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ScienceDirect
Bioorganic & Medicinal Chemistry 17 (2009) 1044–1054
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of a library of resveratrol analogues as inhibitors of COX-1, COX-2 and NF-κB

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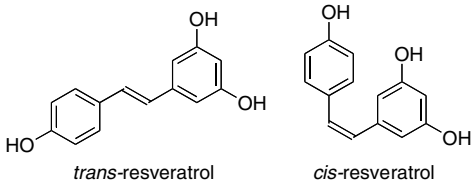
Received 12 December 2007; revised 10 April 2008; accepted 11 April 2008
Available online 16 May 2008

Abstract—Resveratrol (4,3′,5′-trihydroxystilbene) is a naturally occurring antioxidant that inhibits cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and the transcription factor NF-κB. A 78-membered library of resveratrol analogues in which the substituents on the two aryl rings and alkene were varied was synthesized using a solid-phase Wittig olefination reaction. The library contains inhibitors against all three proteins that were more potent than resveratrol itself. Preliminary structure–activity relationships were also obtained from these data that permitted the derivation of pharmacophore models for each of the three targets.

1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene), which occurs in nature as both *cis*- and *trans*-isomers, is a naturally occurring phytoalexin first isolated from the roots of white hellebore in 1940.[1] Since its initial discovery, resveratrol has also been isolated from roughly 70 different plant species, most important of which is in the skin and seeds of red grapes[2] but also in blueberries,[3] peanuts, [4] and rhubarb.[5] Resveratrol is believed to be made by plants to protect them against fungal pathogens,[6] oxidative stress, [7] and/or UV radiation.[8] Within the past decade, however, it has become increasingly clear that resveratrol conveys a number of health benefits to humans and may very well be an important chemopreventive agent for several different disease states, including several cancers,[9–12] cardiovascular disease,[13] and ischemia.[14] Because red wine has been identified as the most important dietary source of resveratrol,[15] it has been suggested that resveratrol is at least partly responsible for the so-called 'French paradox,' which refers to the reduced incidence of cardiovascular disease in regions of France where saturated fats are consumed in even larger quantities than these are in the US, but where red wine is consumed in far larger quantity.[16] Additionally, recent evidence has suggested that resveratrol can extend the lifespan of many organisms, possibly even humans, and also improve the health of older individuals, through a mechanism related to that exhibited by caloric restriction.[17]



trans-resveratrol *cis*-resveratrol

Resveratrol was found to inhibit COX-1 with an ED_{50} of 15 μM during a bioassay-guided fractionation of plant extracts to search for new cancer chemopreventive agents.[18] Subsequent investigations have determined that resveratrol inhibits cyclooxygenase-2 (COX-2) as well, both directly with an IC_{50} of 32 μM and at the transcriptional level.[19] COX-2 is induced during the inflammatory response and has been implicated in the etiology of cancer.[20] Additionally, resveratrol has been shown to inhibit the activation of the oncogenic transcription factor NF-κB by various inflammatory agents with an observed IC_{50} of 30 μM.[21] It is important

Keywords: Resveratrol analogues; Parallel synthesis; Cyclooxygenase; Inhibition; NF-κB .
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doi:10.1016/j.bmc.2008.04.031

to note, however, some COX-2 inhibitors were recently reported to increase the risk of serious cardiovascular events.[22]

Prompted by the promising in vitro data, Jang et al. showed that topical application of resveratrol reduced the incidence of skin cancer in a mouse model by 98%.[17] Systemic administration of resveratrol inhibited tumor growth and initiation in various mouse models.[23] For instance, daily administration of 20 mg/kg of resveratrol to mice with subcutaneous neuroblastomas reduced mortality rates from 100% to 30%.[24] Currently, there are several ongoing Phase I clinical trials of resveratrol as a cancer chemopreventative or chemotherapeutic agent.[25]

Although resveratrol shows tremendous promise as a preventative lead, it is not without some complicating problems. For instance, because of the many targets known to interact with resveratrol, it is very difficult to pinpoint which target is most important for the treatment of a given disease state. The anticancer activity could result from the inhibition of COX-1, COX-2, NF-κB, other targets or any combination thereof. Such an ambiguity makes lead optimization studies difficult, and the broad-spectrum of activities exhibited by resveratrol can lead to side effects. One possible solution to these various problems is to develop resveratrol analogues that exhibit selectivity for only one target.

A second problem with resveratrol is its relative lack of potency. Resveratrol is a micromolar inhibitor of both COX-1 and COX-2, whereas the diarylpyrazole SC-560 inhibits COX-1 with an IC_{50} of 9 nM [26] and the corticosteroid dexamethasone inhibits COX-2 with an IC_{50} of 7 nM.[27] Resveratrol can be administered in high dosage without noticeable problems, but clearly it would be advantageous to find resveratrol analogues with significantly greater potency, thereby reducing the need for large dosages and making them competitive with state of the art cyclooxygenase inhibitors.

Yet another documented problem with resveratrol is its limited bioavailability owing to its metabolism in the liver. Studies have shown that circulating resveratrol has a serum half-life of 8–14 min because it is rapidly metabolized by sulfation[28] and glucuronation.[29] Resveratrol 3-sulfate and resveratrol 3-glucuronide, the two primary metabolites of resveratrol, have both been shown to exhibit far lower affinity for COX-1 and COX-2,[30] thereby suggesting that the observed in vitro activities of resveratrol may not be realized in vivo. To be useful as a chemotherapeutic, resveratrol must have greater bioavailability. A straightforward approach to increasing bioavailability involves finding analogues with comparable activity that lack the hydroxyl groups of resveratrol and consequently cannot be sulfated or glucuronated.

To begin to address these problems with resveratrol, the synthesis and screening of a small library of analogues were envisaged to find lead compounds displaying increased selectivity and/or potency and to begin to elucidate the structure–activity relationships of resveratrol with respect to the inhibition of COX-1, COX-2 and NF-κB. Another goal of these experiments was the discovery of resveratrol analogues with activity comparable to that of the natural product but which lacked the hydroxyl groups known to lead to decreased bioavailability.

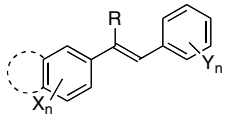
2. Library design

The library (Fig. 1) was designed to probe three different structural features of resveratrol: substitution on each of the two aryl rings (X_n and Y_n) and substitution on the alkene (R). To probe the electronic and steric demands on each of the aryl rings, electron-donating (OH, OMe, and NMe_2) and -withdrawing (F, CF_3, and NO_2) substituents were chosen as well as naphthyl substituents; to probe the required disposition of the two rings and the steric requirements around the central alkene, four small substituents were placed on the alkene. The alkene substituents were kept very minimal to avoid producing molecules with estrogenic activity by avoiding structural similarities to known estrogens such as diethylstilbestrol (Fig. 2).

The substituted aryl groups used in the library are shown in Figure 3. Using the criteria outlined above, these were chosen for their commercial availability as benzaldehyde derivatives to accommodate our synthetic plans (vide infra). Although an exhaustive combination of the 16 aryl rings and four alkene substituents would result in a library with 1008 unique members, a library of 78 members was initially constructed to test the synthetic methodology and to minimally explore the resveratrol structure.

