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Synthesis of Resveratrol Derivatives and *In Vitro* Screening for Potential Cancer Chemopreventive Activities

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New resveratrol *(trans*-3,4′,5-trihydroxystilbene) analogs were synthesized and screened for their *in vitro* cancer chemopreventive potential using various bioassays relevant for the prevention of carcinogenesis in humans: two assays to detect modulators of carcinogen metabolism (Cyp1A inhibition; determination of NAD(P)H/quinone reductase (QR) activity), three assays to identify radical scavenging and antioxidant properties (DPPH, ORAC, superoxide anion radicals in differentiated HL-60 cells), four assays to determine anti-inflammatory and anti-hormonal effects (iNOS, Cox-1 and aromatase inhibition, anti-estrogenic potential). 3,4′,5-Tri-*O*-methyl resveratrol **1a** was about sevenfold more active than resveratrol in inhibiting Cyp1A activity, it was a potent inducer of QR activity, and it showed pure anti-estrogenic activity (whereas resveratrol is a known mixed estrogen (ant)agonist with both estrogenic and anti-estrogenic properties). Dual estrogen ant-/agonist activity was restored in the mono-*O*-benzyl-substituted derivatives **4b** (4′-*O*-benzyl resveratrol) and **5b** (3-*O*-benzyl resveratrol). With respect to aromatase inhibition (Cyp19), which provided the highest number of actives, the benzyl-substituted series was more potent than the methyl-substituted derivatives of resveratrol, and 3-*O*-benzyl resveratrol **5b** was about eightfold more active than resveratrol. Overall, 3,4′,5-tri-*O*-pivaloyl resveratrol oxide **7c** was identified as a potent inducer of phase 2 enzymes concomitant with inhibition of LPS-mediated iNOS induction.

Keywords: Aromatase / Chemoprevention / COX-1 / Drug metabolism / iNOS / Nrf2 / ORAC / Resveratrol

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Introduction

Resveratrol (*trans*-3,4′,5-trihydroxystilbene), a polyphenolic phytoalexin present in grapes, peanuts, and mulberry, has attracted burgeoning attention of the biomedical researchers because of the number of beneficial physiological effects it produces. Resveratrol has been shown to inhibit platelet aggregation [1], lipid peroxidation [2], and to modulate potassium channels [3], all mechanisms involved in coronary artery disease. Neuroprotective properties, such as anticholinesterases, anti-β-amyloid aggregation, and monoamine oxidase inhibition properties against Alzheimer's disease have been recently reported [4].

About 20 years ago, resveratrol had been also identified as a potent cancer chemopreventive agent in assays representing the three major stages of carcinogenesis (i.e., tumor initiation, promotion, and progression) [5]. Investigations in this field evidenced multiple intracellular targets of resveratrol which affects cell growth, inflammation, apoptosis,

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angiogenesis, tumor invasion, and metastasis [6]. This ability to interact with multiple molecular targets, accompanied by the relatively simple structure and a low toxicity, makes resveratrol an ideal candidate in the design of multitargeted drugs for cancer chemoprevention and treatment.

In this field and as a part of an extensive study of stilbene derivatives/analogs with anticancer activity [7], we have synthesized a variety of resveratrol analogs and screened them for *in vitro* cancer chemopreventive potential, using a series of bioassays relevant for the prevention of carcinogenesis in humans (Table 1). Compounds **1–5**, functionalized at the oxygen atoms have been previously reported [3,8a] and some of them have been identified as potassium channel modulators [3].

Results and discussion

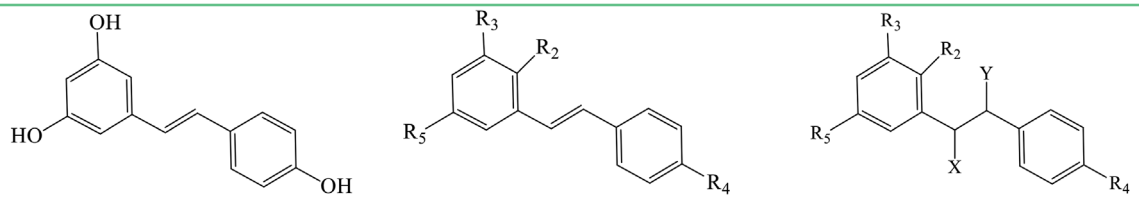
Chemistry

In this work, the attention was focused on the functionalization of the stilbene double bond with multiple aims: (i) modify the geometry and the flexibility of the molecule; (ii) vary its lipophilicity; (iii) build a third heteroatom(s)-containing ring in place of the stilbene double bond; (iv) introduce functional units able to bind peptide chains and/or specific linkers, without modifying the substitution pattern of the aromatic rings.

To this purpose, two synthetic strategies were tested, starting with the introduction of one (*epoxidation*) or two oxygen atoms (*dihydroxylation*), followed by reaction with a nucleophile.

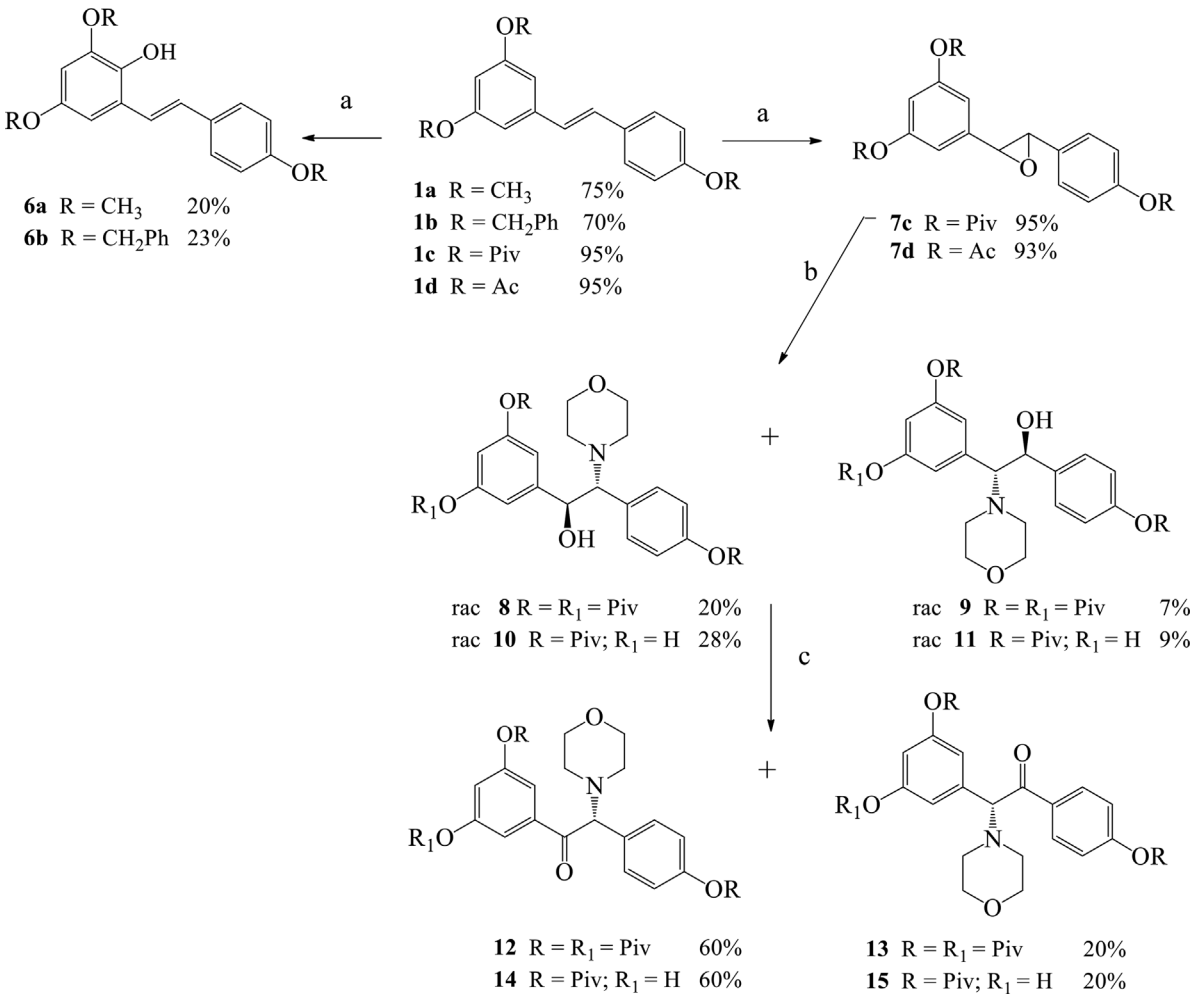
In the first approach, the epoxidation step required protection of the phenolic groups as esters: treatment of the tri-*O*-pivaloyl and tri-*O*-acetyl derivatives **1c** and **1d** with *m*-chloroperbenzoic acid (*m*-CBPA) in methylene chloride afforded the corresponding *rac*-epoxides **7c** and **7d** in about 90% yield (Scheme 1). Epoxidation of the protected phenolic groups with methyl or benzyl ether was unsuccessful: Treatment of tri-*O*-methyl resveratrol **1a** or tri-*O*-benzyl resveratrol **1b** with *m*-chloroperbenzoic acid in methylene chloride gave a mixture of products, the most abundant being the 2-hydroxy derivative, **6a** (as already reported in the

