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Design, synthesis, biological and structural evaluation of functionalized resveratrol analogues as inhibitors of quinone reductase 2

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ABSTRACT

Resveratrol (3,5,4′-trihydroxylstilbene) has been proposed to elicit a variety of positive health effects including protection against cancer and cardiovascular disease. The highest affinity target of resveratrol identified so far is the oxidoreductase enzyme quinone reductase 2 (QR2), which is believed to function in metabolic reduction and detoxification processes; however, evidence exists linking QR2 to the metabolic activation of quinones, which can lead to cell toxicity. Therefore, inhibition of QR2 by resveratrol may protect cells against reactive intermediates and eventually cancer. With the aim of identifying novel inhibitors of QR2, we designed, synthesized, and tested two generations of resveratrol analogue libraries for inhibition of QR2. In addition, X-ray crystal structures of six of the resveratrol analogues in the active site of QR2 were determined. Several novel inhibitors of QR2 were successfully identified as well as a compound that inhibits QR2 with a novel binding orientation.

1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene, Fig. 1) is a naturally occurring phytoalexin that was discovered in 1940, when it was isolated from the roots of white hellebore.[1–3] Resveratrol occurs in nature as both the *cis*- and *trans*-isomers and it can be found in a variety of dietary sources including peanuts, pistachios, and berries.[4,5] Of the more common dietary sources of resveratrol, the skins and seeds of grapes are the most notable with red wine being the most heavily consumed form.[5,6]

Interest in resveratrol increased dramatically in 1992, when it was hypothesized to explain the cardioprotective effects of red wine and the 'French paradox,' the observation of reduced incidence of cardiovascular disease in regions of France where red wine and saturated fats are consumed in greater quantities than in the US.[7,8] Since then, numerous studies have demonstrated the ability of resveratrol to prevent or slow the progression of various disease states including cancer and cardiovascular disease.[9,10] Resveratrol has even been shown to increase the lifespans of several organisms including yeast, worms, fruit flies and fish.[11–13]

A number of direct targets for resveratrol have been discovered in vitro, including cyclooxygenase-1 (COX1), cyclooxygenase-2 (COX2), and the transcription factor NF-κB.[14–16] The highest affinity target of resveratrol identified to date is quinone reductase 2 (QR2), a FAD-dependent cytosolic enzyme that catalyzes the 1-, 2-, or 4-electron reduction of quinones and other compounds using *N*-alkyl- and *N*-ribosylnicotinamides.[17,18] QR2 is an oxidoreductase thought to function in metabolic reduction and detoxification; however, the true physiological role of QR2 is currently unknown.[19] Evidence exists that QR2 is capable of catalyzing the metabolic activation of quinones and anti-tumor drugs, leading to cell toxicity.[20–22] Thus, in some cases, inhibition of QR2 by resveratrol may guard cells against these reactive species that induce DNA damage, which may subsequently lead to cancer.[23]

Resveratrol has been found to bind tightly to the oxidized, FAD-form of QR2 and it acts as a competitive inhibitor against *N*-methyldihydronicotinamide (NMeH) with a K_i value of 88 ± 20 nM, determined by steady-state kinetic studies, and a K_d value of

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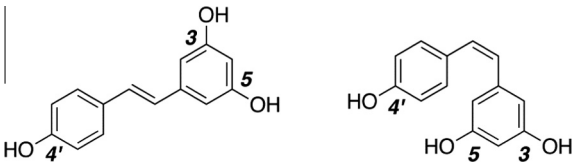


Figure 1. *Trans*- and *cis*-resveratrol.

54 ± 0.6 nM, determined by isothermal titration calorimetry[17,24] Additionally, plasma levels of resveratrol are able to reach concentrations of 500 nM, which suggests that significant inhibition of QR2 by resveratrol in vivo may be achievable.[24] Taken together, these data suggest that the amount of resveratrol consumed from dietary sources may be sufficient for effective inhibition of QR2. However, circulating resveratrol is rapidly metabolized in the liver and gut by sulfation and glucuronation to its 3- and 4′-O-sulfate and 3-O-glucuronide conjugates.[24–27] These primary metabolites of resveratrol have been shown to have far lower affinity for QR2.[24]

The present work was undertaken in an attempt to identify novel analogues of resveratrol that could potently inhibit QR2 with increased affinity and to serve as leads for the development of future QR2 inhibitors as cancer chemopreventive or anticancer drugs. To do this, we first tested a library of 78 previously synthesized resveratrol analogues designed to investigate the effects of different steric and electronic substituents on both the aryl rings and central olefin resveratrol.[28] Based on the inhibition of QR2 by the most active of these compounds, we set out to determine what effect functionalization of the central olefin of resveratrol with electron withdrawing substituents would have on inhibition of QR2 by creating a series of olefin-substituted and benzanilide resveratrol analogues. In addition, to circumvent inhibitory inactivation of resveratrol by its rapid metabolism, identification of effective resveratrol analogues that lacked the 3- and 4′-hydroxyl groups required for sulfation and glucuronation was of interest.

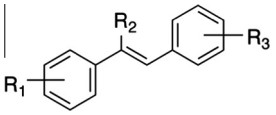
2. Results and discussion

2.1. Inhibition of QR2 by a first-generation resveratrol analogue library

The first-generation library of resveratrol analogues was designed to investigate the effects of substitution on each of the two aryl rings and central alkene of resveratrol. Therefore, to explore the electronic and steric demands of each of the aryl rings, electron-donating (OH, OMe, and NMe_2) and electron-withdrawing (F, CF_3 and NO_2) and naphthyl substitutents were selected. Four substituents were chosen to determine the effect of sterics and electronics on the central olefin (H, Me, Et, CF_3). The synthesis of this first generation library of 78 resveratrol analogues has been previously reported.[28] Twenty-four of the seventy-eight resveratrol analogues were found to effectively inhibit QR2. These results are displayed in Table 1.

