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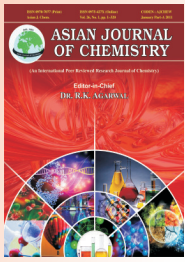

Synthesis and Bioactivity of Resveratrol Analogues

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It has been reported that resveratrol enhanced SIRT1 expression and significantly mimicked calorie restriction by stimulating Sir2 which is the most homologic homologue of SIRT1 of mammalian. A series of novel resveratrol derivatives were designed and synthesized as novel SIRT1 activator candidates. These synthesized compounds were characterized by spectral (^{1}H NMR) analysis and examined for their Sir2 activation against yeast parental strain-BY4743 at a concentration of 100 μM/L by Bioscreen C MBR machine. Several compounds showed a promising Sir2 activation activity compared with resveratrol. Meanwhile, the structure-activity relationships with Sirt2 activation activities were also discussed.

Keywords: Resveratrol analogues, Synthesis, SIRT1 activator, Wittig-horner reaction, Structure-activity-relationship.

INTRODUCTION

In recent years, a number of papers have been published on resveratrol (3,5,4'-trihydroxystilbene)[1,2], which report a wide range of novel discoveries, such as new extraction methods, new applications[3-5] and resveratrol analogs[6-8]. This intrigued us to prepare its analoges and their derivatives. In order to increase its stability and water-solubility, we designed and synthesized some analogues of resveratrol. These changes will influence bioavailability, susceptibility to metabolism and possibly the pharmacological profile of the resulting analogue. There have also been a large of reports about resveratrol exert a variety of biological activities. Among the most significant activities of resveratrol are its cancer chemo-preventive properties[9,10], antioxidant[11], antibacterial and antiinflammatory activities[12-14]. Some of these has been reported to inhibit LDL oxidation in human[15], in addition to its blocking of platelet aggregation[16] and vasorelaxing activities[17].

Especially resveratrol enhanced SIRT1 expression which led to the effect on the proteins. SIRT1, an NAD^+-dependent sirtuin deacetylase, has emerged as potential therapeutic targets for treatment of human illnesses such as type II diabetes, cancer, cardiovascular and neurodegenerative diseases. The nuclear SIRT1 is the closest homolog of yeast Sir2[18]. In yeast assays, resveratrol was also found to extended lifespan by 70 %[19], a mechanistic explanation has been proposed whereby calorie restriction slows aging by activating Sir2.

The budding yeast *Saccharomyces cerevisiae* is a widely used model of cellular and organismal aging[20-23]. A role for sirtuins in longevity determination was first suggested from work showing that deletion of Sir2 shortens replicative life span[24], while overexpression increases replicative life span[25]. We used a yeast-based screening to seek compounds for activating Sir2, leading to optimizable lead compound for SIRT1 activator. Here we report a novel yeast-based screening for measuring yeast to detect the SIRT1 activators by monitoring outgrowth of yeast cells using a Bioscreen C MBR machine. The aim of this work is to explore novel potent SIRT1 activators.

EXPERIMENTAL

The progress of the reaction is monitored by TLC and product are purified through recrystallizationand or flash chromatography. The purity of the compounds was checked by thin layer chromatography (TLC) performed on E. Merck silica gel 60 GF254 precoated plate of 0.5 mm thickness. Flash chromatography was performed with 230-400 mesh silica gel. ^{1}H NMR spectra was obtained at Bruker AM-400 NMR spectrometer using $CDCl_3$ or DMSO as the solvent except where otherwise specified, the chemical shifts are reported in δ values (ppm) relative to Me_4Si line as internal standard and *J* values are reported in Hertz. Unless otherwise stated, reagents and solvents were of reagent grade and used as obtained from commercial sources without further purification. Dichloro-

methane was distilled from CaH_2, THF from sodium prior to use and DMF were dried over anhydrous sodium sulfate. Reaction temperatures were controlled by oil bath with temperature modulator and dewars.

General procedure for synthesis of aromatic esters (5a-6a): To a well-stirred suspension of a mixture of hydroxybenzoic acid (**1a-2a**) (1 eq.), anhydrous potassium carbonate (5 eq.) and tetrabutylammonium bromide (0.5 eq.) in DMF (10 mL) was added dimethyl carbonate (50 mL, 30 eq.) at room temperature. After the mixture was stirred at 140 °C for 58 h. The dimethyl carbonate was removed under reduced pressure and water (80 mL) was added to the residue. The resulted mixture was extracted with ethyl acetate and the organic layer was washed with water and brine, dried over anhydrous sodium sulfate. The solvent was concentrated *in vacuo* to yield the pure product **5a-6a**.

General procedure for synthesis of aromatic alcohol (7a): $LiAlH_4$ (0.5 eq.) was added slowly to a solution of aromatic esters (**3a-6a**) (1 eq.) in THF (50 mL) at 0 °C for 1 h. The reaction mixture was stirred for 10 h at room temperature. Ice-water (10 mL) was added slowly to the reaction mixture and stirred for 10 min, followed by concentrated 2 M HCl to neutralize the base. The reaction mixture was extracted with ethyl acetate and the ethyl acetate layer was washed with water and brine, dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to yield the pure product **7a**.

General procedure for synthesis of benzyl bromine (8a): A solution of tribromophosphine (3 eq.) in CH_2Cl_2 (15 mL) was added dropwise to a solution of aromatic alcohol (**7a**) (1 eq.) in CH_2Cl_2 (30 mL) at 0 °C. The reaction mixture was stirred for 2 h and then warmed up to room temperature. After 4 h, the solution was poured into 80 mL ice-water and stirred for 10 min. Then the organic layer was washed with water and aq $NaHCO_3$ (3 × 20 mL), dried over anhydrous sodium sulfate and evaporated. The crude product on recrystallization from appropriate solvents yield **8a**.