Table 1. **Resveratrol derivatives screened for biological activities.**



Resveratrol | ***O*-Functionalization** | **Double bond Functionalization**

Compound		R_2	R_3	R_5	R_4	X	Y
				***O*-Funtionalization**			
1a[3]		H	OCH_3	OCH_3	OCH_3		
2a[3]		H	OH	OCH_3	OCH_3		
3a[3]		H	OCH_3	OCH_3	OH		
4a[3]		H	OH	OH	OCH_3		
5a[3]		H	OCH_3	OH	OH		
6a[8]		OH	OCH_3	OCH_3	OCH_3		
1b[3]		H	OCH_2Ph	OCH_2Ph	OCH_2Ph		
2b[3]		H	OH	OCH_2Ph	OCH_2Ph		
3b[3]		H	OCH_2Ph	OCH_2Ph	OH		
4b[3]		H	OH	OH	OCH_2Ph		
5b[3]		H	OCH_2Ph	OH	OH		
6b[8]		OH	OCH_2Ph	OCH_2Ph	OCH_2Ph		
				Double bond functionalization			
7c		H	OPiv	OPiv	OPiv	–O–	
16a		H	OCH_3	OCH_3	OCH_3	OH	OH
21		H	OCH_3	OCH_3	OCH_3	–OC(O)O–	
22	Isomer	H	OCH_3	OCH_3	OCH_3	OH	N_3
23	Mixture	H	OCH_3	OCH_3	OCH_3	N_3	OH
24	Isomer	H	OCH_3	OCH_3	OCH_3	OH	NH_2
25	Mixture	H	OCH_3	OCH_3	OCH_3	NH_2	OH



Scheme 1. (a) *m*-Chloroperbenzoic acid, CH_2Cl_2, 0°C, 8 h; (b) morpholine, reflux, 5 h; (c) Jones' reagent (CrO_3, diluted H_2SO_4), acetone, 2 h.

literature [8a]) from **1a**, and **6b** [8b] from **1b**, respectively. The electron-richness of the methoxylated and benzyloxylated derivatives renders the concerned resveratrol phenyl ring more nucleophilic in comparison to that of the acetoxylated and pivaloylated derivatives, so that the *m*-CPBA-mediated *ortho*-selective hydroxylation occurs, to some extent, instead of the epoxidation of the central olefin. In both cases, the 2-hydroxy derivative, obtained in about 20% yield, was accompanied by unreacted starting material and unidentified compounds.

Reaction of **7c–d** with various nucleophiles (amines, azides) was tested. The best results were obtained for the reaction of **7c** with morpholine, which afforded a regioisomeric and stereoisomeric mixture of the expected *anti*-aminoalcohols **8** and **9** (27%), accompanied by products of partial deprotection of the phenolic groups, **10** and **11** (37%). Their ratio was determined as 3:1 by ^{1}H NMR, while the regioselectivity of the reaction was determined through a multibond correlation experiment (HMBC) recorded on the mixture, identifying **8** as the major regioisomer (Fig. 1).

Only a few percent of aminoalcohols (10%), with recovery of unreacted epoxide (70%), was obtained using benzylamine as nucleophile. Attempted epoxide cleavage with sodium azide in DMF failed and gave a mixture of unidentified products.

Oxidation of the regioisomers **8–9** with chromium trioxide and diluted sulfuric acid in acetone (Jones' reagent) proceeded smoothly and afforded the regioisomeric ketones **12–13** in 80% yield. Analogously, the regioisomers **14–15** were obtained from the corresponding aminoalcohols **10–11**, respectively.

In the second approach, the introduction of two oxygen atoms was achieved via asymmetric sharpless dihydroxylation using AD-mix-α (Scheme 2). The reaction, performed on tri-*O*-methyl

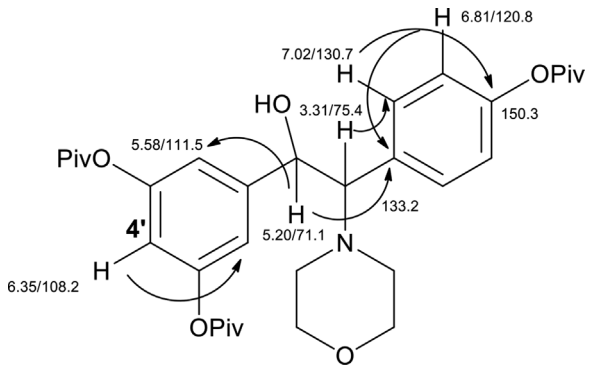


Figure 1. Indicative $^{1}H–^{13}C$ NMR correlations (HMBC) in compound **8**.

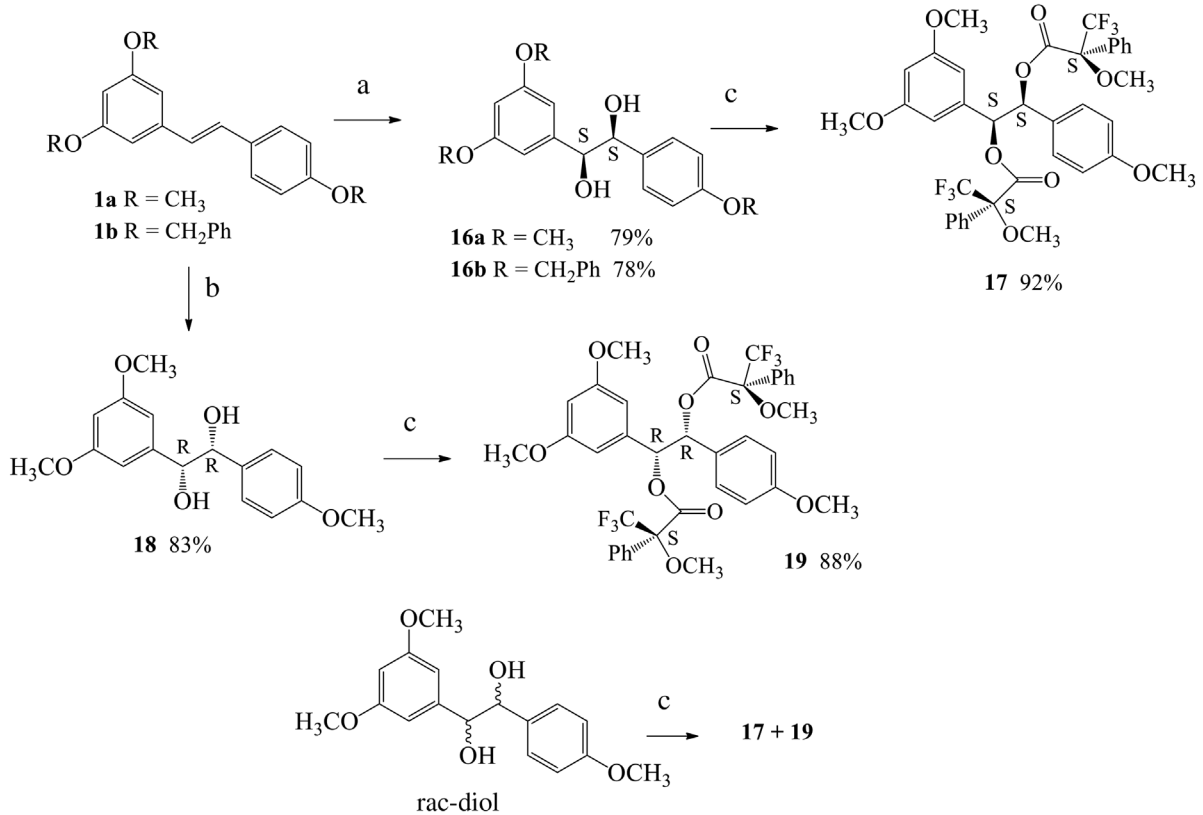
resveratrol **1a**, afforded the diol **16a** in 79% yield. The depicted stereochemistry has been suggested on the basis of the results obtained in the asymmetric dihydroxylation of (*E*)-combretastatin A-1 [9]. Analogously, **16b** was obtained in 78% yield by dihydroxylation of tri-*O*-benzyl resveratrol **1b**.

The enantiomeric purity of the (*S*,*S*)-diol **16a** was assessed by application of the Mosher method [10]. To this purpose, **16a** was treated with an excess of (*R*)-α-methoxy, α-trifluoromethyl phenylacetyl chloride to give the corresponding bis-MTPA-esters **17** (Scheme 2).

The ^{19}F NMR of **17** shows two peaks, at δ_F −72.15 and −71.95, in accordance with the presence of two CF3 groups. This result was confirmed by the ^{1}H NMR spectrum, which shows two singlets at δ_H 3.40 and 3.44 (corresponding to the OCH_3 groups at the ester α-positions), and by the ESIMS spectrum, which shows a molecular ion at 759 *m/z* (M^{+}+Na). In the ^{1}H NMR spectrum, the two vicinal benzylic hydrogens give an AB system at δ_H 6.13 and 6.17 (JAB = 8.0 Hz).

Within the limits of the instrument sensitivity, no traces of the (*R*,*R*,*S*,*S*)-diastereoisomer, which would have been obtained from the enantiomeric (*R*,*R*)-diol, were detected, and the enantiomeric purity was then assessed to be ≥99%.

