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Antioxidant and anti-inflammatory properties of 1,2,4-oxadiazole analogs of resveratrol

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ABSTRACT

The chemopreventive properties of resveratrol are ascribed mostly to its antioxidant activity, in particular its scavenging ability for reactive oxygen species (ROS), and to the inhibition of NF-κB pathway which has also been suggested as an important underlying mechanism of its reported properties. In present study, a small library of nine 1,2,4-oxadiazole-based structural analogs of resveratrol were assayed for their antioxidant and anti-inflammatory activities. Several compounds showed significant inhibitory activities against NF-κB and/or ROS production. Compound **2**, incorporating two *para*-hydroxyphenyl moieties connected by the 1,2,4-oxadiazole ring, was the most active, its potency in inhibiting activation of NF-κB and ROS scavenging abilities surpassing that of resveratrol. Additionally, we elucidated the mechanisms underlying the NF-κB inhibitory activity of compound **2**. Finally, in contrast to resveratrol, compound **2** significantly reduced the LPS-induced release of pro-inflammatory cytokines, indicating its prominent anti-inflammatory potential.

1. Introduction

Resveratrol is a naturally occurring polyphenol present in grapes and certain other plants, best known as the essential antioxidative constituent of red wine and responsible for its chemopreventive properties. It exists in *cis* and *trans* configurations, both of which possess biological activities, although those of *trans*-resveratrol have been ascribed greater potential than its *cis*-isomer [1–4]. Interestingly, on exposure of *trans*-resveratrol to UV light, it is converted to its *cis*-isomer [5]. It has been thoroughly researched due to its presence in many food sources in relatively large quantities (up to 40 μM in red wine) [6]. The discovery of numerous pleiotropic activities, including antioxidant, anti-inflammatory, anticancer, anti-aggregating, heart protecting properties, has attracted much attention [7–13]. These beneficial effects are most prominently displayed in the low to medium micromolar range (1–100 μM) [14]. The chemopreventive properties of resveratrol are ascribed mostly to its antioxidant activity, in particular its scavenging ability for reactive oxygen species (ROS). These free radicals are known to cause oxidative damage to biological macromolecules, leading to various diseases including cardiovascular disease and cancer. However, not all its numerous biological activities can be explained exclusively by its antioxidant properties. Resveratrol has been found to interact with numerous targets within the immune system, such as the nuclear factor κB (NF-κB) pathway that plays a key role in regulating the immune system [7,14].

Resveratrol is a stilbene derivative with a characteristically (*E*) configured double bond and phenolic hydroxyl groups. Much research has focused on the antioxidant and anti-inflammatory properties of resveratrol analogs and several classes of compounds have been synthesized and described [15–25]. In general, the antioxidant and anti-inflammatory activities of these compounds depend on the existence of several structural features: the presence of hydroxy/methoxy groups, the OH substitution pattern and the nature of the heterocycle/ethylenic linker connecting the two phenyl rings [16,17].

1,2,4-oxadiazole is considered to be a privileged scaffold and has

Abbreviations: AP-1, activator protein-1; C12-iE-DAP, lauroyl-γ-D-glutamyl-meso—diaminopimelic acid; ERK, extracellular signal-regulated kinase; IκBα, NF-κB inhibitor alpha; IKK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MTS, ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); NF-κB, nuclear factor κB; NMM, N-methylmorpholine; ROS, reactive oxygen species; SAR, structure/activity relationship; SEAP, secreted embryonic alkaline phosphatase; TBH, tert-butyl hydroperoxide; TBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TNF-α, tumor necrosis factor-α.

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been used extensively in medicinal chemistry [26,27]. In the present study, we have synthesized a small library of nine 1,2,4-oxadiazoles **1**–**9**, based on the structure of resveratrol. The *trans*-stilbene ethylenic bridge of the resveratrol scaffold was replaced with a 3,5-diphenyl 1,2,4-oxadiazole to keep the geometry of these phenyl rings close to that of the *trans*-stilbene template. Modification of the substituents on the two aromatic rings afforded a series of analogs (Fig. 1).

The aim of this study was to investigate their antioxidant and anti-inflammatory properties and compare them to those of *trans*-resveratrol. Their antioxidant capacities were evaluated using the *tert*-butyl hydroperoxide (TBH) assay, and their anti-inflammatory effects against NF-κB by their action on the THP-1 cell line. The immunomodulatory properties of resveratrol and of the best compound of the series, compound **2**, were assessed by evaluating their effect on proinflammatory cytokine production by THP-1 cells in the presence of lipopolysaccharide (LPS). Additionally, resveratrol and compound **2** were docked into the DNA-binding site of NF-κB p50·p65 heterodimer. We report a compound, **2**, that is even more active than resveratrol in inhibiting activation of NF-κB and in scavenging ROS.

