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SYNTHESIS OF NOVEL RESVERATROL-PHTHALIDE HYBRID COMPOUNDS AND EVALUATION OF THEIR INHIBITORY ACTIVITIES OF NITRIC OXIDE PRODUCTION

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Abstract – Four types of novel resveratrol-phthalide hybrid compounds were designed and synthesized systematically by Suzuki-Miyaura cross-coupling reaction. These hybrid compounds were evaluated upon an inhibitory effect of the LPS-stimulated NO production in murine macrophage cell line, RAW264.7. As a result, two of them showed stronger inhibitory activity than the original resveratrol.

The authors dedicate this article to Prof. Tohru Fukuyama in honor of his 70th birthday.

INTRODUCTION

Resveratrol and oxyresveratrol, found in abundance in grapes or peanuts, are well-known polyphenols which have three or four hydroxy groups in the molecules (see Figure 1).[1] For example, resveratrol and oxyresveratrol provide a variety of biological activities such as anti-inflammatory, anti-tumor, anti-angiogenic, melanin-producing restraint, metabolic syndrome restoration, prevention of Alzheimer's disease and the arteriosclerosis prevention effects. Even though there are no reports as for hazardous

effects of resveratrol and oxyresveratrol to date, inactivation of resveratrol and oxyresveratrol by the oxidation and/or related metabolism would be a problem when judged from their structures. Actually, it has been pointed out that phenolic hydroxy groups are biologically exposed to the oxidation reaction.[2] An answer for the specific enhancement of biological activities is the derivatization by the chemical modification of resveratrol and oxyresveratrol. For example, the introduction of a coumarin frame into one of the aromatic rings in resveratrol brought increased anti-tumor activity compared with that of the original resveratrol and coumarin.[3] Furthermore, geometrically fixed resveratrol compounds, forming immobilized bridge by heteroatom, showed increased anti-tumor and vasorelaxing activities (Figure 2).[2] These chemical hybridization methodologies have received much attention since exceptionally improved biologically active compounds can be obtained with respect to the corresponding original compounds. Encouraged by this theory, we designed and synthesized novel resveratrol analogs hybridizing phthalide in the molecule, aiming to obtain enhanced anti-inflammatory compounds better than that of original resveratrol. Phthalides are often found in the edible Hanabiratake mushroom (*Sparassis crispa*) in Japan as bioactive constituents, such as superoxide dismutase (SOD)-like activity and suppression of LPS-induced NO production.[4] Now we report herein the design, synthesis of novel resveratrol-phthalide hybrid compounds and evaluation on their inhibitory activity of the LPS-stimulated NO production in murine macrophage cell line, RAW264.7.

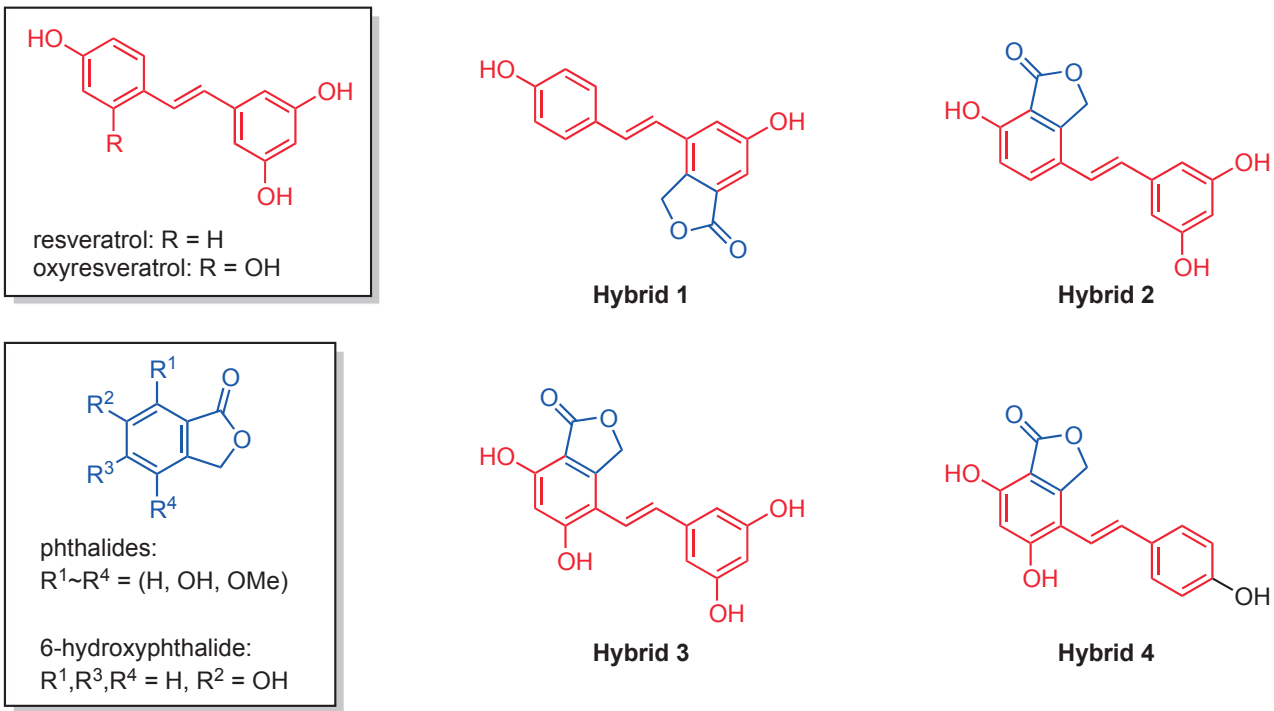
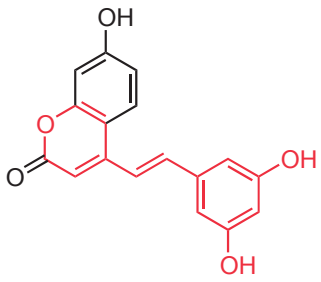
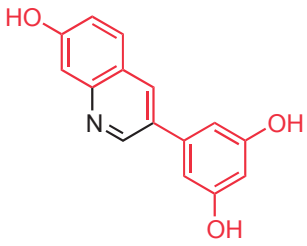
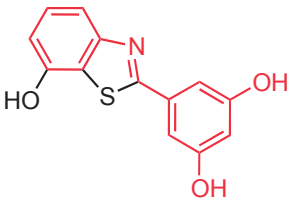