3. Chemistry

The general approach[31] taken to library generation involved the use of a Wittig olefination reaction employing a resin-bound phosphonium ylide to establish the central alkene of resveratrol analogues. In the event, a phosphine-substituted polystyrene resin (**1**) was used to form a phosphonium ion with various substituted ben-



X, Y = *m*-, *p*- OH, OMe, NMe_2, F, CF_3, NO_2
R = H, Me, Et, CF_3

Figure 1. Generic structure of resveratrol library.

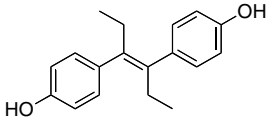


Figure 2. Structure of diethylstibestrol.

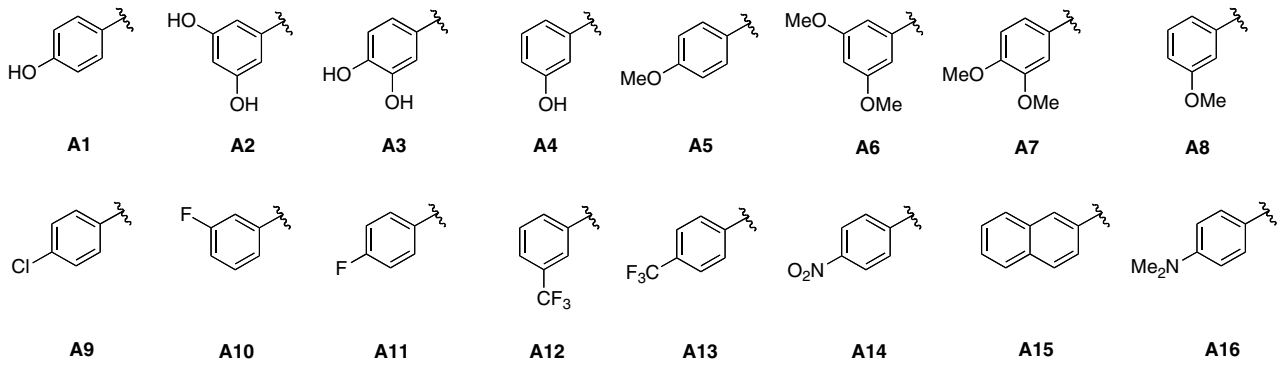
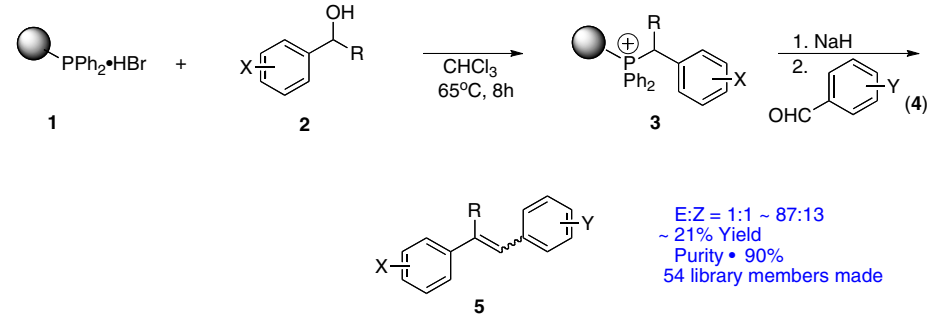


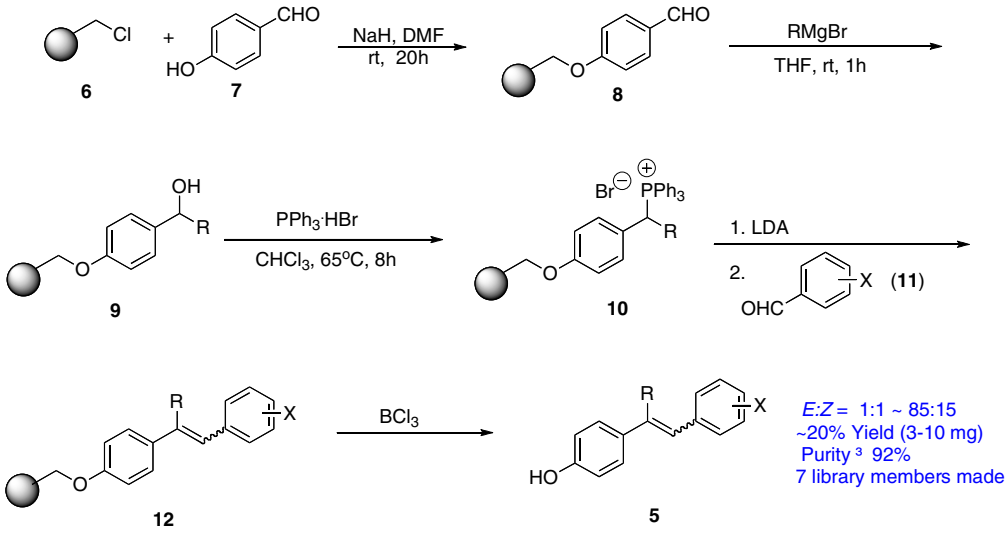
Figure 3. Substituted aryl groups used in the construction of resveratrol library.

zylic alcohols (**2**, Scheme 1). Wittig olefination was accomplished by deprotonation of **3** and reaction with various substituted benzaldehydes (**4**) to form the resveratrol analogues **5**. It was found that a sufficient sample purity was achieved through filtration of the crude product through a silica gel plug. This procedure was used to make the majority of the library, but it was found to afford unsatisfactory results in two classes of compounds: those analogues requiring a 4-hydroxy substituent on alcohol **2**, in which case the phosphonium ion **3** could not be formed; and those analogues bearing a trifluoromethyl substituent on the alkene.

Those library members containing a 4-hydroxyphenyl unit, including resveratrol itself, were synthesized using the procedure shown in Scheme 2. A Merrifield chloromethylstyrene resin (**6**) was used to covalently tether 4-hydroxybenzaldehyde (**7**), which was then reacted with a Grignard to form a secondary alcohol (**9**). Alcohol **9** was then converted to a substituted phosphonium ion reagent (**10**) for use in a Wittig reaction to couple to



Scheme 1. Solid-phase synthesis of resveratrol library (Method A).



Scheme 2. Synthesis of library members containing a 4-hydroxyphenyl unit (Method B).

a second aryl aldehyde (**11**). The completed resveratrol analogue **5** was then cleaved from the resin with BCl_3 and passed through a small plug of silica gel to afford material of >90% purity.

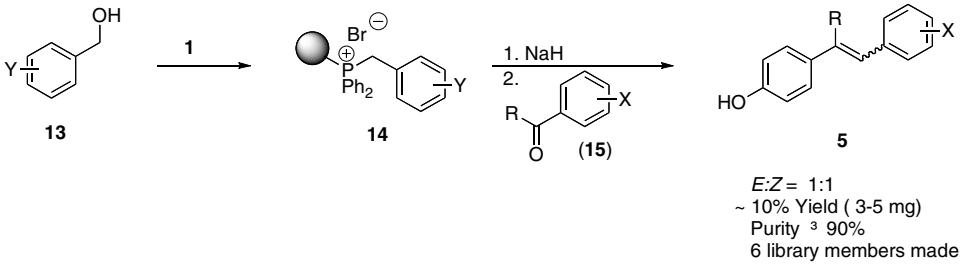
In those cases from Scheme 1 where the Wittig reaction failed, presumably a result of steric hindrance of the ylide intermediate, a primary alcohol corresponding to the less substituted end of the alkene (**13**) was attached to resin **1** (Scheme 3). Deprotonation of the less hindered phosphonium ion **14** proceeded smoothly, permitting clean reaction with a substituted aryl ketone (**15**), thereby affording analogue **5** with concomitant cleavage from the resin. Purification again was achieved to >90% purity by filtration through silica gel.