General procedure for synthesis of methoxylated diethyl benzylphosphonates (9a) (Arbuzov rearrangement)[26]: A mixture of bromide (**8a**) (1 eq.) and triethyl phosphate (2 mL 5 eq.) was stirred at 135 °C for 3 h. Triethyl phosphate (2 mL 5 eq.) was added again and stirred another 4 h. After the reaction was completely finished, the mixture was purified by distillation at 4×10^{-3} bar and 120 °C to yield yellow oils methoxylated diethyl benzylphosphonates (**9a**). The crude product was used directly for the next step without further purification.

General procedure for synthesis of 1c, 2c, 5c-8c, 1d, 2d, 5d-8d, 1e, 2e, 5e-8e, 7f and 8f (Wittig-Hornor condensation)[27]: Methoxylated diethyl benzylphosphonates (**9a**) (1 eq.) in the round-bottomed flask was added dropwise a suspension of NaH (4 eq.) in THF (20 mL) at 0 °C and stirred for 1 h. Then the mixture was treated with aromatic carboxaldehyde (1-6) (1 eq.) at 0 °C for 2 h. The reaction temperature was warmed up to room temperature and further stirred for 15 h. The solution was poured into 150 mL ice-water, neutralized with 2 M HCl and extracted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated. The crude product purified by recrystallization or flash column chromatography to afford compounds **1c**, **2c**, **5c-8c**, **1d**, **2d**, **5d-8d**, **1e**, **2e**, **5e-8e**, **7f**, **8f**.

General procedure for synthesis of the nitro groups and stilbenes (3c, 4c, 3d, 4d, 4e): To a stirred suspension of CH_3ONa (3 eq.) in DMF (25 mL) was added dropwise to the corresponding methoxylated diethyl benzylphosphonate (1 eq.) at 0 °C in a round-bottomed flask and the mixture was stirred for 1 h. Then the corresponding benzaldehyde (**7-8**) (1 eq.) were added. The mixture was stirred at room temperature for 1 h, then heated to 100 °C under argon for 10 h. The solution was poured into 150 mL ice-water, the precipitate was filtered off and recrystallized from appropriate solvents to afford the nitro groups and stilbenes **3c**, **4c**, **3d**, **4d**, **4e**.

3,4,5-Trimethoxy-4'-bromo-*trans*-stilbene (1c): Yield 51.6 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.48 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 16.4 Hz, 1H), 6.93 (d, *J* = 16.4 Hz, 1H), 6.73 (s, 2H), 3.92 (s, 6H), 3.87 (s, 3H).

3,4,5-Trimethoxy-4'-chloro-*trans*-stilbene (2c): Yield 55.5 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.43 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 16.4 Hz, 1H), 6.94 (d, *J* = 16.4 Hz, 1H), 6.73 (s, 2H), 3.92 (s, 6H), 3.87 (s, 3H).

3,4,5-Trimethoxy-3'-nitro-*trans*-stilbene (3c): Yield 58.7 %. Yellow solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 8.36 (s, 1H), 8.09 (d, *J* = 11.2 Hz, 1H), 7.79 (d, *J* = 8 Hz, 1H), 7.52 (t, *J* = 8 Hz, 1H), 7.17 (d, *J* = 16.4 Hz, 1H), 7.04 (d, *J* = 16.4 Hz, 1H), 6.77 (s, 2H), 3.93 (s, 6H), 3.89 (s, 3H).

3,4,5-Trimethoxy-4'-nitro-*trans*-stilbene (4c): Yield 55.5 %. Orange solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 8.22 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 16.4 Hz, 1H), 7.04 (d, *J* = 16.4 Hz, 1H), 6.78 (s, 2H), 3.93 (s, 6H), 3.89 (s, 3H).

3,4,5-Trimethoxy-*trans*-stilbene (5c): Yield 38.4 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.51 (d, *J* = 7.2 Hz, 2H), 7.36 (t, *J* = 7.6 Hz, 2H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.05 (d, *J* = 16.4 Hz, 1H), 7 (d, *J* = 16.4 Hz, 1H), 6.74 (s, 2H), 3.92 (s, 6H), 3.87 (s, 3H).

3,4,5,4'-Tetramethoxy-*trans*-stilbene (6c): Yield 34.2 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.44 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 16.4 Hz, 1H), 6.89 (d, *J* = 16.4 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.71 (s, 2H), 3.91 (s, 6H) 3.86 (s, 3H), 3.82 (s, 3H).

3,4,5,2'-Tetramethoxy-*trans*-stilbene (7c): Yield 45.2 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.57 (d, *J* = 8.8 Hz, 1H), 7.36 (t, *J* = 16.4 Hz, 1H), 7.24 (q, *J* = 8.8 Hz, 1H), 7.04 (d, *J* = 16.4 Hz, 1H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.76 (s, 2H), 3.92 (s, 6H), 3.90 (s, 3H), 3.87 (s, 3H).

3,4,5,2',3'-Pentamethoxy-*trans*-stilbene (8c): Yield 49.4 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.34 (d, *J* = 16.4 Hz, 1H), 7.22 (d, *J* = 9.2 Hz, 1H), 7.06 (t, *J* = 8 Hz, 1H), 7.05 (d, *J* = 16.4 Hz, 1H), 6.84 (d, *J* = 9.2 Hz, 1H), 6.77 (s, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H).

