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# Synthesis and Biological Evaluation of Coumarin-Based Inhibitors of NAD(P)H: Quinone Oxidoreductase-1 (NQO1)†

Karen A. Nolan,‡ Jeremy R. Doncaster,§ Mark S. Dunstan,⊥ Katherine A. Scott,‡ A. David Frenkel,‡ David Siegel,∥ David Ross,∥ John Barnes,§ Colin Levy,⊥ David Leys,⊥ Roger C. Whitehead,§ Ian J. Stratford,‡ and Richard A. Bryce*,‡

‡School of Pharmacy and Pharmaceutical Sciences, §School of Chemistry and ⊥Manchester Interdisciplinary Biocentre, University of Manchester and Manchester Cancer Research Center, Manchester, M13 9PT, United Kingdom, and ∥Department of Pharmaceutical Sciences, University of Colorado Denver School of Pharmacy, Aurora, Colorado

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The synthesis is reported here of two novel series of inhibitors of human NAD(P)H quinone oxidoreductase-1 (NQO1), an enzyme overexpressed in several types of tumor cell. The first series comprises substituted symmetric dicoumarol analogues; the second series contains hybrid compounds where one 4-hydroxycoumarin system is replaced by a different aromatic moiety. Several compounds show equivalent or improved NQO1 inhibition over dicoumarol, both in the presence and in the absence of added protein. Further, correlation is demonstrated between the ability of these agents to inhibit NQO1 and computed binding affinity. We have solved the crystal structure of NQO1 complexed to a hybrid compound and find good agreement with the *in silico* model. For both MIA PaCa-2 pancreatic tumor cells and HCT116 colon cancer cells, dicoumarol shows the greatest toxicity of all compounds. Thus, we provide a computational, synthetic, and biological platform to generate competitive NQO1 inhibitors with superior pharmacological properties to dicoumarol. This will allow a more definitive study of NQO1 activity in cells, in particular, its drug activating/detoxifying properties and ability to modulate oncoprotein stability.

## Introduction

NAD(P)H quinone oxidoreductase 1 (NQO1,[a] DT-diaphorase, EC 1.6.99.2) is an obligate two-electron reductase with the ability to use the cofactors NADH and NADPH with equal efficiency.[1] While the enzyme has a broad structural spectrum of substrates, it has great sensitivity to inhibition by the anticoagulant dicoumarol or 3,3′-methylenebis(4-hydroxycoumarin) (**1**, Table 1).[2] NQO1 is generally considered to be a detoxification enzyme, playing an antioxidant role by preventing the formation of reactive oxygen species.[3] Additional functions of the enzyme include the bioactivation of certain antitumor quinones such as mitomycin C,[4] EO9,[5] and RH1.[5] More recently, studies have shown that NQO1 plays an important role in regulating the stability of the tumor suppressor protein p53[6] and several other short-lived proteins including p73α[7] and ornithine decarboxylase[8] (ODC).

Structural studies have shown that NQO1 exists as a homodimer, with each subunit containing one molecule of flavin adenine dinucleotide (FAD), which is noncovalently attached to the protein.[9] The FAD is held such that its isoalloxazine ring forms the floor of the active site cavity. The two active sites are positioned at opposite ends of the dimer and contain residues from both monomers.[9] The catalytic cycle of NQO1 functions via a "ping-pong" mechanism, which is proposed to occur in two distinct steps: hydride transfer from NAD(P)H to the FAD cofactor, followed by release of $NAD(P)^+$ and hydride transfer from the reduced cofactor to the substrate.[9,10] Compound **1** is a potent competitive inhibitor of NQO1, competing with NAD(P)H for binding to NQO1, thus preventing electron transfer to FAD.[11] Compound **1** is frequently used to study the functional consequences of a lack of NQO1 activity in cells and recently, much of the research involving **1** has centered on its ability to suppress the malignant phenotype of pancreatic cancer cells both in vitro and in vivo.[12−16] NQO1 expression is upregulated in pancreatic cancer and the underlying mechanism by which **1** acts is believed to be due to interference with the ability of NQO1 to defend cells against oxidative damage.[13−16] However, **1** is known to have a variety of other biochemical effects, such as mitochondrial uncoupling, that can confound interpretation of the cellular properties of NQO1.[17]

Therefore, to optimize and achieve a better understanding of the consequences of the pharmacological inhibition of NQO1, in previous work we mined the National Cancer Institute compound database to identify novel coumarin-based, competitive inhibitors of NQO1.[18,19] From these studies, we demonstrated a correlation between a calculated binding affinity and inhibitory potency, and further, provided preliminary evidence that we could uncouple NQO1 inhibitory activity from the "off target" effects associated with **1**. In these initial studies, only one of the coumarin-based compounds showed comparable inhibitory potency to **1**. To gain additional insights, we initiated a much wider investigation of inhibitors based around the structure of **1**. Here we describe

†The atomic coordinates and structure factors have been deposited in the Protein Data Bank for NQO1 in complex with compound **16**; PDB code 3JSX.

*Corresponding author. Tel.: (+44) (0)161 275 8345. E-mail: richard.bryce@manchester.ac.uk.

[a]Abbreviations: hNQO1, human NAD(P)H quinone oxidoreductase-1; QSAR, quantitative structure−activity relationship; BSA, bovine serum albumin; RMSD, root-mean-square deviation.

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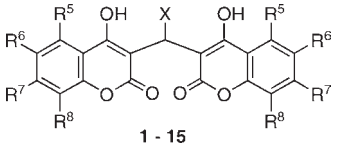
the synthesis of a novel series of substituted symmetric dicoumarol analogues together with a novel hybrid series where one of the 4-hydroxycoumarin ring systems is replaced by an aromatic ring system. The $IC_{50}$ values for these compounds have been determined for their inhibition of NQO1 and found to distribute fairly evenly over four orders of magnitude, with a number of the compounds showing equal or greater potency than **1**. Further, using a QSAR model incorporated into a ChemScore scoring function,[19] the efficiency of enzyme inhibition correlates closely with calculated binding affinity. Additionally, we have solved the crystal structure of NQO1 containing an asymmetric compound, and this supports the QSAR model. Pancreatic cancer has previously been identified as a potential target for the cytotoxic effects of **1**; we therefore used MIA PaCa-2 pancreatic tumor cells to evaluate the toxicity of the dicoumarol analogues. In order to confirm any toxicity trends, we recapitulated these experiments using the colon cancer cell line, HCT 116. From these studies we identify compounds that may be useful for pharmacological evaluation of NQO1 function, since they have a similar ability as **1** to inhibit the enzyme but with lower toxicity and fewer off-target effects.

## Results and Discussion

**Chemistry.** The structures of the symmetric (**S**) analogues of **1** used in this study are depicted in Table 1. The parent compound (**1**) is commercially available and the other analogues (**2**−**15**), which differ from each other in the nature and position of substituents on the aromatic rings, were selected due to their synthetic accessibility.

**Table 1.** Structures of Symmetric Dicoumarol Analogues




1 - 15

| | $R^5$ | $R^6$ | $R^7$ | $R^8$ | X | | $R^5$ | $R^6$ | $R^7$ | $R^8$ | X |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | H | H | H | H | H | **9** | H | Br | H | Br | H |
| **2** | H | $CH_3$ | H | H | H | **10** | H | $CH_3$ | $CH_3$ | H | H |
| **3** | $OCH_3$ | H | H | H | H | **11** | H | $OCH_3$ | $OCH_3$ | H | H |
| **4** | H | $OCH_3$ | H | H | H | **12** | H | H | 7,8-$C_4H_4$ | | H |
| **5** | H | H | $OCH_3$ | H | H | **13** | H | H | $CH_3$ | $CH_3$ | H |
| **6** | H | F | H | H | H | **14** | H | H | H | H | $CO_2Et$ |
| **7** | H | H | F | H | H | **15** | H | H | H | H | $C_3H_7$ |
| **8** | H | Cl | H | H | H | | | | | | |

To probe the importance of intramolecular hydrogen bonding present in the dicoumarols at physiological pH, a second series of asymmetric (**AS**) NQO1 inhibitors was prepared (**16**−**29**; Table 2). These compounds possess a 4-hydroxy-2*H*-chromen-2-one core bearing variable substituents on both the phenyl ring as well as at C3 of the pyrone ring. An important beneficial feature of these compounds is their improved solubility compared with the members of the symmetric series.

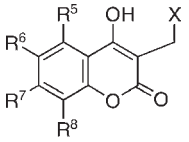
**Symmetric Dicoumarol Analogues.** The symmetrically substituted analogues of **1** (**2**−**13**) were prepared by condensation of the appropriate 4-hydroxy-2*H*-chromen-2-one (**30b**−**30m**) with formaldehyde (Scheme 1). Where necessary, the 4-hydroxy-2*H*-chromen-2-ones were prepared according to the procedure of Barker, by base-mediated cyclization of the corresponding 2-hydroxy acetophenones (**31b**−**31m**) with diethylcarbonate.[20]

Most of the required acetophenones were obtained from commercial sources with the exception of 2-hydroxy-4, 5-dimethoxy acetophenone (**31k**) and 2-hydroxy-3,4-dimethyl acetophenone (**31m**) which were prepared by acetylation of the corresponding phenols (**32k**, **32m**) followed by $AlCl_3$ mediated Fries rearrangement (Scheme 2).[21]

In addition to **1**, unsubstituted 4-hydroxy-2*H*-chromen-2-one (**30a**) is also commercially available. Condensation of the latter compound with either ethyl glyoxalate or butyraldehyde yielded the remaining two symmetric analogues, which possess an ethoxycarbonyl (**14**) or propyl substituent (**15**) on the bridging carbon.

**Asymmetric Dicoumarol Analogues. 3-Substituted 4-Hydroxy-2*H*-chromen-2-ones.** The 3-substituted 4-hydroxy-2*H*-chromen-2-ones (asymmetric analogues **16**−**29**) were prepared by reductive scission of the dicoumarols (**34**−**47**) with sodium cyanoborohydride (Scheme 3). The latter dicoumarols, which possess an aromatic substituent attached to the methylene bridge, were prepared in a similar manner to **2**−**15** by condensation of a 4-hydroxy-2*H*-chromen-2-one with the appropriate aryl aldehyde. Three of the chromen-2-ones used in this study (**30a**, **30j**, and **30l**) are common with those used previously. The fourth, 5,6-benzo-4-hydroxy-2*H*-chromen-2-one (**30n**), was prepared in a similar manner to **30b**−**30m**, by base-mediated cyclization of 2-hydroxy-acetonaphthone with diethylcarbonate (see Scheme 1).

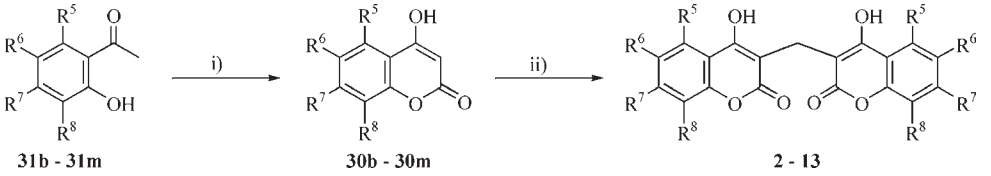
**Table 2.** Structures of Asymmetric Analogues




16 - 29

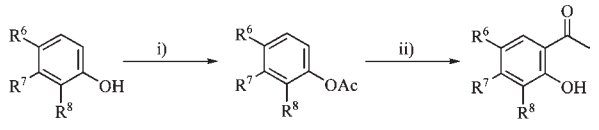
| | $R^5$ | $R^6$ | $R^7$ | $R^8$ | X | | $R^5$ | $R^6$ | $R^7$ | $R^8$ | X |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **16** | H | $CH_3$ | $CH_3$ | H | 1-naphthyl | **23** | 5,6-$C_4H_4$ | | H | H | 2-naphthyl |
| **17** | H | $CH_3$ | $CH_3$ | H | 2-naphthyl | **24** | 5,6-$C_4H_4$ | | H | H | phenyl |
| **18** | H | $CH_3$ | $CH_3$ | H | phenyl | **25** | H | H | H | H | 1-naphthyl |
| **19** | H | H | 7,8-$C_4H_4$ | | 1-naphthyl | **26** | H | H | H | H | 2-naphthyl |
| **20** | H | H | 7,8-$C_4H_4$ | | 2-naphthyl | **27** | H | H | H | H | phenyl |
| **21** | H | H | 7,8-$C_4H_4$ | | phenyl | **28** | H | H | H | H | 3,4-dimethyl phenyl |
| **22** | 5,6-$C_4H_4$ | | H | H | 1-naphthyl | **29** | H | $CH_3$ | $CH_3$ | H | 3,4-dimethyl phenyl |

**Scheme 1**[a]




[a] Reagents and conditions: (i) $(EtO)_2C{=}O$, NaH, rt, 0.5 h then 100 °C, 2 h; (ii) $H_2C{=}O$, EtOH, reflux, 24 h.

**Scheme 2**[a]




$R^8 = H$; $R^6, R^7 = OCH_3$ **32k**
$R^6 = H$; $R^7, R^8 = CH_3$ **32m**

[a] Reagents and conditions: (i) $Ac_2O$, DMAP, pyridine, reflux, 1.5 h; (ii) $AlCl_3$, 135 °C, 0.5 h.

**Biology.** All 29 compounds, **1**−**15** (symmetric) and **16**−**29** (asymmetric), were assayed for their ability to inhibit purified recombinant human NQO1 in the presence and absence of BSA (Table 3). The $IC_{50}$ values for these compounds determined in the absence of BSA were found to extend over four orders of magnitude, with several of the inhibitors being significantly more potent than **1**: the compounds **10**, **12**, and **13** have $IC_{50}$ values of 0.41, 0.18, and 0.42 nM, respectively, whereas the $IC_{50}$ is 2.6 nM for **1**. The pharmacological action of **1** is compromised, to some extent, by protein binding as is evidenced by the close to 200-fold decrease in inhibitory potency to an $IC_{50}$ of 404 nM in the presence of BSA (Table 3). Further, in the presence of protein, some of the symmetric and asymmetric analogues show equal or greater potency than **1**. For example, **3**, **10**, **12**, and **13** show $IC_{50}$ values of 38, 233, 370, and 96 nM, respectively, and **17**, **20**, and **29** have values of 167, 255, and 192 nM, respectively. As mentioned above, the **AS** series of compounds show substantially greater water solubility than the **S** series, including **1**. Therefore, taken together, these results strongly suggest that there are real prospects for developing, for pharmacological use, alternative competitive inhibitors of NQO1.

From the **S** and **AS** series of analogues, we selected a cohort of compounds for more detailed biological analysis. The analogues were chosen to be representative of the different structural classes and the different substitution patterns but importantly to take account of protein binding and the impact this would have when comparing NQO1 inhibitory ability in cells. Initially, we evaluated the dicoumarol analogues for toxicity in MIA Paca-2 and HCT116 tumor cells, by exposing them to the compounds for either 24 or 96 h. Cell killing was determined using the MTT assay (Table 4). Both these cell lines showed significant activity of NQO1 (1252 ± 142 and 545 ± 115 nmol cytochrome c reduced/min/mg protein respectively)

**Molecular Modeling.** To obtain additional insight into the relationship between observed enzyme inhibition of these compounds and the structure of their interactions within the active site of NQO1, the **S** and **AS** series were computationally docked into the active site of NQO1 using the protein X-ray crystal structure from its complex with **1** (PDB code 2F1O).[22] From the crystallography, it is clear that the active site of NQO1 is composed of two distinct but joined pockets: the "FAD pocket" (Figure 1A) is a buried, slot-like cavity bordered by the isoalloxazine ring system and residues Pro68, Trp105, Phe106, Tyr126 and Tyr128 (end on), and Phe178; the second, frontal "access pocket" (Figure 1B) is broader, more-exposed, and bounded by Tyr128, Met131, Gly149, Gly150, Met154, His161, His194, Phe232, Phe236, and the polar edge of the FAD isoalloxazine ring system (Supporting Information, Figure 1S).

Simple visual inspection of these pockets in 2F1O and six other completely homologous NQO1/ligand complexes (1DXO,[10] 1GG5,[23] 1KBO,[24] 1KBQ,[24] 1H66,[23] and 1H69[23]) clearly indicate that the active site of NQO1 is a good binding site for appropriate aromatic structures (Figure 2S).

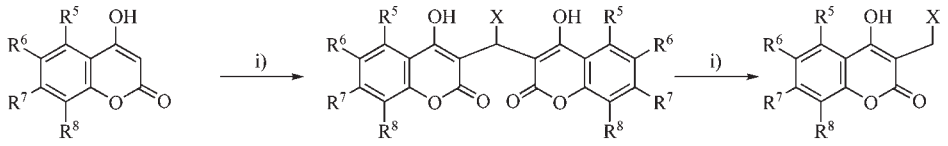
After docking of the **S** and **AS** series into NQO1, the calculated binding affinities (using the ChemScore function[25]) and experimental binding affinities, derived from $IC_{50}$ values obtained in the absence of BSA (Table 3), were compared, alongside a related series of bridge-substituted (**BS**) dicoumarol analogues (Figure 2).[19] Each series of compounds demonstrate a good correlation, with an $r^2$ = 0.8 for the symmetric series, $r^2 = 0.9$ for the asymmetric series and an $r^2 = 0.6$ for the bridge-substituted series suggesting a reliable QSAR model for each series of compounds.