This assumption has been verified by two independent ways: (i) synthesis of the (*R*,*R*)-diol **18** from **1a** and β-AD-mix, followed by conversion to bis-MTPA ester **19** by treatment with an excess of (*R*)-α-methoxy, α-trifluoromethyl phenylacetyl chloride; (ii) synthesis of the racemic diol, followed by treatment with (*R*)-α-methoxy, α-trifluoromethyl phenylacetyl chloride to afford the



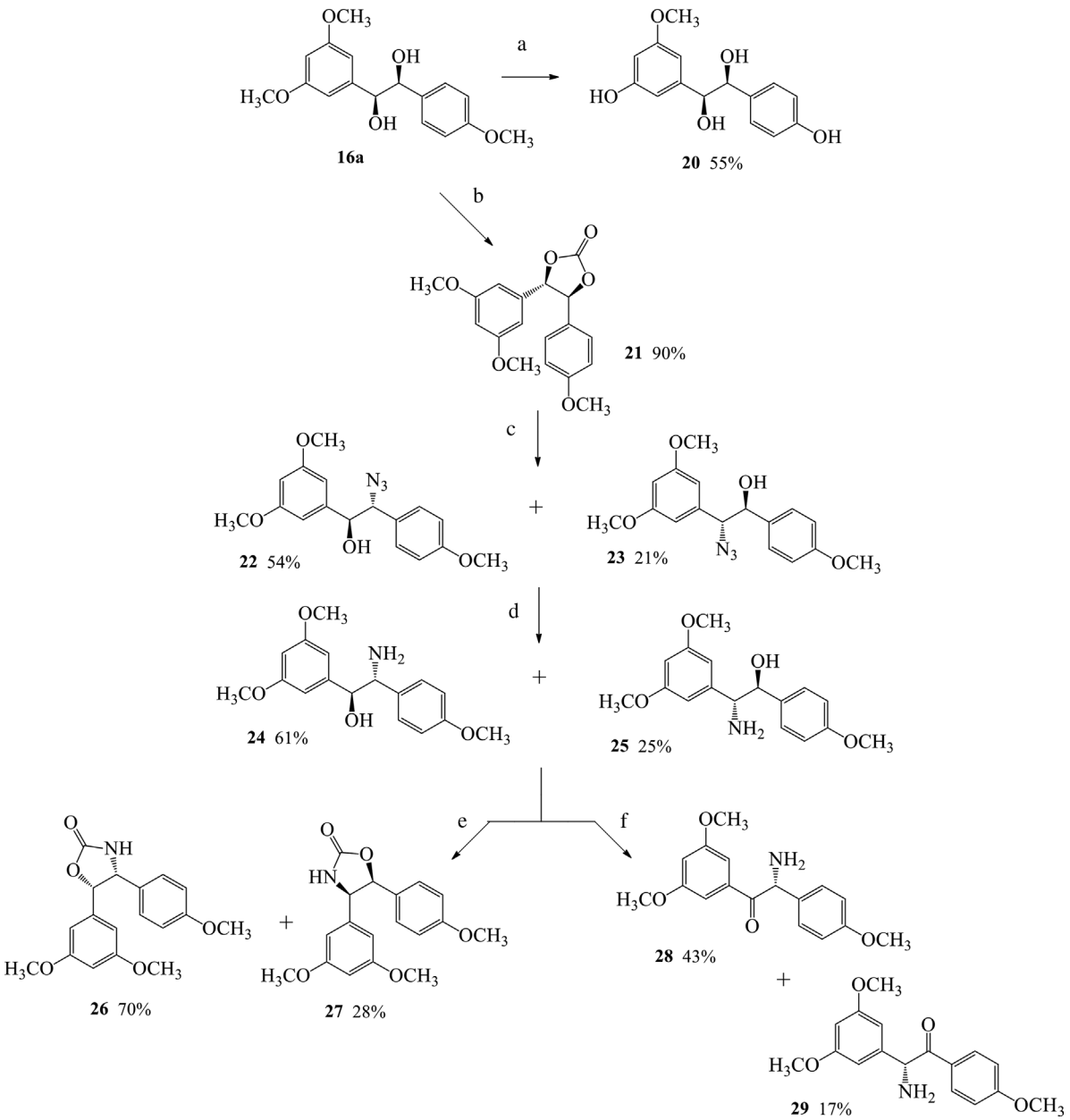
Scheme 2. (a) α-AD-mix, *t*-BuOH:H_2O (1:1), $MeSO_2NH_2$, 0°C to r.t., 24 h; (b) β-AD-mix, *t*-BuOH:H_2O (1:1), $MeSO_2NH_2$, 0°C to r.t; (c) *R*-(–)-α-methoxy-α-(trifluoromethyl)phenyl-acetyl chloride, 4-dimethylamino-pyridine, pyridine, 20 h.

diastereoisomeric mixture **17** + **19**, and comparison of the spectral data.

Attempted exhaustive demethylation of **16a** by treatment with sodium thioethoxide in dimethylformamide failed and gave the partially demethylated derivative **20** (Scheme 3).

With the aim to obtain nitrogen-containing compounds, diol **16a** was first treated with triphosgene, to give the corresponding carbonate **21**, and then with sodium azide in dimethyl formamide (Scheme 3). A 2.5:1 mixture of the regioisomeric azido alcohols **22** and **23** was obtained (and directly used for further transformations, as the chromatographic separation proved quite difficult).

Hydrogenation of the mixture in methanol in the presence of 10% palladium on charcoal afforded the corresponding regioisomeric aminoalcohols **24** and **25** (86% yields) which were purified to give the major regioisomer **24** as a



Scheme 3. (a) EtSNa, DMF, 150°C, overnight; (b) $Cl_3COC(O)OCCl_3$, Py, CH_2Cl_2, r.t., 35 min; (c) NaN_3, DMF, 110°C, 30 min; (d) H_2, 10% Pd/C, 4 h; (e) KOH, triphosgene, toluene, 40 min; (f) DMP (Dess–Martin periodinane), CH_2Cl_2, 2 h.

chromatographically homogeneous compound. The regioselectivity of the reaction was determined through a multibond correlation experiment (HMBC) (Fig. 2).

The regioisomeric mixture **24–25** was then used to build an heterocyclic oxazolidinone ring, by treatment with triphosgene in toluene, in the presence of potassium hydroxide. In a different approach, the regioisomeric mixture **24–25** was oxidized to aminoketones **28** and **29**, *a priori* also useful precursors for the introduction of heterocyclic rings.

As chromatographic separation of the various regioisomers obtained was not simple, preliminary biological tests were initially performed on enriched mixtures of regioisomers, in order to identify the most promising compounds and focus on them.

Screening for *in vitro* cancer chemopreventive activities

Resveratrol has been identified as a broad-spectrum cancer chemopreventive compound [5]. Based on its activities, we selected *in vitro* test systems relevant for cancer prevention to screen a series of 15 derivatives as pure compounds and two mixtures. We included two systems to detect modulators of carcinogen metabolism, three systems to identify antioxidant properties, and four assays to characterize anti-inflammatory and anti-hormonal effects [11].

Modulation of carcinogen metabolism

Modulation of enzymes involved in metabolic activation and elimination of carcinogens is one of the best investigated mechanisms of chemopreventive agents [12]. Phase 1 enzymes (cytochromes P450) metabolize xenobiotics by addition of functional groups which render these compounds more water-soluble. Although phase 1 functionalization might be required for complete detoxification, induction of phase 1 enzymes might increase the risk to produce ultimate carcinogens capable of reacting with DNA and initiating carcinogenesis. Phase 2 enzymes conjugate the activated compounds to endogenous ligands like glutathione (GSH), glucuronic, acetic, or sulfuric acid, thus enhancing their excretion in form of these conjugates. Food-based natural products have been shown to induce cytoprotective enzymes, such as NAD(P)H/QR 1 (also known as NQO1, nicotinamide quinone oxidoreductase 1), superoxide dismutase, and heme oxygenase-1 (HO-1) coordinately with phase 2 enzymes [12]. Comparison of promoter regions has indicated that most of these genes can be induced via the antioxidant response element (ARE), which is activated upon binding of the nuclear factor E2-related protein 2 (Nrf2) transcription factor protein. Nrf2 plays an essential role in mediating the induction of these genes in response to oxidative stress, electrophiles, and various natural products. Natural inducers of ARE-dependent cytoprotective genes include diphenols and quinones, Michael reaction acceptors, isothiocyanates and related sulfur compounds, 1,2-dithiol-3-thiones, hydroperoxides, and carotenoids and related polyenes. Under basal conditions, Nrf2 is sequestered in the cytosol by interaction with the Kelch-like ECH-associated protein 1 (Keap1) and maintained at low levels by degradation via the proteasome. Upon activation, Nrf2 is released from Keap1, translocates to the nucleus, and induces transcription of ARE-dependent genes. The interaction of Nrf2 and Keap1 can be disrupted by decreased ubiquitination/proteasomal degradation of Keap1 and Nrf2, covalent or oxidative thiol modification of Keap1, or phosphorylation of Nrf2 [12].

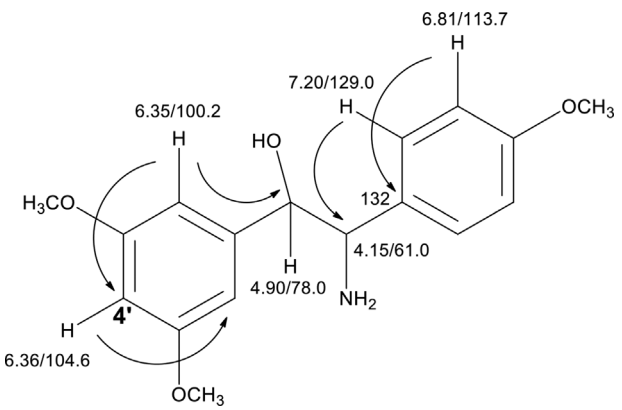


Figure 2. Indicative $^{1}H–^{13}C$ NMR correlations (HMBC) in compound **24**.

Generally, inhibition of phase 1 enzymes concomitantly with induction of cytoprotective enzymes is considered a logical strategy in chemoprevention, which is especially beneficial in early stages of carcinogenesis. Consequently, for the identification of modulators of carcinogen metabolism, we selected inhibition of Cyp1A (cytochrome P450 1A) activity as a representative for carcinogen-activating enzymes, and NAD(P)H/QR activity as a model cytoprotective enzyme.