3,5-Dimethoxy-4'-bromo-*trans*-stilbene (1d): Yield 29.5 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.47 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.01(s, 2H), 6.65 (d, *J* = 2.4 Hz, 2H), 6.41 (t, *J* = 2.2 Hz, 1H), 3.83 (s, 6H).

3,5-Dimethoxy-4'-chloro-*trans*-stilbene (2d): Yield 32.5 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.42 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 1.6 Hz, 2H), 6.65 (d, *J* = 2.4 Hz, 2H), 6.41 (t, *J* = 2.4 Hz, 1H), 3.83 (s, 6H).

3, 5-Dimethoxy-3'-nitro-*trans*-stilbene (3d): Yield 40.3 %. Yellow solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 8.36 (s, 1H), 8.09 (d, *J* = 9.6 Hz, 1H), 7.79 (d, *J* = 8 Hz, 1H), 7.52 (t, *J* = 8 Hz, 1H), 7.16 (d, *J* = 16.4 Hz, 1H), 7.09 (d, *J* = 16.4 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, 2H), 6.45 (t, *J* = 2.4 Hz, 1H), 3.84 (s, 6H).

3,5-Dimethoxy-4'-nitro-*trans*-stilbene (4d): Yield 38.5 %. Orange solid: 1 H NMR (400 MHz $CDCl_3$): δ ppm 8.22 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 2H), 7.20 (d, *J* = 16.4 Hz, 1H), 7.11 (d, *J* = 16.4 Hz, 1H), 6.96 (t, *J* = 7.2 Hz, 1H), 6.70 (s, 2H), 6.46 (t, *J* = 2 Hz, 1H), 3.85 (s, 6H).

3,5-Dimethoxy-*trans*-stilbene (5d): Yield 22.5 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.55 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 17.2 Hz, 1H), 7.14 (d, *J* = 16.4 Hz, 1H), 7.08 (d, *J* = 16.4 Hz, 1H), 6.73 (d, *J* = 2 Hz, 2H), 6.45 (t, *J* = 2.4 Hz, 1H), 3.87 (s, 6H).

3,5,4'-Trimethoxy-*trans*-stilbene (6d): Yield 18.5 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.44 (d, *J* = 8.4 Hz, 2H), 7.04 (d, *J* = 16 Hz, 1H), 6.91 (t, *J* = 6.4 Hz, 3H), 6.65 (s, 2H), 6.37 (s, 1H), 3.83 (s, 9H).

3,5,2'-Trimethoxy-*trans*-stilbene (7d): Yield 23.2 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.57 (d, *J* = 7.6 Hz, 2H), 7.45 (d, *J* = 16.4 Hz, 1H), 7.22-7.26 (m, 1H), 7.04 (d, *J* = 16.4 Hz, 1H), 6.96 (t, *J* = 7.2 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.69 (d, *J* = 2 Hz, 2H), 6.38 (t, *J* = 2.4 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 6H).

3,5,2',3'-Tetramethoxy-*trans*-stilbene (8d): Yield 24.8 %. White solid: 1 H NMR (400 MHz $CDCl_3$): δ ppm 7.42 (d, *J* = 16.4 Hz, 1H), 7.23 (d, *J* = 9.2 Hz, 1H), 7.06 (t, *J* = 9.2 Hz, 1H), 7.05 (d, *J* = 16.4 Hz, 1H), 6.84 (d, *J* = 9.6 Hz, 1H), 6.70 (d, *J* = 2 Hz, 2H), 6.4 (t, *J* = 2.4 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.84 (s, 6H).

4-Methoxy-4'-bromo-*trans*-stilbene (1e): Yield 24.9 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.45 (d, *J* = 6.4 Hz, 2H), 7.43 (d, *J* = 6 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.04 (d, *J* = 16.4 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 16.4 Hz, 1H), 3.83 (s, 3H).

4-Methoxy-4'-chloro-*trans*-stilbene (2e): Yield 31.8 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.43 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 16.4 Hz, 1H), 6.90 (d, *J* = 16.4 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 3.83 (s, 3H).

4-Methoxy-3'-nitro-*trans*-stilbene (3e): Yield 24.3 %. Yellow solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 8.32 (s, 1H), 8.07 (t, *J* = 9.6 Hz, 1H), 7.75 (d, *J* = 8 Hz, 1H), 7.49 (t, *J* = 8.8 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.18 (d, *J* = 16.4 Hz, 1H), 6.98 (d, *J* = 16.4 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H).

4-Methoxy-4'-nitro-*trans*-stilbene (4e): Yield 17.1 %. Orange solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 8.20 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.23 (d, *J* = 16.4 Hz, 1H), 7.01 (d, *J* = 16.4 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H).

4-Methoxy-*trans*-stilbene (5e): Yield 23.4 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.49 (d, *J* = 7.6 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 16 Hz, 1H), 6.97 (d, *J* = 16 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 3.83 (s, 3H).

4,4'-Dimethoxy-*trans*-stilbene (6e): Yield 40.2 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.40-7.44 (m, 4H), 6.93 (s, 2H), 6.87-6.90 (m, 4H), 3.82 (s, 6H).

2,4'-Dimethoxy-*trans*-stilbene (7e): Yield 20.2 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.55 (d, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 16.4 Hz, 1H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.05 (d, *J* = 16.4 Hz, 1H), 6.94 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 3H), 3.85 (s, 3H), 3.79 (s, 3H).