In terms of energetics, closer inspection of the asymmetric series shows some interesting trends in binding affinity and inhibitory potency as a function of structural substitution. Compounds **16**−**18**, **19**−**21** and **25**−**27** are three groups of asymmetric aryl coumarins. The first group of compounds are 6,7-dimethyl coumarins, the second group are 7,8-benzyl coumarins and the third group contains the unsubstituted coumarin fragment (Table 2). The three compounds within each group are substituted with 1-naphthyl, 2-naphthyl and phenyl moieties, respectively. In each group, the measured inhibitory potency ranks 2-naphthyl > 1-naphthyl > phenyl, and this is associated with a trend for calculated binding efficiency to decrease in the same order. This is most likely to be associated with increased hydrophobic interactions seen with the naphthyl versus benzyl groups in the binding pocket of the enzyme. Differences in the 2-naphthyl versus 1-naphthyl are attributable to steric clashes with the active site observed with the latter set of compounds.

When comparative groups of compounds with respect to substituents from the **S** and **AS** series are compared, for example, **10** versus **16**, **17**, and **18**; **12** versus **19**, **20**, and **21**; and **1** versus **25**, **26**, and **27**, the symmetric compounds always demonstrate increased inhibitory potency. A potential explanation for this observation is that at pH 7.4, the symmetric compounds are monoanionic and, hence, form an intramolecular hydrogen bond that holds the bound conformation of the ligand stable.

We now consider the computed geometries of the complexes of NQO1 with **S** and **AS** compounds. Rather than predicting a single energetically favorable binding mode, our docking calculations suggest that preferred poses cluster into four distinct

**Scheme 3**[a]




$R^5,R^6,R^7,R^8 = H$ **30a**
$R^5,R^8 = H$; $R^6,R^7 = CH_3$ **30j**
$R^5,R^6 = H$; $R^7,R^8 = -C_4H_4-$ **30l**
$R^5,R^6 = -C_4H_4-$; $R^7,R^8 = H$ **30n**

[a] Reagents and conditions: (i) XCH=O, EtOH, reflux, 24 h (X = phenyl, 1-naphthyl, 2-naphthyl, 3,4-dimethyl phenyl); (ii) $NaCNBH_3$, $CH_3OH$, reflux, 24 h.

**Table 3.** Calculated and Observed Binding Affinities of **1** and its Symmetric and Asymmetric Analogues, Together with their Ability To Inhibit NQO1 in the Presence and Absence of BSA

| ID | $\Delta G_{calc}$ (kJ/mol) | $\Delta G_{obs}$ (kJ/mol) | $IC_{50}$ (nM) − BSA | $IC_{50}$ (nM) + BSA | preferred binding mode | polar active site interactions |
|---|---|---|---|---|---|---|
| **1** | −45.2 | −49.7 | 2.6 ± 1.6 | 404 ± 184 | a | Tyr 128 and His 161 |
| **2** | −42.6 | −45.1 | 14 ± 3.5 | 5500 ± 1980 | a | Tyr 128 and His 161 |
| **3** | −40.5 | −48.9 | 2.8 ± 0.42 | 38 ± 2.1 | a | Tyr 128 and His 161 |
| **4** | −40.1 | −45.7 | 11 ± 3.9 | 3300 ± 600 | b | Tyr 126, Tyr 128 and His 161 |
| **5** | −40.5 | −47.2 | 6.0 ± 3.2 | 790 ± 355 | b | Tyr 128 and His 161 |
| **6** | −44.6 | −47.7 | 4.5 ± 1.6 | 1600 ± 424 | a | Tyr 128 and His 161 |
| **7** | −40.0 | −48.2 | 3.8 ± 1.5 | 1150 ± 212 | b | Tyr 128 and His 161 |
| **8** | −43.4 | −46.2 | 9.0 ± 6.7 | 6250 ± 4313 | b | Tyr 128 and His 161 |
| **9** | −44.1 | −47.7 | 4.9 ± 2.3 | 63000 ± 36018 | b | Tyr 128 and His 161 |
| **10** | −46.8 | −54.0 | 0.41 ± 0.38 | 233 ± 68 | c | Tyr 128 and His 161 |
| **11** | −40.2 | −41.4 | 62 ± 32 | 1497 ± 442 | d | Tyr 126, Tyr 128 and His 161 |
| **12** | −51.2 | −55.7 | 0.18 ± 0.16 | 370 ± 198 | c | Tyr 128 and His 161 |
| **13** | −47.7 | −46.5 | 0.42 ± 0.14 | 96 ± 75 | d | Tyr 128 and His 161 |
| **14** | −24.7 | −34.0 | 1221 ± 523 | 22333 ± 12660 | bs | Tyr 128 |
| **15** | −33.6 | −35.8 | 588 ± 304 | 5322 ± 8132 | bs | Tyr 128 |
| **16** | −49.6 | −46.5 | 7.7 ± 4.5 | 1095 ± 290 | d | Tyr 128 and His 161 |
| **17** | −49.2 | −48.7 | 2.5 ± 1.9 | 167 ± 83 | d | Tyr 128 and His 161 |
| **18** | −44.7 | −42.6 | 39 ± 12 | 660 ± 108 | d | Tyr 128 and His 161 |
| **19** | −51.2 | −47.1 | 6.3 ± 2.7 | 450 ± 325 | c | Tyr 128 and His 161 |
| **20** | −52.7 | −49.7 | 2.2 ± 1.6 | 255 ± 151 | c | Tyr 128 and His 161 |
| **21** | −46.6 | −42.8 | 35 ± 21 | 880 ± 364 | c | Tyr 128 and His 161 |
| **22** | −48.0 | −46.8 | 6.3 ± 2.3 | 610 ± 156 | c | Tyr 128 and His 161 |
| **23** | −49.6 | −47.2 | 6.0 ± 3.1 | 2366 ± 1401 | c | Tyr 128 and His 161 |
| **24** | −45.9 | −44.9 | 15 ± 5.7 | 465 ± 64 | c | Tyr 128 and His 161 |
| **25** | −47.9 | −43.8 | 24 ± 9.2 | 1522 ± 627 | b | Tyr 128 and His 161 |
| **26** | −47.8 | −45.1 | 14 ± 5.8 | 1452 ± 517 | b | Tyr 128 and His 161 |
| **27** | −42.8 | −39.3 | 144 ± 79 | 3200 ± 566 | b | Tyr 128 and His 161 |
| **28** | −45.9 | −43.1 | 31 ± 13 | 1600 ± 424 | d | Tyr 128 and His 161 |
| **29** | −47.7 | −46.0 | 9.9 ± 4.4 | 192 ± 41 | d | Tyr 128 and His 161 |

categories; these binding modes together with their hydrogen bond contacts are shown in Table 3. For the parent structure **1**, two preferred poses were identified, labeled **a** and **b**, with similar predicted binding affinities. Thus, **1a** has its coumarin ring oxygen oriented in the same direction as in the crystallographic pose (Figure 3A) toward the glycerol moiety of FAD. Conversely, **1b** has O1 pointing in the opposite direction (Figure 3B). Compound **1a** has a predicted binding affinity of −45.2 kJ/mol and superimposes on the X-ray crystal structure[22] with a heavy atom rmsd of 0.23 Å. By comparison, **1b**, with a predicted binding affinity of −45.1 kJ/mol, superimposes with a heavy atom rmsd of 1.98 Å. It appears the **b** pose, predicted as energetically degenerate according to ChemScore, incurs a larger steric clash than **a** to sustain a greater, but possibly overweighted, hydrogen bonding contribution.

It is clear that both poses **a** and **b** are tightly packed against the Trp105 "end wall" in the NQO1 active site (Figure 3C). Trp105 and Phe106 form a hydrophobic pocket of sufficient size to allow only small substituents to interact favorably. The 6-methyl group of **2** creates steric hindrance with Trp105 limiting any hydrophobic contribution to binding. The 5-OMe substituent of **3** causes a slight lateral displacement of the first coumarin ring but the ligand remains in conformation **a** (Figure 4A). The distance from the 6-H atom of **1a** to the face of the Trp105 aromatic ring is 2.83 Å (Figure 3C). The predicted docking solution for its 6-F analogue, **6**, is also found to be in conformation **a**. However, in this case, the F···Trp105 distance is ~2.6 Å, well within the limits at which ChemScore begins to register a steric penalty. Nonetheless, this ligand is still found to dock in orientation **a** (Figure 4B) but clearly bulkier substituents at this same position will incur significantly increased steric potentials and will be unable to occupy low energy poses if aligned in the same way. In fact, the 7-H···Trp105 distance for the docked poses of **1a** and **6a** (Figure 4B) is ~2.4 Å, even less than the distances noted above; therefore, to be consistent with an **a** conformation, no substitution is possible in this position.

**Table 4.** Toxicity of Selected Dicoumarol Analogues in the MIA Paca-2 and HCT116 Cell Lines Expressed as Values of $IC_{50}$ (± Standard Deviation) from at Least Three Independent Experiments [a]

| | | $IC_{50}$ ($\mu$M) | | | |
|---|---|---|---|---|---|
| | | MIA PaCa, 24 h | MIAPaCa, 96 h | HCT116, 24 h | HCT116, 96 h |
| symmetric analogues | **1** | 90 ± 42 | 52 ± 26 | 103 ± 35 | 19 ± 13 |
| | **3** | 390 ± 14 | 350 ± 142 | >400 | 80 ± 27 |
| | **4** | 300 ± 85 | 405 ± 22 | 196 ± 176 | 48 ± 1 |
| | **5** | 448 ± 60 | 303 ± 32 | >400 | 187 ± 17 |
| | **10** | 213 ± 11 | 106 ± 33 | 201 ± 1 | 35 ± 1 |
| | **11** | >400 | >400 | >400 | >400 |
| | **12** | 190 ± 15 | 93 ± 26 | 163 ± 32 | 26 ± 7 |
| | **13** | 359 ± 17 | 119 ± 42 | 178 ± 7 | 35 ± 16 |
| asymmetric analogues | **16** | >400 | 188 ± 2.9 | 160 ± 35 | 56 ± 4.9 |
| | **17** | 390 ± 28 | 162 ± 28 | 208 ± 11 | 180 ± 21 |
| | **18** | >400 | 267 ± 31 | >400 | 178 ± 3 |
| | **19** | 134 ± 69 | 163 ± 20 | 194 ± 3 | 102 ± 18 |
| | **20** | 215 ± 7.1 | 152 ± 16 | 185 ± 7 | 39 ± 3 |
| | **21** | >400 | 222 ± 60 | 263 ± 81 | 40 ± 45 |
| | **29** | >400 | 407 ± 15 | >400 | 176 ± 6 |

[a] It is clear that in both cell lines **1** is the most toxic agent, and there appears to be no systematic relationship between toxicity and NQO1 inhibitory potency.

It follows that **7**, **8**, and the other isosteric (or larger) substituents of **9**, **11**, and **13** have to bind in a different manner. For some of these molecules, pose **b** is preferred (Table 3, Figure 4C), while for **10**, **12**, and **19**−**24**, the preferred docked conformation is denoted as pose **c**, a laterally displaced variant of pose **a** (Figure 4D). Here, the FAD pocket coumarin group moves away from the Trp105 face by 1.2−1.5 Å and is lifted slightly so that the O1 atom is now quite close to where the carbonyl oxygen is in the original X-ray pose of **1**. This shift is accompanied by a slight twist so that the enolate O atom at the 4-position is less displaced. It was also found that compounds in the asymmetric series that have an unsubstituted coumarin core, **25**−**27**, were predicted to have solutions that superimpose very well on conformation **b** with the O1-down core, as illustrated for **26** (Figure 4C), presumably as a consequence of interaction changes at the other end of the ligand. Finally, the preferred bound orientation for symmetric compounds **11** and **13** and asymmetric compounds **16**−**18**, **28**, and **29** was found to be a variant of pose **b**, labeled here as pose **d** (Figure 4E). This docking pose has O1 pointing down with respect to Tyr128 and with some displacement.

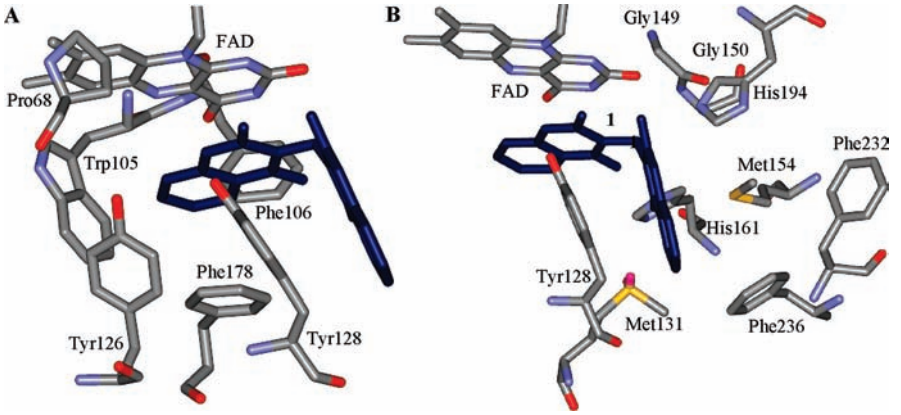



**Figure 1.** (A) "FAD pocket" and (B) "access pocket" from crystal structure of **1**/NQO1 complex.[22]

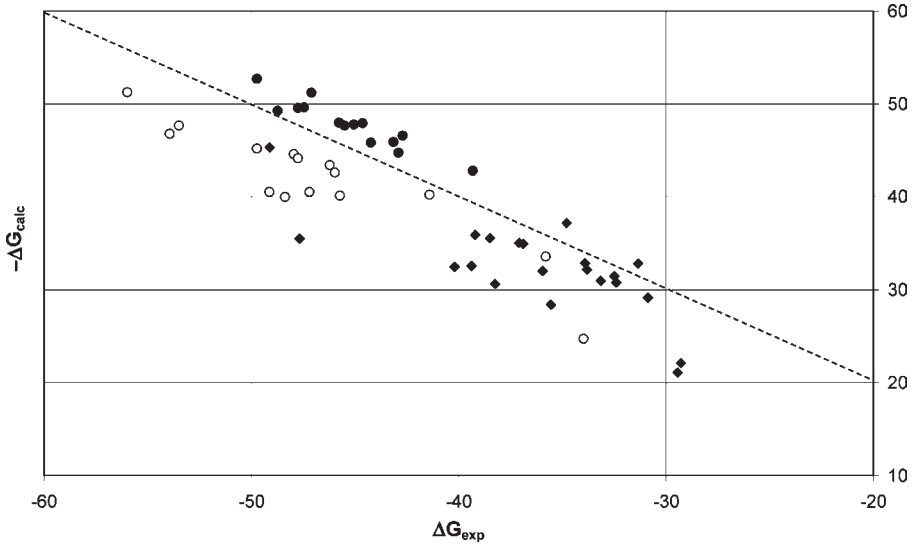



**Figure 2.** Calculated and experimental binding affinity (kJ/mol) in the absence of BSA. The symmetrical (**S**) analogues are shown as white circles, the asymmetric (**AS**) analogues are shown as black circles, and the bridge-substituted (**BS**) derivatives[19] are shown as black diamonds. The theoretical line where $\Delta G_{exp} = \Delta G_{calc}$ is also shown (dashed).

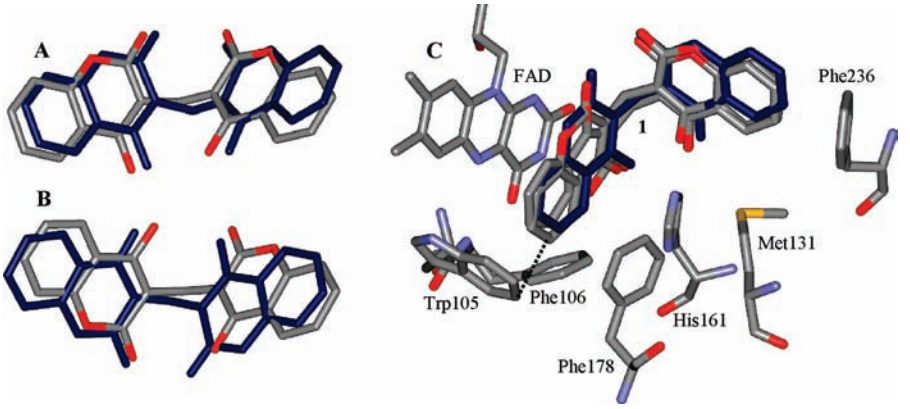



**Figure 3.** Crystal pose of **1** (blue) and the two preferred docked structures: (A) pose **a**, (B) pose **b**, and (C) all three poses in active site of NQO1.