The use of these three synthetic approaches permitted the construction of a small, 78-membered library in sufficient quantities and purity to assay for the inhibition of COX-1, COX-2, and NF-κB. Because these approaches all use substituted benzaldehyde derivatives as their key starting material, all 78 library members could be made from 16 different aldehydes. Additionally, the solid-phase approach chosen for the production of this library proved to be amenable to automation, so most of the library was made using an Argonaut Trident parallel synthesis workstation.

4. Biological activity

The COX-1 and COX-2 assays were performed by first screening all 78 library members for COX inhibition at a concentration of 10 μg/ml. The assays followed established protocols,[32] the details of which can be found in the Experimental section. Those library members that showed at least 50% inhibition at that concentration were then tested in triplicate in a dose–response to determine the IC_{50} value. Resveratrol was also tested because it is a part of the synthetic library. Inhibition of NF-κB was determined using a luciferase reporter gene assay.[33] An initial screen for activity was performed at 20 μg/ml, following which the library members that displayed more than 50% inhibition at that concentration were then tested in duplicate in a dose–response to determine the IC_{50} value.

In the COX-1 inhibition assay, eight of the 35 resveratrol analogues (Fig. 4) for which the IC_{50} was determined were found to have inhibitory potency comparable to or better than that of resveratrol ($IC_{50} = 0.83 \pm 0.44$ μM). In the COX-2 assay, 16 analogues were tested in a dose–response, of which 6 proved to inhibit COX-2 with a comparable or superior potency to that of resveratrol ($IC_{50} = 0.99 \pm 0.40$ μM). In the NF-κB assay, 33 analogues showed sufficient activity



Scheme 3. Alternative method for library generation via Wittig reaction (Method C).

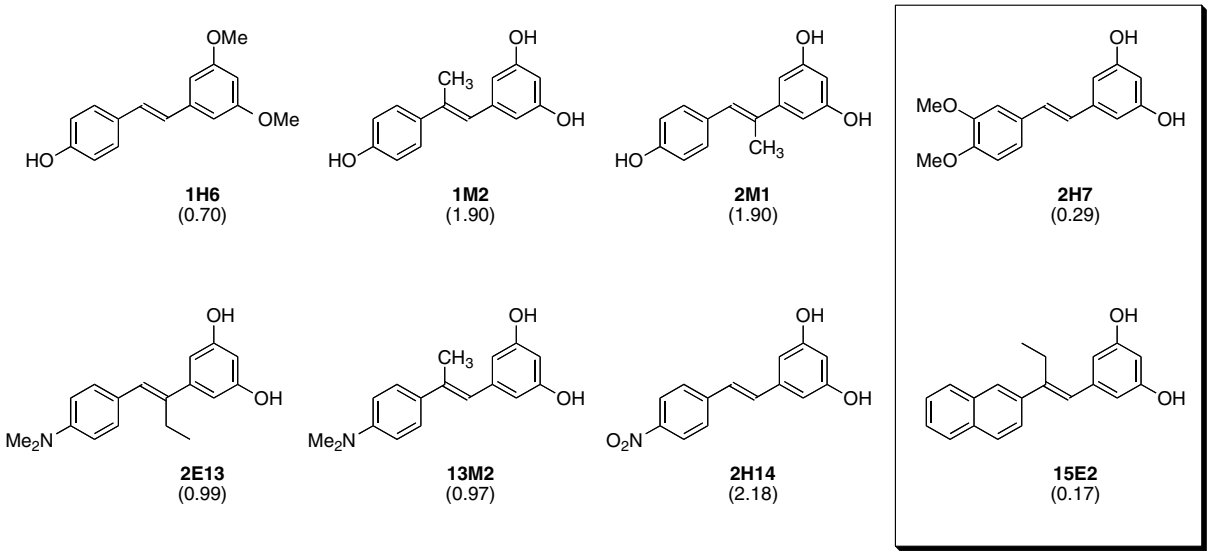
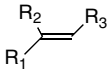


Figure 4. Structures of library members with greatest inhibition of COX-1. IC_{50} values are given in parentheses and the most potent library members are highlighted.

