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Resveratrol analogues as selective cyclooxygenase-2 inhibitors: synthesis and structure–activity relationship

Marek Murias,[a] Norbert Handler,[a] Thomas Erker,[a] Karin Pleban,[a] Gerhard Ecker,[a] Philipp Saiko,[b] Thomas Szekeres[b] and Walter Jäger[a,*]

[a]*Institute of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*
[b]*Clinical Institute for Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Währinger, Gürtel 18–20, A-1090 Vienna, Austria*

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Abstract—Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is found in grapes and various medical plants. Among cytotoxic, antifungal, antibacterial cardioprotective activity resveratrol also demonstrates non-selective cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibition. In order to find more selective COX-2 inhibitors a series of methoxylated and hydroxylated resveratrol derivatives were synthesized and evaluated for their ability to inhibit both enzymes using in vitro inhibition assays for COX-1 and COX-2 by measuring PGE_2 production. Hydroxylated but not methoxylated resveratrol derivatives showed a high rate of inhibition. The most potent resveratrol compounds were 3,3′,4′,5-tetra-*trans*-hydroxystilbene (COX-1: $IC_{50} = 4.713$, COX-2: $IC_{50} = 0.0113\,\mu M$, selectivity index = 417.08) and 3,3′,4,4′,5,5′-hexa-hydroxy-*trans*-stilbene (COX-1: $IC_{50} = 0.748$, COX-2: $IC_{50} = 0.00104\,\mu M$, selectivity index = 719.23). Their selectivity index was in part higher than celecoxib, a selective COX-2 inhibitor already established on the market (COX-1: $IC_{50} = 19.026$, COX-2: $IC_{50} = 0.03482\,\mu M$, selectivity index = 546.41). Effect of structural parameters on COX-2 inhibition was evaluated by quantitative structure–activity relationship (QSAR) analysis and a high correlation was found with the topological surface area TPSA ($r = 0.93$). Docking studies on both COX-1 and COX-2 protein structures also revealed that hydroxylated but not methoxylated resveratrol analogues are able to bind to the previously identified binding sites of the enzymes. Hydroxylated resveratrol analogues therefore represent a novel class of highly selective COX-2 inhibitors and promising candidates for in vivo studies.

1. Introduction

The enzyme cyclooxygenase (COX) catalyzes the first two steps in the biosynthesis of prostaglandins (PGs) from the substrate arachidonic acid. At least two forms of this enzyme exist.[1] One of these forms, COX-1, is constitutively expressed and is responsible for maintaining normal physiologic function and the PGs produced by this enzyme play a protective role. The other known form of the enzyme, cyclooxygenase-2 (COX-2), is an inducible form and its expression is affected by various stimuli such as mitogens, oncogenes, tumor promoters, and growth factors.[2]

COX-2 is the principal isoform that participates in inflammation, and induction of COX-2 is responsible for the production of PGs at the site of inflammation. Consequently, selective inhibition of COX-2 should have therapeutic actions similar to those of non-steroidal anti-inflammatory drugs (NSAIDs), but without gastrointestinal side effects, which are being caused as a consequence of COX-1 inhibition. Several selective COX-2 inhibitors are currently used in the clinics (e.g. celecoxib, valdecoxib, parecoxib, rofecoxib, etoricoxib, and lumiracoxib) which provide effective treatment of inflammatory disease states such as rheumatoid arthritis and osteoarthritis.[3] Several lines of evidence suggest that selective COX-2 inhibitors may also provide an opportunity for both cancer prevention and therapy.[4] Furthermore, promising in vitro data also indicate that treatment with selective COX-2 inhibitors may also reduce the risk of Alzheimer's[5,6] and Parkinson's disease[7,8] and may also be effective in the treatment of asthma.[9]

Keywords: Resveratrol; Nonsteroidal anti-inflammatory drugs; Cyclooxygenase-1; Cyclooxygenase-2; QSAR.
* Corresponding author. Tel.: +43 1 4277 55176; fax: +43 1 4277 9551; e-mail: walter.jaeger@univie.ac.at