With respect to inhibition of Cyp1A activity (Table 2), the tri-methyl-substituted derivative **1a** was about sevenfold more active than resveratrol (IC_{50} values: 0.03 vs. 0.23 μM). Concentration-dependent effects of **1a** in comparison with the known Cyp1A inhibitor, α-naphthoflavone ($IC_{50} = 0.01$ μM) and with resveratrol are shown in Fig. 3A.

Compounds **2a–6a** with methyl substitution were similarly active as resveratrol (IC_{50} values in the range of 0.1–0.2 μM), with the exception of **5a**, which did not show any inhibitory activity at a concentration of 0.5 μM. When Cyp1A inhibitory potential of compounds **1b–6b** with benzyl substitution was compared with that of the methyl-derivatized series **1a–6a**, we observed a differential influence of the substitution. Compounds **1b**, **2b**, **4b**, and **6b** lost activity in comparison with **1a**, **2a**, **4a**, and **6a**, compounds **3a** and **3b** were equally active, and compound **5b** was identified as a more potent Cyp1A inhibitor than compound **5a**. Compounds with a functionalized double bond, i.e., **16a**, **21**, and **7c**, as well as the mixtures **22** + **23** and **24** + **25** demonstrated less than 50% inhibition at a 5 μM concentration.

Table 2. Effects of resveratrol derivatives on carcinogen-metabolizing enzymes.

Compound	Cyp1A	NAD(P)H/QR	
	IC_{50} (μM)[a]	CD (μM)[b]	IC_{50} (μM)[a]
Resveratrol	0.23	12.4	29.8
1a	0.03	>2.6	2.6
2a	0.10	>50 (1.4)[c]	>50
3a	0.19	>50 (1.0)	>50
4a	0.21	>52.9	>52.9
5a	>0.5 (36)[d]	>50 (1.0)	>50
6a	0.17	1.04	47.4
1b	4.3	>50 (1.4)	>50
2b	1.0	>50 (1.5)	>50
3b	0.23	>50 (1.2)	>50
4b	1.7	>39.1 (1.6)	39.1
5b	0.14	4.1	13.2
6b	>5 (27)	>50 (1.1)	>50
16a	>0.5 (15)	>50 (1.5)	>50
21	>5 (37)	>50 (1.1)	>50
22 + 23	>5 (26)	>50 (1.1)	>50
24 + 25	>5 (40)	>19.2	19.2
7c	>5 (49)	0.83	4.2

[a] IC_{50}: halfmaximal inhibitory concentration.
[b] CD: concentration required to double the specific activity of QR.
[c] Values in parentheses indicate the maximum fold induction at the indicated concentration.
[d] Values in parentheses indicate the percentage of inhibition at the indicated concentration.

With respect to phase 2 modulation, two compounds, **6a** and **7c**, were identified as potent inducers of QR activity. QR induction in a concentration range of 0.4–50 μM is shown in Fig. 3B, in comparison with the known phase 2 enzyme inducer β-naphthoflavone used as a positive control. To compare the inducing potential of various derivatives, we computed CD values (concentration required to double the specific activity of QR). For **6a**, a tri-methyl derivative of resveratrol with an additional OH-group in 2-position, a CD value of 1.04 μM was calculated (Table 2). The epoxide **7c** leads to a twofold induction of QR activity at a similar concentration (CD = 0.83 μM), but was about 10-times more toxic (IC_{50} = 4.2 μM). A decline of the induction curve at concentrations above 3 μM was indicative of cytotoxic effects (Fig. 3B). Of the benzyl-substituted series, only **5b** induced the enzymatic activity of QR with a CD value of 4.1 μM. Notably, the therapeutic margin between activity and toxicity calculated from the ratio between the IC_{50} and CD (previously defined as chemopreventive index (CI) [13]) of these three derivatives was about 3- to 40-fold higher than that of resveratrol (CI = 1.2).

Antioxidant activity

Reactive oxygen species (ROS) play an important role in tumor initiation and tumor promotion [14]. We analyzed the general potential of the compounds to scavenge reactive radicals using the DPPH assay. The results are summarized in Table 3. Resveratrol was not very potent in this assay, with an IC_{50} value of 82 μM. Substitution of the free OH-groups reduced the radical scavenging potential even further, whereas one additional OH-group in *ortho* position (**6a**) restored the DPPH-scavenging potential when compared to the trimethylated compound **1a**. This was not the case when comparing the monohydroxylated tribenzylated derivative **6b** with the tribenzylated compound **1b**. Benzyl substitution probably hindered the interaction with the bulky radical DPPH. Functionalization of the double bond in combination with substituted OH-groups completely abrogated radical scavenging activity.

The compounds were tested for antioxidant potential to scavenge superoxide anion radicals, which are generally formed by autoxidation processes. In the HL-60 *in vitro* test system, superoxide anion radicals were generated by phorbol ester stimulation of differentiated HL-60 human promyelocytic leukemia cells. Only the carbonate **21** was active in this assay. Since **21** did not scavenge DPPH radicals, the compound might act on the signal transduction pathway

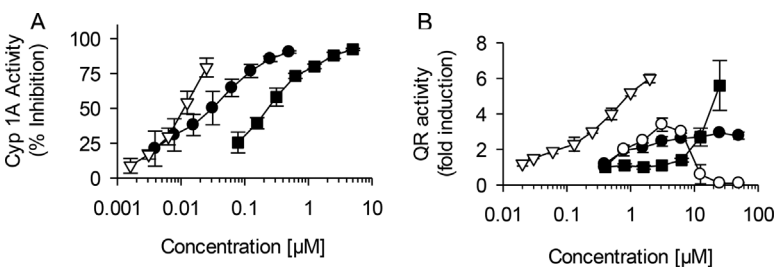


Figure 3. Modulation of carcinogen metabolism. (A) Dose-dependent inhibition of Cyp1A enzymatic activity by **1a** (●), α-naphthoflavone (▽), and resveratrol (■), respectively, measured in β-naphthoflavone-induced H4IIE rat hepatoma cell homogenates by dealkylation of 3-cyano-7-ethoxy-coumarin to fluorescent 3-cyano-7-hydroxy-coumarin. Activity of β-naphthoflavone-induced controls: 18.1 ± 4.3 nmoles/min/mg of protein (n = 4). (B) Induction of QR activity in Hepa 1c1c7 cell culture by **6a** (●), **7c** (○), and resveratrol (■), respectively, in comparison with the positive control β-naphthoflavone (▽). Specific activities of untreated controls: 55 ± 2 nmoles/min/mg protein (n = 4).

Table 3. Summary of radical-scavenging and antioxidant effects.

Compound	DPPH SC_{50} (μM)[a]	HL-60 IC_{50} (μM)	$ORAC_{ROO}$ (units)[b]
Resveratrol	82.0	>100 (29)[c]	2.1
1a	>250 (8)[c]	>100 (0)	0.7
2a	>250 (9)	>100 (0)	1.3
3a	>250 (4)	>100 (0)	0.4
4a	>264.5 (30)	>105.8 (28)	1.4
5a	>250 (4)	>100 (17)	0.8
6a	109.7	>100 (18)	0.4
1b	>250 (0)	>100 (9)	0
2b	>250 (22)	>100 (17)	0.8
3b	>250 (21)	>100 (17)	0.2
4b	>250 (27)	>100 (0)	0.9
5b	>250 (39)	>100 (22)	2.0
6b	>250 (0)	>100 (0)	0.2
16a	>250 (1)	>100 (31)	0.3
21	>250 (0)	24.9	0.1
22 + 23	>250 (0)	>100 (21)	0
24 + 25	>250 (0)	>100 (17)	0.2
7c	>250 (0)	>100 (17)	0

[a] SC_{50}: halfmaximal scavenging concentration.
[b] One ORAC unit equals the net protection of fluorescein produced by 1 μM Trolox.
[c] Numbers in parentheses indicate the percentage of scavenging or inhibition at the indicated concentrations.

between TPA stimulation and superoxide anion radical release.

We also determined the potential to scavenge peroxyl radicals in the oxygen radical absorbance capacity (ORAC) assay. Trolox, a water soluble vitamin E derivative, was used as a reference compound. At a 1 μM concentration, resveratrol was twofold more potent than Trolox to scavenge peroxyl radicals. Chemical modification reduced the peroxyl radical scavenging potential of all derivatives tested. Compound **5b** with two free OH-groups in 4′- and 5-position was only slightly less effective than resveratrol. Of all other compounds tested, only **2a** and **4a** with free OH-groups in 3-position scavenged peroxyl radicals more potently than Trolox.

Anti-inflammatory activity

It is assumed that up to 10% of all cancer cases are related to chronic inflammatory processes. Chronic inflammation and infections stimulate the inducible form of nitric oxide (NO) synthase (iNOS) and consequently, enhance the generation of NO. Long-term elevated levels of NO have been linked to early steps in carcinogenesis via nitrosative deamination of DNA bases and DNA adduct formation [15]. We analyzed the inhibition of lipopolysaccharide (LPS)-induced iNOS induction in Raw 264.7 macrophages via the Griess reaction as an *in vitro* model for inflammation. Results are summarized in Table 4.

Resveratrol is a weak inhibitor of NO release in this model. The epoxy derivative **7c** was identified as a potent inhibitor of LPS-induced NO production, with an IC_{50} of 2.2 μM and no significant toxicity (IC_{50} for inhibition of cell growth >50 μM, data not shown).

In addition to the inhibition of iNOS induction, we tested the potential of all derivatives to inhibit the enzymatic activity of cyclooxygenase 1 (Cox-1) (Table 4).

Excessive production of prostaglandins (PGs), i.e., hormone-like endogenous mediators of inflammation, from arachidonic acid by Cox-1 and the inducible form Cox-2 is thought to be a causative factor of cellular injury and may ultimately lead to carcinogenesis [16]. Resveratrol is a potent inhibitor of Cox-1 with an IC_{50} value of 1.6 μM. Unfortunately, all substitutions led to a decreased anti-inflammatory potential. Only two compounds retained weak Cox-1 inhibitory activity: **4a** with a resorcinol-substitution similar to resveratrol ($IC_{50} = 12.7$ μM, Fig. 4), and **5b** with free OH-groups in 4′- and 5-position ($IC_{50} = 41.5$ μM).