Table 1. COX-1, COX-2, and NF-κB inhibition data for resveratrol library



Code	R_1	R_2	R_3	IC_{50}[a] (μM)		
				COX-1	COX-2	NF-κB
Resveratrol	4-HOPh	H	3,5-$(HO)_2$Ph	0.83 ± 0.44	0.99 ± 0.40	16.1 ± 8.6
1H3	4-HOPh	H	3,4-$(HO)_2$Ph	—	—	**12.7**
1H6	4-HOPh	H	3,5-$(MeO)_2$Ph	**0.70**	**0.82**	**19.5**
2H5	3,5-$(HO)_2$Ph	H	4-MeOPh	25.8	19.6	—
2H7	3,5-$(HO)_2$Ph	H	3,4-$(MeO)_2$Ph	**0.29**	21.3	—
2H10	3,5-$(HO)_2$Ph	H	3-FPh	16.5	—	—
2H11	3,5-$(HO)_2$Ph	H	4-FPh	51.3	—	52.1
2H13	3,5-$(HO)_2$Ph	H	4-CF_3Ph	29.7	—	**12.9**
2H14	3,5-$(HO)_2$Ph	H	4-NO_2Ph	**2.2**	31.6	—
2H15	3,5-$(HO)_2$Ph	H	2-Naphthyl	18.4	—	—
2H16	3,5-$(HO)_2$Ph	H	4-Me_2NPh	17.8	—	—
3H5	3,4-$(HO)_2$Ph	H	4-MeOPh	—	—	**12.2**
3H10	3,4-$(HO)_2$Ph	H	3-FPh	11.5	—	**10.3**
3H11	3,4-$(HO)_2$Ph	H	4-FPh	—	—	**17.9**
3H13	3,4-$(HO)_2$Ph	H	4-CF_3Ph	—	—	**14.8**
3H15	3,4-$(HO)_2$Ph	H	2-Naphthyl	—	—	**6.91**
3H16	3,4-$(HO)_2$Ph	H	4-Me_2NPh	—	—	—
5H6	4-MeOPh	H	3,5-$(MeO)_2$Ph	—	—	28.3
5H7	4-MeOPh	H	3,4-$(MeO)_2$Ph	—	—	52.0
6H7	3,5-$(MeO)_2$Ph	H	3,4-$(MeO)_2$Ph	—	—	—
6H10	3,5-$(MeO)_2$Ph	H	3-FPh	—	—	—
6H11	3,5-$(MeO)_2$Ph	H	4-FPh	—	—	37.5
6H13	3,5-$(MeO)_2$Ph	H	4-CF_3Ph	—	—	—
6H14	3,5-$(MeO)_2$Ph	H	4-NO_2Ph	10.0	5.94	—
6H15	3,5-$(MeO)_2$Ph	H	2-Naphthyl	—	—	**18.8**
6H16	3,5-$(MeO)_2$Ph	H	4-Me_2NPh	—	—	—
7H8	3,4-$(MeO)_2$Ph	H	3-MeOPh	—	—	**17.3**
7H10	3,4-$(MeO)_2$Ph	H	3-FPh	3.2	—	38.8
7H13	3,4-$(MeO)_2$Ph	H	4-CF_3Ph	—	—	—
7H14	3,4-$(MeO)_2$Ph	H	4-NO_2Ph	—	—	—
7H15	3,4-$(MeO)_2$Ph	H	2-Naphthyl	—	—	—
7H16	3,4-$(MeO)_2$Ph	H	4-Me_2NPh	—	—	34.7
1M2	4-HOPh	Me	3,5-$(HO)_2$Ph	**1.9**	**1.57**	—
1M3	4-HOPh	Me	3,4-$(HO)_2$Ph	—	—	21.3
1M6	4-HOPh	Me	3,5-$(MeO)_2$Ph	—	—	—
1M13	4-HOPh	Me	4-CF_3Ph	36.3	**0.47**	—
1M16	4-HOPh	Me	4-Me_2NPh	—	—	—
2M1	3,5-$(HO)_2$Ph	Me	4-HOPh	**1.9**	**1.78**	26.2
2M7	3,5-$(HO)_2$Ph	Me	3,4-$(MeO)_2$Ph	10.6	12.8	—
2M10	3,5-$(HO)_2$Ph	Me	3-FPh	10.5	—	—
2M11	3,5-$(HO)_2$Ph	Me	4-FPh	—	—	—
2M13	3,5-$(HO)_2$Ph	Me	4-CF_3Ph	10.9	—	—
2M15	3,5-$(HO)_2$Ph	Me	2-Naphthyl	—	—	42.3
2M16	3,5-$(HO)_2$Ph	Me	4-Me_2NPh	10.7	**1.74**	—
5M2	4-MeOPh	Me	3,5-$(HO)_2$Ph	12.4	16.0	35.2
5M6	4-MeOPh	Me	3,5-$(MeO)_2$Ph	—	—	27.1
5M7	4-MeOPh	Me	3,4-$(MeO)_2$Ph	—	—	—
5M15	4-MeOPh	Me	2-Naphthyl	—	—	48.2
7M2	3,4-$(MeO)_2$Ph	Me	3,5-$(HO)_2$Ph	11.4	—	**13.1**
7M6	3,4-$(MeO)_2$Ph	Me	3,5-$(MeO)_2$Ph	—	—	—
9M2	4-ClPh	Me	3,5-$(HO)_2$Ph	5.3	—	39.2
9M3	4-ClPh	Me	3,4-$(HO)_2$Ph	—	—	37.5
10M2	3-FPh	Me	3,5-$(HO)_2$Ph	4.9	2.54	—
13M2	4-CF_3Ph	Me	3,5-$(HO)_2$Ph	**0.97**	—	34.2
13M3	4-CF_3Ph	Me	3,4-$(HO)_2$Ph	—	—	—
13M6	4-CF_3Ph	Me	3,5-$(MeO)_2$Ph	32.6	—	—
1F2	4-HOPh	CF_3	3,5-$(HO)_2$Ph	—	—	—
5F2	4-MeOPh	CF_3	3,5-$(HO)_2$Ph	—	—	—
5F6	4-MeOPh	CF_3	3,5-$(MeO)_2$Ph	—	—	37.8
6F5	3,5-$(MeO)_2$Ph	CF_3	4-MeOPh	7.8	—	—

Table 1 (*continued*)

Code	R_1	R_2	R_3	IC_{50}[a] (μM)		
				COX-1	COX-2	NF-κB
11F6	4-FPh	CF_3	3,5-$(MeO)_2$Ph	3.0	—	39.0
12F6	3-CF_3Ph	CF_3	3,5-$(MeO)_2$Ph	—	—	31.9
12F7	3-CF_3Ph	CF_3	3,4-$(MeO)_2$Ph	—	—	25.5
1E2	4-HOPh	Et	3,5-$(HO)_2$Ph	—	—	—
1E6	4-HOPh	Et	3,5-$(MeO)_2$Ph	—	—	—
2E7	3,5-$(HO)_2$Ph	Et	3,4-$(MeO)_2$Ph	9.7	—	—
2E10	3,5-$(HO)_2$Ph	Et	3-FPh	5.4	—	—
2E11	3,5-$(HO)_2$Ph	Et	4-FPh	—	—	—
2E13	3,5-$(HO)_2$Ph	Et	4-CF_3Ph	**0.99**	—	—
2E15	3,5-$(HO)_2$Ph	Et	2-Naphthyl	—	—	—
2E16	3,5-$(HO)_2$Ph	Et	4-Me_2NPh	7.4	**2.2**	—
7E6	3,4-$(MeO)_2$Ph	Et	3,5-$(MeO)_2$Ph	—	—	27.2
9E2	4-ClPh	Et	3,5-$(HO)_2$Ph	11.6	—	38.3
9E3	4-ClPh	Et	3,4-$(HO)_2$Ph	—	—	—
10E2	3-FPh	Et	3,5-$(HO)_2$Ph	3.5	17.7	25.7
15E2	2-Naphthyl	Et	3,5-$(HO)_2$Ph	**0.17**	3.3	—
16E2	4-Me_2NPh	Et	3,5-$(HO)_2$Ph	30.5	14.8	—
16E3	4-Me_2NPh	Et	3,4-$(HO)_2$Ph	—	—	—

[a] The most potent IC_{50} values are highlighted in bold, where no IC_{50} is shown, the IC_{50} > 10 μg/ml in COX-1 or -2 assays or >20 μg/ml in the NF-κB assay.

for a dose–response curve, of which 11 showed activity comparable to or better than that of resveratrol (IC_{50} = 16.1 ± 8.6 μM). All the analogues assayed, and their associated activities, are shown in Table 1.

Several trends emerge from an examination of these data. Firstly, a resorcinol (A2) ring is present in all the potent inhibitors of COX-1, although in **1H6** it is methylated. This suggests that the diol of the resorcinol ring is either involved in specific hydrogen bonding interactions with residues in the COX-1 active site or the active site of COX-1 recognizes a highly electron-rich aryl ring present on the resveratrol skeleton. A second trend is that most of the potent inhibitors feature a substituent on either alkene carbon. This trend, coupled with the observation that the two resveratrol derivatives **1M2** and **2M1** are equipotent, suggests that both rings of resveratrol must rotate out of the plane of the alkene to bind COX-1. The logic behind this conclusion is that molecular mechanics calculations using the MM3 forcefield[34] as implemented in the MacroModel software package[35] show that placing a substituent on the alkene sterically destabilizes the planar conformation of stilbenes such as **1M2** by >20 kcal/mol, forcing the rings out of plane. Since resveratrol itself preferentially adopts a planar conformation to maximize conjugation, it is reasonable to presume that the COX-1 pharmacophore features both rings rotated out of plane. Such a hypothesis is also supported by the SAR of **15E2**, the most potent COX-1 inhibitor in our library: **2H15**, which lacks an ethyl substituent on the alkene, is *100-fold less active* in this assay. The third and last trend that emerges from our COX-1 data is that the phenol ring of resveratrol tolerates a wide variation of substitution, though most of the active members of our library have another electron-rich ring in the R_1 position. More importantly, the two most active members (**2H7** and **15E2**) are those with the largest R_1 substituent. It therefore appears reasonable that either the phenol of resveratrol occupies a large, hydrophobic cavity in the COX-1 active site or that that region of resveratrol is solvent exposed in the complex and that the increased potency of **2H7** and **15E2** owes somewhat to the hydrophobic effect.