2. Materials and methods

2.1. Synthesis

The synthesis of 1,2,4-oxadiazole-based analogs of resveratrol as well as their spectroscopic characterization are described in detail in the Supporting Information.

2.2. Cell culture

Cell line THP-1 was purchased from ATCC (LGC Standards, UK), Ramos-Blue™ cells were from Invivogen (San Diego/CA, USA). THP-1 were cultured in RPMI 1640 medium (Sigma–Aldrich, St. Louis/MO, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island/NY, USA), 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 50 μM 2-mercaptoethanol (all from Sigma–Aldrich) in a humidified chamber at 37 °C and 5% CO_2. Ramos-Blue™ cells were cultured in accordance with the manufacturer's instructions.

2.3. Cytotoxicity assay

Cells (3×10^5 cells/mL) were treated with the appropriate amounts of compounds of interest or corresponding vehicle (control cells), then seeded in triplicate on 96-well plates. The cell viability was assessed using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison/WI, USA), in accordance with the manufacturer's instructions.

2.4. ROS detection

THP-1 (8×10^5 cell/mL) were centrifuged at 1200 rpm and resuspended in PBS buffer with 10 μM H_2DCFDA probe (Sigma–Aldrich). Staining was performed for 15 min at 37 °C and 5% CO_2. Afterwards, cells were once washed with PBS and then resuspended in cell culture media. Compounds of interest were added 1 h prior to 50 μM *tert*-butyl hydroperoxide (TBH) treatment. After 1 h of co-treatment, ROS production was determined on FACSCalibur flow cytometer (BD Biosciences; San Diego, CA, USA).

2.5. Quanti-blue assay/NF-κB transcriptional activity assay

Ramos-Blue™ cells (Invivogen), which stably express an NF-κB/AP-1-inducible secreted embryonic alkaline phosphate (SEAP) reporter construct, were assayed for NF-κB transcriptional activity changes upon pretreating them with the compound of interest (25 μM) for 1 h and subsequently stimulating them with C12-iE-DAP (5 μM). SEAP activity was determined in the supernatant in accordance with the manufacturer's instructions. Briefly, to 180 μL of QUANTI-Blue regent 20 μL of cell supernatant was added and incubated at 37 °C for 3 h. Absorbance was measured on microplate reader Tecan Safire2 at 640 nm.

2.6. Western blot analysis

THP-1 cells were seeded in 6-well culture plates at a concentration of 1×10^6 cells/mL and treated with the compound of interest or corresponding vehicle for 5′, 30′ or 60′. After the indicated time points, cells were harvested, washed in ice-cold PBS and lysed in modified RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.25% Na-deoxycholate, 1 mM EDTA, 1 μg/mL aprotinin,

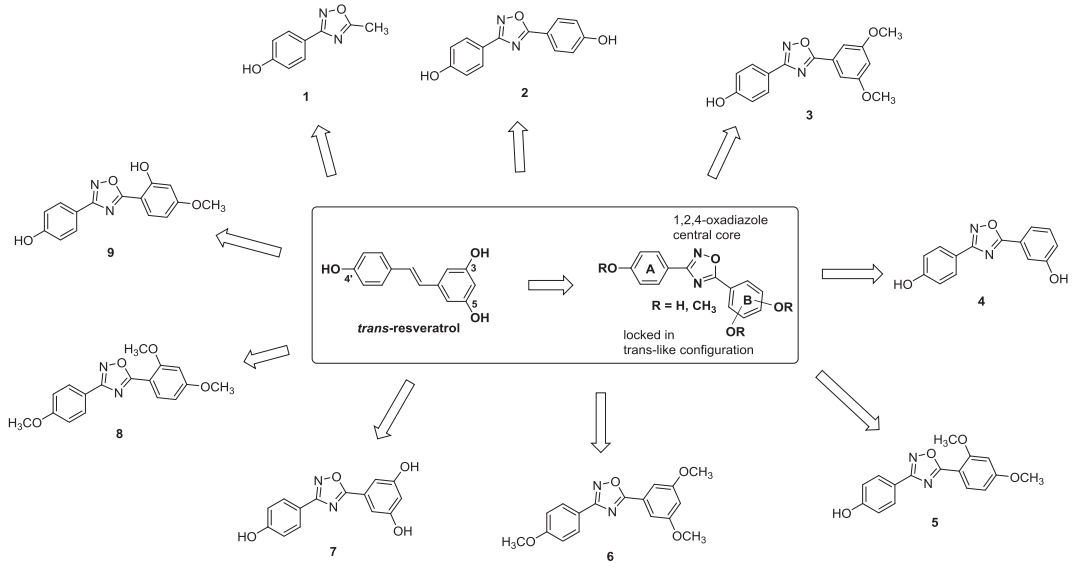

Fig. 1. Design of novel 1,2,4-oxadiazole resveratrol analogs.