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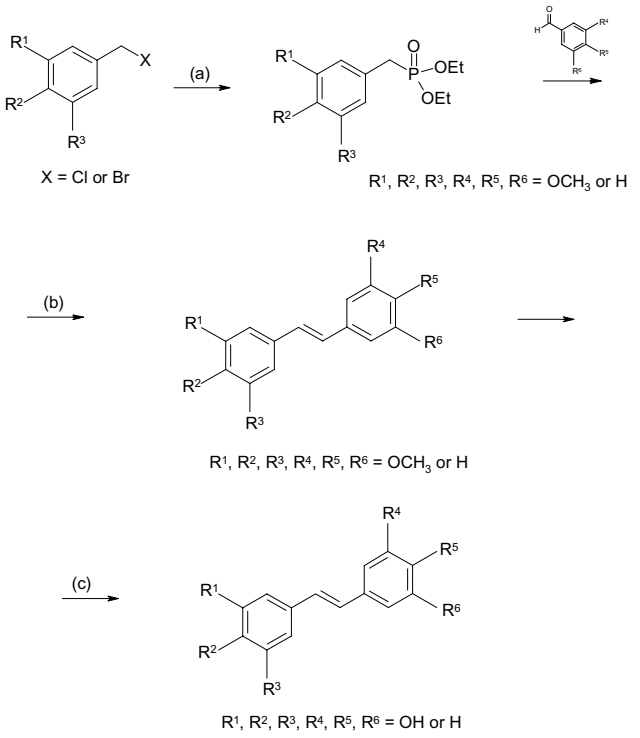
Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) is a phytoalexin found mainly in the skin of grapes and red wine and demonstrates anti-inflammatory, cardiovascular protective, and cancer chemopreventive properties.[10] It has also been shown to be a non-selective inhibitor of COX-1 and COX-2.[11] In vitro and in vivo, resveratrol is extensively metabolized to several glucuronides and sulfates.[12,13] Moreover, resveratrol undergoes cytochrome P450 catalyzed hydroxylation to piceantannol, 3,4,4′,5-tetrahydroxy-*trans*-stilbene, and to two other unidentified mono- and dihydroxyresveratrol analogues.[14] As piceatannol[15] demonstrates a several fold higher antileukaemic activity than resveratrol and as COX-2 is a known target for anticancer activity we investigated whether piceatannol and other hydroxylated resveratrol analogues might achieve a better and more selective COX-2 inhibition than resveratrol. Five hydroxy and six methoxy analogues of resveratrol were therefore synthesized using standard chemical methods. Each analogue was then tested for COX-1 and COX-2 inhibition in an in vitro model and the resulting inhibition values compared with that of resveratrol and the clinically established selective COX-2 inhibitor celecoxib. In addition, a quantitative structure–activity relationship (QSAR) study was being conducted to evaluate the effects of various structural parameters of the molecules on COX-1 and COX-2 inhibition.



Scheme 1. General synthetic route for synthesis of the resveratrol analogues **1**–**6** and **8**–**12**. Reagents and conditions: (a) $P(OEt)_3$, 130 °C; (b) NaOMe, DMF, 100 °C; (c) BBr_3, CH_2Cl_2, rt.

2. Results and discussion

2.1. Chemistry

Methoxylated (**1**–**6**) and hydroxylated (**8**–**12**) resveratrol analogues were synthesized using standard chemical methodologies (resveratrol (**7**) was obtained from a commercial supplier and was of analytical grade). The corresponding benzylphosphonates were obtained from benzylhalides via Michaelis–Arbuzov rearrangement with triethyl phosphite at 130 °C. The following Horner–Wadsworth–Emmons reactions were carried out at 100 °C with the phosphonate, the corresponding methoxybenzaldehyde and sodiummethoxide in DMF to yield the desired methoxystilbenes. Finally, demethylation of the phenolic ethers was successfully established with boron tribromide at room temperature (Scheme 1). Those procedures afforded all compounds in a good yield (Table 1). Melting points of compounds **1**–**6** and **8**–**12** were consistent with those found in the literature.[16–23] However, physicochemical characterization of compounds **1**–**6** and **8**–**12** in the literature was incomplete. Missing data of mass spectroscopy, ^1H NMR or ^{13}C NMR were now included in the chemistry part.

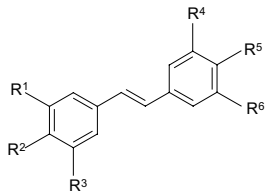
2.2. Inhibition of COX-1 and COX-2

Inhibition of COX-1 and COX-2 by resveratrol analogues was analyzed in a cell-free immunoassay system. Purified ovine enzyme served as the source of COX-1, while the human recombinant enzyme formed the source of COX-2. The inhibition of COX-1 by resveratrol analogues is shown in Table 2. The methoxy derivatives **1**–**6** were weak inhibitors of COX-1 with 2–55 fold lower IC_{50}-values than resveratrol (**7**, IC_{50}: 0.53 μM). On the other hand, hydroxylated resveratrol analogues, especially **9** and **11** were more potent inhibitors with IC_{50}-values of 0.00998 and 0.01027 μM, respectively. Celecoxib showed only a moderate inhibition (IC_{50}: 19.026 μM).

The present study revealed that all hydroxylated analogues were also more potent against COX-2 than resveratrol (**7**), (Fig. 1). Compounds **9**–**12** showed even lower IC_{50}-values (IC_{50s}: of 0.00171, 0.0113, 0.00138, 0.001 and 0.035 μM, respectively) which is up to 35-fold lower than for the selective COX-2 inhibitor celecoxib (Table 2).