Figure 1. Design of resveratrol-phthalide hybrid compounds



trans-stilbene-coumarin hybrid compoud[3]





geometrically *trans*-fixed resveratrol model compounds[2]

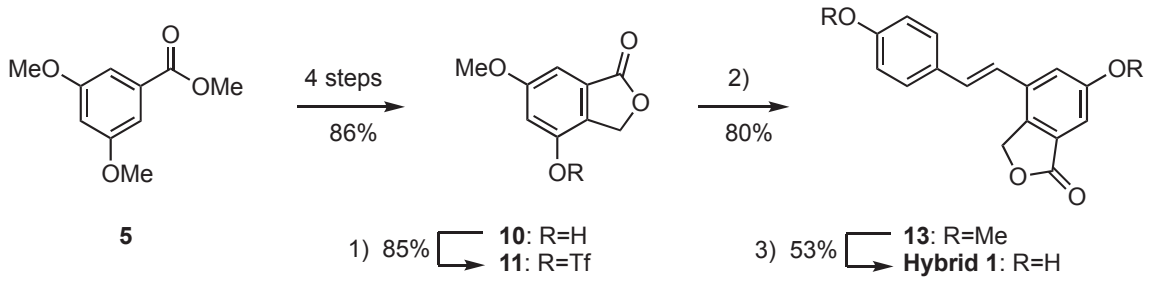
Figure 2. Research background: known stilbene hybrid compounds having improved biological activities

RESULTS AND DISCUSSION

We designed and synthesized the hybrid compounds as close to the chemical structure of resveratrol as possible because the substituted position of the OH group in the molecules seemed crucial for the suppression of NO production (See Figure 1). Indeed, unpublished results on inhibition of melanin production using resveratrol derivatives suggest that the inhibitory effect changed dramatically depending on the position of the OH group.[5] Thus, one of the aromatic rings in resveratrol was replaced by phthalide moiety. We evaluate synthetic hybrid compounds (**Hybrid 1-4**) to assure the importance of the position of phenolic hydroxy groups for their activities to inhibit NO production.

1. Synthesis of hybrid compounds

The hybrid compounds were synthesized by the following strategies. Namely, the phthalide fragment was first constructed, and we subsequently introduced the alkenyl moiety by Suzuki-Miyaura cross-coupling reaction with arylvinylboronic acids to provide the desired resveratrol-phthalide hybrid molecules. This cross-coupling methodology has two advantages: i) in the convenient construction of stilbene frame of **Hybrid 1-4**, ii) reliability in the synthesis of future-designed hybrid analogues when conducting further study on the structure-activity relationships between the steric and/or electronic effects of substituent group on the aromatic rings and the assessment for the inhibition of NO production.

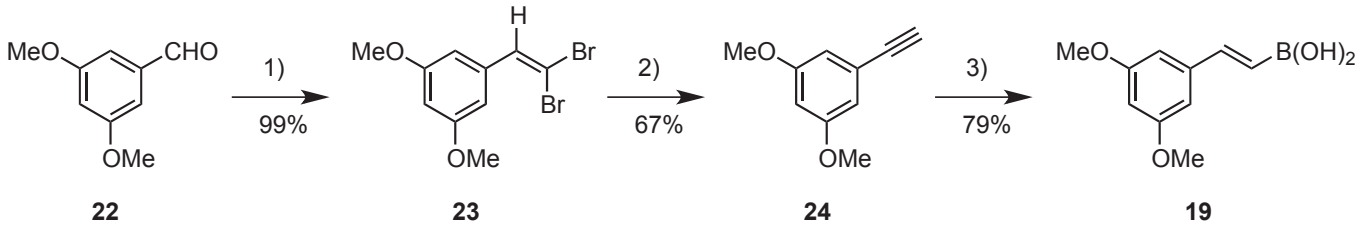


Reagents and conditions: 1) Tf_2O, Py, CH_2Cl_2, -10 to 0 °C. 2) 4-MeOPhCH=CHB(OH)$_2$ (**12**), $PdCl_2(PPh_3)_2$, Cs_2CO_3, DMF, 90 °C, 18 h. 3) BBr_3, CH_2Cl_2, -80 °C to rt, 18 h.

Scheme 1. Synthesis of **Hybrid 1**

4-Hydroxy-6-methoxyphthalide (**10**), prepared from a known procedure[6-8] (four steps synthesized from **5** in 86% overall yield) was converted into the corresponding aryl trifluoromethanesulfonate (**11**) that can be utilized as a starting material for the cross-coupling reactions. Two arylvinylboronic acids were selected for use in this study. Namely, *trans*-2-(4-methoxyphenyl)vinylboronic acid (**12**) was obtained from a commercially available source, and compound **19**[9] was synthesized (*vide infra*). Suzuki-Miyaura cross-coupling of aryl triflate **11** and boronic acid **12** afforded **13** a good chemical yield (80%). The following demethylation by BBr_3 afforded desired **Hybrid 1** a moderate yield (Scheme 1).

Next, we undertook the syntheses of **Hybrid 2** and **Hybrid 3**. Prior to study for the syntheses of those compounds, arylvinylboronic acid **19** that can be used as a common building block for those compounds was prepared. Although boronic acid **19** is commercially available,[10] it is supposedly inadequate to obtain from the commercial source because of its cost. Therefore, the commercially available aldehyde **22** was converted to the desired boronic acid **19** by the known Corey-Fuchs alkynylation method[11] and subsequent hydroboration[12] utilizing catecholborane.