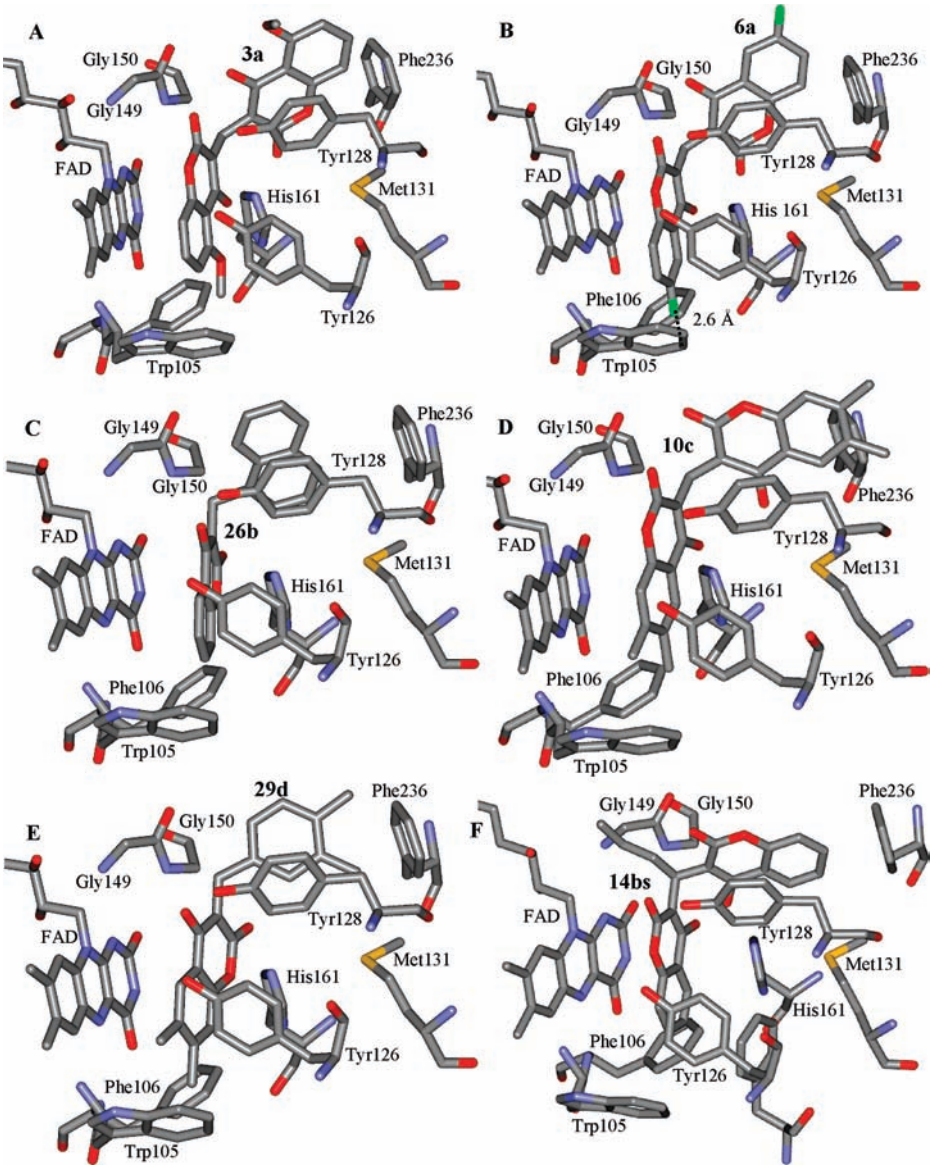



**Figure 4.** Compounds (A) **3a**, (B) **6a**, (C) **26b**, (D) **10c**, (E) **29d**, and (F) **14bs** docked into the active site of NQO1.

Interestingly, **4**, with a 6-methoxy substituent, binds in pose **b**, making a good hydrogen bond between 6-methoxy oxygen atom and the phenolic hydrogen on Tyr126. However, the 6,7-dimethoxy biscoumarol, **11**, binds in pose **d**, due to increased steric clash at the foot of Tyr128 and Phe236 (Figure 3S). This may explain the 5-fold decrease in potency from 11 nM for **4** to 62 nM for **11**.

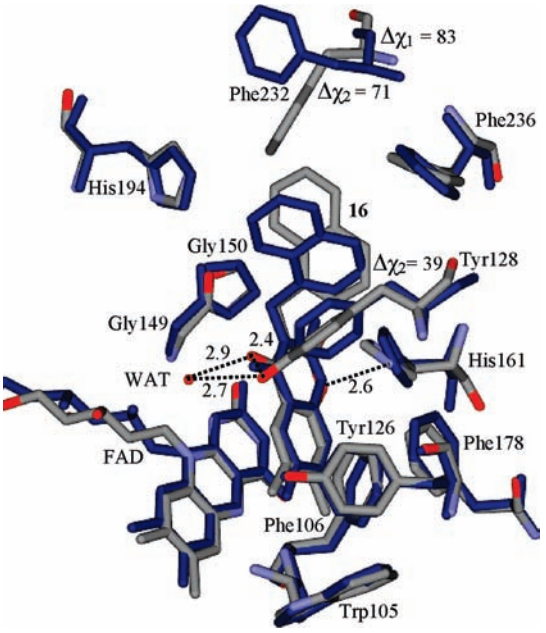



**Figure 5.** Superimposed crystal structures of **16**/NQO1 (from monomer D) and calculated **16**/NQO1 structure based on docking into the 2F1O protein structure (blue). Differences in side-chain torsion angles are also shown. Distances are in Å and angles are in degrees.

The third series of compounds in the SAR (Figure 2), from our previous work,[19] has a biscoumarol structure, substituted with a bridge linker substituent. The biological activity for the **BS** series is rather lower than that found for the **S** and **AS** series. We suggest a number of factors contribute to this. Compound **15** was the smallest compound in this series (Figure 4F), with an *n*-propyl bridge substituent. Modeling suggests that for the two coumarin ring systems of **15** to adopt the same orientation as that seen for **1** in its crystal structure, the *n*-propyl chain has to project up and out of the active site, broadly parallel to the backbone of the FAD cofactor. In this orientation, the *n*-propyl substituent of **15** clashes significantly with the residues Gly149 and Gly150. This steric stress leads to a displacement of the **15** biscoumarol moiety, which results in loss of a hydrogen bond between the ligand and His161. However, analysis of the ChemScore component contributions suggests that the major cost is due to the large internal energy penalty incurred. When this pose is compared with the observed crystal orientations of analogous structures in the Cambridge Crystallographic Structural Database (CCSD), not one of the 18 substituted analogues found there (all obviously nonreceptor bound) showed this to be the preferred conformation (see Supporting Information, **4S**). Indeed calculation via the ChemScore function indicates that to sustain the proposed docking torsion of 14° incurs a penalty of 9.7 kJ/mol for the *n*-propyl compound **15** and for the larger branched carboethoxy derivative, **14**, this torsional penalty rises to 17.2 kJ/mol.

**Crystallography.** To seek to corroborate our predicted docking poses, we have solved the crystal structure of human NQO1 cocrystallized with **16** to 2.45 Å resolution. The orientation and local arrangement of the **16** ligand and associated binding site is highly ordered across four of the eight monomers in the asymmetric unit (one per dimer). These correspond to monomers A, D, G, and H. The alternative positions of the coumarin moiety observed in the remaining monomers reflects a decrease in order of the coumarin section of the ligand in each of these sites. The docked orientation of **16** superimposes on to the more ordered crystal pose of **16** with an rmsd of 0.9 Å (Figure 5). The coumarin ring is nearly identical in both ligands, where a laterally displaced conformation with the FAD-stacked coumarin O1 pointing down is found (binding mode **d**). This adoption of pose **d** is quite clear from the resolved ligand electron density (Figure 5S) and is distinct from the crystallographically observed pose **a** for **1**, where the coumarin O1 points up with respect to Tyr128. Hydrogen bond contacts are formed between the O$\delta$ of Tyr128 and O4 of **16** (2.4 Å) and N$\varepsilon$ of His161 and O1 of **16** (2.6 Å). There is also a highly ordered water molecule within the active site of NQO1, which may be important in the coordination of ligand conformation via the formation of hydrogen bonds between **16** and Tyr128. The largest difference observed in the binding of **16** is caused by a 31° rotation of the $CH_2$ linker group, resulting in a small change in orientation of the naphthyl ring (Figure 5). Additionally, the crystal structure of NQO1 solved here superimposes onto the 2F1O crystal structure of NQO1 from its complex with **1** with an rmsd of 0.6 Å (Figure 5). The docked pose of **16** has a parallel displaced $\pi$-stacking interaction with Tyr128 which in the crystal structure here appears to be more of a T-shaped interaction corresponding to an average rotation of 39° around the $\chi_2$ torsion angle of Tyr128 (Figure 5, Table 1S). The most noticeable difference between the two crystal structures is seen in the orientation of Phe232, corresponding to average rotations of 83° around $\chi_1$ and 71° around $\chi_2$ torsion angles of Phe232 (Figure 5). However, the agreement of computed and observed ligand/protein pose is excellent and supports our in silico rationalization of NQO1 binding specificity for the series of symmetric, asymmetric, and bridge-substituted compounds. That we predict more than one mode generally reflects the hydrophobic nature of the NQO1 binding site.

## Conclusions

We have synthesized a novel series of symmetric dicoumarol analogues and a novel hybrid series where one of the 4-hydroxycoumarol ring systems is replaced by an aromatic ring system. A strong correlation has been established between the ability of these agents to inhibit NQO1 and their computationally derived binding affinity. This structure/activity relationship encompasses both the **S** and the **AS** series as well as a set of bridge-substituted analogues we evaluated previously.[19] It is clear from the docking studies and experimental $IC_{50}$ values that the greatest contribution to inhibitory potency is made by lipophilic interactions within the active site of NQO1. This is supported by the fact that **10**, **12**, and **13** are superior to **1** as a result of increased hydrophobic interactions with active site residues. Additionally, **17** and **20** have comparable $IC_{50}$ values to **1** despite the lack of an intramolecular hydrogen bond. Hence, it is clear that, while the internal hydrogen bond formed by the symmetric compound holds the ligand in a stable conformation in the binding pocket, other interactions can contribute to inhibitory potency. Interestingly, the electronic effects afforded by the addition of functional groups such as fluorine, chlorine, or bromine to **6**, **7**, **8**, and **9** do not impact significantly the ability of the dicoumarol analogues to act as inhibitors of the enzyme.

There have been a number of reports showing that **1** can inhibit growth of pancreatic tumor cells *in vitro* and *in vivo*.[12−16] A property of NQO1 is its ability to directly scavenge superoxide and this led to the proposal that the effect of **1** on pancreatic cell growth was mediated through the inhibition of NQO1, causing a rise in superoxide levels.[12−16] We have measured the generation of superoxide by the compounds reported here and no systematic structure−activity relationship has emerged (data not shown). Thus, the underlying mechanism of toxicity is unlikely to be due to superoxide generation, and this is supported by the observation that a number of the compounds show similar NQO1 inhibitory potency to **1** but show significantly lower toxicity. Second, some analogues that can efficiently inhibit NQO1 generate little or no superoxide. Furthermore, our findings are also consistent with results of Dehn et al.[16] and Reigan et al.[26] who reported the use of mechanism-based irreversible inhibitors of NQO1 and showed that inhibition of growth of MIA Paca-2 cells did not correlate with the extent of NQO1 inhibition.

In conclusion, we have generated a series of compounds that can act as potent inhibitors of NQO1 but with lower toxicity and greater water solubility than **1**. Thus, we have provided a computational, synthetic, and biological platform to generate competitive NQO1 inhibitors that will be useful pharmacological probes to evaluate the cellular effects of NQO1: in particular, its drug activating/detoxifying properties and its ability to modulate the stability of oncoproteins.

## Experimental Section

**Chemistry. General.** Synthetic reagents were purchased from Sigma-Aldrich or Alfa Aesar and used as supplied. Solvents were dried and distilled prior to use. IR spectra were recorded on a Perkin-Elmer 881 spectrometer, an AT1-Matson Genesis Series FTIR spectrometer, or a Perkin-Elmer Spectrum BX FTIR spectrometer. $^{1}$H and $^{13}$C spectra were recorded on a Bruker Avance II 500 MHz spectrometer, a Bruker Avance III 400 MHz spectrometer, or a Bruker Avance 300 MHz spectrometer. Chemical shifts are referenced to the residual solvent peak. Mass spectra were recorded on a Micromass Platform II (electrospray) spectrometer. Melting points were recorded using a Sanyo Gallenkamp MPD350 heater and are uncorrected.

Several of the compounds described below are only sparingly soluble in deuterated NMR solvents and it was not possible to acquire full $^{13}$C NMR data in a small number of cases.

The purity of all compounds was judged to be greater than or equal to 95% based on assessment of TLC, melting point, and $^{1}$H NMR data.

**Synthesis of Symmetric Analogues of 1.** Condensation reactions to give 3,3′-alkylidene-4,4′-dihydroxybis[coumarins] were carried out according to the procedure described by Appendino and co-workers.[27]

**Method A.** A typical condensation reaction involved mixing the appropriate 4-hydroxy-2*H*-chromen-2-one with formaldehyde (37% aqueous solution stabilized with 12% methanol). EtOH was added to give a solution of approximately 0.25 M with respect to the 4-hydroxy-2*H*-chromen-2-one. This reaction mixture was heated at 80 °C for 16 h. The resulting precipitate was collected by filtration, washed with EtOH (3× reaction volume), and dried overnight at 90 °C.

**3,3′-Methylenebis(4-hydroxy-6-methyl-2*H*-chromen-2-one) 2.** Using method A, 4-hydroxy-6-methyl-2*H*-chromen-2-one **30b** (352 mg, 2.00 mmol) and formaldehyde solution (81 mg, 0.07 mL, 1.00 mmol) gave the title compound (206 mg, 56%) as a colorless solid: mp 297−299 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3052 bw (O−H), 2920 w, 1653 s (C═O), 1616 s, 1576 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 7.67 (2H, s, 2 × C*H*), 7.32 (2H, d, $J$ = 7.9, 2 × C*H*), 7.15 (2H, d, $J$ = 7.9, 2 × C*H*), 3.68 (2H, s, C$H_2$), 2.36 (6H, s, 2 × C$H_3$); $\delta_C$ (75 MHz; DMSO-$d_6$) 166.0 (*C*), 164.5 (*C*), 150.6 (*C*), 133.0 (*C*), 132.5 (*C*H), 123.6 (*C*H), 118.1 (*C*H), 115.9 (*C*), 102.3 (*C*), 34.1 (*C*H$_2$), 20.8 (*C*H$_3$); −ES $m/z$ 364 (20%, [M]$^-$), 363 (100, [M − H]$^-$); found by −ES 363.0878, $C_{21}H_{15}O_6$ ([M − H]$^-$) requires 363.0874, error 1.1 ppm.

**3,3′-Methanediylbis(4-hydroxy-5-methoxy-2*H*-chromen-2-one) 3.** Using method A, 4-hydroxy-5-methoxy-2*H*-chromen-2-one **30c** (300 mg, 1.56 mmol) and formaldehyde solution (24 mg, 0.07 mL, 0.78 mmol) gave the title compound (167 mg, 54%) as a colorless solid: mp 261−263 °C; $\nu_{max}$/cm$^{-1}$ 3286 m, 1712 s, 1650 s, 1607, 1465 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 10.08 (2H, bs, 2 × O*H*), 7.53 (2H, dd, $J$ = 8.4, 8.4, 2 × C*H*), 6.99 (4H, dd, $J$ = 8.4, 8.4, 4 × C*H*), 3.97 (6H, s, 2 × OC$H_3$), 3.66 (2H, s, C$H_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) 161.86 (q), 160.66 (q), 155.98 (q), 152.81 (q), 132.12 (2 × *C*H), 109.59 (2 × *C*H), 106.35 (2 × *C*H), 104.52 (q), 102.16 (q), 56.88 (2 × O*C*H$_3$); −ES $m/z$ 396 (22%, [M]$^-$), 395 (100, [M − H]$^-$); found by −ES 395.0768, $C_{21}H_{15}O_8$ [M − H]$^-$ requires 395.0772 error 1.1 ppm.

**3,3′-Methanediylbis(4-hydroxy-6-methoxy-2*H*-chromen-2-one) 4.** Using method A, 4-hydroxy-6-methoxy-2*H*-chromen-2-one **30d** (2.15 g, 11.2 mmol) and formaldehyde solution (0.45 g, 0.41 mL, 5.60 mmol) gave the title compound (2.02 g, 91%) as a colorless solid: mp 288−291 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3080 bw (O−H), 2969 w, 1660 s (C═O), 1573 s; $\delta_H$ (500 MHz; DMSO-$d_6$, 100 °C) 7.40 (2H, d, $J$ = 3.0, 2 × C*H*), 7.27 (2H, d, $J$ = 9.0, 2 × C*H*), 7.16 (2H, dd, $J$ = 9.0, 3.0, 2 × C*H*), 3.83 (6H, s, 2 × OC$H_3$), 3.78 (2H, s, C$H_2$); −ES $m/z$ 396 (13%, [M]$^-$), 395 (48, [M − H]$^-$), 192 (11), 191 (100), 113 (18); found by −ES 395.0756, $C_{21}H_{15}O_8$ ([M − H]$^-$) requires 395.0767, error 2.8 ppm.

**3,3′-Methanediylbis(4-hydroxy-7-methoxy-2*H*-chromen-2-one) 5.** Using method A, 4-hydroxy-7-methoxy-2*H*-chromen-2-one **30e** (3.05 g, 15.9 mmol) and formaldehyde solution (0.64 g, 0.58 mL, 7.95 mmol) gave the title compound (2.62 g, 83%) as a colorless solid: mp 288−291 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 2979 w, 2942 w, 1650 s (C═O), 1616 s, 1598 s; −ES $m/z$ 396 (18%, [M]$^-$), 395 (55, [M − H]$^-$), 369 (8), 368 (23), 263 (42), 113 (100); found by −ES 395.0761, $C_{21}H_{15}O_8$ ([M − H]$^-$) requires 395.0767, error 1.5 ppm.

**3,3′-Methanediylbis(4-hydroxy-6-fluoro-2*H*-chromen-2-one) 6.** Using method A, 4-hydroxy-6-fluoro-2*H*-chromen-2-one **30f** (757 mg, 4.20 mmol) and formaldehyde solution (170 mg, 0.16 mL, 2.10 mmol) gave the title compound (420 mg, 54%) as a colorless solid: mp 300−302 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 2970 w, 2942 w, 1645 s (C═O), 1573 s, 1517 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.56−7.52 (2H, m, 2 × C*H*), 7.38−7.26 (4H, m, 4 × C*H*), 3.66 (2H, s, C$H_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident, 163.1 (*C*), 161.9 (*C*), 157.9 (*C*F, d, $J_{CF}$ = 240.2), 148.3 (*C*), 118.8 (*C*H, d, $J_{CF}$ = 25.0), 118.3 (*C*, d, $J_{CF}$ = 8.6), 118.1 (*C*H, d, $J_{CF}$ = 8.3), 109.0 (*C*H, d, $J_{CF}$ = 25.3), 102.9 (*C*), 19.6 (*C*H$_2$); $\delta_F$ (282 MHz; DMSO-$d_6$) −121.32; −ES $m/z$ 371.2 (55%, [M − H]$^-$); found by −ES 371.0363, $C_{19}H_9F_2O_6$ ([M − H]$^-$) requires 371.0367, error 1.1 ppm.