2,3,4'-Trimethoxy-*trans*-stilbene (8e): Yield 25.3 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.49 (d, *J* = 8 Hz, 1H), 7.32 (t, *J* = 16.4 Hz, 1H), 7.22 (d, *J* = 8 Hz, 1H), 7.08 (d, *J* = 16.4 Hz, 1H), 7.04 (t, *J* = 8 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 3.89 (s, 6H), 3.84 (s, 3H), 3.83 (s, 3H).

3,4,2'-Trimethoxy-*trans*-stilbene (7f): Yield 23.9 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.58 (d, *J* = 9.2 Hz, 1H), 7.33 (d, *J* = 16.4 Hz, 1H), 7.21 (t, *J* = 4.8 Hz, 1H), 7.10 (d, *J* = 1.6 Hz, 1H), 7.06 (d, *J* = 16.4 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.96 (t, *J* = 7.6 Hz, 1H), 6.90 (d, *J* = 8 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 3.95 (s, 3H), 3.90 (d, *J* = 1.2 Hz, 6H).

3,4,2',3'-Tetramethoxy-*trans*-stilbene (8f): Yield 19.6 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.31 (d, *J* = 16.4 Hz, 1H), 7.22 (d, *J* = 9.6 Hz, 1H), 7.09 (d, *J* = 16.4 Hz, 1H), 7.09 (s, 1H), 7.08 (t, *J* = 6.4 Hz, 1H), 7.04 (d, *J* = 8 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 6.83 (d, *J* = 9.6 Hz, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H).

General procedure for the preparation of compounds (2b): The mixture of hydroxybenzoic acid (**1b**) (1 eq.), acetic anhydride (5 eq.) and pyridine (6 mL) was stirred at room temperature for 12 h. Then the cold H_2SO_4 (10 %) was slowly added into the reaction mixture, the precipitate was filtered off to give **2b** which was dried for the next step.

General procedure for the preparation of compounds (4b)[28]: To a stirred solution of 4-acetoxybenzoic acid (**2b**) (1 eq.) was added thionyl chloride (10 mL 5 eq.). Then the mixture was heated to reflux for 3 h. The reaction mixture was removed the excessive thionyl chloride under reduced pressure. The crude product (**4b**) was used directly for the next step without further purification.

General procedure for the preparation of compounds (1f, 2f, 4f-6f): In a microwave reactor vial (20 mL), to solution of styrene (9-12) (1.5 eq.) and reagent (3b-5b) (1 eq.) were added tetrabutylammonium bromide (2 eq.), anhydrous potassium carbonate (2.5 eq.) and $Pd(OAc)_2$ (0.05 eq.) in DMF (15 mL) under argon. The vial was placed into the microwave cavity. The reaction mixture was irradiated at high level for 4 h at 100 °C then cooled to room temperature. The solution was poured into 100 mL H_2O and then extracted with ethyl acetate. The organic layers were combined and washed with water and brine, then dried with sodium sulphate anhydrous. The removal of the solvent under reduced pressure provided the crude which was purified by flash column chromatography

(ether/ethyl acetate 40:1) and recrystallization from appropriate solvents to afford compounds (**1f**, **2f**, **4f**-**6f**).

3,5-Dimethoxy-4'-amino-*trans*-stilbene (1f): Yield 49.6 %. Yellow solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.33 (d, *J* = 8.4 Hz, 2H), 7 (d, *J* = 16.4 Hz, 1H), 6.84 (d, *J* = 16.4 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.63 (d, *J* = 2.4 Hz, 2H), 6.36 (t, *J* = 2.4 Hz, 1H), 3.82 (s, 6H), 3.75 (br, 2H).

4-Amino-*trans*-stilbene (2f): Yield 40.3 %. Yellow solid: ^{1}H NMR (400 MHz DMSO): δ ppm 7.50 (d, *J* = 8 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.18 (t, *J* = 7.2 Hz, 1H), 7.06 (d, *J* = 16.4 Hz, 1H), 6.89 (d, *J* = 16.4 Hz, 1H), 6.57 (d, *J* = 8.8 Hz, 2H), 5.32 (br, 2H).

General procedure for the preparation of compounds 3f: Sodium methoxide (0.1 eq.) was added to a solution of compound (**4f**) (1 eq.) in THF (20 mL). The reaction mixture was stirred for 10 h, followed by concentrated 2 M HCl to acidification and evaporation *in vacuo*. The residue was purified by silica gel column chromatography, eluting with a solution of 1 % methanol in dichloromethane. Then the compound **3f** was obtained in 75.8 % yield. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.51 (d, *J* = 7.2 Hz, 2H), 7.44 (d, *J* = 8 Hz, 2H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.26 (t, *J* = 7.2 Hz, 1H), 7.08 (d, *J* = 16.4 Hz, 1H), 6.99 (d, *J* = 16.4 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.77 (br, 1H).

4-Acetoxy-*trans*-stilbene (4f): Yield 48.8 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.54 (d, *J* = 8.8 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 7.12 (d, *J* = 16.4 Hz, 1H), 7.08 (d, *J* =16.4 Hz, 1H), 2.34 (s, 3H).

3,4-Methylenedioxy-4'-acetoxy-*trans*-stilbene (5f): Yield 48.2 %. White solid: ^{1}H NMR (400MHz $CDCl_3$): δ ppm 7.40 (d, *J* = 8 Hz, 2H), 7.18 (d, *J* = 8 Hz, 2H), 7.08 (s, 1H), 7.00 (d, *J* = 16 Hz, 1H), 6.95 (d, *J* = 8 Hz, 1H), 6.93 (d, *J* = 16 Hz, 1H), 6.81 (d, *J* = 8 Hz, 1H), 6 (s, 2H), 2.38 (s, 3H).