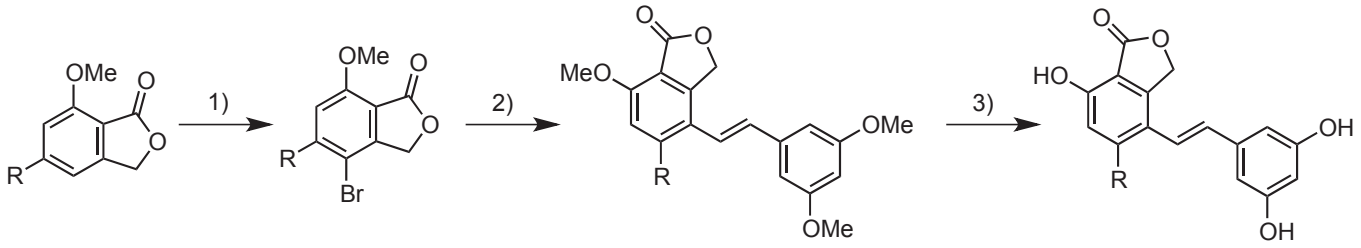
22 **23** **24** **19**

Reagents and conditions: 1) CBr_4, PPh_3, CH_2Cl_2, rt. 2) *n*-BuLi, THF, -80 °C to rt. 3) i) catecholborane, THF, reflux, 4 h. ii) H_2O, 0 °C, 16 h.

Scheme 2. Synthesis of 3,5-dimethoxyphenylvinylboronic acid **19**

Meanwhile, the known phthalides **17**[13] and **28**[14] were selectively introduced to the bromo-substituent group at C4 positions utilizing bromine under low temperature conditions. The structures of the brominated products **18**, **29** were confirmed by the analysis of $\{^1H\}$ NOE spectrum. As a result, the brominated position of both compounds were assigned by NOE between the aromatic C7-H and the methyl-H. The 4-bromo adducts **18**, **29** were used for cross-coupling reaction with boronic acid **19**, respectively. Various reaction conditions were examined to improve the yield, we found that 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos) was suitable as a ligand for the series of cross-coupling reaction.[15] The cross-coupling reaction proceeded smoothly to afford the corresponding products **20** and **30** in good yield, which were then convert to **Hybrid 2** and **Hybrid 3** by the same demethylation reaction (*vide supra*) (Scheme 3).

Finally, the synthesis of **Hybrid 4** was carried out. The cross-coupling of phthalide **29** and boronic acid **12**, that were already synthesized, was conducted in a same condition as described in Scheme 3. The coupling product was afforded in 69% yield, and the following demethylation proceeded easily to provide the desired **Hybrid 4** in high yield. Each spectral data of those hybrid compounds strongly supported their chemical structures (see experimental section).



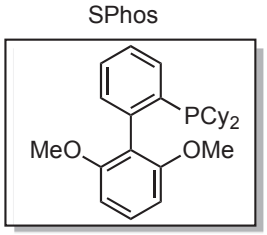
17: R = H
28: R = OMe

18: R = H (67%)
29: R = OMe (96%)

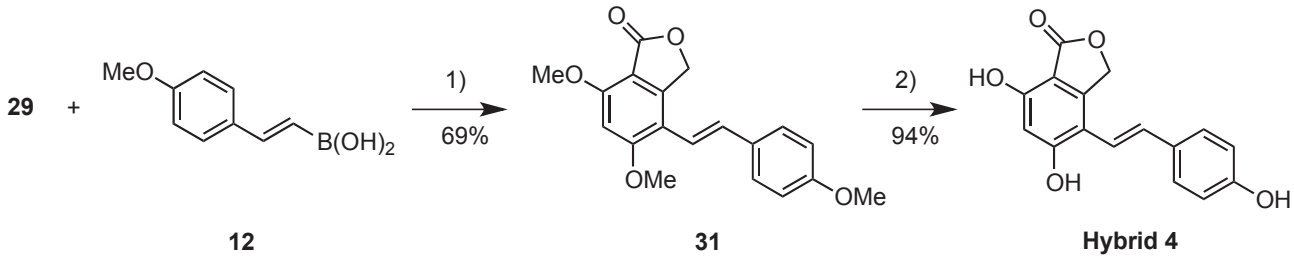
20: R = H (53%)
30: R =OMe (84%)

Hybrid 2: R = H (75%)
Hybrid 3: R = OH (41%)

Reagents and Conditions:1) Br_2, $CHCl_3$. 2) **19**, $Pd_2(dba)_3$, SPhos, K_3PO_4, toluene, 100 °C, 20 h. 3) BBr_3, CH_2Cl_2, -80 °C to rt, 18 h.



Scheme 3. Synthesis of **Hybrid 2** and **Hybrid 3**



12 **31** **Hybrid 4**

Reagents and conditions: 1) $Pd_2(dba)_3$, K_3PO_4, SPhos, toluene, 100 °C, 20 h. 2) BBr_3, CH_2Cl_2, -80 °C to rt, 18 h.

Scheme 4. Synthesis of **Hybrid 4**

2. Bioactive evaluation of **Hybrid 1-4**

RAW264.7 cells cultured in the presence of LPS and **Hybrid 1-4** were used for evaluation. NO amount was measured by the Griess reagent system.[16] Because the amount of this NO production is assumed to be an index of the evaluation on the anti-inflammatory effect of the hybrid compounds, the amount can be analyzed quantitatively. NO production by these cells was strongly suppressed by the treatment with **Hybrid 1** and **Hybrid 4** compared with those of resveratrol and oxyresveratrol in a dose dependent

manner. On the other hand, **Hybrid 2** and **Hybrid 3** did not show significant ability to suppress the NO production. 6-Hydroxyphthalide also had weak inhibitory activity on the NO production (Figure 3). **Hybrid 1-4** had no effect on viability of RAW264.7 cells, indicating that inhibition of NO production by the hybrid compounds is not due to cytotoxicity.

The *p*-hydroxystyrene structure seemed to play an important role, but we need further synthesis of various hybrid compounds and the accumulation of the experimental data to confirm the structure-activity relationship.

