The presence of an electron-withdrawing group (carboxylic acid) in the structure of **C9** and **C10** adjacent to the hydroxyl group at position 4′-, might be attributed to the lower DPPH radical scavenging of these hybrids compared to resveratrol. Nevertheless, a recent report demonstrated (theoretically and experimentally) that salicylate analogs exhibited antioxidant capacity that was thought to be due to electron or hydrogen transfer.[16]

To further confirm the antioxidant potential of resveratrol–salicylate derivatives, we evaluated their potential to reduce H_2O_2-induced intracellular ROS levels in HepG2 cells.[17] We selected **C10** based on its significant inhibitory effect on two enzymes, the DNMT (results of a previous study[13]), and the cyclooxygenase (COX) enzyme (next section). For comparison purposes, we also tested the parent resveratrol and TMS;[18] we summarize these results in Figure 3. Pre-treatment of cells with test drugs (resveratrol, TMS and **C10**) decreased the oxidative stress insult induced by H_2O_2 (1 mM) (Fig. 3). In this regard, 10 μM resveratrol decreased ROS levels by 25% (compared to control cells), TMS exerted a 42% decrease, and **C10** diminished ROS by 33%. It is noteworthy that none of the compounds at the tested concentrations (2.5, 5 and 10 μM) decreased the HepG2 cells viability using the MTT assay (data not shown).

COX-1 and COX-2 enzymes are associated with inflammation and we were interested in determining whether the hybrid resveratrol–salicylate derivatives could inhibit these enzymes using an in vitro screening assay.[19] We calculated the potency of the test compounds as the corresponding IC_{50} values, and we present the results in Table 1. According to literature, both resveratrol and its methoxylated derivative TMS, exert significant inhibitory activity on COX-1 as well as on COX-2 (non-selective COX inhibitors), with selectivity ratios (COX-1/COX-2) = 0.5 and 0.7, respectively.[19] **C8** did not show considerable activity (COX-1 IC_{50} = 421, COX-2 $IC_{50} \geqslant 500$ μM), whereas **C9** and **C10** exerted significant inhibition of both enzymes. Generally speaking, **C9** and **C10** were almost equipotent (fairly similar IC_{50} values on COX-1), but **C10** was more potent (10-fold) than **C9** on COX-2, and more selective toward this enzyme as well (Table 1).

To support the results of the in vitro COX screening assays, we carried out molecular docking simulations. The goal was to understand the COX-2 selectivity exerted by **C10** at the molecular level.

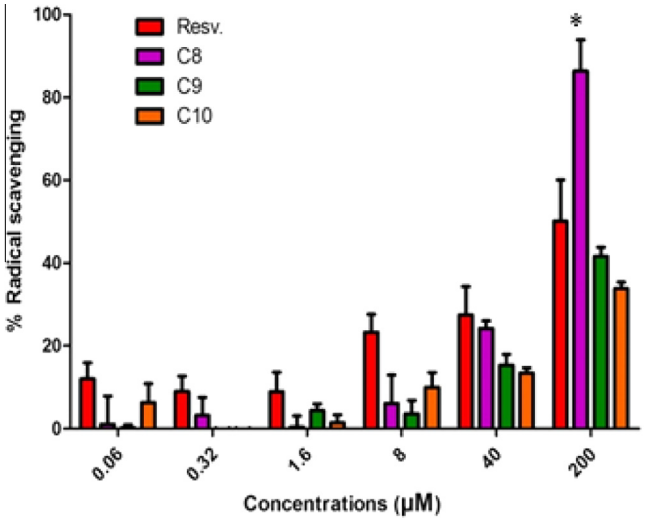

Figure 2. DPPH radical scavenging activities of resveratrol–salicylate analogs. Results are expressed as mean ± SD of three different experiments, in triplicate. *P <0.05 compared to resveratrol at the same concentration.

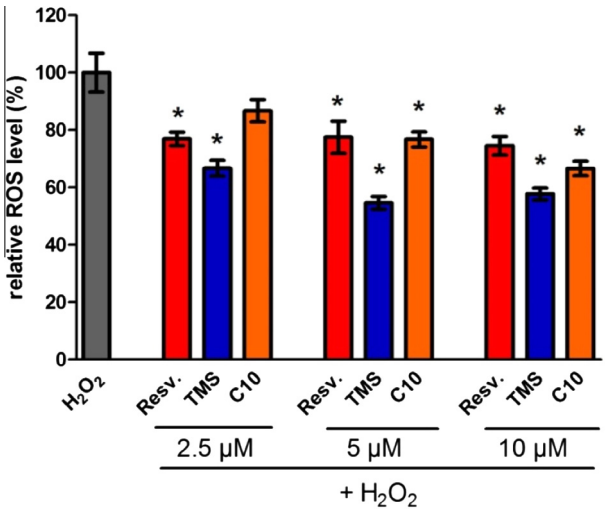

Figure 3. Effects of resveratrol analogs on intracellular reactive oxygen species (ROS) levels in HepG2 cells stimulated with H_2O_2. Results are expressed as mean ± SEM (n = 8). *P <0.05 compared to H_2O_2-only treated cells.