**3,3′-Methanediylbis(4-hydroxy-7-fluoro-2*H*-chromen-2-one) 7.** Using method A, 4-hydroxy-7-fluoro-2*H*-chromen-2-one **30g** (363 mg, 2.00 mmol) and formaldehyde solution (81 mg, 0.07 mL, 1.00 mmol) gave the title compound (134 mg, 18%) as a colorless solid: mp 311−313 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3088 bw (O−H), 2964 w, 1652 s (C═O), 1626 s, 1600 s, 1575 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.96−7.88 (2H, m, 2 × C*H*), 7.26−7.13 (4H, m, 4 × C*H*), 3.69 (2H, s, C$H_2$); −ES $m/z$ 372 (20%, [M]$^-$), 371 (100, [M − H]$^-$); found by −ES 371.0376, $C_{19}H_9F_2O_6$ ([M − H]$^-$) requires 371.0367, error 2.4 ppm.

**3,3′-Methanediylbis(4-hydroxy-6-chloro-2*H*-chromen-2-one) 8.** Using method A, 4-hydroxy-6-chloro-2*H*-chromen-2-one **30h** (589 mg, 3.00 mmol) and formaldehyde solution (121 mg, 0.11 mL, 1.50 mmol) gave the title compound (210 mg, 35%) as a colorless solid: mp 279−281 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3060 bw (O−H), 1650 s (C═O), 1611 s, 1600 s, 1565 s; $\delta_H$ (300 MHz;

DMSO-$d_6$) 7.82 (2H, d, $J$ = 2.6, 2 × C*H*), 7.54 (2H, dd, $J$ = 8.8, 2.6, 2 × C*H*), 7.31 (2*H*, d, $J$ = 8.8, 2 × C*H*), 3.70 (2H, s, C$H_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) 162.7 (*C*), 150.8 (*C*), 130.8 (*C*), 129.6 (*C*), 127.4 (*C*H), 122.8 (*C*H), 119.7 (*C*), 117.9 (*C*H), 102.6 (*C*), 20.4 (*C*$H_2$); −ES *m*/*z* 405 (58%, [M($^{35}$Cl/$^{37}$Cl) − H]$^-$), 403 (100, [M($^{35}$Cl/$^{35}$Cl) − H]$^-$), 369 (10), 368 (30), 197 (16), 195 (43); found by −ES 402.9788, $C_{19}H_9{}^{35}Cl_2O_6$ ([M − H]$^-$) requires 402.9776, error 2.9 ppm.

**3,3′-Methanediylbis(4-hydroxy-6,8-dibromo-2*H*-chromen-2-one) 9.** Using method A, 4-hydroxy-6,8-dibromo-2*H*-chromen-2-one **30i** (319 mg, 1.00 mmol) and formaldehyde solution (41 mg, 0.04 mL, 0.50 mmol) gave the title compound (71 mg, 22%) as a colorless solid: mp 325−328 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3073 bw (O−H), 1669 s (C═O), 1625 s, 1556 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 8.01 (2H, d, $J$ = 2.3, C*H*), 7.92 (2H, d, $J$ = 2.3, C*H*), 3.67 (2H, s, C$H_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) 164.3 (*C*), 161.7 (*C*), 148.3 (*C*), 135.7 (*C*H), 125.6 (*C*H), 121.4 (*C*), 115.1 (*C*), 110.0 (*C*), 102.5 (*C*), 19.9 (*C*$H_2$); −ES *m*/*z* 655 (28%), 653 (37), 651 (56), 649 (40), 647 (20), 391 (45), 389 (40), 371 (26), 368 (100), 321 (20), 319 (44), 281 (48), 145 (38); found by −ES 646.6983, $C_{19}H_7{}^{79}Br_4O_6$ ([M − H]$^-$) requires 646.6976, error 1.1 ppm.

**3,3′-Methanediylbis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) 10.** Using method A, 4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one **30j** (700 mg, 3.68 mmol) and formaldehyde solution (159 mg, 0.15 mL, 1.96 mmol) gave the title compound (670 mg, 87%) as a colorless solid: mp 308−310 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 2996 w, 2954 w, 2893 w, 1663 s (C═O), 1625 s, 1605 m, 1567 m; −ES *m*/*z* 392 (28%, [M]$^-$), 391 (100, [M − H]$^-$), 368 (13), 113 (50); found by −ES 391.1188, $C_{23}H_{19}O_6$ ([M − H]$^-$) requires 391.1182, error 1.5 ppm.

**3,3′-Methanediylbis(4-hydroxy-6,7-dimethoxy-2*H*-chromen-2-one) 11.** Using method A, 4-hydroxy-6,7-dimethoxy-2*H*-chromen-2-one **30k** (600 mg, 2.70 mmol) and formaldehyde solution (109 mg, 0.10 mL, 1.30 mmol) gave the title compound (500 mg, 84%) as a colorless solid: mp 273−275 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3067 bw (O−H), 1651 s (C═O), 1615 s, 1562 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 12.50 (2H, bs, 2 × O*H*), 7.30 (2H, s, 2 × C*H*), 7.08 (2H, s, 2 × C*H*), 3.90 (2H, s, C$H_2$), 3.84 (12H, s, 4 × OC$H_3$); −ES *m*/*z* 455 (48%, [M − H]$^-$), 293 (100); found by −ES 455.0973, $C_{23}H_{19}O_{10}$ ([M − H]$^-$) requires 455.0978, error 1.1 ppm.

**3,3′-Methanediylbis(4-hydroxy-2*H*-benzo[*h*]chromen-2-one) 12.** Using method A, 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **30l** (424 mg, 2.00 mmol) and formaldehyde solution (81 mg, 0.07 mL, 1.00 mmol) gave the title compound (346 mg, 79%) as a colorless solid: mp 279−281 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3049 bw (O−H), 1734 w, 1661 s (C═O), 1610 s, 1559 s; +ES *m*/*z* 442 (80%, [M − OH + Na]$^+$); found by −ES 435.0872, $C_{27}H_{15}O_6$ ([M − H]$^-$) requires 435.0869, error 0.7 ppm.

**3,3′-Methanediylbis(4-hydroxy-7,8-dimethyl-2*H*-chromen-2-one) 13.** Using method A, 4-hydroxy-7,8-dimethyl-2*H*-chromen-2-one **30m** (380 mg, 2.00 mmol) and formaldehyde solution (81 mg, 0.07 mL, 1.00 mmol) gave the title compound (252 mg, 64%) as a colorless solid: mp 276−278 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 2981 b, 2918 b, 2861 w, 1650 s (C═O), 1598 s, 1562 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 7.65 (2H, d, $J$ = 8.2, 2 × C*H*), 7.14 (2H, d, $J$ = 8.2, 2 × C*H*), 3.73 (2H, s, C$H_2$), 2.33 (6H, s, 2 × C$H_3$), 2.24 (6H, s, 2 × C$H_3$); $\delta_C$ (75 MHz; CDCl$_3$) 167.9 (*C*), 161.8 (*C*), 150.3 (*C*), 139.4 (*C*), 131.7 (*C*), 124.9 (*C*H), 120.4 (*C*H), 112.6 (*C*), 101.2 (*C*), 19.9 (*C*$H_2$), 15.5 (*C*$H_3$), 11.3 (*C*$H_3$); −ES *m*/*z* 392 (24%, [M]$^-$), 391 (100, [M − H]$^-$); found by −ES 391.1184, $C_{23}H_{19}O_6$ ([M − H]$^-$) requires 391.1182, error 0.5 ppm.

**Ethyl Bis(4-hydroxy-2-oxo-2*H*-chromen-3-yl)acetate 14.** Using method A, a mixture of 4-hydroxy-2*H*-chromen-2-one **30a** (1.00 g, 6.16 mmol), ethylglyoxalate (50% toluene soln; 0.31 g, 3.08 mmol), and EtOH (20 mL) was stirred at reflux for 16 h to give the title compound (0.51 g, 41%) as a colorless solid: mp 245−247 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 3076 bw (O−H), 2989 w, 2952 w, 1743 s (C═O), 1650 s (C═O), 1619 s, 1601 s, 1566 s, 1495 s; $\delta_H$ (400 MHz; DMSO-$d_6$) 8.55 (2H, b, 2 × O*H*), 7.91 (2H, d, $J$ = 7.9, 2 × C*H*), 7.59−7.54 (2H, m, 2 × C*H*), 7.33−7.28 (4H, m, 4 × C*H*), 5.68 (1H, s, C*H*), 4.06 (2H, q, $J$ = 7.1, C$H_2$), 1.09 (3H, t, $J$ = 7.1, C$H_3$); $\delta_C$ (100 MHz; DMSO-$d_6$) 170.9 (*C*), 165.5 (*C*), 163.8 (*C*), 152.3 (*C*), 131.9 (*C*H), 123.9 (*C*H), 123.7 (*C*H), 118.0 (*C*), 116.0 (*C*H), 101.8 (*C*), 60.5 (*C*$H_2$), 38.1 (*C*H), 14.2 (*C*$H_3$); +ES *m*/*z* 409 (100%, [M + H]$^+$); found by +ES 409.0923, $C_{22}H_{11}O_8$ ([M + H]$^+$) requires 409.0923, error 0.0 ppm; Anal. Calcd from $C_{22}H_{10}O_8$): C, 64.71; H, 3.95. Found: C, 64.00; H, 3.66.

**3,3′-Butane-1,1-diylbis(4-hydroxy-2*H*-chromen-2-one) 15.** Using method A, 4-hydroxy-2*H*-chromen-2-one **30a** (1.00 g, 6.16 mmol) and butyraldehyde (0.33 g, 4.62 mmol) in EtOH (20 mL) gave the title compound as a colorless solid (0.56 g, 47%): mp 120.2−121.1 °C (decomp.); $\nu_{max}$/cm$^{-1}$ 2955 w, 2924 w, 2870 w, 1655 s (C═O), 1649 s, 1618 s, 1603 s, 1563 s, 1553 s; $\delta_H$ (400 MHz; DMSO-$d_6$) 7.95 (2H, d, $J$ = 9.3, 2 × C*H*), 7.61−7.56 (2H, m, 2 × C*H*), 7.36−7.32 (4H, m, 4 × C*H*), 4.92 (1H, t, $J$ = 8.2, C*H*), 2.12−2.06 (2H, m, C$H_2$), 1.28−1.19 (2H, m, C$H_2$), 0.86 (3H, t, $J$ = 7.6, C$H_3$); $\delta_C$ (100 MHz; DMSO-$d_6$) 165.1 (*C*), 163.8 (*C*), 151.9 (*C*), 132.0 (*C*H), 124.0 (*C*H), 123.6 (*C*H), 117.2 (*C*), 116.0 (*C*H), 105.5 (*C*), 31.5 (*C*$H_2$), 31.4 (*C*H), 21.0 (*C*$H_2$), 13.9 (*C*$H_3$); −ES *m*/*z* 378 (15%, [M]$^-$), 377 (100, [M − H]$^-$); found by +EI 379.1176, $C_{22}H_{19}O_6$ ([M + H]$^+$) requires 379.1173, error 0.8 ppm.

**3,3′-(1-Naphthylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) 34.** Using method A, 4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one **30j** (1.00 g, 5.26 mmol) and 1-naphthaldehyde (0.41 g, 2.63 mmol) gave the title compound as a cream colored powder (0.72 g, 52%): mp 204−206 °C; $\nu_{max}$/cm$^{-1}$ 2942 b, 1660 s (C═O), 1619 s, 1560 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.88−7.73 (3H, m, 3 × C*H*), 7.59 (2H, 2s, 2 × C*H*), 7.42−7.34 (4H, m, 4 × C*H*), 7.15 (2H, 2s, 2 × *CH*), 6.70 (1H, s, *CH*), 2.29 (6H, s, 2 × C$H_3$), 2.23 (6H, s, 2 × C$H_3$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident 165.8 (*C*), 164.4 (*C*), 162.2 (*C*), 152.0 (*C*), 150.5 (*C*), 142.4 (*C*), 141.6 (*C*), 133.7 (*C*), 132.3 (*C*), 132.2 (*C*), 131.4 (*C*), 128.7 (*C*H), 126.9 (*C*H), 125.7 (*C*H), 125.3 (*C*H), 125.2 (*C*H), 123.6 (*C*H), 123.0 (*C*H), 116.7 (*C*H), 116.4 (*C*H), 114.9 (*C*), 113.3 (*C*), 104.5 (*C*), 35.0 (*C*H), 19.7 (*C*$H_3$), 19.6 (*C*$H_3$), 18.9 (*C*$H_3$), 18.8 (*C*$H_3$); −ES *m*/*z* 518 (28%, [M]$^-$), 517 (100, [M − H]$^-$); found by −ES 517.1638, $C_{33}H_{25}O_6$ ([M − H]$^-$) requires 517.1651, error 2.5 ppm.

**3,3′-(2-Naphthylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) 35.** Using method A, 4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one **30j** (1.00 g, 5.26 mmol) and 2-naphthaldehyde (0.41 g, 2.63 mmol) gave the title compound as a cream colored powder (1.19 g, 87%): mp 253−254 °C; $\nu_{max}$/cm$^{-1}$ 3053 bw (O−H), 2971 w, 2928 w, 1660 s (C═O), 1619 s, 1561 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.84−7.81 (1H, m, C*H*), 7.77−7.73 (2H, m, 2 × C*H*), 7.64 (2H, s, 2 × C*H*), 7.58 (1H, s, C*H*), 7.43−7.40 (2H, m, C*H*), 7.27 (1H, dd, $J$ = 8.8, 1.5, C*H*), 7.20 (2H, s, 2 × C*H*), 6.46 (1H, s, C*H*), 2.32 (6H, s, 2 × C$H_3$), 2.27 (6H, s, 2 × C$H_3$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 165.5 (*C*), 165.2 (*C*), 150.7 (*C*), 141.6 (*C*), 137.9 (*C*), 133.0 (*C*), 132.2 (*C*), 131.6 (*C*), 127.6 (*C*H), 127.5 (*C*H), 127.2 (*C*H), 125.8 (*C*H), 125.2 (*C*H), 124.3 (*C*H), 123.8 (*C*H), 116.4 (*C*H), 115.3 (*C*), 103.4 (*C*), 36.2 (*C*H), 19.7 (*C*$H_3$), 18.9 (*C*$H_3$); −ES *m*/*z* 519 (28%), 518 (100); found by −ES 517.1638, $C_{33}H_{25}O_6$ ([M − H]$^-$) requires 517.1657 error −3.6 ppm.

**3,3′-(Phenylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) 36.** Using method A, 4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one **30j** (1.00 g, 5.26 mmol) and benzaldehyde (0.28 g, 2.63 mmol) gave the title compound as a cream colored powder (0.89 g, 72%): mp 289−290 °C; $\nu_{max}$/cm$^{-1}$ 3069 bw (O−H), 3032 w, 1653 s (C═O), 1603 s, 1567 m, 1496 m; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.64 (2H, s, 2 × C*H*), 7.23−7.09 (5H, m, 5 × C*H*), 7.18 (2H, 2s, 2 × C*H*), 6.32 (1H, s, C*H*), 2.31 (6H, s, 2 × C$H_3$), 2.27 (6H, s, 2 × C$H_3$); $\delta_C$ (125 MHz; DMSO-$d_6$) some signals coincident: 165.6 (*C*), 164.9 (*C*), 152.3 (*C*), 150.6 (*C*), 142.1 (*C*), 140.2 (*C*), 132.7 (*C*), 131.9 (*C*H), 128.1 (*C*H), 126.7 (*C*H), 125.6 (*C*H), 124.0 (*C*H), 123.7 (*C*H), 118.1 (*C*), 116.5

(CH), 116.0 (CH), 104.1 (C), 103.6 (C), 36.1 (C), 19.7 ($CH_3$), 19.0 ($CH_3$); −ES *m/z* 468 (28%, $[M]^-$), 467 (100, $[M - H]^-$); found by −ES 467.1497, $C_{29}H_{23}O_6$ ($[M - H]^-$) requires 467.1495, error 0.4 ppm.

**3,3′-(1-Naphthylmethanediyl)bis(4-hydroxy-2*H*-benzo[*h*]chromen-2-one) 37.** Using method A, 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **30l** (424 mg, 2.00 mmol) and 1-napthaldehyde (156 mg, 1.00 mmol) gave the title compound as a cream colored powder (363 mg, 64%): mp 243−244 °C; $\nu_{max}/cm^{-1}$ 3051 bw (O−H), 2970 w, 1734 w, 1655 m (C═O), 1597 s, 1558 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 8.40−8.36 (2H, m, 2 × C*H*), 8.11−8.07 (1H, m, C*H*), 8.00−7.97 (2H, m, 2 × C*H*), 7.92−7.83 (2H, m, 2 × C*H*), 7.86 (2H, d, *J* = 8.8, 2 × C*H*), 7.72 (2H, d, *J* = 8.8, 2 × C*H*), 7.68−7.65 (4H, m, 4 × C*H*), 7.58 (1H, d, *J* = 7.2, C*H*), 7.42−7.35 (3H, m, 3 × C*H*), 6.85 (1H, s, C*H*); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 168.4 (*C*), 164.2 (*C*), 148.8 (*C*), 137.9 (*C*), 134.3 (*C*), 133.7 (*C*), 131.7 (*C*), 128.6 (*C*H), 128.0 (*C*H), 126.9 (*C*H), 126.4 (*C*H), 125.8 (*C*H), 125.6 (*C*H), 125.1 (*C*H), 125.0 (*C*H), 124.1 (*C*H), 122.8 (*C*H), 122.3 (*C*H), 121.6 (*C*H), 120.5 (*C*), 114.5 (*C*), 104.5 (*C*), 35.1 (*C*H); −ES *m/z* 561 (100%, $[M - H]^-$), 367 (90), 248 (68); found by −ES 561.1354, $C_{37}H_{21}O_6$ ($[M - H]^-$) requires 561.1338, error 2.8 ppm).