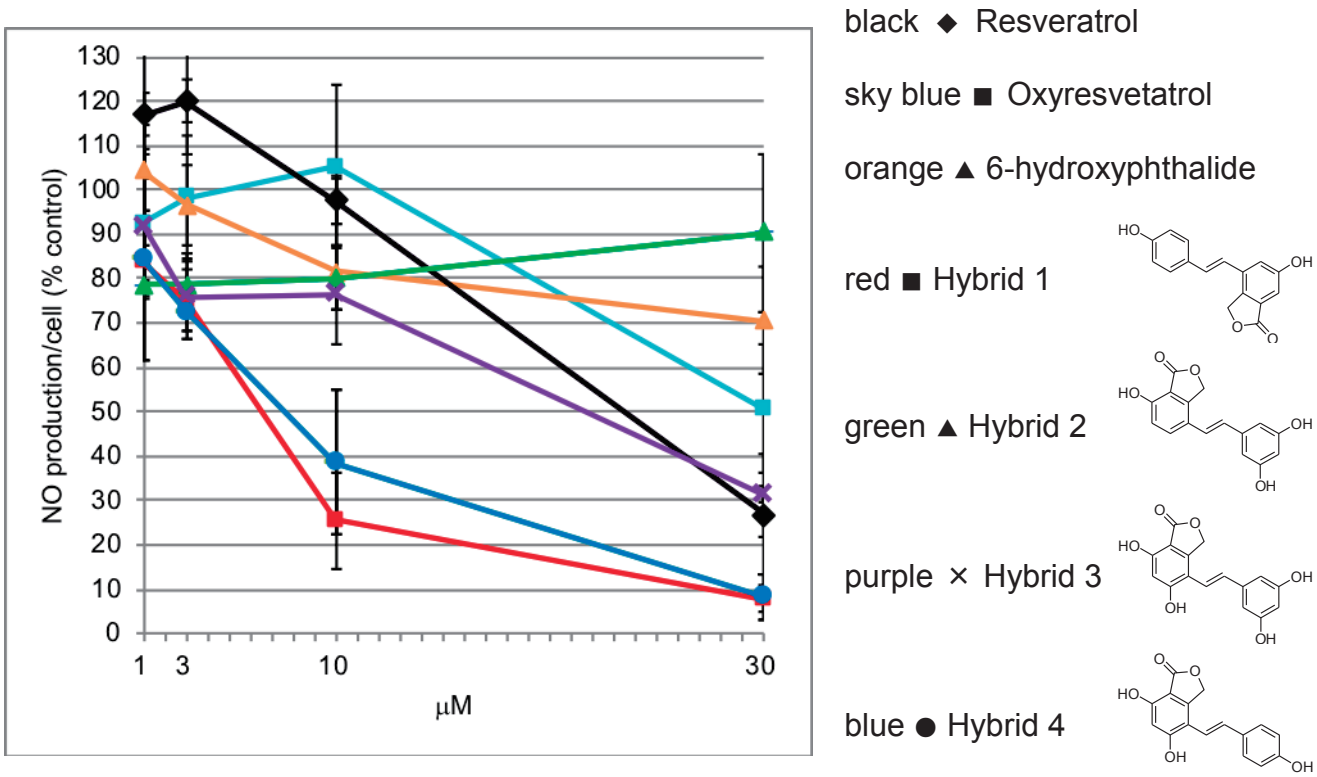


Figure 3. Suppression of LPS-induced NO production by RAW264.7 cells when resveratrol, oxyresveratrol, 6-hydroxyphthalide and **Hybrid 1-4** were added.

CONCLUSION

We have successfully synthesized four types of novel resveratrol-phthalide hybrid compounds (**Hybrid 1-4**), and found that two of them showed stronger inhibitory activity than the original resveratrol by the evaluation of using the LPS-stimulated NO production in murine macrophage cell line. Further study for the synthesis of other hybrid compounds and more detailed experiment on the inhibitory activity are in progress.

EXPERIMENTAL

Part 1: Synthesis of hybrid compounds

General: All materials for chemical synthesis not explicitly mentioned were purchased from Wako Pure Chemical Products Co. (Osaka, Japan), Tokyo Kasei Kogyo Co. (Tokyo, Japan), Nacalai Tesque Co. (Kyoto, Japan), and Aldrich Chemical Co. (USA). Materials for biological assay: Resveratrol, oxyresveratrol, RPMI1640, *E. coli* lipopolysaccharide (LPS), fetal bovine serum (FBS) (Invitrogen; Carlsbad, USA); Live/Dead cell staining kit II (Promokine, Heidelberg, Germany). Reagents and synthesized compounds were dissolved in dimethyl sulfoxide, with a final concentration in the medium at less than 0.1%. All other chemicals were of reagent grade. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a JEOL JNM-ECP400 spectrometer. Chemical shift values were expressed in ppm relative to an internal reference of tetramethylsilane (0 ppm) in ^{1}H NMR and $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm), or DMSO-d_6 (39.5 ppm) in ^{13}C NMR. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Coupling constant (*J*) values were expressed in Hz. MS were obtained on JEOL JMS-700 instruments. Melting points were measured on a Yanaco micro melting point apparatus without correction. Flash column chromatography was performed with silica gel (Wakosil C-200) obtained from Wako Pure Chemical Products Co. Analytical thin layer chromatography was performed on Merck Silica gel 60 F_{254} aluminum sheets and visualization was accomplished with a UV lamp.

2-Formyl-3,5-dimethoxybenzoic acid methyl ester: 6[17]

According to a literature procedure,[6] to a solution of 3,5-dimethoxybenzoic acid methyl ester (588 mg, 3 mmol) in 8 mL of anhydrous CH_2Cl_2 was added dropwise titanium tetrachloride (1.13 g, 6 mmol) at 0 °C (in an ice-water bath) under an argon atmosphere. Dichloromethyl methyl ether (449 mg, 3.9 mmol) was added slowly to the reaction mixture, and was warmed up to rt. After stirring for 45 min, the resulting dark red solution was carefully quenched by the addition of 1 M HCl under ice-water bath cooling, reaction mixture was extracted twice with $CHCl_3$. The combined organic layer was washed twice with water, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with EtOAc and hexane in 1/3 to 1/1 ratio to give 659 mg of the title compound **6** as a white solid. This material was used in the next step without further purification at this stage. For analysis,[17] a portion of the sample was picked and was recrystallized from MeOH gave colorless needles of mp 108.5–110.0 °C. ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H), 3.90 (s, 3H), 3.91 (s, 3H), 6.52 (d, 1H, J = 2.2), 6.57 (d, 1H, J = 2.2), 10.30 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 52.8, 55.8, 56.0, 99.6, 105.3, 116.8, 136.6, 163.2, 165.0, 169.4, 187.6. HR-MS (EI+) *m/z*: Calcd for $C_{11}H_{12}O_5$ 224.0685; Found 224.0683.