Four analogues that showed good COX-1 inhibition (**1H6**, **1M2**, **2M1** and **2E7**) also displayed potent inhibition of COX-2 (Fig. 5). Also, as with COX-1, all but one inhibitor bore an alkene substituent, again suggesting that COX-2 may recognize a conformation of resveratrol in which both rings are twisted out of the plane of the alkene. However, unlike COX-1, COX-2 recognizes a variety of substitution patterns on R_3, all of which are very electron-rich, suggesting that the active site of COX-2 does not make any specific hydrogen bonding contacts with the right-hand ring. Instead, it would appear that the COX-2 active site preferentially recognizes electron-rich R_3 substituents, possibly through a cation–pi interaction.[36] As with COX-1, R_1 of the potent COX-2 inhibitors displays no particular electronic preference and can accommodate the steric bulk of a naphthalene ring, as is shown by **2E7**. Also of note is that, of the COX-2 inhibitors found in our assay, only **1M13**, **3M16** and **10M2** showed high (>15:1) selectivity for COX-2 over COX-1.

In the inhibition studies of NF-κB, the standard deviations associated with the luciferase gene reporter assay had standard deviations of 20–50% associated with them, so that drawing conclusions from the data was less certain than in the other cases. Nonetheless, it is instructive to consider those compounds that exhibited an IC_{50} < 20 μM as 'actives' (Fig. 6) and the most potent NF-κB inhibitor (**3H15**) as significantly more potent than resveratrol. Viewing the actives collectively, three general conclusions can be drawn. Firstly, all but one active (**7M2**) have unsubstituted alkenes, implying that a coplanar conformation may be needed for an effective

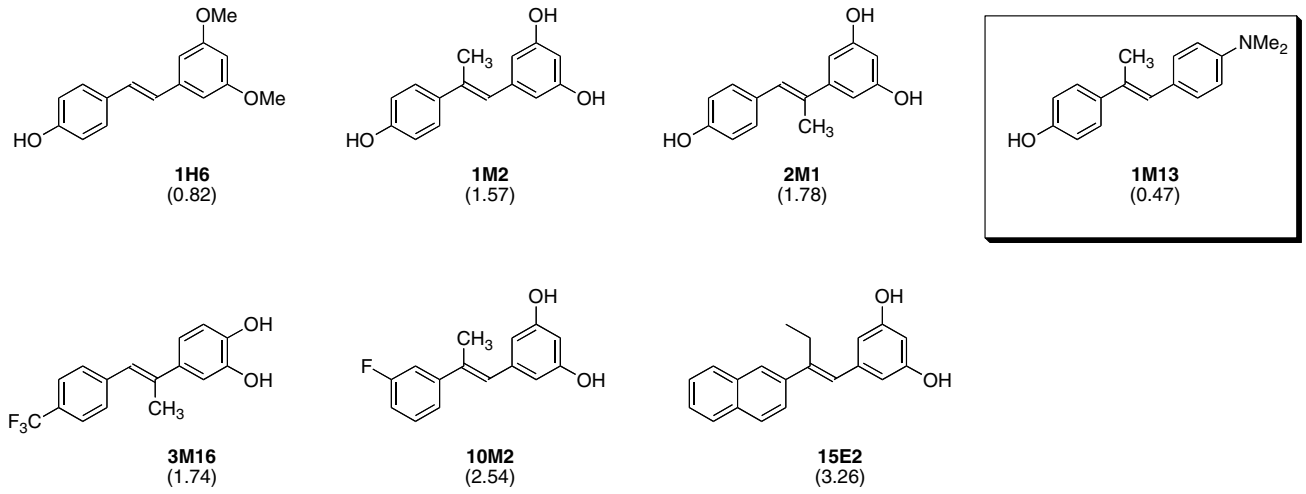


Figure 5. Structures of library members with the greatest inhibition of COX-2. IC_{50} values are given in parentheses and the most potent library member is highlighted.

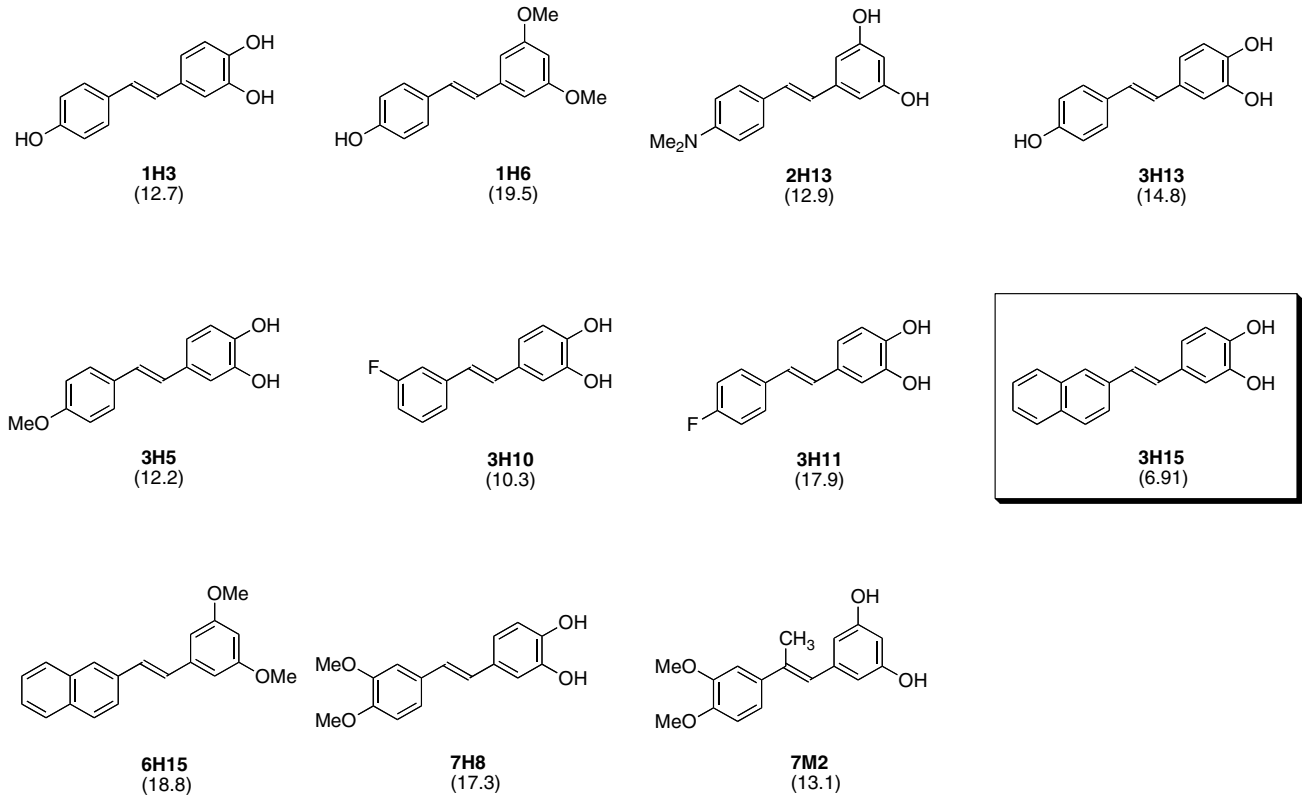


Figure 6. Structures of library members with greatest inhibition of COX-2. IC_{50} values are given in parentheses and the most potent library member is highlighted.