**3,3′-(2-Naphthylmethanediyl)bis(4-hydroxy-2*H*-benzo[*h*]chromen-2-one) 38.** Using method A, 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **30l** (424 mg, 2.00 mmol) and 2-napthaldehyde (156 mg, 1.00 mmol) gave the title compound as a cream colored powder (385 mg, 68%): mp 273−274 °C; $\nu_{max}/cm^{-1}$ 3046 bw (O−H), 1734 w, 1654 s (C═O), 1621 s, 1597 s, 1559 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 8.42−8.37 (2H, m, 2 × C*H*), 8.10−8.01 (2H, m, 2 × C*H*), 7.94−7.91 (2H, m, 2 × C*H*), 7.84−7.68 (10H, m, 10 × C*H*), 7.41−7.37 (3H, m, 3 × C*H*), 6.57 (1H, s, C*H*); $\delta_C$ (125 MHz; DMSO-$d_6$) some signals coincident: 168.2 (*C*), 164.8 (*C*), 149.1 (*C*), 139.4 (*C*), 134.4 (*C*), 133.1 (*C*), 131.5 (*C*), 128.1 (*C*H), 128.0 (*C*H), 127.6 (*C*H), 127.4 (*C*H), 127.2 (*C*H), 127.0 (*C*H), 126.3 (*C*H), 125.7 (*C*H), 125.0 (*C*H), 124.3 (*C*H), 122.8 (*C*H), 122.4 (*C*), 121.6 (*C*H), 120.7 (*C*H), 114.6 (*C*), 103.6 (*C*), 36.6 (*C*H); −ES *m/z* 562 (35%, $[M]^-$), 561 (100, $[M - H]^-$), 367 (40), 248 (40); found by −ES 561.1353, $C_{37}H_{21}O_6$ ($[M - H]^-$) requires 561.1338, error 2.8 ppm.

**3,3′-(Phenylmethanediyl)bis(4-hydroxy-2*H*-benzo[*h*]chromen-2-one) 39.** Using method A, 4-hydroxy-2*H*-benzo[*h*]chromen-2-one **30l** (424 mg, 2.00 mmol) and benzaldehyde (100 mg, 1.00 mmol) gave the title compound as a cream colored powder (355 mg, 69%): $\nu_{max}/cm^{-1}$ 3047 bw (O−H), 2971 b, 1733 w, 1656 s (C═O), 1599 s, 1562 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 8.40−8.37 (2H, m, 2 × C*H*), 8.03−8.00 (2H, m, 2 × C*H*), 7.91 (2H, d, *J* = 8.8, 2 × C*H*), 7.77 (2H, d, *J* = 8.8, 2 × C*H*), 7.70−7.67 (4H, m, 4 × C*H*), 7.22−7.20 (4H, m, 4 × C*H*), 7.15−7.10 (1H, m, C*H*), 6.44 (1H, s, C*H*); $\delta_C$ (125 MHz; DMSO-$d_6$) some signals coincident: 167.3 (*C*), 164.7 (*C*), 149.0 (*C*), 141.1 (*C*), 136.1 (*C*), 134.4 (*C*), 130.2 (*C*), 128.3 (*C*H), 128.1 (*C*H), 128.0 (*C*H), 128.0 (*C*H), 127.9 (*C*H), 127.0 (*C*H), 126.8 (*C*H), 125.3 (*C*H), 124.2 (*C*H), 122.9 (*C*H), 122.3 (*C*H), 121.6 (*C*H), 120.5 (*C*H), 114.3 (*C*), 103.7 (*C*) 36.2 (*C*H); −ES *m/z* 512 (30%, $[M]^-$), 511 (100, $[M - H]^-$); found by −ES 511.1177, $C_{33}H_{19}O_6$ ($[M - H]^-$) requires 511.1182, error 1.0 ppm.

**2,2′-(1-Naphthylmethanediyl)bis(1-hydroxy-3*H*-benzo[*f*]chromen-3-one) 40.** Using method A, 4-hydroxy-3*H*-benzo[*f*]chromen-3-one **30n** (424 mg, 2.00 mmol) and 1-naphthaldehyde (156 mg, 1.00 mmol) gave the title compound as a cream colored powder (291 mg, 51%): mp 242−243 °C; $\nu_{max}/cm^{-1}$ 3055 bw (O−H), 3012 b, 2970 w, 1636 s (C═O), 1550 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 9.65 (2H, d, *J* = 8.7, 2 × C*H*), 8.06−8.01 (3H, m, 3 × C*H*), 7.93 (2H, d, *J* = 8.7, 2 × C*H*), 7.84−7.81 (1H, m, C*H*), 7.71 (1H, d, *J* = 9.1, C*H*), 7.64 (1H, d, *J* = 9.1, C*H*), 7.58−7.33 (9H, m, 9 × C*H*), 6.86 (1H, s, C*H*); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 152.7 (*C*), 134.0 (*C*), 133.8 (*C*), 132.3 (*C*H), 131.7 (*C*), 130.2 (*C*), 130.0 (*C*), 128.5 (*C*H), 128.5 (*C*H), 127.4 (*C*H), 126.7 (*C*H), 126.2 (*C*H), 126.0 (*C*H), 125.3 (*C*H), 125.1 (*C*H), 124.9 (*C*H), 117.0 (*C*H), 111.9 (*C*), 107.8 (*C*), 105.3 (*C*), 36.3 (*C*H); −ES *m/z* 562 (13%, $[M]^-$), 561 (13, $[M - H]^-$), 419 (40), 359 (80), 289 (100), 248 (65); found by −ES 561.1330, $C_{37}H_{21}O_6$ ($[M - H]^-$) requires 561.1338, error 1.4 ppm.

**2,2′-(2-Naphthylmethanediyl)bis(1-hydroxy-3*H*-benzo[*f*]chromen-3-one) 41.** Using method A, 4-hydroxy-3*H*-benzo[*f*]chromen-3-one **30n** (424 mg, 2.00 mmol) and 2-naphthaldehyde (156 mg, 1.00 mmol) gave the title compound as a cream colored powder (298 mg, 53%): mp 275−278 °C; $\nu_{max}/cm^{-1}$ 3049 bw (O−H), 1654 s (C═O), 1556 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 9.66 (2H, d, *J* = 8.9, 2 × C*H*), 8.10 (2H, d, *J* = 9.0, 2 × C*H*), 7.96 (2H, d, *J* = 8.8, 2 × C*H*), 7.82−7.79 (1H, m, C*H*), 7.74−7.68 (3H, m, 3 × C*H*), 7.59−7.48 (6H, m, 6 × C*H*), 7.42−7.37 (3H, m, 3 × C*H*), 6.52 (1H, s, C*H*); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 164.0 (*C*), 162.5 (*C*), 153.0 (*C*), 141.1 (*C*), 138.6 (*C*), 133.2 (*C*), 133.1 (*C*), 132.5 (*C*H), 130.3 (*C*), 130.0 (*C*), 128.5 (*C*H), 127.5 (*C*H), 127.3 (*C*H), 127.2 (*C*H), 126.6 (*C*H), 126.4 (*C*H), 126.3 (*C*H), 125.6 (*C*H), 125.5 (*C*H), 125.0 (*C*H), 124.8 (*C*H), 124.2 (*C*H), 120.8 (*C*), 117.1 (*C*H), 109.2 (*C*H), 104.3 (*C*), 36.2 (*C*H); −ES *m/z* 562 (5%, $[M]^-$), 561 (12, $[M - H]^-$), 447 (43), 445 (100), 381 (86), 271 (21); found by −ES 561.1344, $C_{37}H_{21}O_6$ ($[M - H]^-$) requires 561.1338, error 1.1 ppm.

**2,2′-(Phenylmethanediyl)bis(1-hydroxy-3*H*-benzo[*f*]chromen-3-one) 42.** Using method A, 4-hydroxy-3*H*-benzo[*f*]chromen-3-one **30n** (424 mg, 2.00 mmol) and benzaldehyde (100 mg, 1.00 mmol) gave the title compound as a cream colored powder (265 mg, 51%): $\nu_{max}/cm^{-1}$ 1645 s (C═O), 1551 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 9.69 (2H, d, *J* = 8.6, 2 × C*H*), 8.09 (2H, d, *J* = 9.1, 2 × C*H*), 7.97 (2H, d, *J* = 7.9, 2 × C*H*), 7.60 (2H, t, *J* = 7.4, 2 × C*H*), 7.54−7.48 (4H, m, 4 × C*H*), 7.24−7.11 (5H, m, 5 × C*H*), 6.39 (1H, s, C*H*); $\delta_C$ (125 MHz; DMSO-$d_6$) some signals coincident: 171.8 (*C*), 168.5 (*C*), 164.1 (*C*), 152.9 (*C*), 150.5 (*C*), 148.4 (*C*), 143.4 (*C*), 132.5 (*C*H), 132.0 (*C*), 130.2 (*C*), 130.1 (*C*H), 128.5 (*C*H), 127.8 (*C*H), 127.5 (*C*H), 126.7 (*C*H), 124.9 (*C*H), 117.0 (*C*H), 111.9 (*C*), 104.3 (*C*), 36.9 (*C*H); −ES *m/z* 511 (4%, $[M]^-$), 468 (20), 467 (78), 331 (100), 279 (88); found by −ES 511.1190, $C_{33}H_{19}O_6$ ($[M - H]^-$) requires 511.1182, error 1.6 ppm.

**3,3′-(1-Naphthylmethanediyl)bis(4-hydroxy-2*H*-chromen-2-one) 43.** Using method A, 4-hydroxy-2*H*-chromen-2-one **30a** (648 mg, 4.00 mmol) and 1-naphthaldehyde (312 mg, 2.00 mmol) gave the title compound as a yellow powder (565 mg, 61%): $\nu_{max}/cm^{-1}$ 3059 bw (O−H), 2971 b, 1654 s (C═O), 1604 s, 1559 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.83 (2H, d, *J* = 8.8, 2 × C*H*), 7.72 (1H, d, *J* = 8.8, C*H*), 7.56−7.47 (3H, m, 3 × C*H*), 7.40−7.35 (4H, m, 4 × C*H*), 7.31−7.22 (5H, m, 5 × C*H*), 6.73 (1H, s, C*H*); $\delta_C$ (75 MHz; DMSO-$d_6$) 166.7 (*C*), 164.7 (*C*), 153.0 (*C*), 138.0 (*C*), 134.4 (*C*), 132.3 (*C*H), 129.4 (*C*H), 127.3 (*C*H), 126.4 (*C*H), 126.3 (*C*H), 126.1 (*C*H), 125.9 (*C*H), 125.9 (*C*H), 124.6 (*C*H), 124.2 (*C*H), 120.0 (*C*), 119.1 (*C*), 116.6 (*C*H), 105.7 (*C*), 38.1 (*C*H); −ES *m/z* 462 (33%, $[M]^-$), 461 (100, $[M - H]^-$); found by −ES 461.1017, $C_{29}H_{17}O_6$ $[M - H]^-$ requires 461.1025, error 1.7 ppm.

**3,3′-(2-Naphthylmethanediyl)bis(4-hydroxy-2*H*-chromen-2-one) 44.** Using method A, 4-hydroxy-2*H*-chromen-2-one **30a** (1.00 g, 6.17 mmol) and 2-naphthaldehyde (0.48 g, 3.08 mmol) gave the title compound as a cream colored solid (1.24 g, 86%): mp 286−287 °C; $\nu_{max}/cm^{-1}$ 3050 bw (O−H), 1655 s (C═O), 1603, 1559 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.86 (2H, d, *J* = 8.8, 2 × C*H*), 7.82−7.79 (1H, m, C*H*), 7.76−7.71 (2H, m, 2 × C*H*), 7.59−7.53 (3H, m, 3 × C*H*), 7.41−7.38 (2H, m, 2 × C*H*), 7.35 (1H, s, C*H*), 7.32−7.25 (4H, m, 4 × C*H*), 6.45 (1H, s, C*H*); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 165.7 (*C*), 164.8 (*C*), 152.3 (*C*), 138.1 (*C*), 133.0 (*C*), 131.8 (*C*H), 131.6 (*C*), 127.6 (*C*H), 127.5 (*C*H), 127.2 (*C*H), 126.1 (*C*H), 125.7 (*C*H), 125.1 (*C*H), 124.4 (*C*H), 124.0 (*C*H), 123.7 (*C*H), 118.2 (*C*) 116.0 (*C*H), 104.1 (*C*), 36.4 (*C*); −ES *m/z* 462 (35%, $[M]^-$), 461 (100, $[M - H]^-$); found by −ES 461.1040, $C_{29}H_{17}O_6$ $[M - H]^-$ requires 461.1025, error 3.3 ppm.

**3,3′-(Phenylmethanediyl)bis(4-hydroxy-2*H*-chromen-2-one) 45.** Using method A, 4-hydroxy-2*H*-chromen-2-one **30a** (3.07 g, 19.0 mmol) and benzaldehyde (1.00 g, 9.47 mmol) gave the title

compound as a colorless solid (2.55 g, 65%): $\nu_{max}/cm^{-1}$ 2947 w, 2920 w, 2899 w, 1652 s (C=O), 1620 s, 1561 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.86 (2H, d, $J$ = 7.9, 2 × C*H*), 7.56 (2H, t, $J$ = 8.8, 2 × C*H*), 7.34−7.26 (4H, m, C*H*), 7.19−7.11 (5H, m, C*H*), 6.32 (1H, s, C*H*); $\delta_C$ (125 MHz; DMSO-$d_6$) some signals coincident: 166.0 (*C*), 165.3 (*C*), 165.0 (*C*), 164.8 (*C*), 152.6 (*C*), 150.6 (*C*), 141.9 (*C*), 140.6 (*C*), 132.5 (*C*), 131.7 (*C*H), 128.2 (*C*H), 128.0 (*C*H), 126.7 (*C*H), 126.6 (*C*H), 125.7 (*C*H), 125.4 (*C*H), 124.0 (*C*H), 123.8 (*C*H), 123.6 (*C*H), 118.5 (*C*), 116.5 (*C*H), 115.9 (*C*H), 114.9 (*C*H), 103.9 (*C*), 35.9 (*C*H); −ES *m*/*z* 412 (25%, $[M]^-$), 411.4 (100, $[M - H]^-$); found by −ES 411.0863, $C_{25}H_{15}O_6$ ($[M - H]^-$) requires 411.0869, error 1.5 ppm.

**3,3′-(3,4-Dimethyl-phenylmethanediyl)bis(4-hydroxy-2*H*-chromen-2-one) 46.** Using method A, 4-hydroxy-2*H*-chromen-2-one **30a** (484 mg, 2.98 mmol) and 3,4-dimethylbenzaldehyde (200 mg, 1.49 mmol) gave the title compound as a colorless solid (264 mg, 40%): mp 268−270 °C; $\nu_{max}/cm^{-1}$ 2919 w, 1673 s (C=O), 1631 m, 1608 m, 1566 m; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.88 (2H, d, $J$ = 9.2, 2 × C*H*), 7.60−7.50 (2H, m 2 × C*H*), 7.36−7.24 (4H, m, 4 × C*H*), 6.96 (1H, d, $J$ = 8.2, C*H*), 6.85 (1H, s, C*H*), 6.84 (1H, d, $J$ = 8.2, C*H*), 6.26 (1H, s, C*H*), 2.14 (3H, s, C$H_3$), 2.10 (3H, s, C$H_3$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 165.3 (*C*), 164.7 (*C*), 152.3 (*C*), 150.5 (*C*), 141.9 (*C*), 136.6 (*C*), 135.7 (*C*), 133.4 (*C*), 132.5 (*C*), 129.3 (*C*H), 127.7 (*C*H), 124.0 (*C*H), 123.7 (*C*H), 116.4 (*C*H), 114.8 (*C*), 103.7 (*C*), 35.3 (*C*H), 19.7 (*C*H$_3$), 18.9 (*C*H$_3$); −ES *m*/*z* 440 (35%, $[M]^-$), 439 (100, $[M - H]^-$); found by −ES 439.1197, $C_{27}H_{19}O_6$ ($[M - H]^-$) requires 439.1182, error 3.4 ppm.

**3,3′-(3,4-Dimethyl-phenylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) 47.** Using method A, 4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one **30j** (285 mg, 1.50 mmol) and 3,4-dimethylbenzaldehyde (100 mg, 0.75 mmol) gave the title compound as a colorless solid (258 mg, 69%): mp 283−284 °C; $\nu_{max}/cm^{-1}$ 2937 b, 2916 b, 1668 s (C=O), 1603 s, 1560 m; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.62 (2H, s, 2 × C*H*), 7.16 (2H, s, 2 × C*H*), 6.95 (1H, d, $J$ = 8.0, C*H*), 6.84 (1H, s, C*H*), 6.80 (1H, d, $J$ = 8.0, C*H*), 6.22 (1H, s, C*H*), 2.31 (6H, s, 2 × C$H_3$), 2.26 (6H, s, 2 × C$H_3$), 2.14 (3H, s, C$H_3$), 2.09 (3H, s, C$H_3$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 165.9 (*C*), 165.1 (*C*), 164.8 (*C*), 152.2 (*C*), 138.5 (*C*), 137.1 (*C*), 135.6 (*C*), 133.5 (*C*), 133.3 (*C*), 131.9 (*C*H), 129.2 (*C*H), 127.8 (*C*H), 124.2 (*C*H), 123.9 (*C*H), 123.8 (*C*H), 117.9 (*C*), 116.0 (*C*H), 104.3 (*C*), 35.6 (*C*H), 19.6 (*C*H$_3$), 18.9 (*C*H$_3$); −ES *m*/*z* 495 (100%, $[M - H]^-$); found by −ES 495.1823, $C_{31}H_{27}O_6$ ($[M - H]^-$) requires 495.1808 error 3.0 ppm.