2-Formyl-3-hydroxy-5-methoxybenzoic acid methyl ester: 7[7]

According to a procedure found in the literature,[7] a solution of **6** (659 mg, 2.94 mmol) in anhydrous CH_2Cl_2 (9.4 mL) was cooled to -70 °C under an argon atmosphere. 1 M boron trichloride solution in

heptane (4.7 mL) was added dropwise to the solution, and the resulting pale yellow suspension was warmed up to rt and then stirred for 3.5 h. After complete consumption of the starting material which was checked by TLC, the reaction mixture was poured into ice-cooled 1 M HCl to quench the reaction. The whole mixture was extracted twice with $CHCl_3$. The combined organic extracts were washed thrice with water, once with brine successively, dried over Na_2SO_4, filtered and concentrated to give a crude colorless solid. The solid was purified by flash column chromatography eluting with EtOAc and hexane in 1/3 ratio to give 604 mg (96%) (2 steps from **5**) of **7** as a white solid of mp 87–88 °C. ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H), 3.94 (s, 3H), 6.55 (d, 1H, J = 2.6), 7.02 (d, 1H, J = 2.6), 10.43 (s, 1H), 12.71 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 52.8, 55.9, 103.9, 111.8, 112.8, 135.2, 165.4, 166.0, 166.4, 195.4. HR-MS (EI+) *m/z*: Calcd for $C_{10}H_{10}O_5$ 210.0528; Found 210.0530.

2-Formyl-5-methoxy-3-methoxymethoxybenzoic acid methyl ester: 8[8]

To a solution of **7** (420 mg, 2 mmol) in anhydrous DMF (10 mL) was slowly added diisopropylethylamine (516 mg, 4 mmol) under ice-water bath cooling, and was stirred at the same temperature for 10 min. Methoxymethyl chloride (480 mg, 6 mmol) was added to the resulting yellow solution, and was stirred at rt for 1 h. After complete consumption of the starting material, the reaction mixture was quenched by an addition of water at 0 °C, and the mixture was extracted thrice with EtOAc. The combined organic layer was washed with brine, and then dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with EtOAc and hexane in 1/2 to 1/1 ratio to give 506 mg (99%) of **8** as a white solid of mp 76.5–78.0 °C. ^{1}H NMR ($CDCl_3$) δ 3.51 (s, 3H), 3.86 (s, 3H), 3.92 (s, 3H), 5.27 (s, 2H), 6.65 (d, 1H, J = 2.2), 6.79 (d, 1H, J = 2.2), 10.33 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 52.8, 55.8, 56.5, 94.9, 102.5, 107.2, 117.5, 136.3, 160.9, 164.7, 169.2, 187.7. HR-MS (EI+) *m/z*: Calcd for $C_{12}H_{14}O_6$ 254.0790; Found 254.0789.

4-Hydroxy-6-methoxy-3*H*-isobenzofuran-1-one (4-hydroxy-6-methoxyphthalide): 10

According to a previously reported procedure,[8] to a solution of **8** (2.54 g, 10 mmol) in MeOH (20 mL) was added sodium borohydride (1.11 g, 30 mmol) at 0 °C. The reaction mixture was gradually warmed to rt, and stirred for 16 h. To a reaction mixture including alcohol **9** was added 6 M HCl (6 mL), and was heated at 80 °C for 1 h. The reaction was quenched immediately if the color of the reaction mixture turned orange or red. After heating the reaction mixture, a cream-colored slurry was formed, and this slurry was collected by suction filter under a vacuum, and the solid was thoroughly washed with water. The solid was dried at 50 °C under reduced pressure gave 1.64 g, 91% of **10** as a cream-colored powder of mp >230 °C (decomp). ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 3H), 5.20 (s, 2H), 6.65 (d, 1H, J = 2.2), 6.78 (d, 1H, J = 2.2), 10.37 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 55.6, 67.9, 98.5, 107.7, 126.6, 127.3, 152.8, 161.5, 170.6. HR-MS (EI+) *m/z*: Calcd for $C_9H_8O_4$ 180.0423; Found 180.0418.

6-Methoxy-1-oxo-1,3-dihydroisobenzofuran-4-yl trifluoromethanesulfonate: 11

To a solution of **10** (90 mg, 0.5 mmol) in dry CH_2Cl_2 (5 mL) was added pyridine (198 mg 2.5 mmol) at -10 °C. Triflic anhydride (164 μL, 1.0 mmol) was added slowly to the resulting yellow solution over 2 min at the same temperature, and was then warmed up to 0 °C and stirred for 30 min. The reaction mixture turned into a yellow solution, and the reaction was quenched by the addition of water. After acidifying with 10 w/w% HCl, the reaction mixture was extracted twice with EtOAc, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by flash column chromatography eluting with EtOAc and hexane in 1/10 to 1/5 ratio gave 133 mg (85%) of **11** as a white solid of mp 75–77 °C. ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H), 5.35 (s, 2H), 7.16 (d, 1H, *J* = 2.2), 7.42 (d, 1H, *J* = 2.2). ^{13}C NMR ($CDCl_3$) δ 56.5, 67.1, 108.6, 115.4, 118.6 (d, *J* = 320), 130.0, 130.2, 143.5, 162.3, 168.9. HR-MS (EI+) *m/z*: Calcd for $C_{10}H_7F_3O_6S$ 311.9915; Found 311.9919.

(*E*)-6-Methoxy-4-(4-methoxystyryl)isobenzofuran-1(3*H*)-one: 13

A dry, 20 mL round bottom flask equipped with a magnetic stirrer was charged with *trans*-2-(4-methoxyphenyl)vinylboronic acid: **12** (231 mg, 1.3 mmol), **11** (312 mg, 1.0 mmol), cesium carbonate (652 mg, 2.0 mmol), anhydrous DMF (4 mL), and dichlorobis(triphenylphosphine)palladium (II) (35 mg, 0.05 mmol). The reaction mixture was heated to 90 °C in an oil bath, stirred for 21 h, then cooled to rt and quenched with water (5 mL) and EtOAc (5 mL). The layers were partitioned and the aqueous layer was extracted twice with EtOAc (15 mL). The combined organic layers were washed with 10 w/w% aqueous citric acid solution, water, brine, and the separated organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography eluting with EtOAc and hexane in 1/2 to afford 235 mg (80%) of the title compound **13** as a white solid of mp 153–154 °C. ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 3.90 (s, 3H), 5.41 (s, 2H), 6.84 (d, 1H, *J* = 16.5), 6.92 (d, 2H, *J* = 8.4), 6.96 (d, 1H, *J* = 16.5), 7.23 (d, 1H, *J* = 1.8), 7.34 (d, 1H, *J* = 1.8), 7.45 (d, 2H, *J* = 8.4). ^{13}C NMR ($CDCl_3$) δ 55.4, 55.9, 69.6, 106.5, 114.4, 119.5, 121.4, 127.5, 128.2, 129.0, 132.6, 133.9, 136.4, 160.0, 161.1, 171.1. HR-MS (EI+) *m/z*: Calcd for $C_{18}H_{16}O_4$ 296.1049; Found 296.1048.