Based on the IC_{50} values for COX-1 and COX-2, the relative ratios of IC_{50} COX-1/IC_{50} COX-2 were calculated and the data are presented in Table 2. Resveratrol (**7**) showed a weak COX-2 inhibition with a selectivity index (COX-1/COX-2) of 0.5. The COX-2/COX-1 ratios obtained for compounds **10** and **12** (417 and 719), however, are comparable or significantly better than that obtained for the clinically used selective COX-2 inhibitor celecoxib (selectivity index: 546) (Table 2). Compounds **2**–**6**, **8**, **9**, and **11** were also more selective against COX-2 than to COX-1 with COX-1/COX-2 selectivity index values up to 45. The selectivity index of 0.54 for resveratrol (**7**) is comparable with data found by others.[11] Piceatannol (**10**), a natural compound found in grapes and red wine, showed the second best selectivity index from all compound tested. This data, however, strongly differs from investigations performed

Table 1. Structures of the resveratrol analogues **1**–**12**, **DL1**, and **DL2**



Compd.	Pos. 3 (= R^1)	Pos. 4 (= R^2)	Pos. 5 (= R^3)	Pos. 3′ (= R^4)	Pos. 4′ (= R^5)	Pos. 5′ (= R^6)	Yield (%)
1	$-OCH_3$	–H	$-OCH_3$	–H	$-OCH_3$	–H	59
2	$-OCH_3$	$-OCH_3$	$-OCH_3$	–H	$-OCH_3$	–H	55
3	$-OCH_3$	–H	$-OCH_3$	$-OCH_3$	–H	$-OCH_3$	52
4	$-OCH_3$	–H	$-OCH_3$	$-OCH_3$	$-OCH_3$	–H	55
5	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	–H	$-OCH_3$	59
6	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	49
7	–OH	–H	–OH	–H	–OH	–H	Ns
8	–OH	–OH	–OH	–H	–OH	–H	55
9	–OH	–H	–OH	–OH	–H	–OH	60
10	–OH	–H	–OH	–OH	–OH	–H	51
11	–OH	–OH	–OH	–OH	–H	–OH	57
12	–OH	–OH	–OH	–OH	–OH	–OH	45
DL1[a]	–OH	–H	$-OCH_3$	–H	–OH	–H	Ns
DL2[a]	$-OCH_3$	–H	$-OCH_3$	–H	–OH	–H	Ns

Ns: not synthesized.
[a] Structures were taken from Ref. 11.

Table 2. Inhibitory effect of **1**–**12**, **DL1**, **DL2**, and the reference compound celecoxib on COX-1 and COX-2 activity

Compound	IC_{50} [μM]		Selectivity index COX-1/COX-2
	COX-1	COX-2	
1	1.228	1.667	0.74
2	9.099	7.797	1.17
3	27.783	1.575	17.64
4	2.834	0.796	3.56
5	7.247	0.514	14.11
6	11.348	0.355	31.97
7	0.535	0.996	0.54
8	2.072	0.04537	45.67
9	0.00998	0.00171	5.83
10	4.713	0.0113	417.08
11	0.01027	0.00138	7.44
12	0.748	0.00104	719.23
Celecoxib	19.026	0.03482	546.41
DL1	4.92[a]	2.21[a]	2.23[a]
DL2	4.84[a]	1.19[a]	4.07[a]

[a] Values were taken from Ref. 11.

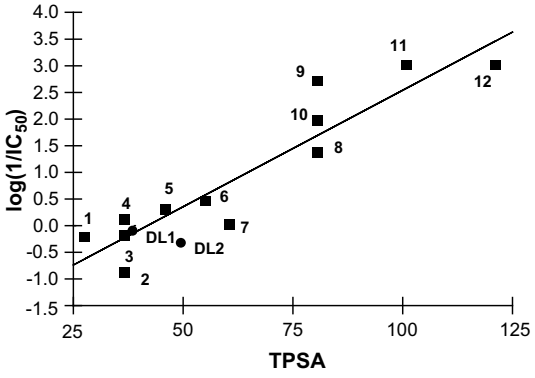


Figure 1. Plot of TPSA-values of compounds **1**–**12**, **DL1** and **DL2** versus log(l/EC_{50})-values for COX-2 activity.

by Lee et al.[24] who described piceatannol as inactive (IC_{50} values >100lg/mL) in both the COX-1 and the COX-2 inhibition assay. This discrepancy might be explained by the fact that the authors used extracted (from the seed of a Peruvian plant) and not unambiguously identified piceatannol. Furthermore, assay conditions might also strongly effect COX-1 and COX-2 inhibition as indicated for the COX-2 selectivity index of celecoxib. While assays based on human whole blood,[25,26] oxidation of TMPD[27] or thin layer chromatography[28] showed values of 7.3, 26.9, and 67, respectively, the selectivity of celecoxib measured by an ELISA assay was 404, which is close to our result of 546.