**3-Substituted 4-Hydroxy-2*H*-chromen-2-ones. Method B.** The preparation of 3-substituted 4-hydroxy-2*H*-chromen-2-ones was carried out according to the procedure described by Appendino and co-workers: please note the caution;[27] in the majority of cases, purification was achieved by flash column chromatography on silica using $CH_2Cl_2$/MeOH 95:5 as the eluent.

**4-Hydroxy-6,7-dimethyl-3-(1-naphthylmethyl)-2*H*-chromen-2-one 16.** Using method B, 3,3′-(1-naphthylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) **34** (500 mg, 0.96 mmol) and $NaCNBH_3$ (182 mg, 2.89 mmol) gave the title compound (167 mg, 53%) after purification by flash column chromatography as a colorless solid: mp 253−255 °C (decomp.); $\nu_{max}/cm^{-1}$ 3048 bw (O−H), 2970 w, 2920 w, 1737 w, 1653 m (C=O), 1619 s, 1561 m; $\delta_H$ (500 MHz; DMSO-$d_6$) 8.27 (1H, d, $J$ = 8.2, C*H*), 7.94 (1H, d, $J$ = 8.2, C*H*), 7.77 (1H, d, $J$ = 8.2, C*H*), 7.75 (1H, s, C*H*), 7.62−7.53 (2H, m, 2 × C*H*), 7.38−7.32 (1H, m, C*H*), 7.23 (1H, s, C*H*), 7.09 (1H, d, $J$ = 6.3, C*H*), 4.31 (2H, s, C$H_2$), 2.34 (3H, s, C$H_3$), 2.30 (3H, s, C$H_3$); $\delta_C$ (125 MHz; DMSO-$d_6$) 164.4 (*C*), 163.2 (*C*), 150.6 (*C*), 141.6 (*C*), 134.9 (*C*), 133.3 (*C*), 132.3 (*C*), 131.8 (*C*), 128.4 (C*H*), 126.4 (C*H*), 125.9 (C*H*), 125.6 (C*H*), 125.5 (C*H*), 123.9 (*C*), 123.7 (C*H*), 123.6 (C*H*), 123.2 (C*H*), 116.6 (C*H*), 101.5 (*C*), 26.4 (C$H_2$), 19.6 (C$H_3$), 18.9 (C$H_3$); −ES *m*/*z* 330 (18%, $[M]^-$), 329 (100, $[M - H]^-$); found by +ES 330.1252, $C_{22}H_{18}O_3$ ($[M]^+$) requires 330.1256, error 1.2 ppm.

**4-Hydroxy-6,7-dimethyl-3-(2-naphthylmethyl)-2*H*-chromen-2-one 17.** Using method B, 3,3′-(2-naphthylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) **35** (500 mg, 0.96 mmol) and $NaCNBH_3$ (182 mg, 2.89 mmol) gave the title compound (139 mg, 43%) as a colorless solid after purification by flash column chromatography: mp 246−247 °C (decomp.); $\nu_{max}/cm^{-1}$ 3084 bw (O−H), 3053 b, 3010 b, 2966 b, 1677 w, 1652 m (C=O), 1619 s, 1561 m; $\delta_H$ (500 MHz; DMSO-$d_6$) 7.84 (1H, d, $J$ = 8.2, C*H*), 7.81 (2H, d, $J$ = 8.2, 2 × C*H*), 7.75 (1H, s, C*H*), 7.67 (1H, s, C*H*), 7.46−7.41 (3H, m, 3 × C*H*), 7.20 (1H, s, C*H*), 4.03 (2H, s, C$H_2$), 2.32 (3H, s, C$H_3$), 2.29 (3H, s, C$H_3$); $\delta_C$ (125 MHz; DMSO-$d_6$) 163.3 (*C*), 161.1 (*C*), 150.6 (*C*), 141.7 (*C*), 137.8 (*C*), 133.1 (*C*), 132.4 (*C*), 131.7 (*C*), 127.5 (*C*H), 127.5 (*C*H), 127.4 (*C*H), 126.1 (*C*H), 125.8 (*C*H), 125.4 (*C*H), 123.4 (*C*H), 123.3 (*C*H), 116.7 (*C*H), 113.8 (*C*), 103.1 (*C*), 29.4 (*C*H$_2$), 19.7 (*C*H$_3$), 19.1 (*C*H$_3$); −ES *m*/*z* 330 (18%, $[M]^-$), 329 (100, $[M - H]^-$); found by +EI 330.1262, $C_{22}H_{18}O_3$ ($[M]^+$) requires 330.1256, error 1.8 ppm; Anal. Calcd for $C_{22}H_{18}O_3$): C, 79.98; H, 5.49. Found: H, 79.46; C, 5.18.

**3-Benzyl-4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one 18.** Using method B, 3,3′-(phenylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) **36** (500 mg, 1.07 mmol) and $NaCNBH_3$ (201 mg, 3.20 mmol) gave the title compound (120 mg, 40%) as a colorless solid after purification by flash column chromatography: mp 242−245 °C (decomp.); $\nu_{max}/cm^{-1}$ 3130 bw (O−H), 3064 w, 2969 w, 2921 w, 1666 s (C=O), 1624 s, 1605 s, 1563 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 11.47 (1H, broad s, O*H*), 7.70 (1H, s, C*H*), 7.22−7.27 (4H, m, 4 × C*H*), 7.12−7.17 (2H, m, 2 × C*H*), 3.86 (2H, s, C$H_2$), 2.27 (3H, s, C$H_3$), 2.25 (3H, s, C$H_3$); $\delta_C$ (75 MHz; DMSO-$d_6$) 163.1 (*C*), 160.7 (*C*), 150.4 (*C*), 141.4 (*C*), 140.0 (*C*), 132.2 (*C*), 128.2 (*C*H), 128.1 (*C*H), 125.8 (*C*H), 123.2 (*C*H), 116.6 (*C*H), 113.7 (*C*), 103.3 (*C*), 29.1 (*C*H$_2$), 19.6 (*C*H$_3$), 18.9 (*C*H$_3$); −ES *m*/*z* 280 (14%, $[M]^-$), 279 (100, $[M - H]^-$); found by +ES 280.1094, $C_{18}H_{16}O_3$ ($[M]^+$) requires 280.1099, error 1.8 ppm.

**4-Hydroxy-3-(1-naphthylmethyl)-2*H*-benzo[*h*]chromen-2-one 19.** Using method B, 3,3′-(1-naphthylmethanediyl)bis(4-hydroxy-2*H*-benzo[*h*]chromen-2-one) **37** (106 mg, 0.19 mmol) and $NaCNBH_3$ (59 mg, 0.95 mmol) gave (15 mg, 22%) after chromatography: mp 258−260 °C (decomp.); $\nu_{max}/cm^{-1}$ 3203 bw (O−H), 3056 w, 1646 s (C=O), 1609 s, 1566 m; $\delta_H$ (500 MHz; DMSO-$d_6$) 8.40−8.38 (1 H, m, C*H*), 8.27 (1 H, d, $J$ = 8.2 C*H*), 8.07−8.05 (1H, m, C*H*), 8.01 (1H, d, $J$ = 8.2 C*H*), 7.95 (1H, d, $J$ = 8.2 C*H*), 7.88 (1H, d, $J$ = 8.2 C*H*), 7.77 (1H, d, $J$ = 8.2 C*H*), 7.74−7.72 (2H, m, 2 × C*H*), 7.63−7.54 (2H, m, 2 × C*H*), 7.38−7.34 (1H, m, C*H*), 7.15 (1H, d, $J$ = 6.9, C*H*), 4.38 (2H, s, C$H_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 162.9 (*C*), 162.4 (*C*), 149.1 (*C*), 134.8 (*C*), 134.4 (*C*), 133.4 (*C*), 131.8 (*C*), 128.6 (*C*H), 128.5 (*C*H), 128.0 (*C*H), 126.4 (*C*H), 126.0 (*C*H), 125.7 (*C*H), 125.6 (*C*H), 123.7 (*C*H), 123.7 (*C*H), 122.2 (*C*), 121.6 (*C*H), 119.4 (*C*H), 111.6 (*C*), 102.1 (*C*), 26.6 (*C*H$_2$); −ES *m*/*z* 352 (22%, $[M]^-$), 351 (100, $[M - H]^-$); found by −ES 352.1094, $C_{24}H_{16}O_3$ ($[M]^-$) requires 352.1099, error 1.4 ppm.

**4-Hydroxy-3-(2-naphthylmethyl)-2*H*-benzo[*h*]chromen-2-one 20.** Using method B, 3,3′-(2-naphthylmethanediyl)bis(4-hydroxy-2*H*-benzo[*h*]chromen-2-one) **38** (220 mg, 0.39 mmol) and $NaCNBH_3$ (123 mg, 1.95 mmol) gave (35 mg, 25%) as a colorless solid after chromatography: mp 260−263 °C (decomp.); $\nu_{max}/cm^{-1}$ 3166 bw (O−H), 3051 b, 1651 s (C=O), 1608 s, 1563 m; $\delta_H$ (500 MHz; DMSO-$d_6$) 8.40−8.32 (1H, m, C*H*), 8.10−8.00 (1H, m, C*H*), 8.02 (1H, d, $J$ = 8.8, C*H*), 7.86 (1H, d, $J$ = 8.8, C*H*), 7.86−7.85 (3H, m, 3 × C*H*), 7.82 (1H, d, $J$ = 8.2, C*H*), 7.73−7.69 (2H, m, 2 × C*H*), 7.49 (1H, d, $J$ = 10.1, C*H*), 7.45−7.41 (2H, m, 2 × C*H*), 4.12 (2H, s, C$H_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) some *C*H signals coincident: 162.6 (*C*), 161.7 (*C*), 149.0 (*C*), 137.4 (*C*), 134.3 (*C*), 133.1 (*C*), 131.7 (*C*), 128.5 (*C*H), 127.7 (*C*H), 127.4 (*C*H), 127.3 (*C*H), 126.0 (*C*H), 125.8 (*C*H), 125.2 (*C*H), 123.6 (*C*H), 122.1 (*C*), 121.6 (*C*H), 119.4 (*C*H), 111.6 (*C*), 103.7 (*C*),

29.4 ($CH_2$); −ES *m/z* 352 (10%, $[M]^-$), 351 (100, $[M - H]^-$), 248 (68); found by −ES 352.1095, $C_{24}H_{16}O_3$ ($[M]^-$) requires 352.1099, error 1.1 ppm.

**3-Benzyl-4-hydroxy-2*H*-benzo[*h*]chromen-2-one 21.** Using method B, 3,3′-(phenylmethanediyl)bis(4-hydroxy-2*H*-benzo-[*h*]chromen-2-one) **39** (210 mg, 0.41 mmol) and $NaCNBH_3$ (129 mg, 2.05 mmol) gave (60 mg, 95%) as a colorless solid after chromatography: mp 251−254 °C (decomp.); $\nu_{max}/cm^{-1}$ 3028 bw (O−H), 2939 b, 1666 m, 1639 m, 1610 s, 1559 m; $\delta_H$ (300 MHz; DMSO-$d_6$) 11.82 (1H, s, O*H*), 8.37−8.33 (1H, m, C*H*), 8.02−7.97 (2H, m, 2 × C*H*), 7.77−7.70 (1H, m, C*H*), 7.67−7.65 (2H, m, 2 × C*H*), 7.34−7.22 (3H, m, 3 × C*H*), 7.20−7.10 (1H, m, C*H*), 3.86 (2H, s, $CH_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 162.9 (*C*), 161.7 (*C*), 148.9 (*C*H), 139.9 (*C*), 134.4 (*C*), 128.3 (*C*H), 128.2 (*C*H), 126.0 (*C*H), 122.2 (*C*H), 111.7 (*C*H), 103.9 (*C*H), 29.2 ($CH_2$); −ES *m/z* 302 (7%, $[M]^-$), 301 (100, $[M - H]^-$); found by +EI 302.0931, $C_{20}H_{14}O_3$ ($[M]^+$) requires 302.0943, error 4.0 ppm.

**1-Hydroxy-2-(1-naphthylmethyl)-3*H*-benzo[*f*]chromen-3-one 22.** Using method B, 2,2′-(1-naphthylmethanediyl)bis(1-hydroxy-3*H*-benzo[*f*]chromen-3-one) **40** (400 mg, 0.71 mmol) and $NaCNBH_3$ (134 mg, 2.13 mmol) gave (77 mg, 31%) as a colorless solid after chromatography: mp 261−263 °C (decomp.); $\nu_{max}/cm^{-1}$ 3086 bw (O−H), 3061 b, 1646 s (C═O), 1625 m, 1550 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 9.43 (1H, d, $J$ = 8.8, C*H*), 8.27 (1H, d, $J$ = 8.2, C*H*), 8.21 (1H, d, $J$ = 8.8, C*H*), 8.06 (1H, d, $J$ = 8.2, C*H*), 7.96 (1H, d, $J$ = 8.2, C*H*), 7.77 (1 H, d, $J$ = 8.2, C*H*), 7.71−7.68 (1H, m, C*H*), 7.64−7.55 (4H, m, 4 × C*H*), 7.37−7.34 (1H, t, $J$ = 7.7, C*H*), 7.10 (1H, d, $J$ = 6.9, C*H*), 4.45 (2H, s, $CH_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) 165.3 (*C*), 162.4 (*C*), 153.2 (*C*), 134.5 (*C*), 133.6 (*C*H), 133.4 (*C*), 131.9 (*C*), 130.7 (*C*), 129.1 (*C*H), 128.5 (*C*H), 128.2 (*C*H), 126.4 (*C*H), 126.3 (*C*H), 126.0 (*C*H), 125.7 (*C*H), 125.6 (*C*H), 125.5 (*C*H), 123.7 (*C*H), 123.0 (*C*H), 117.1 (*C*H), 111.2 (*C*), 108.6 (*C*), 102.3 (*C*), 26.5 ($CH_2$); −ES *m/z* 352 (23%, $[M]^-$), 351 (100, $[M - H]^-$); found by +EI 352.1092, $C_{24}H_{16}O_3$ ($[M]^+$) requires 352.1099, error 1.9 ppm.

**1-Hydroxy-2-(2-naphthylmethyl)-3*H*-benzo[*f*]chromen-3-one 23.** Using method B, 2,2′-(2-naphthylmethanediyl)bis(1-hydroxy-3*H*-benzo[*f*]chromen-3-one) **41** (224 mg, 0.40 mmol) and $NaCNBH_3$ (125 mg, 2.00 mmol) gave (67 mg, 47%) as a colorless solid after chromatography: mp 265−267 °C (decomp.); $\nu_{max}/cm^{-1}$ 3081 bw (O−H), 3043 b, 2923 w, 1650 s (C═O), 1627 m, 1549 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 9.45 (1H, d, $J$ = 8.9, C*H*), 8.19 (1H, d, $J$ = 8.2, C*H*), 8.05 (1H, d, $J$ = 7.2, C*H*), 7.85−7.81 (3H, m, 3 × C*H*), 7.72−7.69 (2H, m, 2 × C*H*), 7.62−7.58 (1H, m, C*H*), 7.55 (1H, d, $J$ = 9.2, C*H*), 7.47 (1H, d, $J$ = 10.1, C*H*), 7.44−7.41 (2H, m, 2 × C*H*), 4.20 (2H, s, $CH_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 164.5 (*C*), 162.4 (*C*), 153.0 (*C*), 137.1 (*C*), 133.6 (*C*H), 133.1 (*C*), 131.7 (*C*), 130.7 (*C*), 129.1 (*C*H), 128.2 (*C*H), 127.7 (*C*H), 127.4 (*C*H), 127.4 (*C*H), 127.3 (*C*H), 126.3 (*C*H), 126.0 (*C*H), 125.5 (*C*H), 125.3 (*C*H), 117.0 (*C*H), 109.4 (*C*), 103.9 (*C*), 29.2 ($CH_2$); −ES *m/z* 352 (22%, $[M]^-$), 351 (100, $[M - H]^-$), 212 (12); found by +EI 352.1086, $C_{24}H_{16}O_3$ ($[M]^+$) requires 352.1099, error 3.7 ppm.

**2-Benzyl-1-hydroxy-3*H*-benzo[*f*]chromen-3-one 24.** Using method B, 2,2′-(phenylmethanediyl)bis(1-hydroxy-3*H*-benzo-[*f*]chromen-3-one) **42** (303 mg, 0.59 mmol) and $NaCNBH_3$ (186 mg, 2.95 mmol) gave (81 mg, 89%) as a colorless solid after chromatography: mp 212−214 °C (decomp.); $\nu_{max}/cm^{-1}$ 3060 bw (O−H), 3029 b, 2970 w, 1705 w, 1644 s (C═O), 1624 m, 1546 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 11.94 (1H, broad s, O*H*), 9.42 (1H, d, $J$ = 8.8, C*H*), 8.17 (1H, d, $J$ = 8.8, C*H*), 8.05 (1H, d, $J$ = 7.2, C*H*), 7.72−7.68 (1H, m, C*H*), 7.61−7.58 (1H, m, C*H*), 7.54 (1H, d, $J$ = 8.8, C*H*), 7.27−7.25 (4H, m, 4 × C*H*), 7.20−7.15 (1H, m, C*H*), 4.04 (2H, s, $CH_2$); $\delta_C$ (125 MHz; DMSO-$d_6$) 162.6 (*C*), 153.2 (*C*), 137.4 (*C*), 133.2 (*C*), 131.8 (*C*), 130.8 (*C*), 129.3 (2 x *C*H), 127.9 (2 x *C*H), 127.5 (*C*H), 127.4 (*C*H), 126.2 (*C*H), 125.7 (*C*H), 125.6 (*C*H), 125.4 (*C*H), 117.2 (*C*H), 109.6 (*C*), 104.0 (*C*), 29.4 ($CH_2$); −ES *m/z* 302 (17%, $[M]^-$), 301 (100, $[M - H]^-$); found by +EI 302.0929, $C_{20}H_{14}O_3$ ($[M]^+$) requires 302.0943, error 4.6 ppm.