Of the 78 resveratrol analogues tested, twenty-four were found to actively inhibit QR2. Of the twenty-four active analogues, ten were more potent inhibitors of QR2 than resveratrol, five of which lacked both the 3- and 4′-hydroxyl substituents that undergo metabolic sulfation and glucuronation (**1h**, **1i**, **1j**, **1r**, and **1v**). Interestingly, two of the analogues found to be more active inhibitors of QR2 than resveratrol had a naphthyl substituted for an aryl ring (**1i** and **1l**), possibly allowing for a greater pi–stacking interaction with the oxidized isoalloxazine ring of the FAD in the active site of QR2. The most potent inhibitor of QR2 identified from the first generation library was compound **1v**, which has both a highly electron-deficient aryl ring and central olefin as a result of the trifluoromethyl substituents. Unfortunately, attempts at obtaining an X-ray crystal structure of our most active compound **1v** in the active site of QR2 were unsuccessful; however, as a result we were determined to further investigate the effect of substitution at the central olefin. Therefore, we set out to synthesize a second-generation library comprised of analogues with electron-withdrawing substituents on the central olefin as well as a set of resveratrol analogues where the central olefin had been replaced by an amide.

Table 1
Inhibition of QR2 by first generation resveratrol analogue library



Analogue	R_1	R_2	R_3	IC_{50}[a] (μM)
Resveratrol	4-OH	H	3,5-$(OH)_2$	11.5 ± 3.2
1a	4-OH	H	3,4-$(OH)_2$	**6.0 ± 1.2**
1b	4-OH	H	3,5-$(OMe)_2$	**5.1 ± 1.2**
1c	3,4-$(OH)_2$	H	4-OMe	9.3 ± 3.7
1d	3,4-$(OH)_2$	H	3-F	20.8 ± 8.6
1e	3,4-$(OH)_2$	H	4-CF_3	14.2 ± 4.3
1f	4-OMe	H	3,5-$(OMe)_2$	14.6 ± 4.3
1g	3,5-$(OMe)_2$	H	3,4-$(OMe)_2$	37.1 ± 6.8
1h	3,5-$(OMe)_2$	H	4-F	**4.6 ± 1.0**
1i	3,5-$(OMe)_2$	H	2-Naphthyl	**0.73 ± 0.12**
1j	3,4-$(OMe)_2$	H	3-OMe	**6.8 ± 1.2**
1k	3,4-$(OMe)_2$	H	4-NMe_2	13.0 ± 3.5
1l	3,4-$(OMe)_2$	H	2-Naphthyl	**5.5 ± 1.3**
1m	4-OH	Me	3,5-$(OH)_2$	39.0 ± 17.2
1n	4-OH	Me	4-CF_3	**2.8 ± 1.2**
1o	3,5-$(OH)_2$	Me	4-OH	**4.8 ± 1.2**
1p	4-OMe	Me	3,5-$(OH)_2$	12.0 ± 8.4
1q	4-OMe	Me	3,5-$(OMe)_2$	20.1 ± 0.1
1r	4-OMe	Me	3,4-$(OMe)_2$	**6.7 ± 3.0**
1s	3,4-$(OMe)_2$	Me	3,5-$(OH)_2$	33.7 ± 32.4
1t	3,4-$(OMe)_2$	Me	3,5-$(OMe)_2$	9.9 ± 4.0
1u	3,5-$(OMe)_2$	CF_3	4-OMe	13.0 ± 3.1
1v	3-CF_3	CF_3	3,4-$(OMe)_2$	**0.18 ± 0.3**
1w	3,5-$(OH)_2$	Et	4-NMe_2	10.9 ± 2.2
1x	3,4-$(OMe)_2$	Et	3,5-$(OMe)_2$	16.3 ± 7.0

[a] The lowest IC_{50} values are highlighted in bold.

2.2. Synthesis of olefin-substituted resveratrol analogues

To investigate the effect of substitution of both the central olefin and the phenols, as well as the conformational effect of *E*/*Z*-isomerism of resveratrol on its inhibition of QR2, a small library of 21 olefin-substituted resveratrol analogues was synthesized. The synthesis of (*Z*)-cyano resveratrol analogues **4a**, **4b**, and **4c** was accomplished by condensation of the appropriate aldehyde **2** with the appropriate 2-phenylacetonitrile **3**, as shown in Scheme 1[29,30] With these easily functionalized nitriles in hand, the remaining eighteen resveratrol structural analogues could be synthesized. Deprotection of **4b** with boron tribromide yielded (*Z*)-nitrile analogue **4d**. Protection of the phenols of **4d** as *tert*-butyldimethylsilyl ethers followed by the reduction of the nitrile using diisobuytlaluminum hydride yielded a mixture of (*E*)- and (*Z*)-aldehydes **5**. Treatment of **5** with tetra-*n*-butylammonium fluoride resulted in the isomerically pure (*E*)-acrylaldehyde **4e**, where the (*Z*)-isomer was not detected. Further reduction of **5** with diisobutylaluminum followed by deprotection of the hydroxyls with dilute hydrochloric acid in methanol yielded the isomerically pure (*Z*)-alcohol **4f** (Scheme 1).

Reduction of (*Z*)-nitriles **4b** and **4c** using diisobutylaluminum hydride resulted in (*Z*)-aldehyde analogues **4g** and **4h** in moderate to good yields. Further reduction of the aldehydes **4g** and **4h** with sodium borohydride yielded mixtures of both (*Z*)- and (*E*)-alcohols, which could be separated by flash chromatography to give the four