inhibition of NF-κB. Secondly, six of the 11 actives (**3H15**, **3H10**, **3H5**, **1H3**, **3H13**, and **3H11**) contain a catechol subunit (A3), including the four most potent inhibitors (**3H15**, **3H10**, **3H5**, and **1H3**), compared to only two, less potent inhibitors that preserve resveratrol's resorcinol (A2) ring. Although the pseudo-symmetry of the resveratrol analogues lacking an alkene substituent complicates drawing firm conclusions, it is fairly clear that the catechol occupies the R_3 (resorcinol) site. That being the case, it again appears that there is little selectivity for the R_1 ring, beyond a general preference for large and electron-rich rings, again suggesting either a hydrophobic or a solvent-exposed binding site for R_1.

5. Conclusions

The data obtained in these studies provide some initial SAR for resveratrol's interactions with COX-1, COX-

2, and NF-κB. In each instance, at least one analogue was identified with activity superior to that of resveratrol, and in each case a hypothetical pharmacophore model was developed. Interestingly, both COX-1 and COX-2 appear to bind a conformation of resveratrol in which both aryl rings are rotated out of plane with respect to the alkene. COX-1 would appear to require a resorcinol ring in the R_3 site (where resveratrol places a resorcinol) but tolerate various aryl rings in the R_1 site (occupied by the phenol of resveratrol), including large and hydrophobic aryl rings; COX-2, however, permits greater variation in the R_3 site, accepting various electron-rich aryl rings there and also tolerates large and hydrophobic aryl rings in the R_3 site. NF-κB inhibition appears to require a planar conformation of resveratrol, preferring large, hydrophobic R_1 rings and a catechol at the R_3 site. These observations suggest possible second-generation structures to either increase potency or provide greater selectivity. Studies are ongoing in our group to explore these possibilities.

6. Experimental

6.1. Chemistry

6.1.1. General experimental—synthesis. Merrifield resin (1% DVB crosslinked, 100–200 mesh, 0.9 mmol/g) was purchased from Advanced Chemtech. Wang Bromo resin (polymer bound 4-benzyloxybenzyl bromide, 1% DVB crosslinked, 100–200 mesh, 0.92 mmol/g) was purchased from Sigma–Aldrich. Polystyrene resin (1% DVB crosslinked, 100–200 mesh) was purchased from Sigma–Aldrich and modified to triphenylphosphine·HBr resin (1.8 mmol/g) using a literature method.[37] All other reagents were purchased from Aldrich, Alfa Aesar, or TCI and used without further purification. The solvents were purified by passage through an activated column prior to use.[38] *tert* -Butyldimethylsilyl (TBS) or isopropyl (*i*-Pr) protected hydroxyl benzaldehydes were prepared by literature methods using TBSCl[39] or *i*-PrBr.[40] α-Alkyl benzyl alcohols were prepared from benzaldehyde by literature methods.[41] Solid-phase syntheses were preformed on an Argonaut Trident™ Synthesizer. ^{1}H NMR spectra were recorded at 300 MHz.

6.1.2. Method A. Triphenylphosphine·HBr resin (100 mg, 0.18 mmol) placed in an Argonaut Trident™ reaction vessel was swelled with $CHCl_3$ and treated with a solution of an α-alkyl benzyl alcohol (0.26 mmol) in $CHCl_3$ (3 ml). The resin was shaken for 8 h at 65 °C, cooled to room temperature, and sequentially washed with $CHCl_3$ (2× 3 ml), MeOH (2× 3 ml), DMF (2× 3 ml), and Et_2O (3 ml).

The resin was swelled in DMF, and benzaldehyde (0.05 mmol) in DMF (2.5 ml) was added to the resin, followed by the addition of NaH (10 mg, 0.23 mmol). The resin was shaken for 10 h at room temperature and the reaction was quenched by the addition of MeOH (0.5 ml). The solution was removed from the reaction vessel and the remaining resin was washed with EtOAc (2× 3 ml). The combined filtrates were washed with water (6 ml) and the organic layer was dried with $MgSO_4$, and passed through a 2 × 0.5 cm silica gel plug. The solvent was evaporated to afford the desired product.

Those products protected with TBS or *i*-Pr ethers were deprotected; the organic residue was dissolved in CH_2Cl_2 (5 ml) and BCl_3 (2.0 M solution in CH_2Cl_2, 0.45 ml, 0.9 mmol) or TBAF (1.0 M solution in THF, 0.6 ml, 0.6 mmol) was added to the solution. The reaction mixture was stirred for 2 h at room temperature, and washed with water (5 ml). The organic layer was dried with $MgSO_4$, and passed through a 3 × 0.5 cm silica gel plug. The solvent was evaporated under reduced pressure to afford the desired product.

6.1.3. Method B. Merrifield resin (200 mg, 0.18 mmol) placed in a Trident™ reaction vessel was washed with anhydrous DMF (3 ml) and treated with 4-hydroxybenzaldehyde (0.27 mmol) and NaH (15 mg, 0.36 mmol) in DMF (2.5 ml). The resin was shaken for 9 h and sequentially washed with MeOH (2× 3 ml), DMF (2× 3 ml), CH_2Cl_2 (2× 3 ml), and Et_2O (3 ml).

The resin was swelled in THF (2.5 ml), cooled to 0 °C, and the alkyl magnesium bromide (0.2 ml, 1.4 M in THF, 0.27 mmol) was added to the resin. The resin was shaken for 2 h and sequentially washed with MeOH (2× 3 ml), DMF (2× 3 ml), CH_2Cl_2 (2× 3 ml), and Et_2O (3 ml).

PPh_3·HBr (74 mg, 0.216 mmol) in $CHCl_3$ (4 ml) was added to the resin and the resin was heated to 65 °C, and shaken for 8 h. The resin was cooled to room temperature and sequentially washed with MeOH (2× 3 ml), CH_2Cl_2 (2× 3 ml), and Et_2O (3 ml).

The resin was swelled in THF (2.5 ml), cooled to 0 °C, and *n*-BuLi (0.54 ml, 0.5 M solution in THF, 0.27 mmol) was added to the resin, followed by the addition of a substituted benzaldehyde (0.18 mmol) in THF (0.2 ml). The resin was shaken for 2 h and sequentially washed with MeOH (2× 3 ml), DMF (2× 3 ml), CH_2Cl_2 (2× 3 ml) and Et_2O (3 ml).

The resin was swelled in CH_2Cl_2 (2.5 ml) and BCl_3 (0.45 ml, 2.0 M solution in CH_2Cl_2, 0.9 mmol) was added. After shaking for 2 h, the reaction mixture was removed by filtration and the resin was washed with ethyl acetate (2× 3 ml), and quenched by the addition of water (6 ml). The organic layer was separated, dried with $MgSO_4$, and passed through a 2 × 0.5 cm silica gel plug. The solvent was evaporated to afford the desired product.