2.3. QSAR-study

To address the question, whether there exists a quantitative relationships between chemical structure and biological activity, a small set of physicochemical descriptors (log *P*, lipophilicity index; TPSA, topological surface area; MR, molar refractivity; APOL, atom polarizability) were calculated on basis of 2D-structures. Then multiple linear regression analyses were performed. An intercorrelation matrix was calculated and shown in Table 2. A close correlation between MR and APOL was found. This strong relationship was also observed for other data sets analyzed (G. Ecker,

unpublished). However, regarding the biological activity, only for COX-2 a clear relationship to one of the descriptors was found (TPSA; $r = 0.93$). Multiple linear regression analysis with subsequent reduction of the dimensionality of the model via stepwise elimination of statistically insignificant descriptors ($P > 0.05$) confirmed this relationship (Eq. 1).

$$\log(1/IC_{50})_{COX\text{-}2} = 0.044(\pm 0.0056)TPSA - 1.827(\pm 0.392)$$
$$n = 12,\ r = 0.93,\ F = 60.4 \quad (1)$$

As indicated in Eq. 1 and shown in Figure 1, high TPSA values are favorable for high COX-2 activity. In case of COX-1, either APOL or MR are present in the final equation. The MR-based model leads to slightly higher correlation coefficients (0.650 vs 0.649; Eq. 2). In both cases, higher values of the descriptor leads to lower pharmacological activity.

$$\log(1/IC_{50})_{COX\text{-}1} = -0.586(\pm 0.216)MR + 4.449(\pm 1.706)$$
$$n = 12,\ r = 0.65,\ F = 7.34 \quad (2)$$

Figure 2 shows the corresponding plot of MR versus pharmacological activity. Obviously, the relationship is by far less significant than in the case of COX-2. Additionally, the plot of observed versus calculated log(1/EC_{50}) values shows a slope of only 0.44 (Fig. not shown). Interestingly, for the calculation of log(selectivity), TPSA showed to be the most predictive parameter (Eq. 3; Fig. 3).

$$\log(IC_{50}COX\text{-}1/IC_{50}COX\text{-}2) = 0.022(\pm 0.008)TPSA - 0.314(\pm 0.571)$$
$$n = 12,\ r = 0.64,\ F = 7.05 \quad (3)$$

Generally, all significant descriptors, which explain the variance in the data sets describe preferentially polar type of receptor interactions. It has to be noted, that none of the final equations contained log P as descriptor.

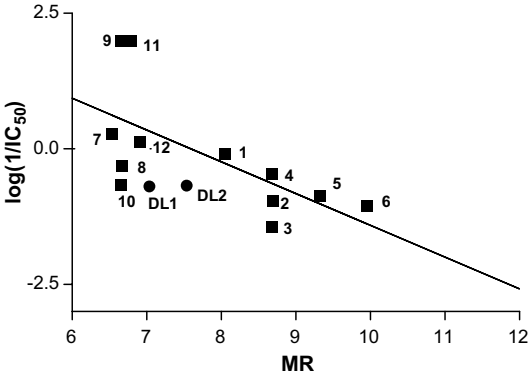


Figure 2. Plot of calculated MR-values of compounds **1**–**12**, **DL1** and **DL2** versus log(1/EC_{50})-values for COX-1 activity.

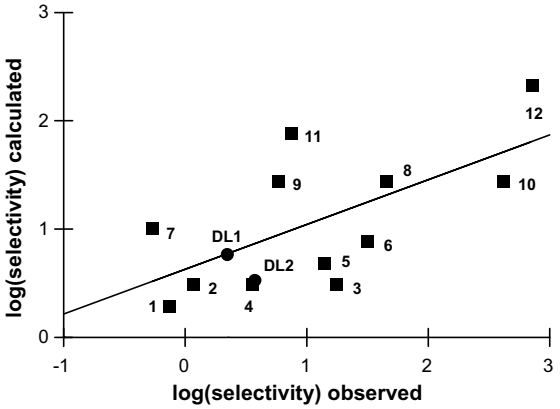


Figure 3. Plot of calculated versus observed log(selectivity) as obtained by Eq. 3.

Thus, for the present data set lipophilicity does not show a significant contribution to either activity or selectivity of the compounds.

In order to further validate our models, two resveratrol analogues (DL1 and DL2), carrying both hydroxy- and methoxy-groups were taken from the literature.[11] These compounds, previously isolated from the stem wood of *Dracaena Loureiri*, also demonstrate COX-1 and COX-2 inhibition. Predictions of biological activities are in good agreement with measured values which gives additional support for the general validity of the model (Figs. 1–3 and Table 3).