(*E*)-6-Hydroxy-4-(4-hydroxystyryl)isobenzofuran-1(3*H*)-one: Hybrid 1

The solution of **13** (191 mg, 0.65 mmol) in anhydrous CH_2Cl_2 (8 mL) was stirred at -80 °C under an argon atmosphere. BBr_3 (3.9 mL) (1 M solution in heptane) was slowly added at this temperature. After removal from the dry ice bath, the mixture was stirred for 18 h at rt and was monitored by TLC developing with MeOH in $CHCl_3$ 1/10. The mixture was worked up as follows. The water was added to the mixture, and then the solvent was removed to a small volume under reduced pressure. The water was

added again and the precipitate was filtered off. The derived powder was collected. The recrystallization by MeOH and hexane afforded 160 mg (92%) of **Hybrid 1** as a pale brown solid of mp 276–277 °C. ^{1}H NMR (CD_3OD) δ 5.45 (s, 2H), 6.78 (d, 2H, *J* = 8.4), 6.91 (d, 1H, *J* = 16.5), 7.01 (d, 1H, *J* = 16.5), 7.06 (d, 1H, *J* = 2.2), 7.31 (d, 1H, *J* = 2.2), 7.43 (d, 2H, *J* = 8.4). ^{13}C NMR (CD_3OD) δ 71.3, 108.3, 115.3, 118.7, 120.4, 128.0, 128.2, 129.7, 132.4, 135.8, 136.9, 159.2, 160.2, 173.7. HR-MS (EI+) *m/z*: Calcd for $C_{16}H_{12}O_4$ 268.0736; Found 268.0735.

1-(2,2-Dibromovinyl)-3,5-dimethoxybenzene: 23[11,18]

According to a procedure found in the literature,[18] to a solution of carbon tetrabromide (14.6 g, 43.2 mmol) in 79 mL of anhydrous CH_2Cl_2 was added portionwise triphenylphosphine (23.4 g, 86 mmol) under ice-water bath cooling so that the reaction temperature was maintained less than 5 °C. The mixture was stirred at 0 °C for an additional 30 min, and a solution of 3,5-dimethoxybenzaldehyde (3.6 g, 21.6 mmol) in 25 mL of dry CH_2Cl_2 was added dropwise via syringe. The stirring was continued at rt for 16 h. At completion, CH_2Cl_2 was removed on a rotary evaporator, and the residue was purified by flash column chromatography ($CHCl_3$ as eluent) to afford 6.61 g (95%) of dibromide **23** as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 6H), 6.44 (br-s, 1H), 6.68 (d, 2H, *J* = 2.1), 7.41 (s, 1H).

1-Ethynyl-3,5-dimethoxybenzene: 24[11]

Dibromide **23** (6.6 g, 20.5 mmol) was dissolved in anhydrous THF (97 mL) and cooled to -78 °C under an argon atmosphere. A 1.6 M solution of *n*-butyllithium in hexane (35.5 mL, 57.5 mmol) was added over 30 min, and the reaction mixture was stirred for 16 h at 0 °C. The reaction mixture was quenched by the addition of a satd. aqueous solution of NH_4Cl. The THF was removed under reduced pressure and EtOAc was added to the mixture. The organic layer was separated and the aqueous layer was extracted further twice with EtOAc and combined. The organic phase was washed with water, brine and dried over $MgSO_4$ and concentrated. The crude residue was purified by flash column chromatography eluting with hexane and EtOAc (5/1) to provide 2.06 g (62%) of the acetylenic compound **24** as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 3.03 (s, 1H), 3.78 (s, 6H), 6.47 (t, 1H, *J* = 2.2), 6.65 (d, 2H, *J* = 2.2).

(*E*)-2-(3,5-Dimethoxyphenyl)vinylboronic acid: 19[10]

To a solution of 3,5-dimethoxyphenylacetylene (**24**) (1.8 g, 11.1 mmol) in dry THF (8.5 mL) was added dropwise catecholborane (16.7 mL, 16.7 mmol, 1 M solution in THF) at rt under an argon atmosphere. The mixture was heated under reflux for 3 h, and then partially concentrated *in vacuo*. Cold water (17 mL) was added and the white suspension was stirred for 2 h at 0 °C to hydrolyze the ester. The resulting solution was extracted thrice with $CHCl_3$. The chloroform layer was washed with water and dried over $MgSO_4$. The concentrated residue was purified by flash column chromatography eluting with $CHCl_3$ to

give 1.83 g (79%) of target compound **19** as a pale yellow wax. ^{1}H NMR (CD_3OD) δ 3.77 (s, 6H), 6.30 (d, 1H, *J* = 18.0), 6.42 (t, 1H, *J* = 2.0), 6.65 (d, 2H, *J* = 2.0), 7.22 (d, 1H, *J* = 18.0).

4-Bromo-7-methoxyphthalide: 18[19]

A stirred solution of the 7-methoxyphthalide (**17**)[12] (1.1 g, 6.7 mmol) in CH_2Cl_2 (25 mL) was treated dropwise at 0 °C with a solution of bromine (0.38 mL, 7.4 mmol) in CH_2Cl_2 (10 mL). The solution was then stirred at 0 °C for 2 h. The reaction mixture was poured into an ice cold solution of satd. aqueous NH_4Cl and separated. The aqueous layer was extracted twice with $CHCl_3$ and the organic layer was combined. The organic phase was washed with 1w/w% $Na_2S_2O_3$, brine, and dried over $MgSO_4$. Removal of the solvent gave the crude product. The crude product was recrystallized from $CHCl_3$-hexane and formed colorless needles (1.1 g, 67%) of mp 196–198 °C. ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 3H), 5.12 (s, 2H), 6.87 (d, 1H, *J* = 9.0), 7.69 (d, 1H, *J* = 9.0).