Anti-estrogenic potential

Hormones like 17β-estradiol (E2) are regarded as endogenous tumor promoters. They interact with the estrogen receptor, stimulate cell growth, and increase the risk for hormone-dependent tumor types like breast and uterine cancer. Recent reports indicate that E2 or its metabolites also might be involved in tumor initiation and act as mutagens that contribute to the formation of DNA adducts and chromosomal changes [17]. For investigation of (anti-)estrogenic properties of resveratrol derivatives, we utilized the human Ishikawa endometrial adenocarcinoma cell line. Treatment with E2 or estrogens leads to an enhanced expression of alkaline phosphatase (ALP) activity as a marker for estrogenic properties, whereas coordinate treatment with test samples and E2 allowed the detection of anti-estrogens.

Resveratrol is a known mixed estrogen (ant)agonist with both estrogenic and anti-estrogenic properties (Table 4). The methyl derivative **1a** was identified as a more potent anti-estrogen than resveratrol ($IC_{50} = 3.4$ μM) without demonstrating estrogenic activity. Dose-dependent inhibition in comparison with the positive control substance tamoxifen ($IC_{50} = 0.26$ μM) and with resveratrol are shown in Fig. 5A. Mixture **24** + **25** also demonstrates pure anti-estrogenic activity, whereas mono-benzyl-substituted **4b** and **5b** were identified as dual estrogen ant-/agonists.

17β-Estradiol is formed from testosterone by the enzymatic activity of aromatase (Cyp19). As aromatase is expressed at a higher level in breast cancer tissue than in surrounding non-cancer tissue, inhibition of aromatase activity is a strategy to lower estrogen levels *in situ* and to prevent the proliferation of estrogen-dependent tumor cells [18]. With the exception of Cyp1A activity, aromatase inhibition provided the highest number of actives (Table 4). Interestingly, the benzyl substituted series was more potent than the methyl-

Table 4. Summary of anti-inflammatory and anti-tumor promoting effects.

Compound	Inhibition of iNOS induction	COX-1 inhibition	Anti-estrogenic potential	Aromatase inhibition
	IC_{50} (μM)[a),b)]	IC_{50} (μM)	IC_{50} (μM)[a),b)]	IC_{50} (μM)[a)]
Resveratrol	18.7	1.6	12.6[d)]	22.5
1a	>50 (6)[c)]	>100 (24)[c)]	3.4	13.6
2a	>50 (9)	>100 (27)	>50 (1)[c)]	>50 (22)[c)]
3a	>50 (14)	>100 (6)	>50 (17)	>50 (14)
4a	>52.9 (23)	12.7	>52.9 (0)	>50 (43)
5a	>50 (0)	>100 (20)	>50 (0)	>50 (13)
6a	>50 (0)	>100 (27)	>50 (14)	21.9
1b	>50 (0)	>100 (3)	>50 (0)	14.2
2b	>50 (0)	>100 (8)	>50 (0)	15.2
3b	>50 (0)	>100 (5)	>50 (0)[d)]	9.0
4b	>50 (4)	>100 (20)	28.3[d)]	6.5
5b	>50 (28)	41.5	12.8[d)]	2.7
6b	>50 (9)	>100 (0)	>50 (1)	>50 (23)
16a	>50 (0)	>100 (2)	>50 (6)[d)]	>50 (45)
21	>50 (13)	>100 (0)	>50 (4)	>50 (0)
22 + 23	>50 (0)	>100 (4)	>50 (0)	>50 (0)
24 + 25	>50 (27)	>100 (4)	40.3	>50 (0)
7c	2.2	>100 (7)	>50 (46)	>50 (33)

[a)] IC_{50}: halfmaximal inhibitory concentration.
[b)] No signs of toxicity up to a concentration of 50 μM.
[c)] Numbers in parentheses indicate the percentage of inhibition at the indicated concentration.
[d)] Indication of estrogenic potential.

substituted derivatives of resveratrol. **5b** with free OH-groups in 4′- and 5-position ($IC_{50} = 2.7$ μM) was about eightfold more active than resveratrol ($IC_{50} = 22.5$ μM). Dose-dependent effects are demonstrated in Fig. 5B. Since this compound also possessed estrogenic potential in the Ishikawa cell line (data not shown), benzyl-substitution in 3-position does not seem to hinder the interaction with the estrogen receptor or the active site of aromatase. Ketoconazole, a broad-spectrum imidazole antimycotic and inhibitor of cytochrome P-450 enzymes including aromatase was used as a positive control ($IC_{50} = 0.9$ μM, Fig. 5B) [19].

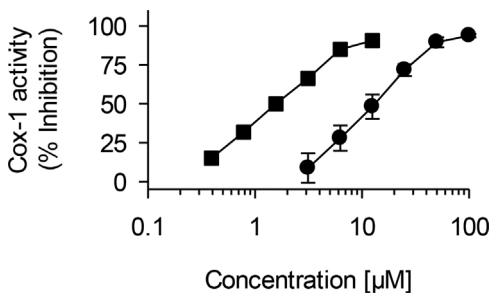


Figure 4. Anti-inflammatory activity. Inhibition of oxygen consumption during *in vitro* prostaglandin formation by Cox-1 mediated by **4a** (●) in comparison with resveratrol (■).

Overall, the epoxide **7c** was identified as the most interesting derivative of resveratrol, acting by coordinate upregulation of phase 2 cytoprotective enzymes and concomitant inhibition of pro-inflammatory enzyme induction at concentrations below 2.5 μM. We postulate that similar to the reactivity of Michael acceptors (compounds with an α,β-unsaturated carbonyl functionality) known to induce cytoprotective enzymes [12], the epoxide functionality facilitates interaction with sulfhydryl residues, e.g., of the cytosolic protein Keap1, thereby releasing transcription factor Nrf2 for translocation to the nucleus. Interestingly, phase 2 enzyme induction has repeatedly been shown to correlate with anti-inflammatory potency. It has been suggested that Nrf2 signaling and phase 2 induction may play an important role in suppressing inflammation. Most convincingly, Liu et al. demonstrated correlation of both mechanisms by comparing activities of seven different chemical classes of phase 2 inducers [20]. Based on these consistent observation, both activities could indeed be mechanistically linked. As a matter of fact, several recent reports have demonstrated that inhibition of iNOS induction requires Nrf2 signaling and the phase 2 enzyme heme oxygenase-1 (HO-1) [21]. HO-1 activity

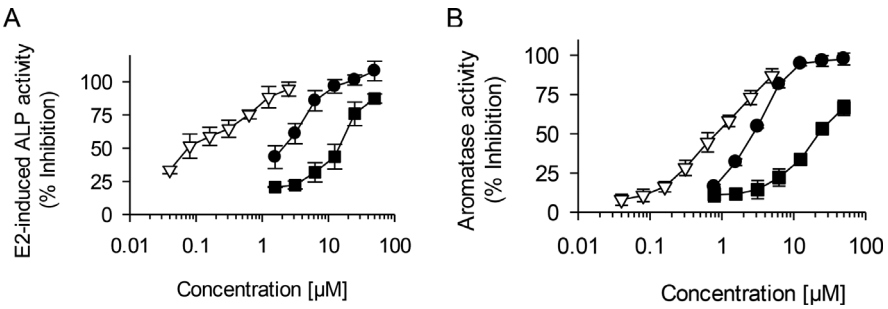


Figure 5. Anti-hormonal effects. (A) Anti-estrogenic potential of **1a** (●), resveratrol (■), and tamoxifen used as a positive control substance (▽) in Ishikawa cell culture. Control levels of ALP activity in untreated Ishikawa cells: 0.8 ± 0.1 pmoles 4-methyl-umbelliferone/min/mg protein ($n = 5$); after stimulation with 5 nM E2: 17.7 ± 3.5 pmoles 4-methylumbelliferone/min/mg protein ($n = 5$). (B) Inhibition of aromatase activity by **5b** (●), resveratrol (■), and with ketoconazole (▽) used as a positive control, using human recombinant aromatase (Cyp19 supersomes) and *O*-benzylfluorescein benzyl ester as a substrate.

is induced in an ARE-dependent manner in parallel with other cytoprotective enzymes such as QR. Mechanistically, the contribution of HO-1 to anti-inflammatory activity has been attributed to the release of carbon monoxide (CO), which is formed during the oxidative degradation of heme through HO-1. To demonstrate a functional link between both activities, overexpression of HO-1 in transfection experiments, induction of HO-1 by Co-protoporphyrin, or treatment of Raw 264.7 macrophages with CO was sufficient to significantly reduce LPS-mediated pro-inflammatory cytokine production. These findings indicate that induction of HO-1 via Nrf2 negatively regulates iNOS gene expression. Further support comes from investigations on HO-1 induction contributing to the inhibition of LPS-mediated iNOS induction in Raw 264.7 macrophages by a series of structurally distinct compounds, such as low dose 15-deoxy-Δ12,14-prostaglandin J2, the flavonoids 3-OH-flavone, baicalein, kaempferol and quercetin, 2′-hydroxychalcone, the mushroom *Phellinus linteus*, propyl gallate, low dose curcumin, and *Amomum compactum* [22]. Blockage of HO-1 activity by an inhibitor, downregulation of HO-1 expression by siRNA, or co-treatment with hemoglobin as a scavenger of CO restored LPS-induced NO production reduced by these inhibitors. Taken together, these experiments consistently point to a functional link between the induction of cytoprotective enzymes and anti-inflammatory potential.