2.4. Docking studies

In order to identify possible binding sites of reservatrol analogues, the ligands were docked into the predefined binding sites of COX-1 and COX-2. Several residues have previously been shown to be involved in binding of selective COX-2 inhibitors, including His90, Arg120, Phe518, Tyr385, and Ser530.[29] For none of the methoxyl compounds, satisfactory solutions into the putative binding sites of either COX-1 or COX-2 were found Table 4.

Table 3. Physicochemical parameters for compounds **1**–**12**, **DL1**, and **DL2**

Compound	MR	APOL	TPSA	log P
1	8.048	44.328	27.690	4.489
2	8.688	48.224	36.920	3.907
3	8.681	48.224	36.920	4.556
4	8.685	48.224	36.920	4.232
5	9.319	52.119	46.150	3.974
6	9.955	56.015	55.380	3.393
7	6.404	34.246	40.460	4.005
8	6.665	35.850	80.920	3.697
9	6.658	35.850	80.920	3.500
10	6.715	37.183	80.920	3.424
11	6.784	36.652	101.150	3.151
12	6.906	37.454	121.380	2.802
DL1	7.040	38.141	49.690	3.961
DL2	7.544	41.235	38.690	4.225

Table 4. Intercorrelation matrix for physicochemical parameters, log(potency) and log(selectivity) values

	APOL	MR	TPSA	log *P*	log COX-1	log COX-2	log Sel
APOL	1						
MR	0.999	1					
TPSA	−0.675	−0.678	1				
log *P*	0.499	0.502	−0.920	1			
log COX-1	−0.649	−0.650	0.566	−0.450	1		
log COX-2	−0.642	−0.645	0.926	−0.793	0.700	1	
log Sel	−0.163	−0.165	0.643	−0.588	−0.152	0.598	1

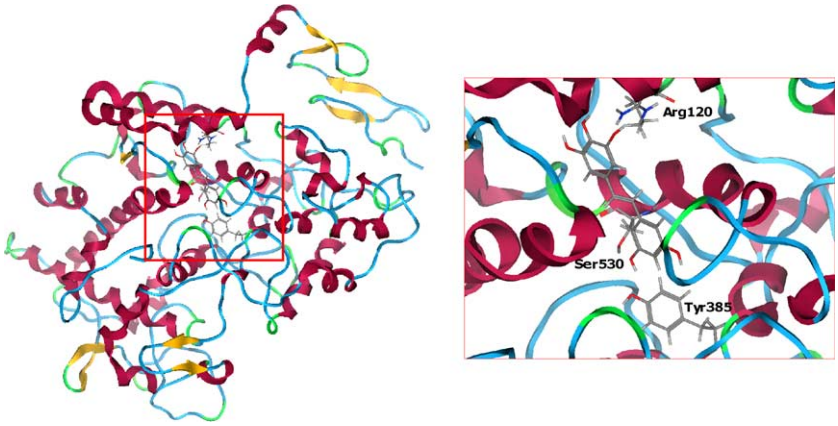


Figure 4. Ribbon drawing of the structure of COX-2 with docked ligand **11**. The helices are colored in red, strands in yellow, loops in blue, and turns in green. Amino acid residues interacting with the ligand are shown. Graph was generated using the MOE software package (Chemical Computing Group, Montreal).

In contrast, all for hydroxylated analogues docked into the binding site of both enzymes. Figure 4 shows a representative ribbon drawing of the crystal structure of COX-2 with compound **11**. The binding mode of hydroxylated resveratrol analogues involves 1–3 hydrogen bonds to the amino acid residues Arg120, Ser530, and Tyr385, which is in accordance to results observed for other NSAIDs.[30] Contrary to the selective COX-2 inhibitor SC-558, the *p*-bromo analogue of celecoxib,[31] compounds **8**–**12** are not able to have access to the additional subpocket (substitution of a valine for an isoleucine in the active site) which is reported to be responsible for COX-2 selectivity. The observed pronounced COX-2 selectivity of compounds **10** and **12** may therefore result either from a different binding mode to COX-2 and/or inhibition of COX-2 related enzymes, for example, peroxidases.[32]

3. Conclusions

In summary, we have demonstrated that hydroxylated resveratrol analogues especially compound **10** and **12** are selective COX-2 inhibitors with potency comparable or better than the clinically established celecoxib. Moreover, hydroxylated resveratrol derivatives also show significantly lower IC_{50} values against COX-2 than celecoxib. This should result in lower doses necessary to achieve the same efficacy in clinical studies. The methoxylated analogues are poor inhibitors of COX-2 activity and do not exhibit COX-2 specificity.