6.1.4. Method C. Triphenylphosphine·HBr resin (100 mg, 0.18 mmol) in a Trident™ reaction vessel was swelled with CH_3CN and treated with a substituted benzyl alcohol (0.26 mmol) in CH_3CN (3 ml). The resin was shaken for 15 h at 85 °C, cooled to room temperature and sequentially washed with $CHCl_3$ (2× 3 ml), MeOH (2× 3 ml), DMF (2× 3 ml), and Et_2O (3 ml).

The resin was swelled in DMF and benzaldehyde or acetophenone (0.05 mmol) in DMF (2.5 ml) was added to the resin, followed by the addition of NaH (10 mg, 0.23 mmol). The resin was shaken for 40 h at room temperature after which the reaction was quenched by the addition of MeOH (0.5 ml). The reaction mixture was removed by filtration and the resin was washed with ethyl acetate (2× 3 ml), and the combined organic layers were washed with water (6 ml). The organic layer was dried with $MgSO_4$, and passed through a 3×0.5 cm silica gel plug. The solvent was evaporated to afford the desired product. If the product was protected with TBS or *i*-Pr group, the deprotection procedure in Method A was performed.

6.1.5. Synthesis and characterization of selected resveratrol analogues. *Compound* **1H3**. Method A was used in combination with Wang Bromo resin and 3,4-diisopropoxybenzaldehyde (7%, isomer ratio: 50/50). ^{1}H NMR (300 MHz, CD_3OD) δ 7.43 (d, 2H, 8.7 Hz), 7.19 (m, 1H), 7.10 (m, 1H), 6.99 (m, 5H), 6.82 (m, 3H), 6.70 (m, 5H), 6.47 (m, 1H).

Compound **1H6**. Method A was used in combination with Wang Bromo resin and 3,5-dimethoxybenzaldehyde (20%, isomer ratio: 68/32). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, 2H, 8.7 Hz), 6.96 (dd, 2H, 15.9, 41.1 Hz), 6.84 (d, 2H, 8.7 Hz), 6.66 (d, 2H, 2.4 Hz), 6.44 (d, 1H, 2.4 Hz), 3.95 (s, 6H).

Compound **2H4**. Method C was used in combination with 4-nitrobenzylalcohol, and 3,5-bis(*tert*-butyldimethylsilyloxy)benzaldehyde (23%, isomer ratio: 67/33). ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ *8.30 (d, 2H, 8.7 Hz), 8.20 (d, 2H, 8.7 Hz), *7.91 (d, 2H, 8.7 Hz), 7.60 (d, 2H, 8.7 Hz), *7.38 (dd, 2H, 16.2, 37.8 Hz), 6.76 (dd, 2H, 12.3, 24.6 Hz), *6.72 (d, 2H, 2.1 Hz), *6.45 (t, 1H, 2.1 Hz), 6.34 (d, 1H, 2.1 Hz), 6.31 (d, 2H, 2.1 Hz) * denotes signals arising from a minor isomer.

Compound **2H7**. Method B was used in combination with 3,4-dimethoxybenzyl alcohol and 4-(*tert*-butyldimethylsilyloxy)benzaldehyde (20%, isomer ratio: 67/33). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99–6.69 (m, 3H), 6.53 (d, 1H, 1.5 Hz), 6.42 (dd, 2H, 12.3, 28.5 Hz), 6.35 (s, 2H), 3.79 (s,3H), 3.62 (s, 3H).

Compound **2H13**. Method C was used in combination with 4-(*N*,*N*-dimethylamino)benzyl alcohol, and 3,5-diisopropoxybenzaldehyde (20%, isomer ratio: 63/37). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, 2H, 8.4 Hz), 7.23 (d, 2H, 8.4 Hz), 6.95 (dd, 2H, 16.5, 52.1 Hz), 6.67 (s, 2 H), 6.50 (s, 1H), 3.00 (s, 6H).

Compound **3H5**. Method B was used in combination with 4-isopropoxybenzyl alcohol, and 3,4-diisopropoxybenzaldehyde (23%, isomer ratio: 95 > 5). ^{1}H NMR (300 MHz, CD_3OD) δ 7.47 (d, 2H, 8.7 Hz) 7.03 (d, 1H, 1.8 Hz), 6.94 (d, 2H, 8.7 Hz), 6.92 (m, 2H), 6.88 (d, 1H, 2.4 Hz), 6.80 (s, 1H), 3.86 (s, 3H).

Compound **3H10**. Method B was used in combination with 3-isopropoxybenzyl alcohol, and 3-fluorobenzaldehyde (24%, isomer ratio: 50/50). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (m, 2H), 6.91 (m, 4H), 6.70 (m, 2H), 6.45 (m, 1H).

Compound **3H11**. Method B was used in combination with 3-isopropoxybenzyl alcohol, and 4-fluorobenzaldehyde (24%, isomer ratio: 50/50). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (m, 2H), 7.06 (m, 3H), 6.98 (m, 2H), 6.93 (m, 1H), 6.80 (m, 1H).

Compound **3H13**. Method B was used in combination with 3,4-diisopropoxybenzyl alcohol, and 4-(*N*,*N*-dimethylamino)benzaldehyde (5%, isomer ratio: 95 > 5). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.93 (s, 1H), 7.50 (d, 2H, 8.7 Hz), 7.14 (d, 1H, 1.8 Hz), 6.98 (s, 2H), 6.92 (dd, 2H, 8.1, 27.0 Hz), 3.06 (s, 6H).

Compound **3H15**. Method B was used in combination with 3-isopropoxybenzyl alcohol, and 2-naphthylaldehyde (21%, isomer ratio: 50/50). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (m, 6H), 7.67 (dd, 2H, 10.8, 19.2 Hz), 7.42 (m, 6H), 7.12 (m, 1H), 7.10 (s, 2H), 7.02 (m, 1H), 6.87 (m, 1H), 6.78 (s, 1H), 6.74 (s, 2H), 6.60 (dd, 2H, 12.3, 33.5 Hz).

Compound **6H15**. Method B was used in combination with 3,5-dimethoxybenzylalcohol, and 2-naphthylaldehyde (18%, isomer ratio: 59/41). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87–7.40 (m, 14H), 7.23 (dd, 2H, 16.2, 30.3 Hz), *7.22 (dd, 2H, 12.0, 42.6 Hz), 6.74 (d, 2H, 2.1 Hz), 6.46 (1H, d, 2.1 Hz), *6.44 (d, 2H, 2.1 Hz), *6.35 (1H, d, 2.1 Hz), 3.86 (s, 6H), *3.61 (s, 6H) * denotes signals arising from a minor isomer.

Compound **7H8**. Method B was used in combination with 3-methoxybenzyl alcohol, and 3,4-dimethoxybenzaldehyde (21%, isomer ratio: 62/38). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.06 (m, 3H), 7.00–6.76 (m, 4H), 6.55 (s, 2H), 3.89 (s, 3H), 3.73 (s, 3H), 3.65 (s, 3H).