Conclusions

In summary, a variety of resveratrol analogs was designed, synthesized, and tested in a series of bioassays related to cancer chemoprevention. The most critical structural elements for displaying biological activity are summarized in Fig. 6.

With respect to modulation of carcinogen metabolism, the tri-*O*-methyl-substituted derivative **1a** was about sevenfold more active than resveratrol in inhibiting Cyp1A activity, and it was also identified as a potent inducer of QR activity. With respect to anti-inflammatory activity, all modifications performed on resveratrol led to a decresed anti-inflammatory potential. With respect to anti-estrogenic potential, whereas

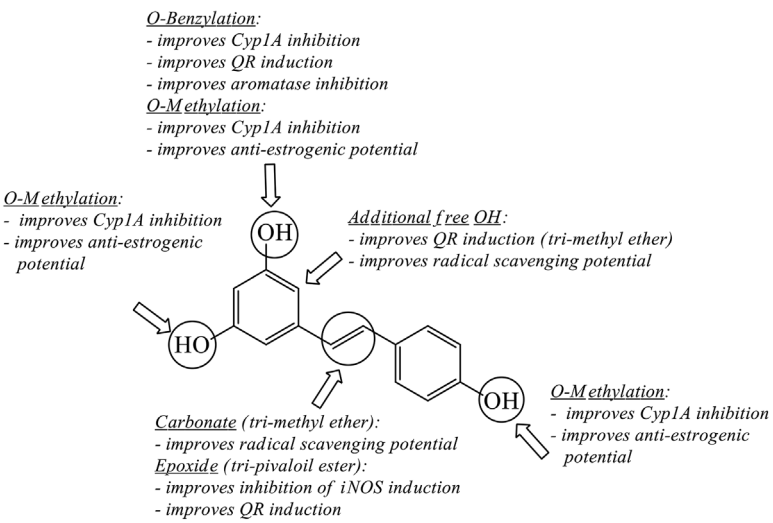


Figure 6. Structural features that improve the biological activity of resveratrol.

resveratrol is a known mixed estrogen (ant)agonist with both estrogenic and anti-estrogenic properties, the tri-methyl derivative **1a** as well as the mixture **24** + **25** demonstrate pure anti-estrogenic activity. Dual estrogen ant-/agonist activity was restored in the mono-benzyl-substituted **4b** and **5b**. The latter, with free OH-groups in 4′- and 5-position, was about eightfold more active than resveratrol in aromatase (Cyp19) inhibition and it also possessed estrogenic potential in the Ishikawa cell line. Interestingly, with respect to aromatase inhibition (which provided the highest number of actives), the benzyl-substituted series was more potent than the methyl-substituted derivatives of resveratrol. Overall, the epoxide derivative **7c** was identified as a potent inducer of phase 2 enzymes concomitant with inhibition of LPS-mediated iNOS induction. Our study points to potential cancer chemopreventive activity of this resveratrol derivative compound and warrants its further investigation.

Experimental

Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and at 400 MHZ on Bruker Instruments (AC 300 Ultrashield™ 400), using deuterochloroform solutions, unless otherwise specified. EIMS spectra were recorded with a Fison-VG Autospec-M246 spectrometer. ESIMS spectra were recorded with a Q-TofMicro (Waters). Melting points were obtained by using a Buchi 535 apparatus. Elemental analyses were determined on a PerkinElmer 240 Elemental Analyzer. Column chromatographies were performed on silica gel Merck Kieselgel 60 (230–400 mesh ASTM). Thin-layer chromatographies were performed on silica gel plates (60 F_{254}, Merck): zones were detected visually by ultraviolet irradiation (254 nm) or by spraying with methanol/H_2SO_4 9:1, followed by heating at 100°C. Compounds **1–5** [3] and **6a [**8a] were synthesized as reported in the literature.

Experimental protocols for the synthesis of compounds **6**–**29** and for the determination of cancer chemopreventive activities [23–25] are reported in the Supporting Information. The InChI codes of the investigated compounds are also provided in the Supporting Information.

*2-Hydroxy-3,4′,5-tribenzyloxy stilbene (**6a**) [8a]*
Colorless crystals; yield: 20%; mp = 99–101°C; ^{1}H NMR δ 7.49 (2H, d, *J* = 8.5 Hz), 7.4 (1H, d, *J* = 16.0 Hz), 7.05 (1H, d, *J* = 16.0 Hz), 6.9 (2H, d, *J* = 8.5 Hz), 6.7 (1H, d, 2.5 Hz), 6.45 (1H, d, *J* = 2.5 Hz), 5.5 (1H, bs), 3.8 (s, 9 H); EI-MS, *m/z*: 286 (M^+). Spectroscopic data are in agreement with those reported in the literature.

*2-Hydroxy-3,4′,5-tribenzyloxy stilbene (**6b**) [8b]*
Colorless amorphous powder; yield: 23%; ^{1}H NMR ($CDCl_3$) δ (ppm) δ 7.49 (2H, d, *J* = 8.5 Hz), 7.3 (2H, d, *J* = 16.5 Hz), 7.03 (1H, d, *J* = 16.5 Hz), 6.9 (2H, d, *J* = 8.5 Hz), 6.7 (1H, d, 2.5 Hz), 6.45 (1H, d, *J* = 2.5 Hz), 5.4 (1H, bs), 4.91 (s, 4H), 4.61 (s, 2H). EI-MS, *m/z*: 514 (M^+).

*3,4′,5-Tri-O-pivaloyl resveratrol oxide (**7c**)*
Colorless crystals; yield: 95%; mp: 63°C; ^{1}H NMR δ 7.48 (d, 2H, *J* = 9.0 Hz), 7.1 (d, 2H, *J* = 9.0 Hz), 6.99 (d, 2H, *J* = 1.9 Hz), 6.83 (t, 1H, *J* = 1.9 Hz), 3.86 (s, 2H), 1.4 (s, 27H). ^{13}C NMR δ 9 × 27.3 (q), 3 × 39.1 (s), 2 × 67.0 (d), 115.0 (s), 2 × 116.0 (s), 2 × 122.0 (d), 2 × 126.0 (s), 134.0 (s), 2 × 139.0 (s), 152.0 (s), 153.9 (s), 3 × 154 (s); EI-MS, *m/z*: 496 (M^+), 383 ($M^+ - Me_3C - Me_2C{=}CH_2$). Anal. calcd. for $C_{29}H_{36}O_7$: C, 70.00; H, 7.00. Found: C, 69.74; H, 7.02.

*3,4′,5-Tri-O-acetyl resveratrol oxide (**7d**)*
Colorless crystals; yield: 93%; mp = 165–167°C; ^{1}H NMR δ 7.4 (2H, d, *J* = 9.0 Hz), 7.15 (2H, d, *J* = 9.0 Hz), 7.01 (2H, d, *J* = 2.0 Hz), 6.92 (1H, t, *J* = 2.0 Hz), 3.86 (2H, s), 2.33 (9H, s). EI-MS, *m/z*: 354 ($M^+ - O$). Anal. calcd. for $C_{28}H_{37}O_7$: C, 64.86; H, 4.86. Found: C, 65.04; H, 4.87.

*1-(3′,5′-Dipivaloyloxyphenyl)-2-(4″-pivaloyloxyphenyl)-1-hydroxy-2-morpholinoethane (**8**)*
Yield: 20%; ^{1}H NMR ($CDCl_3$): δ 7.01 (d, 2H, *J* = 8.8 Hz), 6.86 (d, 2H, *J* = 8.8 Hz), 6.65 (dd, 1H, *J* = 2.0, 2.0 Hz), 6.51 (d, 2H, *J* = 2.0 Hz), 5.28 (d, 1H, *J* = 4.0 Hz), 3.73 (m, 4H), 3.32 (d, 1H, *J* = 4.0 Hz), 2.61 (m, 4H), 1.35 (s, 9H), 1.34 (s, 18 H); EI-MS, *m/z*: 565 ($M^+ - H_2O$), 480 ($M^+ - H_2O - (CH_3)_3CC(O)$), 396 ($M^+ - 3 \times (CH_3)_3CC(O)$), 376. Anal. calcd. for $C_{33}H_{45}O_8N$: C, 67.93; H, 7.72. Found: C, 67.64; H, 7.75.

*1-(4″-Pivaloyloxyphenyl)-2-(3′,5′-dipivaloyloxyphenyl)-1-hydroxy-2-morpholinoethane (**9**)*
(Minor regioisomer, detected in the ^{1}H NMR spectrum). Yield: 7%; ^{1}H NMR ($CDCl_3$) δ (ppm) δ 7.00 (d, 2H, *J* = 8.8 Hz), 6.85 (d, 2H, *J* = 8.8 Hz), 6.71 (dd, 1H, *J* = 2.0, 2.0 Hz), 6.59 (d, 2H, *J* = 2.0 Hz), 5.31 (d, 1H, *J* = 4.0 Hz), 3.73 (m, 4H), 3.28 (d, 1H, *J* = 4.0 Hz), 2.61 (m, 4H), 1.35 (s, 9H), 1.34 (s, 18H).

*1-(3′-Pivaloyloxy-5′-hydroxyphenyl)-2-(4″-pivaloyloxyphenyl)-1-hydroxy-2-morpholinoethane (**10**)*
Yield: 28%, ^{1}H NMR ($CDCl_3$) δ 7.01 (d, 2H, *J* = 8.8 Hz), 6.76 (d, 2H, *J* = 8.8 Hz), 6.35 (d, 2H, *J* = 2.0 Hz), 5.85 (dd, 1H, *J* = 2.0, 2.0 Hz), 5.2 (d, 1H, *J* = 5.8 Hz), 3.73 (m, 4H), 3.30 (d, 1H, *J* = 5.8 Hz), 2.63 (m, 4H), 1.35 (s, 18 H); EI-MS, *m/z*: 481 ($M^+ - H_2O$), 396 ($M^+ - H_2O - (CH_3)_3CC{=}O$), 376. Anal. calcd. for $C_{28}H_{37}O_7N$: C, 67.34; H, 7.42. Found: C, 67.54; H, 7.44.