3,4-Methylenedioxy-4'-methyl-*trans*-stilbene (6f): Yield 44.2 %. White solid: ^{1}H NMR (400 MHz $CDCl_3$): δ ppm 7.40 (d, *J* = 8 Hz, 2H), 7.18 (d, *J* = 8 Hz, 2H), 7.08 (s, 1H), 7.00 (d, *J* = 16.4 Hz, 1H), 6.95 (d, *J* = 8 Hz, 1H), 6.93 (d, *J* = 16.4 Hz, 1H), 6.81 (d, *J* = 8 Hz, 1H), 5.99 (s, 2H), 2.38 (s, 3H).

Strains and media: Yeast strains used throughout this study were Yeast Parental strain-BY4743 (Yeast Gene Sir2) ordered from Thermoscientific, USA. Rich YPDA medium was used for liquid culture of yeast strains, which were incubated at 30 °C and handled by standardized methods of yeast genetics[15,16]. Permanent stocks of yeast strains are maintained in 50 % (v/v) glycerol at -80 °C. For routine use, an aliquot of the frozen stock was streaked onto a YPDA agar plate and incubated for 2 days to form single colonies. The genotype of one independent colonies was tested by replica plating to selective drop-out media and a single verified clonal isolate to be used as a working stock was maintained on a YPDA agar plate at 4 °C for no longer than 2 week. A loop full of cells was taken from the working stock (agar plate), resuspended in a small volume of medium and titered by hemacytometer count for inoculation of a shake flask culture. Shake flask cultures were incubated with orbital shaking at 300 rpm in an Innova Model refrigerated, shaking incubator from New Brunswick Scienti Wc Co. (New Brunswick, NJ) in a volume that was 1/10 th of the total volume of the Erlenmeyer growth flask. These conditions provide maximal aeration for cell growth. Inoculum titers were chosen to allow overnight incubation (16-18 h) and harvesting of cells in mid-logarithmic phase; balanced growth was attained at a titer of 1 × 107 cells/mL. Only the starting flask culture that had been in balanced exponential growth for at least three generations and that had a budding frequency of 65-75 % were used to inoculate microculture experiments.

Bioscreen C MBR Outgrowth: A Bioscreen C MBR (Growth Curves USA, Piscataway, NJ) machine was used for all outgrowth assays. For outgrowth of aged cells, 5 µL of the starting flask culture was inoculated into 295 µL of rich YPD (2 % glucose, 2 % bactopeptone, 1 % yeast extract) medium in a Bioscreen honeycomb 100-well plate (cat no. 9502550). Incubation of the plate is kept constant at 30 °C, with the shaking module set to high continuous shaking. Absorbance readings at 600 nm (wideband range) are taken every 0.5 h for 24 h. OD data were normalized for background prior to presentation by subtracting the initial OD value at t = 0 from each subsequent OD reading.

Yeast-based drug screening assay: The synthesized compounds **1c-8f** were screened for SIRT1 activators against Yeast Parental strain-BY4743 at a concentration of 100 µM/L by Bioscreen C MBR machine. For comparison, resveratrol was used as the standard drugs. Compounds were added to corresponding number of well to a final concentration of 100 µM/L. DMSO served as control and there was no visible change in yeast growth due to this. The doubling time for each well in a Bioscreen assay, δ_w was determined by the maximal slope of the semilog plot of OD as a function of time. This value was defined as the median of the three lowest δ values obtained for every consecutive pair of OD measurements for that well in that experiment (Fig. 1). The cell doubling times (in hours) are measured at the maximal growth rate (μ_m), $\mu = Ln (OD_n/OD_{n-1})/t_n-t_{n-1}$, μ_m is maximum, doubling time (δ_w) = $Ln (2)/\mu_m$ (Fig. 2). The OD_{24h} value show the total biomass in stable phase (Fig. 3).

RESULTS AND DISCUSSION

In this work, we use the Wittig-Horner and Heck reaction to synthesis of resveratrol derivatives. A series of novel resveratrol analogues were synthesized. Compounds **1c-8e**,**7f**, **8f**. Starting from hydroxybenzoic acid and methoxybenzoic acid, the aromatic esters were prepared in moderate yield, followed by reduction with $LiAlH_4$. Subsequent treatment of aromatic alcohol with tribromophosphine afforded benzyl bromine. Using Wittig-Horner reaction, methoxylated diethyl benzylphosphonates were obtained by refluxing benzyl bromine with $P(OEt)_3$, which were condensed with ArCHO in CH_3ONa/DMF or NaH/THF (**Scheme-I**). Compounds **1f-6f** were prepared by aromatic alkenes was reacted with the benzonic acid chloride or halogenated aromatic under palladium acetate catalysis with Heck reaction (**Scheme-II**). Wittig-Horner and Heck reaction reaction is considered as the key route to prepare a series of resveratrol analogues, only the trans isomer was obtained, the coupling constants of the vinylic protons of the trans-stilbenes were about 16.4 Hz. Structures of these compounds were listed in Table-1.