4. Experimental

4.1. Cyclooxygenase assay

The effect of the test compound on COX-1 and COX-2 were determined by measuring prostaglandin E_2 (PGE_2) using a COX Inhibitor Screening Kit (Catalog No 560131) from Cayman Chemicals, Ann Arbor Michigan USA. Reactions mixtures were prepared in 100 mM Tris–HCl buffer, pH 8.0 containing 1 μM heme and COX-1 (ovine) or COX-2 (human recombinant) and preincubated for 10 min in a waterbath (37 °C). The reaction was initiated by the addition of 10 μL arachidonic acid (final concentration in reaction mixture 100 μM). After 2 min the reaction was terminated by adding 1 M HCl and PGE_2 was quantitated by an ELISA method. The test compounds were dissolved in DMSO and diluted to the desired concentration with 100 mM potassium phosphate buffer (pH 7.4). Following transfer to a 96-well plate coated with a mouse anti-rabbit IgG, the tracer prostaglandin acetylcholine esterase and primary antibody (mouse anti PGE_2) were added. Plates were then incubated at room temperature overnight, reaction mixtures were removed, and wells were washed with 10 mM potassium phosphate buffer containing 0.05% Tween 20. Ellman's reagent (200 μL) was added to each well and the plate was incubated at room temperature (exclusion of light) for 60 min, until the control wells yielded an OD = 0.3–0.8 at 412 nm. A standard curve with PGE_2 was generated from the same plate, which was used to quantify the PGE_2 levels produced in the presence of test

samples. Results were expressed as a percentage relative to a control (solvent-treated samples). All determinations were performed in duplicate, and values generally agreed within 10%. Dose–response curves were generated for the calculation of IC_{50} values.

4.2. Molecular modeling studies

Compounds were draw with ISIS Draw and an Accord for Excel database was built. The database was exported as sdf-file and imported into MOE (version 2003.02, Chemical Computing Group). Multiple linear correlation analysis was performed using Microsoft Excel 2000. Descriptors contributing significantly to the equation were retrieved via stepwise selection on the basis of *t*-values (Student *t*-test). As dependent variable log(1/IC_{50}) values were used. In case of selectivity indices, the log[IC_{50}(COX-1)/IC_{50}(COX-2)] was used. Thus, higher values indicate higher selectivity for COX-2.

Docking studies were performed using FlexX[33] as implemented in Sybyl 6.9.[34] The X-ray crystal structures of SC-558 bound to the active site of COX-2 and ibuprofen bound to COX-1 were available in the RCSB Protein Data Bank[35] and used as templates to construct the three-dimensional models of all test compounds. Water molecules and ligands were removed and hydrogen atoms were added to the enzymes. The active sites of COX-1 and COX-2 were defined by a sphere of 7.4 Å around the amino acid residues His90, Leu352, Leu359, and Ala527. Experiments were performed using formal charges whereby the best 30 solutions were inspected.

4.3. Chemistry

All chemicals obtained from commercial suppliers were used as received and were of analytical grade. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian UnityPlus 200 (200 and 50 MHz). Chemical shifts are reported in δ values (ppm) relative to Me_4Si line as internal standard and *J* values are reported in Hertz. Mass spectra were obtained by a Shimadzu GC/MS QP 1000 EX or Hewlett Packard (GC: 5890; MS: 5970) spectrometer. Solutions in organic solvents were dried over anhydrous sodium sulfate.

4.3.1. General procedure for synthesis of methoxylated diethyl benzylphosphonates. The corresponding methoxybenzylhalide (50 mmol) was heated in a flame-dried three-necked-flask with an excess of triethyl phosphite (9.5 mL, 55 mmol) at 130 °C with a $CaCl_2$ tube. After 24 h the mixture was cooled to room temperature and purified by distillation at 4×10^{-3} bar and 110 °C to yield pale oils.

4.3.2. Diethyl (4-methoxybenzyl)phosphonate. Yield: 12.5 g (96%); ^{1}H NMR ($CDCl_3$): δ 7.23–7.18 (m, 2H), 6.84 (d, J = 8.6 Hz, 2H), 4.15–3.93 (m, 4H), 3.78 (s, 3H), 3.09 (d, $J_{H,P}$ = 21.0 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 158.5 ($J_{C,P}$ = 3.4 Hz), 130.6 ($J_{C,P}$ = 6.5 Hz), 123.2 ($J_{C,P}$ = 9.6 Hz), 113.9 ($J_{C,P}$ = 2.7 Hz), 62.1 ($J_{C,P}$ = 6.9 Hz), 55.1, 32.6 ($J_{C,P}$ = 139.1 Hz), 16.2 ($J_{C,P}$ = 6.1 Hz); MS *m*/*z* 258 [M^+, 10%], 121 [100%].