(*E*)-4-(3,5-Dimethoxystyryl)-7-methoxyisobenzofuran-1(3*H*)-one: 20

A dry 30 mL round bottom flask equipped with a magnetic stirrer was charged with **18** (846 mg, 3.48 mmol), **19** (1.09 g, 5.22 mmol), K_3PO_4 (2.62 g, 10.4 mmol), toluene (7 mL), 2-dicyclohexyl phosphino-2',6'-demethoxybiphenyl (SPhos) (28.7 mg, 0.07 mmol) and tris(dibenzylideneacetone)-dipalladium(0) [$Pd_2(dba)_3$] (32.0 mg, 0.035 mmol). The reaction mixture was heated to 100 °C in an oil bath, stirred for 20 h, then cooled to rt and quenched with water (5 mL) and $CHCl_3$ (5 mL). The layers were partitioned and the aqueous layer was extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were washed with 10w/w% aqueous citric acid solution and water, the layers were separated and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography eluting with EtOAc and hexane in 1/2 to afford 606 mg (53%) of the title compound as a white solid of mp 185–186 °C. ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 6H), 4.01 (s, 3H), 5.36 (s, 2H), 6.42 (t, 1H, *J* = 1.8), 6.63 (d, 2H, *J* = 1.8), 6.79 (d, 1H, *J* = 16.5), 6.91 (d, 1H, *J* = 16.5), 6.96 (d, 1H, *J* = 8.4), 7.75 (d, 1H, *J* = 8.4). ^{13}C NMR ($CDCl_3$) δ 55.4, 56.2, 68.6, 100.3, 104.7, 111.3, 113.4, 123.6, 124.3, 130.3, 133.7, 138.7, 146.6, 158.1, 161.1, 168.9. HR-MS (EI+) *m/z*: Calcd for $C_{19}H_{18}O_5$ 326.1154; Found 326.1154.

(*E*)-4-(3,5-Dihydroxystyryl)-7-hydroxyisobenzofuran-1(3*H*)-one: Hybrid 2

The solution of **20** (127 mg, 0.39 mmol) in anhydrous CH_2Cl_2 (4 mL) was stirred at -80 °C under an argon atmosphere. BBr_3 (3.5 mL) (1 M solution in heptane) was slowly added. The reaction flask was removed from the dry ice bath and then stirred for 18 h at rt. The mixture was monitored by TLC developing with MeOH in $CHCl_3$ 1/50. The mixture was worked up as follows. Water was added to the mixture, and then the solvent was removed to the small volume under reduced pressure. Water was added again and the precipitate was filtered off. The derived powder was collected. The recrystallization by

EtOAc afforded 83 mg (75%) of pure **Hybrid 2** as a pale brown solid of mp 257–258 °C. ^{1}H NMR (CD_3OD) δ 5.44 (s, 2H), 6.17 (s, 1H), 6.45 (s, 2H), 6.76 (d, 1H, *J* = 16.5), 6.88 (d, 1H, *J* = 8.1), 6.92 (d, 1H, *J* = 16.5), 7.72 (d, 1H, *J* = 8.1). ^{13}C NMR (CD_3OD) δ 70.9, 103.5, 106.2, 112.3, 117.3, 124.2, 125.2, 131.2, 135.1, 140.5, 147.2, 157.6, 159.8, 172.6. HR-MS (EI+) *m/z*: Calcd for $C_{16}H_{12}O_5$ 284.0685; Found 284.0685.

4-Bromo-5,7-dimethoxyphthalide: 29[20]

A stirred solution of the 5,7-dimethoxyphthalide (**28**)[14] (2.15 g, 11 mmol) in CH_2Cl_2 (40 mL) was treated dropwise at 0 °C with a solution of bromine (0.5 mL, 12 mmol) in CH_2Cl_2 (15 mL). The solution was then stirred at 0 °C for 40 min. The reaction mixture was poured into ice cold solution of satd. aqueous NH_4Cl and separated. The aqueous layer was extracted twice with $CHCl_3$ and the organic layer was combined. The organic phase was washed with 1 w/w% $Na_2S_2O_3$, brine, and dried over $MgSO_4$. Removal of the solvent gave the crude product. The combined crude product was recrystallized from $CHCl_3$-hexane and formed pale brown needles (2.86 g, 96%). ^{1}H NMR (DMSO-d_6) δ 3.97 (s, 3H), 4.02 (s, 3H), 5.14 (s, 2H), 6.81 (s, 1H).

(*E*)-4-(3,5-Dimethoxystyryl)-5,7-dimethoxyisobenzofuran-1(3*H*)-one: 30

A dry, 50 mL round bottom flask equipped with a magnetic stirrer was charged with bromophthalide derivative (**29**), *trans*-3,5-dimethoxyphenylvinylboronic acid (**19**) (624 mg, 3.0 mmol), K_3PO_4 (1.3 g, 6.0 mmol), toluene (4 mL), SPhos (16.4 mg, 0.04 mmol) and $Pd_2(dba)_3$ (18.3 mg, 0.02 mmol). The reaction mixture was heated to 100 °C in an oil bath, stirred for 24 h, then cooled to rt and quenched with water (10 mL) and $CHCl_3$ (10 mL). The layers were partitioned and the aqueous layer was extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were washed with 10% aqueous citric acid solution and water, the layers were separated and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography eluting with EtOAc and hexane in 1/2 to give 598 mg (84%) of **30** as a white solid of mp 201 °C. ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 6H), 4.01 (s, 3H), 4.03 (s, 3H), 5.37 (s, 2H), 6.41 (t, 1H, *J* = 2.2), 6.49 (s, 1H), 6.63 (d, 2H, *J* = 2.2), 6.72 (d, 1H, *J* = 16.8), 7.18 (d, 1H, *J* = 16.8). ^{13}C NMR ($CDCl_3$) δ 55.4, 56.2, 56.4, 69.1, 94.9, 100.0, 104.5, 106.1, 113.2, 120.5, 131.0, 139.5, 148.1, 159.2, 161.0, 163.5, 168.8. HR-MS (EI+) *m/z*: Calcd for $C_{20}H_{20}O_6$ 356.1260; Found 356.1260.