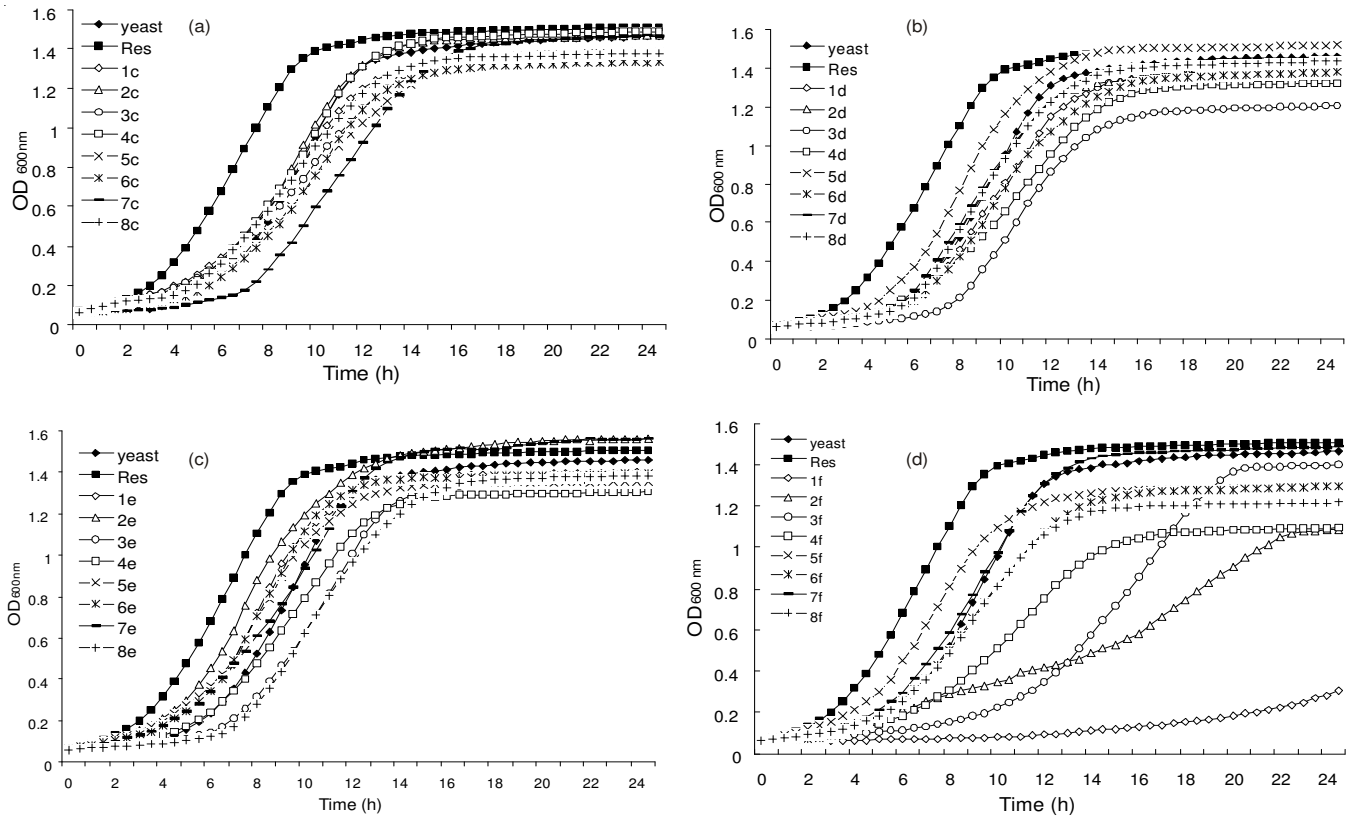


Fig. 1. Exponentially growing cells were resuspended in yeast parental strain-BY4743 media containing all compounds concentration (100 μM/L) and incubated for 24 h at 30 °C. Analysis of cell concentration by OD_{600}, in the honeycomb 100-well plate, cells were deposited in triplicate and cell growth was measured every 0.5 h for 24 h; (a) Growth curve of compound **1c -8c**; (b) Growth curve of compound **1d -8d**; (c) Growth curve of compound **1e-8e**; (d) Growth curve of compound **1f-8f**

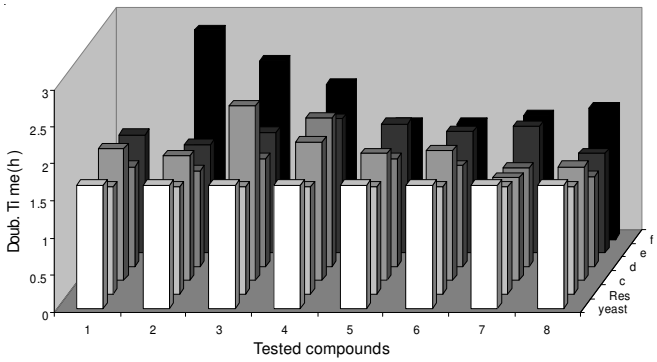


Fig. 2. Cell doubling times of tested compounds compared to the resveratrol and yeast measured at the maximal growth rate