4.3.3. Diethyl (3,5-dimethoxybenzyl)phosphonate. Yield: 13.25 g (92%); ^{1}H NMR ($CDCl_3$): δ 6.47–6.45 (m, 2H), 6.36–6.33 (m, 1H), 4.13–3.96 (m, 4H), 3.77 (s, 6H), 3.09 (d, $J_{H,P}$ = 21.6 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$): 160.6 ($J_{C,P}$ = 3.0 Hz), 133.5 ($J_{C,P}$ = 3.0, 8.8 Hz), 107.7 ($J_{C,P}$ = 6.5 Hz), 99.0 ($J_{C,P}$ = 3.4 Hz), 62.1 ($J_{C,P}$ = 6.5 Hz), 55.2, 33.8 ($J_{C,P}$ = 138.0 Hz), 16.3 ($J_{C,P}$ = 6.1 Hz); MS *m*/*z* 288 [M^+, 40%], 151 [100%].

4.3.4. Diethyl (3,4,5-trimethoxybenzyl)phosphonate. Yield: 14.94 g (94%); ^{1}H NMR ($CDCl_3$): δ 6.54 (d, $J_{H,P}$ = 2.5 Hz, 2H), 4.11–3.97 (m, 4H), 3.85 (s, 6H), 3.82 (s, 3H), 3.09 (d, $J_{H,P}$ = 21.5 Hz, 2H), 1.27 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$): 153.0 ($J_{C,P}$ = 3.0 Hz), 126.9 ($J_{C,P}$ = 9.2 Hz), 106.7 ($J_{C,P}$ = 6.5 Hz), 62.1 ($J_{C,P}$ = 6.5 Hz), 60.8, 55.9, 33.8 ($J_{C,P}$ = 138.7 Hz), 16.3 ($J_{C,P}$ = 6.1 Hz); MS *m*/*z* 318 [M^+, 35%], 181 [100%].

4.3.5. General procedure for synthesis of compounds 1–6. In a flame-dried three-necked-flask the corresponding methoxylated diethyl benzylphosphonate (10 mmol) was cooled to 0 °C under argon. Then 10 mL dry DMF, sodiummethoxide (1.12 g, 20 mmol) and the corresponding methoxylated benzaldehyde (10 mmol) were added. The mixture was stirred at room temperature for 1 h, then heated to 100 °C under argon for 1.5 h. The solution was poured into 250 mL ice-water, the precipitate was filtered off and recrystallized from diluted or pure ethanol to yield white crystals.

4.3.6. 3,4′,5-Trimethoxystilbene (1). Yield: 1.59 g (59%), mp 55–57 °C; ^{1}H NMR ($CDCl_3$): δ 7.43 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 16.3 Hz, 1H), 6.92–6.85 (m, 3H), 6.65–6.64 (m, 2H), 6.38–6.36 (m, 1H), 3.81 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 160.9, 159.3, 139.6, 129.8, 128.6, 127.7, 126.5, 114.0, 104.2, 99.5, 55.3; MS *m*/*z* 270 [M^+, 100%].

4.3.7. 3,4,4′,5-Tetramethoxystilbene (2). Yield: 1.65 g (55%), mp 157 °C; ^{1}H NMR ($CDCl_3$): δ 7.44 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 16.3 Hz, 1H), 6.91–6.83 (m, 3H), 6.71 (s, 2H), 3.90 (s, 6H), 3.86 (s, 3H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 159.2, 153.3, 137.7, 133.3, 129.9, 127.6, 127.5, 126.4, 114.1, 103.2, 60.9, 56.0, 55.2; MS *m*/*z* 300 [M^+, 100%].

4.3.8. 3,3′,5,5′-Tetramethoxystilbene (3). Yield: 1.56 g (52%), mp 130–132 °C; ^{1}H NMR ($CDCl_3$): δ 7.00 (s, 2H), 6.66 (d, J = 2.2 Hz, 4H), 6.40–6.38 (m, 2H), 3.81 (s, 12H); ^{13}C NMR ($CDCl_3$): δ 160.9, 139.0, 129.1, 104.5, 100.0, 55.3; MS *m*/*z* 300 [M^+, 100%].

4.3.9. 3,3′,4′,5-Tetramethoxystilbene (4). Yield: 1.65 g (55%), mp 67 °C; ^{1}H NMR ($CDCl_3$): 7.08–7.00 (m, 3H), 6.93–6.83 (m, 2H), 6.66 (d, J = 2.2 Hz, 2H), 6.39–6.37 (m, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 160.9, 149.0, 148.9, 139.5, 130.1,

128.9, 126.7, 119.9, 111.1, 108.7, 104.2, 99.6, 55.9, 55.8, 55.3; MS *m/z* 300 [M^+, 100%].

