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Induction of Quinone Reductase Activity by Stilbene Analogs in Mouse Hepa 1c1c7 Cells

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Based on the potential cancer chemopreventive activity of resveratrol, a trihydroxystilbene with the induction of quinone reductase activity, this study was designed to determine if stilbene-related compounds were inducers of phase II detoxifying metabolic enzyme quinone reductase (QR) in the mouse hepatoma Hepa 1c1c7 cells. Among the thirteen compounds tested, several compounds including 3,4,5,3′,5′-pentamethoxy-*trans*-stilbene were found to potentially induce QR activity in this cell line. In addition, substitution with 3-thiofurane ring instead of phenyl ring in the stilbene skeleton also exhibited potential induction of QR activity. This result will give primary information to design the potential inducers of QR activity in the stilbene analogs.

Key words: Stilbene analogs, Quinone reductase, 3,4,5,3′,5′-Pentamethoxy-*trans*-stilbene

INTRODUCTION

The induction of phase II detoxification enzymes, such as NAD(P)H:quinone reductase (QR) and glutathione *S*-transferase (GST), is one plausible cancer chemopreventive mechanism (Talalay *et al.*, 1981; Wattenberg *et al.*, 1986). Several naturally occurring or synthetic compounds including sulforaphane (Zhang *et al.*, 1994), brassinin (Mehta *et al.*, 1995), flavonoids (Cheng *et al.*, 1997; Song *et al.*, 1999), sulforamate (Gerhäuser *et al.*, 1997), withanolides (Kennelly *et al.*, 1997), and resveratrol (Jang *et al.*, 1997) have been identified as the potential anticarcinogenic agents based on the capability to induce phase II enzymes.

QR is a cytosolic flavoprotein that catalyzes the reduction of a wide variety of quinones and quinoneimines (Prochaska and Santamaria, 1988; Prochaska and Talalay, 1988). QR protects cells against the toxicity of xenobiotics by promoting the obligatory two-electron reduction of quinones to hydroquinone formation, and thus facilitating excretion of quinoids from human body.

With the usefulness of QR induction activity assay for the anticarcinogenic properties, the present study was undertaken to evaluate the potential of synthetic stilbene analogs for the induction of QR activity in Hepa1c1c7 cells.

Resveratrol, a trihydroxystilbene found in grapes and other plants, has been reported as a potential cancer chemopreventive agent by modulating initiation, promotion, and progression in the carcinogenic process (Jang *et al.*, 1997). Resveratrol also induced the QR in hepatoma 1c1c7 cells (Jang *et al.*, 1997). Based on this information, the objective of this study was to determine if structurally modified stilbenes were the inducers of QR in Hepa1c 1c7 cells.

MATERIALS AND METHODS

Chemicals and cell cultures

Crystal violet, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Tween-20, menadione, digitonin, glucose 6-phosphate, β-NADP, FAD, dicoumarol, glucose-6-phosphate dehydrogenase, sodium dodesyl sulfate and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). Cell culture media (α-MEM), fetal bovine serum (FBS) and supplements were purchased from Life Technologies, Inc. (Grand Island, NY). Hepa 1c1c7 murine hepatoma cells were cultured in α-MEM with 10% FBS, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate (37°C, 5% CO_2). The

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stilbene analogs were synthesized, and provided from Dr. Sanghee Kim (Natural Products Research Institute, Seoul National University) (Fig. 1).

Quinone reductase (QR) assay

QR activity was assessed in 96-well plates with Hepa 1c1c7 murine hepatoma cells as described previously (Gerhäuser *et al.*, 1997). Briefly, the cells were plated at 5,000 cells/microtiter 96-well plate (200 μl of medium/well) and cultured for 24 h. Each test sample was added as dissolved in 0.5% DMSO to fresh medium. After the plates were exposed for 48 h, the media were decanted, and the cells were lysed by incubation with 50 μl of a solution containing 0.8% digitonin and 2 mM EDTA, pH 7.8. The plates were agitated on a plate shaker for an additional 10 min after addition of 200 μl of the reaction mixture containing 25 μM Tris-HCl, pH 7.4, 0.067% bovine serum albumin, 0.01% Tween-20, 5 μM FAD, 1 mM glucose 6-phosphate, 0.03 mM NADP, 2 U/ml glucose 6-phosphate dehydrogenase and 0.03 % MTT to each well. A blue color developed and the reaction was stopped after 5 min by the addition of 50 μl of a solution containing 0.3 mM dicoumarol in 0.5% DMSO and 5 mM potassium phosphate, pH 7.4. The plates were then scanned at 595 nm. The protein contents of each well were determined by the crystal violet protein staining methods, and the specific activity was defined as nmol of MTT blue formazan formed per mg protein per min as described by Prochaska and Santamaria (Prochaska and Santamaria, 1988). A plot of the ratio of QR-specific activities of treated cells to control cells as a function of inducer concentration. When necessary, the CD-value (Concentration required to Double the specific activity of QR) was determined to compare the relative potential.