**4-Hydroxy-3-(1-naphthylmethyl)-2*H*-chromen-2-one 25.** Using method B, 3,3′-(1-naphthyldiyl)bis(4-hydroxy-2*H*-chromen-2-one) **43** (240 mg, 0.52 mmol) and $NaCNBH_3$ (98 mg, 1.56 mmol) gave (165 mg, 68%) as a colorless solid after chromatography: mp 258−260 °C (decomp.); $\nu_{max}/cm^{-1}$ 3042 bw (O−H), 2971 b, 1681 w, 1651 s (C═O), 1599 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 11.70 (1H, s, O*H*), 8.29 (1H, d, $J$ = 8.2, C*H*), 8.01 (1H, dd, $J$ = 8.0, 1.4, C*H*), 7.95 (1H, dd, $J$ = 8.0, 1.4, C*H*), 7.77 (1H, d, $J$ = 8.2, C*H*), 7.68−7.52 (3H, m, C*H*), 7.44−7.34 (3H, m, C*H*), 7.12 (1H, d, $J$ = 8.8, C*H*), 4.35 (2H, s, $CH_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 162.9 (*C*), 161.4 (*C*), 152.2 (*C*), 134.7 (*C*), 133.3 (*C*), 132.0 (*C*H), 131.8 (*C*), 128.5 (*C*H), 126.4 (*C*H), 126.0 (*C*H), 125.6 (*C*H), 125.6 (*C*H), 124.0 (*C*H), 123.7 (*C*H), 123.4 (*C*H), 116.3 (*C*H), 116.2 (*C*), 102.5 (*C*), 26.5 ($CH_2$); −ES *m/z* 302 (13%, $[M]^-$), 301 (100, $[M - H]^-$); found by +ES 302.0930, $C_{20}H_{14}O_3$ ($[M]^+$) requires 302.0943, error 4.3 ppm.

**4-Hydroxy-3-(2-naphthylmethyl)-2*H*-chromen-2-one 26.** Using method B, 3,3′-(2-naphthyldiyl)bis(4-hydroxy-2*H*-chromen-2-one) **44** (231 mg, 0.50 mmol) and $NaCNBH_3$ (210 mg, 3.34 mmol) gave (131 mg, 80%) as a colorless solid after chromatography: mp 202−204 °C (decomp.); $\nu_{max}/cm^{-1}$ 3047 bw (O−H), 1666 m, 1628 m, 1586 m, 1499 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 7.83−7.65 (5H, m, 5 × C*H*), 7.51 (1H, dd, $J$ = 8.8, 1.5, C*H*), 7.42−7.31 (3H, m, 3 × C*H*), 7.12−7.05 (2H, m, 2 × C*H*), 3.80 (2H, s, $CH_2$); $\delta_C$ (75 MHz; DMSO-$d_6$) some signals coincident: 165.1 (*C*), 163.7 (*C*), 152.7 (*C*), 139.6 (*C*), 133.1 (*C*), 131.5 (*C*), 130.7 (*C*H), 127.7 (*C*H), 127.3 (*C*H), 127.2 (*C*H), 125.7 (*C*H), 125.7 (*C*H), 124.9 (*C*H), 124.0 (*C*H), 122.8 (*C*H), 119.5 (*C*), 115.9 (*C*H), 101.8 (*C*), 29.8 ($CH_2$); +ES *m/z* 325 (100, $[M + Na]^+$); found by +ES 325.0843, $C_{20}H_{14}O_3Na$ ($[M + Na]^+$) requires 325.0841, error 0.6 ppm.

**3-Benzyl-4-hydroxy-2*H*-chromen-2-one 27.** Using method B, 3,3′-(phenylmethanediyl)bis(4-hydroxy-2*H*-chromen-2-one) **45** (412 mg, 2.00 mmol) and $NaCNBH_3$ (125 mg, 2.00 mmol) gave (12 mg, 5%) as a colorless solid after chromatography: mp 205−207 °C; $\nu_{max}/cm^{-1}$ 3099 bw (O−H), 2971 w, 1654 s (C═O), 1604 s, 1559; $\delta_H$ (500 MHz; DMSO-$d_6$) 11.69 (1H, s, O*H*), 7.98 (1H, d, $J$ = 7.9, 1.2, C*H*), 7.62−7.59 (1H, m, C*H*), 7.38−7.34 (2H, m, 2 × C*H*), 7.25−7.24 (4H, m, 4 × C*H*), 7.11−7.18 (1H, m, C*H*), 3.89 (2H, s, $CH_2$); $\delta_C$ (125 MHz; DMSO-$d_6$) 163.0 (*C*), 160.6 (*C*), 152.1 (*C*), 139.9 (*C*), 132.09, (*C*H), 132.0 (*C*H), 128.3 (*C*H), 128.2 (*C*H), 126.0 (*C*H), 124.1 (*C*H), 123.5 (*C*H), 123.4 (*C*H), 116.3 (*C*H), 116.3 (*C*), 104.3 (*C*), 29.3 ($CH_2$); −ES *m/z* 251 (100%, $[M - H]^-$); Found by +EI 251.0708, $C_{16}H_{12}O_3$ $[M - H]^-$ requires 251.0703 error 2.1 ppm.

**3-(3,4-Dimethylbenzyl)-4-hydroxy-2*H*-chromen-2-one 28.** Using method B, 3,3′-(3,4-dimethyl-phenylmethanediyl)bis-(4-hydroxy-2*H*-chromen-2-one) **46** (220 mg, 0.50 mmol) and $NaCNBH_3$ (126 mg, 2.00 mmol) gave (10 mg, 7%) as a colorless solid after chromatography: $\nu_{max}/cm^{-1}$ 3092 bw (O−H), 2934 w, 1652 s (C═O), 1633 s, 1607 m, 1496 m; $\delta_H$ (500 MHz; DMSO-$d_6$) 7.98−7.96 (1H, m, C*H*), 7.62−7.58 (1H, m, C*H*), 7.37−7.34 (2H, m, 2 × C*H*), 7.00−6.98 (2H, m, 2 × C*H*), 6.94−6.93 (1H, m, C*H*), 3.80 (2H, s, $CH_2$), 2.14 (3H, s, $CH_3$), 2.13 (3H, s, $CH_3$); $\delta_C$ (125 MHz; DMSO-$d_6$) 162.9 (*C*), 160.3 (*C*), 152.0 (*C*), 137.1 (*C*), 135.8 (*C*), 133.5 (*C*), 131.9 (*C*H), 129.3 (*C*H), 125.5 (*C*H), 124.0 (*C*H), 123.4 (*C*H), 116.2 (*C*), 116.3 (*C*H), 116.2 (*C*H), 104.6 (*C*), 28.7 ($CH_2$), 19.5 ($CH_3$), 19.0 ($CH_3$); −ES *m/z* 280 (21%, $[M]^-$), 279 (100, $[M - H]^-$); Found by −ES 279.1023, $C_{18}H_{15}O_3$ ($[M - H]^-$) requires 279.1021, error 0.7 ppm.

**3-(3,4-Dimethylbenzyl)-4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one 29.** Using method B, 3,3′-(3,4-dimethyl-phenylmethanediyl)bis(4-hydroxy-6,7-dimethyl-2*H*-chromen-2-one) **47** (248 mg, 0.50 mmol) and $NaCNBH_3$ (126 mg, 2.00 mmol) gave (11.5 mg, 8%) as a colorless solid after chromatography: mp 218−219.5 °C (decomp.); $\nu_{max}/cm^{-1}$ 2915 w, 2854 w, 1669 w, 1645 s (C═O), 1631 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 11.40 (1H, s, O*H*),

7.71 (1H, s, C*H*) 7.16 (1H, s, C*H*), 6.98−6.97 (2H, m, 2 × C*H*), 6.93−6.91 (1H, m, C*H*), 3.77 (2H, s, C$H_2$), 2.30 (3H, s, C$H_3$), 2.27 (3H, s, C$H_3$), 2.14 (3H, s, C$H_3$), 2.13 (3H, s, C$H_3$); $\delta_C$ (125 MHz; DMSO-$d_6$) some signals coincident: 163.2 (*C*), 160.5 (*C*), 150.4 (*C*), 141.4 (*C*), 137.4 (*C*), 135.7 (*C*), 133.5 (*C*), 132.3 (*C*), 129.4 (*C*H), 129.3 (*C*H), 125.6 (*C*H), 123.2 (*C*H), 116.6 (*C*H), 113.8 (*C*) 103.7 (*C*), 28.7 (C$H_2$), 19.6 (C$H_3$), 19.5 (C$H_3$), 19.0 (C$H_3$); −ES *m*/*z* 307 (100%, [M − H]$^-$), 189 (22); found by −ES 307.1340, $C_{20}H_{19}O_3$ ([M − H]$^-$) required 307.1334 error 1.9 ppm.

**Acetylated Phenols. Method C.** An acetylation of substituted phenols was achieved by following the representative procedure described by Knölker and co-workers for the synthesis of 2, 3-dimethyl acetate.[21]

**3,4-Dimethoxyphenyl Acetate 33k.** Using method C, 3,4-dimethoxyphenol **32k** (3.25 g, 27.0 mmol), acetic anhydride (8.56 g, 7.92 mL, 84.0 mmol), pyridine (0.1 mL, 0.10 g, 1.2 mmol), and DMAP (0.33 g, 2.70 mmol) followed by purification by distillation gave the title compound as a colorless oil (3.60 g, 68%); $\nu_{max}$/cm$^{-1}$ 3003 w, 2938 w, 2837 w, 1757 s (C═O), 1603 m, 1507 s; $\delta_H$ (500 MHz; $CDCl_3$) 6.85 (1H, d, $J$ = 8.5, C*H*), 6.65 (1H, dd, $J$ = 8.5, 2.5, C*H*), 6.64 (1H, d, $J$ = 2.5, C*H*), 3.88 (3H, s, OC$H_3$), 3.87 (3H, s, OC$H_3$), 2.29 (3H, s, C(═O)C$H_3$); CI *m*/*z* ($NH_3$) 214 (100%, [M + $NH_4$]$^+$).

**2,3-Dimethylphenyl Acetate 33m.** Using method C, 2,3-dimethylphenol **32m** (4.00 g, 32.7 mmol), acetic anhydride (10.5 g, 9.7 mL, 102 mmol), pyridine (0.1 mL, 0.10 g, 1.2 mmol), and DMAP (0.29 g, 2.37 mmol) followed by purification by distillation gave the title compound as a colorless oil (4.10 g, 76%): $\nu_{max}$/cm$^{-1}$ 2923 w, 1762 s (C═O); $\delta_H$ (300 MHz; $CDCl_3$) 7.14−7.04 (2H, m, 2 × C*H*), 6.87 (1H, d, $J$ = 8.8, C*H*), 2.34 (3H, s, C$H_3$), 2.31 (3H, s, C$H_3$), 2.09 (3H, s, C$H_3$); $\delta_C$ (75 MHz; $CDCl_3$) 169.4 (*C*═O), 149.1 (*C*), 138.4 (*C*), 128.6 (*C*), 127.5 (*C*H), 126.0 (*C*H), 119.3 (*C*H), 20.8 (C$H_3$), 20.0 (C$H_3$), 12.3 (C$H_3$); CI *m*/*z* ($NH_3$) 182 (100%, [M + $NH_4$]$^+$).

**2-Hydroxy-acetophenones. Method D.** Fries rearrangement of acetylated phenols was achieved by following the procedure described by Knölker and co-workers for the synthesis of 1-(2-hydroxy-3,4-dimethylphenyl)ethanone.[2]

**1-(2-Hydroxy-4,5-dimethoxyphenyl)ethanone 31k.** Using method D, 3,4-dimethoxyphenyl acetate **33k** (3.00 g, 14.0 mmol) and aluminum trichloride (3.00 g, 22.4 mmol) gave the title compound as a cream solid (1.80 g, 66%): $\nu_{max}$/cm$^{-1}$ 3003 w, 2938 w, 2852 w, 1617 s, 1507 s; $\delta_H$ (500 MHz; $CDCl_3$) 12.63 (O*H*), 7.02 (1H, s, C*H*), 6.42 (1H, s, C*H*), 3.88 (3H, s, OC$H_3$), 3.84 (3H, s, OC$H_3$), 2.53 (3H, s, (C(═O)C$H_3$); $\delta_C$ (125 MHz; $CDCl_3$) 202.0 (*C* = O), 160.0, 156.7, 141.8, 111.6, 111.4, 100.4, 56.5 (OC$H_3$), 56.1 (OC$H_3$), 26.3 (C$H_3$); CI *m*/*z* ($NH_3$) 197 (100%, [M + H]$^+$); found by +ES 197.0814, $C_{10}H_{13}O_4$ [M + H]$^+$ requires 197.0814, error 0.0 ppm.

**1-(2-Hydroxy-3,4-dimethylphenyl)ethanone 31m.** Using method D, 2,3-dimethylphenyl acetate **33m** (4.05 g, 24.7 mmol) and aluminum trichloride (3.94 g, 29.6 mmol) gave the title compound as a cream solid (2.30 g, 57%): $\nu_{max}$/cm$^{-1}$ 2972 w, 2922 w, 2864 w, 1628 s (C═O); $\delta_H$ (300 MHz; $CDCl_3$) 12.70 (1H, bs, O*H*), 7.50 (1H, d, $J$ = 8.2, C*H*), 6.72 (1H, d, $J$ = 8.2, C*H*), 2.61 (3H, s, C$H_3$), 2.32 (3H, s, C$H_3$), 2.18 (3H, s, C$H_3$); $\delta_C$ (75 MHz; $CDCl_3$) 204.2 (*C*═O), 160.6 (*C*), 146.1 (*C*), 127.5 (*C*H), 125.3 (*C*), 120.3 (*C*H), 117.2 (*C*), 26.5 (C$H_3$), 20.7 (C$H_3$), 10.9 (C$H_3$); CI *m*/*z* ($NH_3$) 182 (3%, [M + $NH_4$]$^+$), 165 (100, [M + H]$^+$).

**4-Hydroxy-2*H*-chromen-2-ones.** Annulation reactions to give 4-hydroxy-2*H*-chromen-2-ones were carried out following the procedure described by Barker and co-workers.[20]

**Method E.** Representative procedure: a solution of 1-(2-hydroxy-3,4-dimethylphenyl)ethanone in diethylcarbonate (1 g/5 mL) was added dropwise to a suspension of NaH (60% dispersion in mineral oil) in diethylcarbonate (1 g/5 mL) at 0 °C. The resulting mixture was heated at 100 °C for 3 h when it was cooled first to rt and then to 0 °C. Water was then carefully added dropwise to quench residual NaH. The remaining diethylcarbonate was extracted into diethyl ether (3× total amount of diethylcarbonate used). The aqueous phase was carefully acidified to pH 3 with 2 N HCl (significant foaming occurred). The resulting precipitated solid was collected by filtration, washed sequentially with water followed by petroleum ether (40−60: 3× total amount of diethylcarbonate used), and then dried in a 90 °C oven overnight. Typical yields from this procedure ranged between 54 and 89%.

**4-Hydroxy-6-methyl-2*H*-chromen-2-one 30b.** Using method E, 5-methyl-2-hydroxy-acetophenone **31b** (1.00 g, 6.67 mmol), diethylcarbonate (5.4 mL), and sodium hydride (1.34 g, 33.3 mmol) gave the title compound as a colorless solid (1.08 g, 92%): mp 243−246 °C; $\nu_{max}$/cm$^{-1}$ 3082 bw (O−H), 2924 b, 1681 s (C═O), 1633 s, 1605 s, 1575 s, 1508 m; $\delta_H$ (500 MHz; DMSO-$d_6$) 12.47 (1H, bs, O*H*), 7.61 (1H, s, C*H*), 7.45 (1H, d, $J$ = 8.4, C*H*), 7.26 (1H, d, $J$ = 8.4, C*H*), 5.59 (1H, s, C*H*), 2.37 (3H, s, C$H_3$); $\delta_C$ (75 MHz; $CDCl_3$) 165.7 (*C*), 162.1 (*C*), 151.7 (*C*), 133.5 (*C*H), 133.1 (*C*), 122.8 (*C*H), 116.2 (*C*H), 115.5 (*C*), 91.0 (*C*H), 20.4 (C$H_3$); −ES *m*/*z* 176 (8%, [M]$^-$), 175 (100, [M − H]$^-$); found by −ES 175.0398, $C_{10}H_7O_3$ ([M − H]$^-$) requires 175.0395, error 1.7 ppm.