4.3.10. 3,3′,4,5,5′-Pentamethoxystilbene (5). Yield: 1.94 g (59%), mp 136–138 °C; ^{1}H NMR ($CDCl_3$): δ 7.02 (d, J = 16.1 Hz, 1H), 6.91 (d, J = 16.1 Hz, 1H), 6.73 (s, 2H), 6.66 (d, J = 2.2 Hz, 2H), 6.40–6.38 (m, 1H), 3.91 (s, 6H), 3.87 (s, 3H), 3.82 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 160.9, 153.3, 139.1, 137.9, 132.7, 129.0, 128.0, 104.4, 103.5, 99.8, 60.9, 56.0, 55.2; MS *m/z* 330 [M^+, 100%].

4.3.11. 3,3′,4,4′,5,5′-Hexamethoxystilbene (6). Yield: 1.76 g (49%), mp 215 °C; ^{1}H NMR ($CDCl_3$): δ 6.94 (s, 2H), 6.74 (s, 4H), 3.92 (s, 12H), 3.87 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 153.3, 137.8, 132.8, 128.0, 103.3, 60.9, 56.0; MS *m/z* 360 [M^+, 100%].

4.3.12. General procedure for synthesis of compounds 8–12. In a flame-dried three-necked-flask the corresponding methoxystilbene (2.5 mmol) was solved in dry methylenchloride under argon and cooled to −30 °C with cardice. Then boron tribromide (1 M-solution in methylenchloride, 1.5 mmol per methoxy-group) was added via syringe. The solution was warmed to room temperature and stirred for 2 h. Then the reaction was quenched by adding 20 mL water slowly. After stirring for another 30 min the methylenchloride was evaporated and the water-phase was extracted three times with ethyl acetate. The organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo. The resulting crystals were recrystallized from ethanol/water or pure water to yield brown crystals.

4.3.13. 3,4,4′,5-Tetrahydroxystilbene (8). Yield: 0.33 g (55%), mp 240 °C; ^{1}H NMR (d_6-DMSO): δ 8.78 (br s, 4H), 7.35 (d, J = 8.6 Hz, 2H), 6.76–6.72 (m, 4H), 6.47 (s, 2H); ^{13}C NMR (d_6-DMSO): δ 156.7, 146.1, 132.9, 128.5, 128.2, 127.4, 125.9, 125.1, 115.5, 105.2; MS *m/z* 244 [M^+, 100%].

4.3.14. 3,3′,5,5′-Tetrahydroxystilbene (9). Yield: 0.37 g (60%), mp >320 °C; ^{1}H NMR (d_6-DMSO): δ 9.24 (br s, 4H), 6.84 (s, 2H), 6.41 (d, J = 2.0 Hz, 4H), 6.16 (t, J = 1.9 Hz, 2H); ^{13}C NMR (d_6-DMSO): δ 158.5, 138.7, 128.4, 128.4, 104.6, 102.2; MS *m/z* 244 [M^+, 100%].

4.3.15. 3,3′,4′,5-Tetrahydroxystilbene (10). Yield: 0.31 g (51%), mp 236 °C; ^{1}H NMR (d_6-DMSO): δ 8.70 (br s, 4H), 6.97 (s, 1H), 6.87–6.63 (m, 4H), 6.40 (d, 1.9 Hz, 2H), 6.20–6.19 (m, 1H); ^{13}C NMR (d_6-DMSO): δ 158.0, 144.9, 144.8, 138.9, 128.6, 127.8, 125.4, 118.3, 115.3, 112.7, 104.1,101.6; MS *m/z* 244 [M^+, 100%].

4.3.16. 3,3′,4,5,5′-Pentahydroxystilbene (11). Yield: 0.370 g (57%), mp 252 °C; ^{1}H NMR (d_6-acetone): δ 8.00 (br s, 5H), 6.89 (d, J = 16.3 Hz, 1H), 6.76 (d, J = 16.3 Hz, 1H), 6.64 (s, 2H), 6.51 (d, J = 2.1 Hz, 2H), 6.28–6.25 (m, 1H); ^{13}C NMR (d_6-acetone): δ 160.2, 147.4, 141.4, 134.7, 130.5, 130.3, 127.7, 107.3, 106.3, 103.3; MS *m/z* 260 [M^+, 100%].

4.3.17. 3,3′,4,4′,5,5′-Hexahydroxystilbene (12). Yield: 0.31 g (45%), mp 270 °C; ^{1}H NMR (d_6-DMSO): δ 8.66 (br s, 6H), 6.57 (s, 2H), 6.44 (s, 4H); ^{13}C NMR (d_6-DMSO): δ 146.1, 132.9, 128.1, 125.8, 105.2; MS *m/z* 276 [M^+, 100%].

4.3.18. Purity of compounds 1–12. The purity of compounds **1**–**6** and **8**–**12** obtained by elemental analysis was within ±0.4% of the theoretical values for the formulas given. Compound **7** (resveratrol) was purchased from Sigma (Munich, Germany) with a purity of approximately 99%.

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