Compound **1M2**. Method A was used in combination with Merrifield resin, 4-hydroxybenzaldehyde, methyl magnesium bromide, and 3,5-diisopropoxylbenzaldehyde (21%, isomer ratio: 63/37). ^{1}H NMR (300 MHz, CD_3OD) δ 7.42 (d, 2H, 6.9 Hz), 6.84 (d, 2H, 6.9 Hz), 6.66 (s, 1H), 6.36 (d, 2H, 2.4 Hz), 6.22 (d, 1H, 2.4 Hz), 2.81 (s, 3H).

Compound **1M13**. Method A was used in combination with Merrifield resin, 4-hydroxybenzaldehyde, methyl magnesium bromide, and 4-*N*,*N*-dimethylaminobenzaldehyde (10%, isomer ratio: 86/14). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (d, 2H, 8.7 Hz), 6.83 (d, 2H, 8.7 Hz), 6.77 (s, 2H), 6.74 (s, 1H), 6.70 (s, 1H), 2.98 (s, 6H), 2.27 (s, 3H).

Compound **2M1**. Method B was used in combination with 3,5-diisopropoxybenzyl alcohol, and 4-isopropoxybenzaldehyde (5%, isomer ratio: 53/47). ^{1}H NMR (300 MHz, CD_3OD) δ *7.27(d, 2H, 8.7 Hz), 6.91 (d, 2H, 8.7 Hz), *6.84 (d, 2H, 8.7 Hz), *6.77 (s, 1H), 6.59 (d, 2H, 8.7 Hz), 6.52 (d, 1H, 2.4 Hz), 6.36 (s, 1H), 6.26 (t, 1H, 2.4 Hz), *6.22 (d, 1H, 2.4 Hz), *6.19 (d, 2H,

2.4 Hz), *2.24 (s, 3H), 2.16 (s, 3H) * denotes signals arising from a minor isomer.

Compound **2M16**. Method C was used in combination with 4-(trifluoromethyl)benzyl alcohol, and 1-[3,5-bis(*tert*-butyldimethylsilyloxy)phenyl]ethanone (10%, isomer ratio: 55/45). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, 2H, 7.5 Hz), 7.44 (d, 2H, 7.5 Hz), *7.37 (d, 2H, 8.1 Hz), *7.10 (d, 2H, 8.1 Hz), 6.99 (s, 1H), 6.82 (s, 1H), 6.58 (s, 2H), *6.43 (s, 1H), *6.29 (s, 1H), *6.21 (s, 2H), 2.22 (s, 3H), *2.06 (s, 3H) * denotes signals arising from a minor isomer.

Compound **7M2**. Method B was used in combination with 3-methoxybenzyl alcohol, and 3,5-diisopropoxybenzaldehyde (6%, isomer ratio: 65/35). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (m, 2H), 6.83 (m, 1H), 6.60 (s, 1H), 6.41 (s, 2H), 6.35 (s, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 2.03 (s, 3H).

Compound **13M2**. Method B was used in combination with 1-[4-(*N*,*N*-dimethylamino)phenyl]ethanol, and 3,5-bis(*tert*-butyldimethylsilyloxy)benzaldehyde (10%, isomer ratio: 95 > 5). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, 2H, 8.7 Hz), 6.60 (d, 2H, 8.7 Hz), 6.40 (m, 1H), 6.37 (s, 2H), 6.21 (s, 1H), 2.95 (s, 6H), 2.49 (m, 3H).

Compound **2E13**. Method C was used in combination with 4-(*N*,*N*-dimethylamino)benzyl alcohol, and 1-[3,5-bis(*tert*-butyldimethylsilyloxy)phenyl]propanone (6%, isomer ratio: 56/44). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89 (d, 2H, 8.7 Hz), 6.61 (s, 1H), 6.52 (s. 1H), 6.51 (d, 2H, 8.7 Hz), 6.25 (s, 2H), 2.89 (s, 6H), 2.41 (q, 2H, 7.8 Hz), 1.02 (t, 3H, 7.8 Hz).

Compound **15E2**. Method B was used in combination with 1-(2-naphthalenyl)propanol and 3,5-bis(*tert*-butyldimethylsilyloxy)benzaldehyde (6%, isomer ratio: 95 > 5). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (m, 4H), 7.61 (d, 1H, 8.4 Hz), 7.48 (m, 2H), 6.69 (s, 1H), 6.44 (s, 2H), 6.30 (s, 1H), 2.86 (q, 2H, 7.5, 7.8 Hz), 1.25 (t, 3H, 7.5 Hz).

6.2. Biological protocols

6.2.1. Evaluation of COX-1 and COX-2 activity by quantitation of PGE_2. The effect of test compounds on COX activity was determined by measuring PGE_2 production as described previously.[30] Reaction mixtures were prepared in 100 mM Tris–HCl buffer (pH 8.0) containing 1 μM heme, 500 μM phenol, 300 μM epinephrine, sufficient amounts of COX-1 or COX-2 to generate 150 ng of PGE_2/ml, and various concentrations of test samples. The reaction was initiated by the addition of arachidonic acid (final concentration, 10 μM) and incubated for 10 min at room temperature (final volume, 200 μl). Then, the reaction was terminated by adding 20 μl of the reaction mixture to 180 μl of 27.8 μM indomethacin, and PGE_2 was quantitated by an ELISA method. The samples were diluted to the desired concentration with 100 mM potassium phosphate buffer (pH 7.4) containing 2.34% NaCl, 0.1% bovine serum albumin, 0.01% sodium azide and 0.9 mM Na_4EDTA. Following transfer to a 96-well plate (Nunc-Immuno Plate Maxisorp, Fisher Scientific, Pittsburgh, PA) coated with a goat anti-mouse IgG (Jackson Immuno Research Laboratories, West Grove, PA), the tracer (PGE_2-acetylcholinesterase, Cayman Chemical, Ann Arbor, MI) and primary antibody (mouse anti-PGE_2, Monsanto, St. Louis, MO) were added. Plates were then incubated at room temperature overnight, reaction mixtures were removed, and wells were washed with a solution of 10 mM potassium phosphate buffer (pH 7.4) containing 0.01% sodium azide and 0.05% Tween 20. Ellman's reagent (200 μl) was added to each well and the plate was incubated at 37 °C for 3–5 h, until the control wells yielded an OD = 0.5–1.0 at 412 nm. A standard curve with PGE_2 (Cayman Chemical, Ann Arbor, MI) was generated on 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 control (solvent-treated) samples, and dose–response curves were constructed for the determination of IC_{50} values. IC_{50} values were generated from the results of four serial dilutions of test compounds and are the mean of two different experiments.

6.2.2. Determination of TNFα-induced NF-κB activity in 293 cells. Two hundred and ninety-three cells stably transfected with NF-κB-luciferase plasmid (Panomics, Fremont, CA) were treated with test compounds and the determination of luciferase activity was performed as described previously.[32] In brief, transfected cells were incubated for 24 h in 96-well plates. After 24 h incubation with TNFα (20 ng/ml) and test compounds, cells were analyzed for luciferase activity. Cells were washed with PBS, lysed using 50 μl 1X Reporter Lysis Buffer (Promega, Madison, WI) for 10 min, and the luciferase determination was performed according to the manufacturer's protocol. Results were expressed as a percentage, relative to control (TNFα-treated) samples, and dose–response curves were constructed for the determination of IC_{50} values. IC_{50} values were generated from the results of five serial dilutions of test compounds and are the mean of two different experiments. With the experimental conditions used, no signs of overt cellular toxicity were observed.

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