*1-(4″-Pivaloyloxyphenyl)-2-(3′-pivaloyloxy-5′-hydroxyphenyl)-1-hydroxy-2-morpholinoethane (**11**)*
(Minor regioisomer, detected in the ^{1}H NMR spectrum). Yield: 9%; ^{1}H NMR ($CDCl_3$): δ 7.00 (d, 2H, *J* = 8.8 Hz), 6.85 (d, 2H, *J* = 8.8 Hz), 6.42 (dd, 1H, *J* = 2.0 Hz), 6.38 (dd, 1H, *J* = 2.0, 2.0 Hz), 6.00 (dd, 1H, *J* = 2.0 Hz), 5.3 (d, 1H, *J* = 5.8 Hz), 3.73 (m, 4H), 3.18 (d, 1H, *J* = 5.8 Hz), 2.63 (m, 4H), 1.35 (s, 18H).

*1-(3′,5′-Dipivaloyloxyphenyl)-2-(4″-pivaloyloxyphenyl)-1-oxo-2-morpholinoethane (**12**)*
Yield: 60%; ^{1}H NMR ($CDCl_3$): δ 7.68 (d, 2H, *J* = 2.0 Hz), 7.48 (d, 2H, *J* = 8.8 Hz), 7.05 (d, 2H, *J* = 8.8 Hz), 7.03 (dd, 1H, *J* = 2.0, 2.0 Hz), 4.90 (s, 1H), 3.73 (m, 4H), 2.52 (m, 4H), 1.35 (s, 9H), 1.34 (s, 18H); EI-MS, *m/z*: 581 (M^+), 292 (1-(3,5-dipivaloyloxyphenyl)-1-

morpholino-CH), 208 (292–CH_2=CMe_2–Me–CO). Anal. calcd. for $C_{33}H_{43}O_8N$: C, 68.16; H, 7.40. Found: C, 68.34; H, 7.42.

*1-(4″-Pivaloyloxyphenyl)-2-(3′,5′-dipivaloyloxyphenyl)-1-oxo-2-morpholinoethane (**13**)*
Yield: 20%; ^{1}H NMR ($CDCl_3$): δ 8.09 (d, 2H, *J* = 8.8 Hz), 7.12 (d, 2H, *J* = 8.8 Hz), 7.05 (d, 2H, *J* = 2.0 Hz), 6.85 (dd, 1H, *J* = 2.0, 2.0 Hz), 4.81 (s, 1H), 3.73 (m, 4H), 2.52 (m, 4H), 1.35 (s, 9H), 1.34 (s, 18H); EI-MS, *m/z*: 581 (M^+), 376 (1-(3,5-dipivaloyloxyphenyl)-1-morpholino-CH), 305 (376–CH_2=CMe_2–Me), 276 (376–O=$CCMe_3$–Me).

*1-(3′-Pivaloyloxy-5′-hydroxyphenyl)-2-(4″-pivaloyloxyphenyl)-1-oxo-2-morpholinoethane (**14**)*
Colorless solid; Yield: 60%; ^{1}H NMR ($CDCl_3$): δ 7.48 (d, 2H, *J* = 8.8 Hz), 7.05 (d, 2H, *J* = 8.8 Hz), 6.78 (d, 2H, *J* = 2.0 Hz), 6.5 (dd, 1H, *J* = 2.0, 2.0 Hz) 4.91 (s, 1H), 3.75 (m, 4H), 2.52 (m, 4H), 1.35 (s, 18 H); EI-MS, *m/z*: 497 (M^+). Anal. calcd. for $C_{28}H_{35}O_7N$: C, 67.61; H, 7.04. Found: C, 67.84; H, 7.06.

*1-(4″-Pivaloyloxyphenyl)-2-(3′-pivaloyloxy-5′-hydroxyphenyl)-1-oxo-2-morpholinoethane (**15**)*
Yield: 20%; ^{1}H NMR ($CDCl_3$): δ 8.07 (d, 2H, *J* = 8.8 Hz), 7.42 (d, 2H, *J* = 2.0 Hz), 7.12 (d, 2H, *J* = 8.8 Hz), 6.72 (dd, 1H, *J* = 2.0, 2.0 Hz), 4.81 (s, 1H), 3.73 (m, 4H), 2.52 (m, 4H), 1.34 (s, 18H).

*(1S,2S)-1-(3′,5′-Dimethoxyphenyl)-2-(4″-methoxyphenyl)-1,2-dihydroxyethane (**16a**)*
Colorless oil. Yield: 79%; $[\alpha]_D^{25}$ −90.57° ($CHCl_3$, c 0.1); ^{1}H NMR ($CDCl_3 + D_2O$): δ 7.10 (d, 2H, *J* = 8.8 Hz), 6.80 (d, 2H, *J* = 8.8 Hz), 6.32 (dd, 1H, *J* = 2.0, 2.0 Hz), 6.29 (d, 2H, *J* = 2.0 Hz), 4.64 (bs, 2H), 3.78(s, 3H), 3.70 (s, 6H). ^{13}C NMR ($CDCl_3$) δ_C (ppm): 2 × 160.42 (s), 159.262 (s), 142.55 (s), 132.19 (s), 2 × 128.12 (d), 2 × 113.562 (d), 2 × 104.90 (d), 100.05 (d), 78.02 (d), 77.75 (d), 3 × 55.23 (q); EI-MS, *m/z*: 304 (M^+). Anal. calcd. for $C_{17}H_{20}O_5$: C, 67.10; H, 6.58. Found: C, 67.36; H, 6.60.

*(1S,2S)-1-(3′,5′-Benzyloxyphenyl)-2-(4″-benzyloxyphenyl)-1,2-dihydroxyethane (**16b**)*
Colorless oil. Yield: 78%; ^{1}H NMR ($CDCl_3$): δ 7. 48 (m, 15H), 7.06 (d, 2H, *J* = 8.8 Hz), 6.87 (d, 2H, *J* = 8.8 Hz), 6.51 (dd, 1H, *J* = 2.0, 2.0 Hz), 6.40 (d, 2H, *J* = 2.0 Hz), 5.04 (s, 2H), 4.91 (s, 4H), 4.61 (s, 2H). ^{13}C NMR ($CDCl_3$): δ 2 × 159.67 (s), 158.56 (s), 2 × 142.39 (s), 2 × 136.85 (s), 3 × 132.35 (s), 15 × 127.99 (d), 2 × 127.48 (d), 2 × 114.55 (d), 2 × 106.12 (d), 101.96 (d), 79.1 (d), 78.5 (d), 3 × 70.06 (t); EI-MS, *m/z*: 532 (M^+). Anal. calcd. for $C_{35}H_{32}O_5$: C, 78.95; H, 6.02. Found: C, 78.65; H, 6.02.

*bis-MTPA-ester of (1S,2S)-1-(3′,5′-dimethoxyphenyl)-2-(4″-methoxyphenyl)-1,2-dihydroxyethane (**17**)*
Colorless amorphous solid; yield: 92%; $[\alpha]_D = -35.46$ ($CHCl_3$, c = 1.71); ^{1}H NMR ($CDCl_3$): δ 7.38 (2H, t, *J* = 8 Hz), 7.30–7.21 (6H), 7.10 (d, 2H, *J* = 8.0 Hz), 6.77 (d, 2H, *J* = 8.0 Hz), 6.37 (t, 1H, *J* = 4.0 Hz), 6.31 (d, 2H, *J* = 4.0 Hz), 6.17 and 6.13 (AB system, 2H, *J* = 8.0 Hz), 3.78 (s, 3H), 3.70 (s, 6H), 3.44 (s, 3H), 3.40 (s, 3H). ^{13}C NMR ($CDCl_3$), 166.52 (s), 161.35 (s), 160.56 (s), 137.94 (s), 132.25 (s), 130.30 (d), 129. 36 (d), 129.00 (d), 128.20 (d), 127.7 (s), 125.39 (s), 122.52 (s), 114.51 (d), 105.64 (d), 102.04 (d), 80.19 (d), 79.95 (d), 55.93 (q). ^{19}F NMR ($CDCl_3$), −72.15 (s, 3F), −71.95 (s, 3F). ESIMS *m/z*: 759 (M^++Na), 525 [759–PhC(CF_3)(OCH_3)C(O)OH)].

*bis-MTPA-ester of (1R,2R)-1-(3′,5′-dimethoxyphenyl)-2-(4″-methoxyphenyl)-1,2-dihydroxyethane (**19**)*
Colorless amorphous solid; yield: 88%; $[\alpha]_D = -28.41$; ^{1}H NMR ($CDCl_3$); δ 7.4–7.25 (10 H), 7.09 (d, 2H, *J* = 8.0 Hz), 6.76 (d, 2H, *J* = 8.0 Hz), 6.33 (t, 1H, *J* = 4.0 Hz), 6.30 (d, 2H, *J* = 4.0 Hz), 6.29 and 6.25 (AB system, 2H, *J* = 8.0 Hz), 3.78 (s, 3H), 3.70 (s, 6H), 3.38 (s, 3H), 3.36 (s, 3H). ^{13}C NMR ($CDCl_3$), 166.24 (s), 161.28 (s), 160.55 (s), 137.53 (s), 132.44 (s), 130.23 (d), 130.18 (d), 129.52 (d), 128.98 (d), 128.29 (d), 128.19 (d), 127.32 (s), 125.24 (s), 122.38 (s), 114.42 (d), 105.83 (d), 102.00 (d), 79.34 (d), 79.09 (d), 55.89 (q). ^{19}F NMR ($CDCl_3$), −71.87 (s, 3F), −71.90 (s, 3F). ESIMS *m/z*: 759 (M^++Na), 525 [759–PhC(CF_3)(OCH_3)C(O)OH)].