(*E*)-4-(3,5-Dihydroxystyryl)-5,7-dihydroxyisobenzofuran-1(3*H*)-one: Hybrid 3

The solution of **30** (178 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at -80 °C in an argon atmosphere. BBr_3 (6 mL) (1 M solution in heptane) was slowly added. The reaction flask was removed from the dry ice bath and then stirred for 18 h at rt. The mixture was monitored by TLC developing with MeOH in $CHCl_3$ 1/50. The mixture was worked up as follows. Water was added to the mixture, and then

the solvent was removed under reduced pressure. Water was added again and the precipitate was filtered off and collected. The recrystallization by EtOAc afforded 62 mg (41%) of pure **Hybrid 3** as a colorless solid of mp 288 °C. ^{1}H NMR (CD_3OD) δ 5.35 (s, 2H), 6.09 (t, 1H, *J* = 2.2), 6.32 (s, 1H), 6.36 (d, 2H, *J* = 2.2), 6.63 (d, 1H, *J* = 16.5), 7.07 (d, 1H, *J* = 16.5). ^{13}C NMR (CD_3OD) δ 71.3, 95.4, 97.8, 103.1, 103.6, 105.9, 113.3, 116.8, 121.4, 131.5, 141.5, 149.4, 159.7, 164.4. HR-MS (EI+) *m/z*: Calcd for $C_{16}H_{12}O_6$ 300.0634; Found 300.0636.

(*E*)-6-Hydroxy-4-(4-hydroxystyryl)isobenzofuran-1(3*H*)-one: 31

A dry, 20 mL round bottom flask equipped with a magnetic stirrer was charged with **29** (273 mg, 1.0 mmol), *trans*-2-(4-methoxyphenyl)vinylboronic acid (**12**) (267 mg, 1.5 mmol), potassium phosphate (637 mg, 3 mmol), toluene (2 mL), SPhos (8.2 mg, 0.02 mmol) and $Pd_2(dba)_3$ (9.2 mg, 0.01 mmol). The reaction mixture was heated to 100 °C in an oil bath, stirred for 20 h, then cooled to rt and quenched with water (5 mL) and $CHCl_3$ (5 mL). The layers were partitioned and the aqueous layer was extracted twice with $CHCl_3$. The combined $CHCl_3$ layers were washed with 10 w/w% aqueous citric acid solution and water, the layers were separated and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with MeOH and $CHCl_3$ 1/200 to afford 223 mg (69%) of **31** as a white solid of mp 191–194 °C. ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3H), 4.01 (s, 3H), 4.03 (s, 3H), 5.36 (s, 2H), 6.48 (s, 1H), 6.75 (d, 1H, *J* = 16.8), 6.90 (d, 2H, *J* = 8.8), 7.06 (d, 1H, *J* = 16.8), 7.42 (d, 2H, *J* = 8.8). ^{13}C NMR ($CDCl_3$) δ 55.3, 56.1, 56.3, 69.1, 95.0, 106.1, 113.8, 114.2, 118.0, 127.6, 130.3, 130.7, 147.8, 158.8, 159.5, 163.3, 168.9. HR-MS (EI+) *m/z*: Calcd for $C_{19}H_{18}O_5$ 326.1154; Found 326.1160.

(*E*)-5,7-Dihydroxy-4-(4-hydroxystyryl)isobenzofuran-1(3*H*)-one: Hybrid 4

The solution of **31** (130 mg, 0.4 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at -80 °C under an argon atmosphere. BBr_3 (3.6 mL) (1 M solution in heptane) was slowly added. The reaction vessel was removed from the dry ice bath and then stirred for 18 h at rt. The mixture was monitored by TLC developing with MeOH in $CHCl_3$ 1/50. The mixture was worked up as follows. The water was added to the mixture, and then the solvent was removed to the small volume under reduced pressure. The water was added again and the precipitate was filtered off and collected. The recrystallization by EtOAc afforded 107 mg (94%) of pure **Hybrid 4** as a colorless solid of mp 255 °C. ^{1}H NMR (CD_3OD) δ 5.42 (s, 2H), 6.41 (s, 1H), 6.76 (d, 2H, *J* = 8.4), 6.79 (d, 1H, *J* = 16.8), 7.05 (d, 1H, *J* = 16.8), 7.34 (d, 2H, *J* = 8.4). ^{13}C NMR (CD_3OD) δ 71.4, 103.6, 104.6, 113.9, 116.5, 118.7, 128.5, 131.1, 131.4, 148.9, 158.1, 158.3, 164.0, 173.0. HR-MS (EI+) *m/z*: Calcd for $C_{16}H_{12}O_5$ 284.0685; Found 284.0690.

Part 2: Bioactive evaluation of hybrid compounds

Cell culture RAW264.7 cells, murine macrophage cell line, were maintained in RPMI1640 supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in 5% CO_2 and 95% air.

NO production NO production was determined as generation of nitrite.[16] RAW264.7 cells ($2x10^5$ cells/well) were seeded on 48-well plates and allowed to grow to confluence. The cells were pretreated with vehicle (control), resveratrol, oxyresveratrol, dexamethasone, L-NAME or synthesized compounds for 30 min and then incubated with 1 μg/mL LPS for 24 h. After the conditioned medium was sampled, NO released into the medium was converted to nitrate when left on ice, and then nitrate was completely reduced by nitrate reductase to nitrite. Nitrite was determined by colorimetry at 570 nm following the Griess reaction.[16] Sodium nitrite was used as a standard.

Cell viability RAW264.7 cells were seeded on 48-well plates and allowed to grow to confluence. Cell viability was assessed by Live/Dead cell staining kit II according to the manufacturer's instruction.

Statistical Analysis Data are presented as means ± S.E. Statistical differences in the dose-response study were evaluated by one-way ANOVA followed by Dunnett's multiple comparison test. Student's *t*-test was used for comparing two groups. A p value of <0.05 was regarded as significant.

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