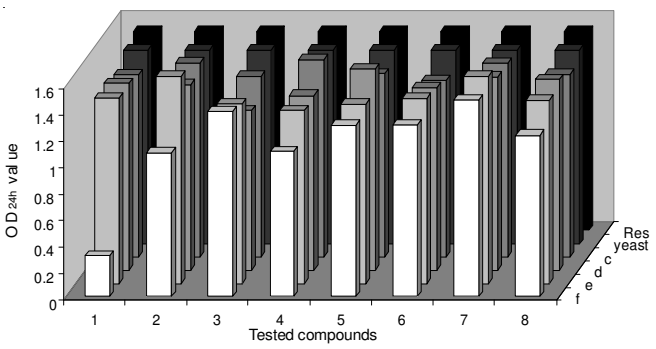
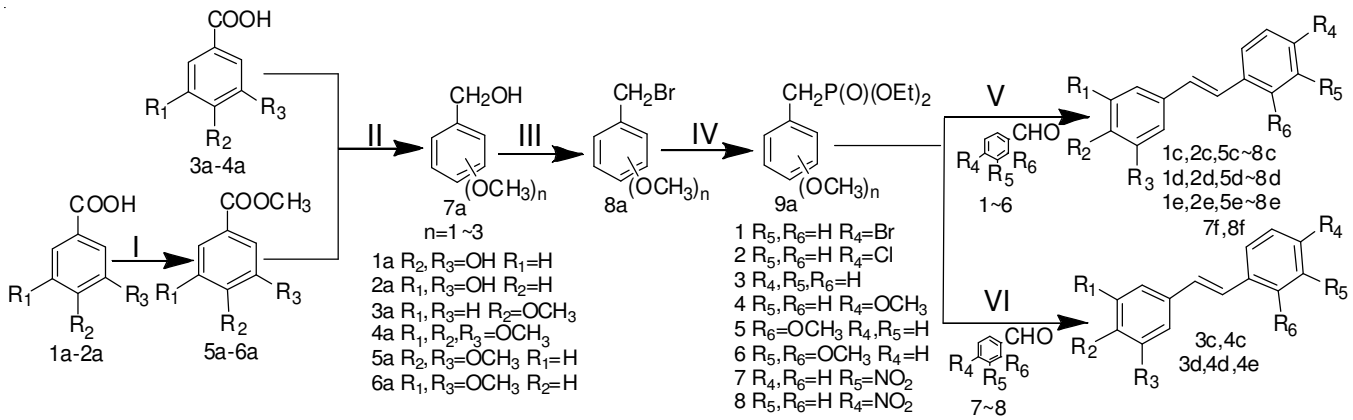
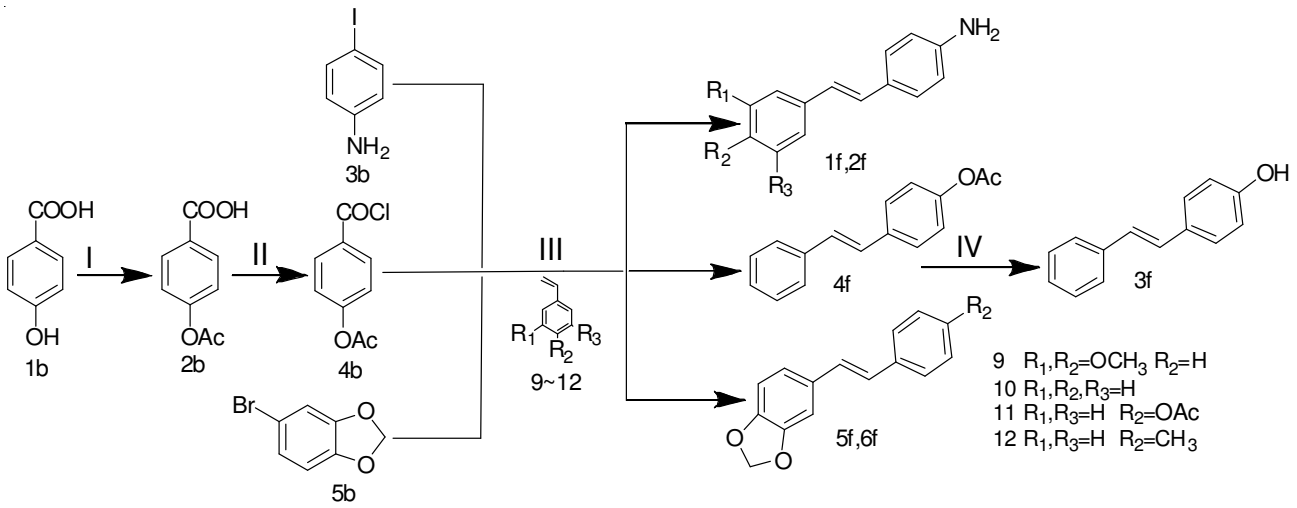


Fig. 3. OD value of 24h of tested compounds compared to the resveratrol and yeast measured at the total biomass

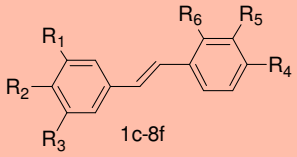


Scheme-I: Synthesis of stilbenes **7f**, **8f** and **1c-8e**. Reagents and conditions: (i) $(CH_3O)_2CO$, Bu_4NBr, DMF, K_2CO_3, 140 °C; (ii) $LiAlH_4$, THF, 0 °C; (iii) PBr_3, CH_2Cl_2, rt; (iv) $P(O)(OEt)_3$, 135 °C; (v) NaH, ArCHO, THF, 0 °C; (vi) CH_3ONa, ArCHO, DMF, 100 °C



Scheme-II: Synthetic pathway for compounds **1f-6f**. Reagents and conditions: (i) Ac_2O, pyridine, rt, 1.5 h; (ii) $SOCl_2$, 80 °C, 3h; (iii) Bu_4NBr, K_2CO_3, $Pd(OAc)_2$, DMF, 80 °C, 4h; (iv) NaOMe, THF, rt, 10h