*(1S,2S)-1-(3′-Hydroxy,5′-methoxyphenyl)-2-(4″-hydroxyphenyl)-1,2-dihydroxyethane (**20**)*
Colorless oil; yield: 55%; ^{1}H NMR (DMSO-d_6): δ 9.75 (bs, 1H, exchanged with D_2O), 9.23 (bs, 1H, exchanged with D_2O), 6.97 (s, 1H), 6.93 (s, 1H), 6.88 (d, 2H, *J* = 8.8 Hz), 6.51 (d, 2H, *J* = 8.8 Hz), 6.49 (dd, 1H, *J* = 2.0, 2.0 Hz), 5.17 (d, 2H, *J* = 2.0 Hz), 3.68 (s, 3H); EI-MS, *m/z*: 276 (M^+). Anal. calcd. For $C_{15}H_{16}O_5$: C, 65.22; H, 5.80. Found: C, C, 65.45; H, 5.81.

*(1S,2S)-1-(3′,5′-Dimethoxyphenyl)-2-(4″-methoxyphenyl)-1,2-dihydroxyethane,1,2-carbonate (**21**)*
Colorless oil. Yield: 90%; $[\alpha]_D^{25}$ −18.44° ($CHCl_3$, c 0.01); ^{1}H NMR ($CDCl_3$): δ 7.26 (d, 2H, *J* = 8.8 Hz), 6.97 (d, 2H, *J* = 8.8 Hz), 6.48 (dd, 1H, *J* = 2.0, 2.0 Hz), 6.40 (d, 2H, *J* = 2.0 Hz), 5.32 and 5.38 (2H, AB system, *J* = 10.7 Hz), 3.84 (s, 3H), 3.77 (s, 6H). ^{13}C NMR ($CDCl_3$) δ_C (ppm): 3 × 55.28 (q), 84.95 (d), 85.29 (d), 94.76 (d), 101.06 (d), 2 × 103.48 (d), 2 × 114.15 (s), 2 × 114.49 (d), 127.97 (d), 128,30. MS, *m/z*: 330 (M^+). Anal. calcd. for $C_{18}H_{18}O_6$: C, 65.46; H, 546. Found: C, 65.35, H, 5.44.

*(1S,2R)-1-(3′,5′-Dimethoxyphenyl)-2-(4″-methoxyphenyl)-1-hydroxy,2-azidoethane (**22**)*
Yield: 54%; ^{1}H NMR ($CDCl_3$): δ 7.24 (d, 2H, *J* = 8.8 Hz), 6.92 (d, 2H, *J* = 8.8 Hz), 6.46 (d, 2H, *J* = 2.0 Hz), 6.42 (dd, 1H, *J* = 2.0, 2.0 Hz), 4.73 (bd, 1H, *J* = 10.0 Hz), 4.63 (d, 1H, *J* = 10.0 Hz), 3.84 (s, 3H), 3.78 (s, 6H), 2.1 (bs, 1H, disappears with D_2O). EI-MS (positive mode): 286 ($M^+ - HN_3$), 283 ($M^+ - H_2O - N_2$), 270 ($M^+ - OH - N_3$), 257 ($M^+ - 2 \times Me - N_3$). Anal. calcd. for $C_{17}H_{19}N_3O_4$: C, 62.01; H, 5.78. Found: C, 62.25; H, 5.80.

*(1S,2R)-1-(4″-Methoxyphenyl)-2-(3′,5′-dimethoxyphenyl)-1-hydroxy,2-azidoethane (**23**)*
Yield: 21%; ^{1}H NMR ($CDCl_3$): δ 7.22 (d, H, *J* = 8.8 Hz), 6.91 (d, 2H, *J* = 8.8 Hz), 6.46 (d, 2H, *J* = 2.0 Hz), 6.42 (dd, 1H, *J* = 2.0, 2.0 Hz), 4.73 (bd, 1H, *J* = 10.0 Hz), 4.55 (d, 1H, *J* = 10.0 Hz), 3.84 (s, 3H), 3.76 (s, 6H), 1.6 (bs, 1H, disappears with D_2O).

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EI-MS (positive mode): 286 ($M^+ - HN_3$), 283 ($M^+ - H_2O - N_2$), 270 ($M^+ - OH - N_3$), 257 ($M^+ - 2 \times Me - N_3$).

*(1S,2R)-1-(3′,5′-Dimethoxyphenyl)-2-(4″-methoxyphenyl)-1-hydroxy,2-aminoethane (**24**)*
Colorless amorphous solid. Yield: 61%; $[\alpha]_D^{25}$ −27.5 ($CHCl_3$, c 0.1); ^{1}H NMR ($CDCl_3$): δ 7.19 (d, 2H, $J = 8.8$ Hz), 6.84 (d, 2H, $J = 8.8$ Hz), 6.38 (d, H, $J = 2.0$ Hz), 6.37 (dd, 1H, $J = 2.0$, 2.0 Hz), 4.68 (d, 1H, $J = 6.0$ Hz), 4.69 (d, 1H, $J = 6.0$ Hz), 3.79 (s, 3H), 3.71 (s, 6H), 1.92 (1H, bs, disappears with D_2O); ^{13}C NMR ($CDCl_3$): δ 3 × 55.18 (s), 61.06 (s), 78.0 (s), 100.11 (d), 2 × 104.74 (d), 2 × 113.67 (d), 2 × 128.69 (d), 123.67 (s), 143.11 (s), 159.19 (s), 2 × 160.52 (s); EI-MS, *m/z*: 136 ($M^+ - (MeO)_2C_6H_3CHOH$)). Anal. calcd. for $C_{17}H_{21}NO_4$: C, 67.33; H, 6.93. Found: C, 67.59; H, 6.95.

*(1S,2R)-1-(4″-Methoxyphenyl)-2-(3′,5′-dimethoxyphenyl)-1-hydroxy,2-aminoethane (**25**)*
(Minor isomer, detected in the NMR spectrum). Yield: 25%; ^{1}H NMR ($CDCl_3$): δ 7.19 (2H, dd, $J = 8.6$, 1.0 Hz), 6.83 (2H, dd, $J = 8.6$, 1.0 Hz), 6.45 (1H, d, $J = 2.0$ Hz), 6.35 (1H, dd, $J = 2.0$, 2.0 Hz), 4.66 (1H, d, $J = 7.1$ Hz), 4.10 (1H, d, $J = 7.1$ Hz), 3.78 (3H, s), 3.74 (3H, s), 1.92 (1H, bs, disappears with D_2O).

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(Major regioisomer). Yield: 70%; ^{1}H NMR ($CDCl_3$): δ 6.91 (d, 2H, $J = 8.8$ Hz), 6.68 (d, 2H, $J = 8.8$ Hz), 6.20 (dd, 1H, $J = 2.0$, 2.0 Hz), 6.13 (d, 2H, $J = 2.0$ Hz), 5.82 (d, 1H, $J = 8.0$ Hz), 5.09 (d, 1H, $J = 8.0$ Hz), 3.72 (s, 3H), 3.62 (s, 6H); EI-MS, *m/z*: 329 (M^+). Anal. calcd. for $C_{18}H_{19}NO_5$: C, 65.65; H, 5.78. Found: C, 65.83; H, 5.80.

27
(Minor regioisomer). Yield: 28%; ^{1}H NMR ($CDCl_3$): δ 6.90 (d, 2H, $J = 8.8$ Hz), 6.65 (d, 2H, $J = 8.8$ Hz), 6.20 (dd, 1H, $J = 2.0$, 2.0 Hz), 6.13 (d, 2H, $J = 2.0$ Hz), 5.80 (d, 1H, $J = 8.0$ Hz), 5.00 (d, 1H, $J = 8.0$ Hz), 3.72 (s, 3H), 3.62 (s, 6H).

*(2R)-1-(3′,5′-Dimethoxyphenyl)-2-(4″-methoxyphenyl)-1-oxo-2-aminoethane (**28**)*
Colorless oil; yield: 43%; ^{1}H NMR ($CDCl_3$) δ: 7.60 (d, 2H, $J = 8.8$ Hz), 6.98 (d, 2H, $J = 2.0$ Hz), 6.96 (d, 2H, $J = 8.8$ Hz), 6.72 (dd, 1H, $J = 2.0$, 2.0 Hz), 3.90 (s, 1H), 3.86 (s, 3H), 3.83 (s, 6H), 1.64 (bs, 2H, disappears with D_2O). EI-MS, *m/z*: 301 (M^+), 286 ($M^+ - Me$), 166 ($(MeO)_2C_6H_3C{=}O$), 135 ($MeOC_6H_4CHNH_2$). Anal. calcd. for $C_{17}H_{19}NO_4$: C, 67.77; H, 6.31. Found: C, 67.63; H, 6.33.

*(2R)-1-(4″-Methoxyphenyl)-2-(3′,5′-dimethoxyphenyl)-1-oxo,2-aminoethane (**29**)*
(Minor regioisomer). Yield: 17%; ^{1}H NMR ($CDCl_3$): δ 7.84 (d, 2H, $J = 8.8$ Hz), 6.94 (d, 2H, $J = 8.8$ Hz), 6.77 (d, 2H, $J = 2.0$ Hz), 6.66 (dd, 1H, $J = 2.0$, 2.0 Hz), 3.86 (s, 1H), 3.85 (s, 3H), 3.81 (s, 6H), 1.64 (bs, 2H, disappears with D_2O).

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