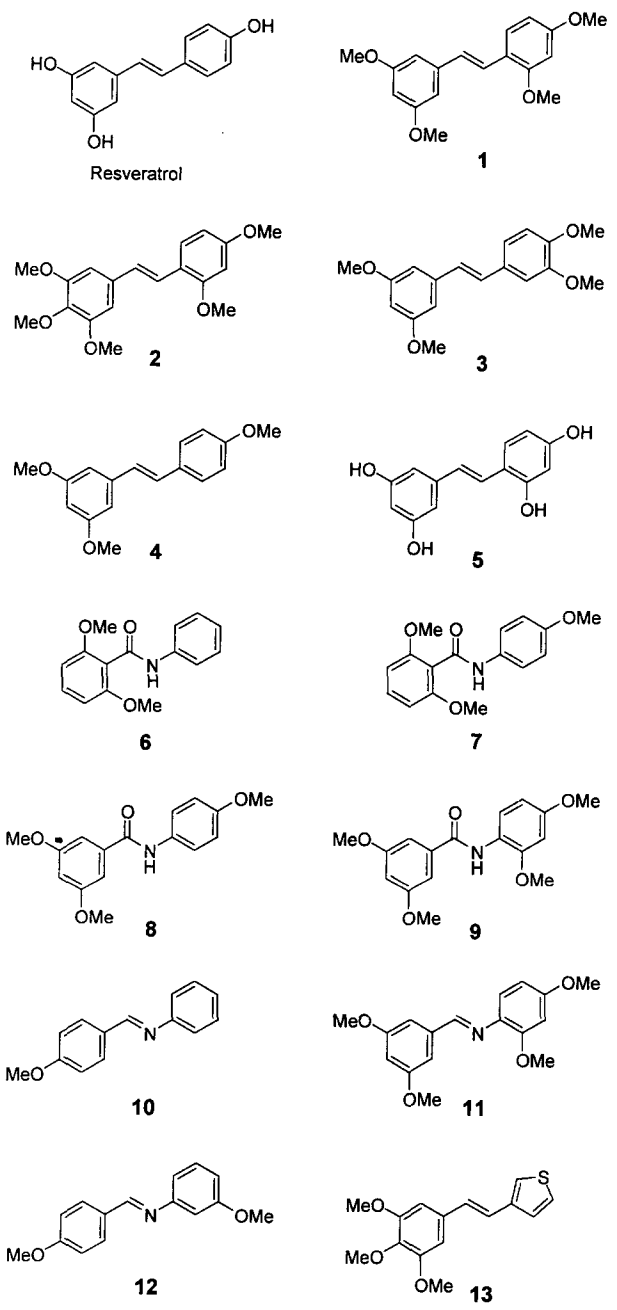

Fig. 1. Chemical structures of stilbene analogs.

RESULTS AND DISCUSSION

Various compounds derived from natural products or synthetic origins, such as β-naphthoflavone, sulforaphane, indole-3-carbinol, sulforamate, dithiolethione, and ethoxyquin, have been reported to exhibit broad-based anticarcinogenic activity against a variety of chemical carcinogens at multiple target sites in animal models through induction of phase II detoxification enzymes (Zhang *et al.*, 1994; De Long *et al.*, 1985; Grubbs *et al.*, 1995; Gurtoo *et al.*, 1985), such as QR and GST. These inducible enzymes facilitate the metabolic detoxification of xenobiotics in mammals and can achieve chemopreventive activity by modification of carcinogen metabolism through increased carcinogen excretion and decreased carcinogen-DNA interactions.

In our continuing effort of searching for novel cancer chemopreventive agents from natural products or synthetic compounds, the Hepa 1c1c7 QR assay was used to identify potent detoxification enzyme inducers because the specific activity of QR rises concomitantly with other phase II detoxification enzymes in many animal tissues in response to various chemopreventive agents (De Long *et al.*, 1985; Prochaska *et al.*, 1985). In this study, based on the induction of phase II enzymes by resveratrol, thirteen stilbene analogs were primarily evaluated for potential to induce QR activity in Hepa 1c1c7 cells. As a result, most of compounds induced QR specific activity in the range of 130~260% compared to control at the test concentration of 50 μg/ml (Table I). Especially, 3,4,5,3′,5′-penta-

Table 1. Effects of stilbene analogs on induction of quinone reductase (QR) activity in cultured mouse Hepa 1c1c7 cells

Compounds	QR activity[a] (Fold induction at 50 μg/ml)
1	1.3 ± 0.10
2	2.6 ± 0.20
3	1.4 ± 0.15
4	1.3 ± 0.05
5	1.6 ± 0.15
6	1.0 ± 0.05
7	1.4 ± 0.10
8	1.6 ± 0.15
9	1.3 ± 0.10
10	1.0 ± 0.10
11	1.4 ± 0.05
12	2.0 ± 0.15
13	2.9 ± 0.15
Resveratrol	1.9 ± 0.20 (at test of 25 μM)

[a]QR activity was determined by MTT reduction potential and expressed fold induction ratio of specific activity as mean ± S.D. compared to vehicle-treated control values in triplicate tests at the test concentration of 50 μg/ml.

methoxy-*trans*-stilbene (**2**) showed more potential induction of QR activity compared to resveratrol. It also seems that the exact locations and numbers of methoxy groups are important feature for QR activity. Replacement of the ethylene bridge of the stilbene with an amide linkage (compound **8**) showed maintenance the QR induction activity. Further, replacement with an imine linkage also exhibited the capacity of QR induction (compound **12**). In additional modification, replacement of the dimethoxy phenyl ring of **2** with 3-thiophenyl ring (compounds **13**) potentiated the QR activity.

In summary, in terms of structure-activity relationships of stilbene on induction of QR activity, the position and numbers of methoxy groups in the stilbene skeleton effected the QR activity significantly, and replacement of the ethylene bridge with an amide or imine linkage with methoxy groups in the certain position also exhibited the QR induction capacity. Our results are of interest for establishing the preliminary structure-activity relationships of stilbenes as QR inducers and allowing the design and synthesis of a stilbene derivative with a superior activity. Additionally, replacement of phenyl ring with 3-thiophenyl ring potentiates the QR activity, and thus it will be helpful for further development of potential QR inducers as cancer chemopreventive agents.

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