TABLE-1
CHEMICAL STRUCTURES OF COMPONUD **1c-8f**



1c-8f

Comp.	R_1	R_2	R_3	R_4	R_5	R_6
1c	OCH_3	OCH_3	OCH_3	Br	H	H
2c	OCH_3	OCH_3	OCH_3	Cl	H	H
3c	OCH_3	OCH_3	OCH_3	H	NO_2	H
4c	OCH_3	OCH_3	OCH_3	NO_2	H	H
5c	OCH_3	OCH_3	OCH_3	H	H	H
6c	OCH_3	OCH_3	OCH_3	OCH_3	H	H
7c	OCH_3	OCH_3	OCH_3	H	H	OCH
8c	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3
1d	OCH_3	H	OCH_3	Br	H	H
2d	OCH_3	H	OCH_3	Cl	H	H
3d	OCH_3	H	OCH_3	H	NO_2	H
4d	OCH_3	H	OCH_3	NO_2	H	H
5d	OCH_3	H	OCH_3	H	H	H
6d	OCH_3	H	OCH_3	OCH_3	H	H
7d	OCH_3	H	OCH_3	H	H	OCH_3
8d	OCH_3	H	OCH_3	H	OCH_3	OCH_3
1e	H	OCH_3	H	Br	H	H
2e	H	OCH_3	H	Cl	H	H
3e	H	OCH_3	H	H	NO_2	H
4e	H	OCH_3	H	NO_2	H	H
5e	H	OCH_3	H	H	H	H
6e	H	OCH_3	H	OCH_3	H	H
7e	H	OCH_3	H	H	H	OCH_3
8e	H	OCH_3	H	H	OCH_3	OCH_3
1f	OCH_3	H	OCH_3	NH_2	H	H
2f	H	H	H	NH_2	H	H
3f	H	H	H	OH	H	H
4f	H	H	H	AcO	H	H
5f	H	AcO	H	—OCH_2—		H
6f	H	CH_3	H	—OCH_2—		H
7f	OCH_3	OCH_3	H	H	H	OCH_3
8f	OCH_3	OCH_3	H	H	OCH_3	OCH_3

Biological activities: Yeast as model of drug screening for SIRT1actovators is convenient based on homology of yeast Sir2 and human SIRT1. Here we reported a novel yeast-based method for measuring yeast growth curves by monitoring outgrowth of yeast cells using a Bioscreen C MBR machine. This method provides growth curves comparable to traditional one. If compounds can activate Sir2, leading to increase replicative life span which could be measured through the visible light absorption of cultural yeast strain, the results could be recorded and showed by its growth curves.

We found that the lag time of most of the yeast is about 4 h from the growth curve. The lag phase of resveratrol was significantly faster than blank control. Compound **5d**, **1e, 2e**, **4e**, **6e**, **7e**, **7f** and **5f** exerted a stronger promoting effect than blank control respectively, indicating that these compounds can shorten the lag time of the yeast, but much slower than resveratrol; Compound **1c**, **2c**, **3c**, **4c**, **5c**, **6c**, **8c**, **1d**, **2d**, **4d**, **6d**, **7d**, **8d**, **4e**, **6f** and **8f** showed no activity than blank control, respectively. Compound **7c**, **3d**, **3e**, **8e**, **2f**, **4f**, **3f** and **1f** obviously inhibited the growth of yeast, significantly prolonging the lag time of yeast, respectively.

Most of the tested compounds began to enter the logarithmic phase after 4 h. Each compound influenced the doubling time of yeast shown in Fig. 2. The doubling time of resveratrol obviously was less than blank control. Compound **7c**, **1d**, **2d**, **6d**, **7d**, **8d** and **8e** was less than blank but faster than resveratrol, respectively, indicating that these compounds exerted a stronger promoting effect than resveratrol; Compounds **8c**, **3d**, **5d**, **1e**, **2e**, **3e**, **6e**, **5f** and **6f** exerted moderate effect but less activity compared to resveratrol, respectively. Compound **1c**, **2c**, **3c**, **4c**, **5c**, **6c**, **4d**, **4e**, **5e**, **7e**, **7f**, **8f**, **2f**, **4f**, **3f** and **1f** restrained the growth of logarithmic phase of yeast, respectively.

Most of compounds came into stationary phase after 12 h. OD value of between 12 and 24 h show stationary phase (Fig. 3). It was shown that the $OD_{24\,h}$ value of resveratrol was higher than blank. The microbial content within compound **2c**, **4c**, **5c**, **2e**, **7e** and **7f** was more than blank while the microbial content of **5c**, **2e**, **7e** and **7f** was a little more than resveratrol, respectively. Compound **3d**, **4e**, **8f**, **1f**, **2f**, **4f**, **5f** and **6f** showed a stronger suppressive effect than blank control, respectively as the microbial content of yeast was obviously less than blank. The rest of the compound exerted moderate activities compared to blank since the microbial count was about equal with the blank control.

Conclusion

Compound **5d** and **2e** indeed significantly improved growth among tested compounds because they improved the lag phase, logarithmic phase and stationary phase of yeast, respectively. The compound **1f**, **2f** and **4f** showed strong inhibition of the three period of yeast, respectively. Other compounds affected the certain period of yeast more or less, but was close to blank overall. Regarding structure-activity relationships (SAR): methoxy group of 4 position at a ring except compound **3e**, **4e**, **8e** shorten the lag time and 3,5 position of A ring except compound **4d** shorten the doubling time most over the blank control. In the B ring: amino, acetyl and hydroxyl group strongly inhibited the yeast growth while nitro group played weaker inhibition activity. The chloride and methoxy at 1',2' positions promoted the yeast growth. In view of a and b ring, the a ring may affect a certain period of yeast but b ring may influence the whole period of yeast.

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