**4-Hydroxy-6-methoxy-2*H*-chromen-2-one 30d.** Using method E, 5-methoxy-2-hydroxy-acetophenone **31d** (1.90 g, 11.4 mmol), diethylcarbonate (6 mL), and sodium hydride (2.28 g, 57.0 mmol) gave the title compound as a colorless solid (2.05 g, 94%): $\nu_{max}$/cm$^{-1}$ 2957 w, 2890 w, 1680 s (C═O), 1577 s, 1549 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 12.53 (1H, bs, O*H*), 7.32 (1H, d, $J$ = 10.0, C*H*), 7.23 (1H, d, $J$ = 10.0, C*H*), 7.23 (1H, s, C*H*), 5.59 (1H, s, C*H*), 3.80 (3H, s, OC$H_3$); $\delta_C$ (75 MHz; DMSO-$d_6$) 165.4 (*C*), 162.1 (*C*), 155.3 (*C*), 147.9 (*C*), 120.5 (*C*H), 117.7 (*C*H), 116.2 (*C*), 105.0 (*C*H), 91.3 (*C*H), 55.7 (OC$H_3$); −ES *m*/*z* 192 (8%, [M]$^-$), 191.2 (100, [M − H]$^-$); found by −ES 191.0356, $C_{10}H_7O_4$ ([M − H]$^-$) requires 191.0344, error 6.3 ppm.

**4-Hydroxy-7-methoxy-2*H*-chromen-2-one 30e.** Using method E, 4-methoxy-2-hydroxy-acetophenone **31e** (2.50 g, 15.1 mmol), diethylcarbonate (7.5 mL), and sodium hydride (3.00 g, 75.0 mmol) gave the title compound as a colorless solid (2.78 g, 95%): $\nu_{max}$/cm$^{-1}$ 3122 bw (O−H), 3072 w, 2924 w, 1687 s (C═O), 1644 s, 1600 s; $\delta_H$ (400 MHz; DMSO-$d_6$) 12.39 (1H, bs, O*H*), 7.70 (1H, d, $J$ = 8.5, C*H*), 6.94−6.90 (2H, m, 2 × C*H*), 5.44 (1H, s, C*H*), 3.84 (3H, s, OC$H_3$); $\delta_C$ (100 MHz; DMSO-$d_6$) 166.0 (*C*), 163.0 (*C*), 162.3 (*C*), 155.4 (*C*), 124.4 (*C*H), 111.9 (*C*H), 108.9 (*C*), 100.5 (*C*H), 88.5 (*C*H), 55.9 (OC$H_3$); −ES *m*/*z* 192 (10%, [M]$^-$), 191 (100, [M − H]$^-$); found by −ES 191.0353, $C_{10}H_7O_4$ ([M − H]$^-$) requires 191.0344, error 4.7 ppm.

**6-Fluoro-4-hydroxy-2*H*-chromen-2-one 30f.** Using method E, 5-fluoro-2-hydroxy-acetophenone **31f** (1.05 g, 6.81 mmol), diethylcarbonate (4 mL), and sodium hydride (2.28 g, 57.0 mmol) gave the title compound as a colorless solid (1.12 g, 91%): mp 240−241 °C; $\nu_{max}$/cm$^{-1}$ 3091 bw (O−H), 2947 w, 1702 s (C═O), 1571 s; $\delta_H$ (400 MHz; DMSO-$d_6$) 7.99−7.97 (1H, m, C*H*), 7.91−7.87 (1H, m, C*H*), 7.74−7.71 (1H, m, C*H*), 5.71 (1H, s, C*H*); $\delta_C$ (125 MHz; DMSO-$d_6$) 164.9 (*C*, d, $J_{CF}$ = 1.9), 161.8 (*C*), 157.9 (*C*F, d, $J_{CF}$ = 240.6), 149.9 (*C*), 120.2 (*C*H, d, $J_{CF}$ = 25.4), 118.7 (*C*H, d, $J_{CF}$ = 9.1), 117.0 (*C*, d, $J_{CF}$ = 10.0), 108.7 (*C*H, d, $J_{CF}$ = 25.4), 91.5 (*C*H); −ES *m*/*z* 180 (8%, [M]$^-$), 179 (100, [M − H]$^-$); found by −ES 179.0155, $C_9H_4FO_3$ ([M − H]$^-$) requires 179.0144, error 6.1 ppm.

**7-Fluoro-4-hydroxy-2*H*-chromen-2-one 30g.** Using method E, 4-fluoro-2-hydroxy-acetophenone **31g** (1.10 g, 7.14 mmol), diethylcarbonate (4 mL), and sodium hydride (1.43 g, 35.7 mmol) gave the title compound as a colorless solid (0.363 g, 52%): mp 217−218 °C; $\nu_{max}$/cm$^{-1}$ 3088 bw (O−H), 2971 w, 1687 s (C═O), 1635 s, 1615 s, 1570 s, 1521 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 12.69 (1H, bs, O*H*), 7.88−7.86 (1H, m, C*H*), 7.37−7.75 (1H, m, C*H*), 7.25−7.23 (1H, m, C*H*), 5.56 (1H, s, C*H*); $\delta_C$ (75 MHz; DMSO-$d_6$) 166.0 (*C*), 163.6 (*C*F, d, $J_{CF}$ = 270.9), 162.6 (*C*), 154.8 (*C*, d, $J_{CF}$ = 13.8), 125.5 (*C*H, d, $J_{CF}$ = 10.4), 112.8 (*C*, d, $J_{CF}$ = 2.5), 111.8 (*C*H, d, $J_{CF}$ = 23.0), 103.9 (*C*H, d, $J_{CF}$ = 25.6), 90.0 (*C*H); $\delta_F$ (282 MHz; DMSO-$d_6$) −106.31; −ES *m*/*z* 180 (8%, [M]$^-$), 179 (100, [M − H]$^-$); found

by −ES 179.0152, $C_9H_4FO_3$ ([M − H]$^-$) requires 179.0144, error 4.5 ppm.

**6,8-Dibromo-4-hydroxy-2*H*-chromen-2-one 30i.** Using method E, 3,5-dibromo-2-hydroxyacetophenone **31i** (1.37 g, 4.65 mmol), diethylcarbonate (4 mL), and sodium hydride (0.93 g, 23.3 mmol) gave the title compound as a colorless solid (0.463 g, 30%): mp 275−280 °C; $\nu_{max}$/cm$^{-1}$ 3080 bw (O−H), 1722 s (C═O), 1606 m, 1543 s; $\delta_H$ (400 MHz; DMSO-$d_6$) 8.18 (1H, d, $J$ = 2.4, C*H*), 7.89 (1H, d, $J$ = 2.4, C*H*), 5.70 (1H, s, C*H*); $\delta_C$ (125 MHz; DMSO-$d_6$) 164.4 (*C*), 160.6 (*C*), 149.5 (*C*), 137.4 (*C*H), 125.1 (*C*H), 118.9 (*C*), 115.9 (*C*), 110.7 (*C*), 91.9 (*C*H); −ES $m/z$ 321 (46, [M($^{81}$Br$_2$)]$^-$), 319 (100, [M($^{81}$Br$^{79}$Br)]$^-$), 317 (48, [M($^{79}$Br$_2$)]$^-$); found by −ES 316.8461, $C_9H_3{}^{79}Br_2O_3$ ([M − H]$^-$) requires 316.8454, error 2.1 ppm.

**6,7-Dimethoxy-4-hydroxy-2*H*-chromen-2-one 30k.** Using method E, 4,5-dimethoxy-2-hydroxyacetophenone **31k** (1.80 g, 9.18 mmol), diethylcarbonate (9.0 mL), and sodium hydride (1.90 g, 47.5 mmol) gave the title compound as a colorless solid (1.11 g, 54%): $\delta_H$ (500 MHz; DMSO-$d_6$) 12.55 (1H, bs, O*H*), 7.18 (1H, s, C*H*), 7.01 (1H, s, C*H*), 5.54 (1H, s, C*H*), 3.85 (3H, s, OC$H_3$), 3.80 (3H, s, OC$H_3$); $\delta_C$ (125 MHz; DMSO-$d_6$) 166.0 (*C*), 162.5 (*C*), 153.0 (*C*), 149.4 (*C*), 145.6 (*C*), 107.5 (*C*), 103.4 (*C*H), 100.1 (*C*H), 88.7 (*C*H), 56.1 (O*C*H$_3$), 55.8 (O*C*H$_3$); −ES $m/z$ 221 (100%, [M − H]$^-$); found by −ES 221.0463 $C_{11}H_9O_5$ ([M − H]$^-$) requires 221.0450, error 5.9 ppm.

**4-Hydroxy-2*H*-benzo[*h*]chromen-2-one 30l.** Using method E, 1-hydroxy-2-acetonaphthone **31l** (20.0 g, 107 mmol), diethylcarbonate (140 mL), and sodium hydride (16.2 g, 400 mmol) gave the title compound as a buff solid (12.3 g, 54%): mp 272−273 °C; $\nu_{max}$/cm$^{-1}$ 3090 bw (O−H), 3049 b, 2943 b, 1716 m, 1687 s, 1637 m, 1601 s, 1497 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 12.95 (1H, broad s, O*H*), 8.36−8.34 (1H, m, C*H*), 8.05−8.03 (1H, m, C*H*), 7.82 (2H, s, 2 × C*H*), 7.74−7.69 (2H, m, 2 × C*H*), 5.85 (1H, s, C*H*); $\delta_C$ (125 MHz; DMSO-$d_6$) 166.8 (*C*), 161.9 (*C*), 150.7 (*C*), 134.8 (*C*), 128.8 (*C*H), 128.1 (*C*H), 127.4 (*C*H), 123.5 (*C*H), 122.3 (*C*), 121.7 (*C*H), 119.0 (*C*H), 111.3 (*C*), 90.7 (*C*H); −ES $m/z$ 212 (10%, [M]$^-$), 211 (100, [M − H]$^-$); found by −ES 211.0394, $C_{13}H_7O_3$ ([M − H]$^-$) requires 211.0395, error 0.5 ppm.

**4-Hydroxy-7,8-dimethyl-2*H*-chromen-2-one 30m.** Using method E, 3,4-dimethyl-2-hydroxy-acetophenone **31m** (2.30 g, 14.0 mmol), diethylcarbonate (13.0 mL), and sodium hydride (2.80 g, 70.0 mmol) gave the title compound as a colorless solid (2.41 g, 89%): mp 232−236 °C; $\nu_{max}$/cm$^{-1}$ 2971 w, 2923 w, 1640 s (C═O), 1593 s, 1551 s, 1513 s; $\delta_H$ (500 MHz; DMSO-$d_6$) 12.40 (1H, bs, O*H*), 7.56 (1H, d, $J$ = 8.2, C*H*), 7.17 (1H, d, $J$ = 8.2, C*H*), 5.55 (1H, s, C*H*), 2.36 (3H, s, C$H_3$), 2.27 (3H, s, C$H_3$); $\delta_C$ (125 MHz; DMSO-$d_6$) 166.2 (*C*), 162.2 (*C*), 151.6 (*C*), 141.7 (*C*), 125.1 (*C*H), 123.5 (*C*), 119.9 (*C*H), 113.5 (*C*), 89.9 (*C*H), 20.0 (*C*H$_3$), 11.2 (*C*H$_3$); −ES $m/z$ 190 (7%, [M]$^-$), 189 (100, [M − H]$^-$); found by −ES 189.0552, $C_{11}H_9O_3$ ([M − H]$^-$) requires 189.0552, error 0.0 ppm.

**1-Hydroxy-3*H*-benzo[*f*]chromen-3-one 30n.** Using method E, 2-hydroxy-1-acetonaphthone (5.03 g, 21.8 mmol), diethylcarbonate (35 mL), and sodium hydride (4.05 g, 100 mmol) gave the title compound as a buff solid (4.20 g, 91%): mp 238−241 °C; $\nu_{max}$/cm$^{-1}$ 3063 bw (O−H), 3011 b, 2969 b, 2937 b, 1683 s (C═O), 1562 s; $\delta_H$ (300 MHz; DMSO-$d_6$) 12.99 (1H, broad s, O*H*), 9.27 (1H, d, $J$ = 8.6, C*H*), 8.19 (1H, d, $J$ = 9.1, C*H*), 8.03 (1H, dd, $J$ = 8.1, 1.5, C*H*), 7.72−7.66 (1H, m, C*H*), 7.61−7.56 (1H, m, C*H*), 7.53 (1H, d, $J$ = 9.1, C*H*), 5.79 (1H, s, C*H*); $\delta_C$ (75 MHz; DMSO-$d_6$) 169.4 (*C*), 161.4 (*C*), 154.9 (*C*), 134.2 (*C*H), 130.3 (*C*), 128.9 (*C*H), 128.9 (*C*), 128.3 (*C*H), 126.0 (*C*H), 125.6 (*C*H), 117.2 (*C*H), 108.6 (*C*), 91.8 (*C*H); −ES $m/z$ 211 (100%, [M − H]$^-$), 113 (28); found by +EI 212.0472, $C_{13}H_7O_3$ ([M]$^+$) requires 212.0468, error 1.9 ppm.

**Molecular Modeling.** For docking purposes, the crystallographic coordinates of the human NQO1/**1** complex were obtained from the Brookhaven Database (PDB code 2F1O[22] and resolution 2.75 Å). This structure, including the protein, the FAD, the **1** ligand and identified solvent oxygen atoms (none of which were within the active site), was protonated allowing for appropriate ionization at physiological pH. The protonated complex was then minimized within Sybyl 7.3 (Tripos Ltd., St Louis) while holding all heavy atoms stationary. The ligand was then removed to leave the receptor complex which was used for the subsequent docking studies. Inspection of the active site, vide infra, showed a perfectly plausible internal hydrogen bond network within the protein. A second receptor model, with an alternative tautomeric pose for His161, was also prepared and the interactions with all putative inhibitors were subsequently evaluated.

For preparation of ligand structures, fragments from Sybyl 7.3 were used to construct the compounds and all symmetric compounds were prepared as monoanionic ligands.[19] Ligands were subject to 1000 iterations of energy minimization using the steepest descent algorithm using the Tripos force field. For computational docking, the GOLD 3.0 software[28] was used in combination with the ChemScore[25] scoring function. GOLD uses a genetic algorithm (GA), whereby the molecular features of a protein−ligand complex are encoded as a chromosome. Each ligand was docked into the two receptor models to assess both ligand potency and binding pose. Given that GOLD's internal definition of "lipophilic atoms" are nonaccepting sulfurs, nonpolar carbon atoms, and nonionic Cl, Br, or I, adoption of the default setup seems likely to underestimate the aromatic/lipophilic nature of the receptor in the immediate vicinity of the isoalloxazine fragment of FAD, consequently causing a systematically low prediction of binding affinities for known active aromatic ligands. To avoid this probability we reatom-typed the fragment as shown in Supporting Information, Figure 6S.

The active site was defined as being any volume within 15 Å of the surface of **1** in its crystal pose in 2F1O. Each GA run comprised 100000 genetic operations on an initial population of 100 members divided into five subpopulations. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively. GOLD allows a user-definable number of GA runs per ligand, each of which starts from a different orientation. For these experiments, the number of GA runs was set to 10, and scoring of the docked poses was performed with the ChemScore scoring function. Each GOLD run saved the five strongest scoring binding poses of each ligand (subject to a rmsd distance threshold of 0.75 Å) and the preferred pose was taken to be the lowest energy conformation subject to the following constraints: the steric clash term had to be less than 6 kJ/mol, and the centroids of the two rings in the coumarol fragment adjacent to the FAD isoalloxazine were not permitted to be displaced more than 2.5 Å from the reference positions observed in the crystal structure of **1**.

**Crystallography.** NQO1 (10 mg/mL and prepared in 25 mM tricene, pH 7.4 containing 5 $\mu$M FAD) was cocrystallized in the presence of **16**. Crystallization conditions were identified using the JCSG III core screen (Qiagen) on a mosquito 96-well crystallization robot. Crystals used in diffraction experiments were obtained by hanging drop vapor diffusion in 400 nL drops containing equal volumes of protein and a solution containing 2.4 M ammonium sulfate and 0.1 M Tris buffer pH 8.5. Crystals reached their maximum dimensions (~100 × 100 × 100 $\mu$m) in 2−3 days. Crystals were cryoprotected by transferring to the same solution supplemented with 15% glycerol and flash cooled by immersion in liquid nitrogen. Diffraction data were collected on IO2 at the Diamond light source (DLS) and processed with the XDS package.[29] The NQO1−**16** structure was solved by molecular replacement (MR) with the CCP4 program Phaser,[30] using the NQO1-**1** structure (PDB code 2F1O) as a starting model. To reduce model bias the NQO1−**16** structure was subjected to initial rounds of simulated annealing followed by rigid body and restrained refinement with Phenix.refine[31] Ordered solvent molecules were added using Phenix.refine and

manual model building was carried out with COOT.[32] Data and final model statistics are given in Table S2. Coordinates and structure factors have been deposited into the protein databank (PDB access code 3JSX).

**Biology. Inhibition of NQO1.** Recombinant human NQO1 was obtained from Sigma and diluted in 50 mM phosphate buffer to give an absorbance of 0.1 at 550 nm; 5 $\mu$L of this solution was then mixed with 495 $\mu$L of 50 mM phosphate buffer at pH 7.5 containing 200 $\mu$M NADH, 70 $\mu$M cytochrome *c*, 20 $\mu$M menadione, with or without 2 $\mu$M BSA, and various concentrations of the potential inhibitor dissolved in DMSO (final concentration 1.0% v/v). On some occasions, potential inhibitors were dissolved in 0.13 M NaOH. Reactions were carried out at 25 °C and cytochrome *c* reduction was monitored at 550 nm in a Beckman DU 650 spectrophotometer. $IC_{50}$ values were determined using nonlinear curve fitting as implemented in the program Excel for which a 50% reduction of the initial rate was attained.

**Proliferation Assays.** MIA PaCa-2 cells were exposed to varying concentrations of each compound for 24 or 96 h. The number of surviving cells was then determined by the use of the MTT assay.[33] Values of $IC_{50}$ are the drug concentrations required to reduce cell number by 50% over this time period relative to untreated and vehicle only control cells.

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**Supporting Information Available:** Further details of NQO1 active site, scoring function and docked poses; details of crystallographic data collection, analysis and structural features. This material is available free of charge via the Internet at http://pubs.acs.org.

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