# Structural Studies on Bioactive Compounds. 40.[1] Synthesis and Biological Properties of Fluoro-, Methoxyl-, and Amino-Substituted 3-Phenyl-4*H*-1-benzopyran-4-ones and a Comparison of Their Antitumor Activities with the Activities of Related 2-Phenylbenzothiazoles

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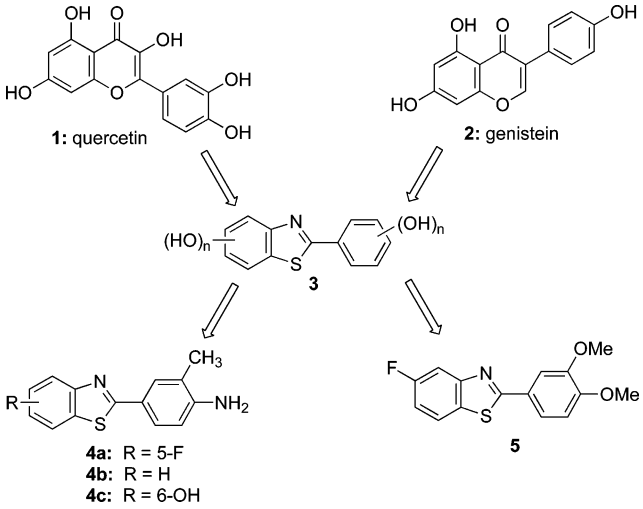
A new series of fluoro-, methoxyl-, and amino-substituted isoflavones have been synthesized as potential antitumor agents based on structural similarities to known flavones and isoflavones (quercetin and genistein respectively) and antitumor 2-phenylbenzothiazoles. Target compounds were synthesized using palladium-catalyzed coupling methodologies to construct the central aryl carbon−carbon single bond. The new isoflavone derivatives were tested for in vitro activity in human breast (MDA-MB-468 and MCF-7) and colon (HT29 and HCT-116) cancer cell lines. Low micromolar $GI_{50}$ values were obtained in a number of cases, with the MDA-MB-468 cell line being the most sensitive overall. Notably, significant potentiation of growth inhibitory activity ($GI_{50} < 1$ $\mu$M for **12d**, **12f**, **12h**, **12k**, **12l**, **12o** but not the methylene-bridged derivative **12i**) was observed when MDA-MB-468 cells were co-incubated with TBDD, a powerful inducer of cytochrome P450 (CYP)-1A1 activity, suggesting that isoflavone derivatives can act as substrates for CYP1A1 bioactivation.

## Introduction

Flavanoids are plant polyphenols (e.g., quercetin, **1**; Chart 1) that are widely represented in nature[2] and exert promiscuous biological effects on mammalian systems[3] that might be exploitable in therapeutic or chemopreventive stategies.[4] Recently, an SAR study of a library of natural and synthetic flavonoids related to quercetin has shown that antiproliferative activity against the human HT29 cell line is associated with caspase activation.[5] Less abundant in nature are the isoflavones, represented by the soy constituent genistein (**2**), which perturb a number of cancer-relevant molecular targets and have attracted recent interest particularly in its potential role in preventing mammary tumor progression.[4,6]

In an earlier paper we reported the synthesis of polyhydroxylated 2-phenylbenzothiazoles (**3**) and compared their cytotoxicities and pharmacological properties with those of quercetin and genistein but were unable to identify any compounds with exploitable antitumor activities.[7] However, providentially, we identified two related series of 2-arylbenzothiazoles with uniquely selective properties. Thus, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole **4a** (5F 203, NSC 703786), like other agents of this planar arylamine class, exploits the arylhydrocarbon receptor (AhR) to translocate the drug to cell nuclei[8] wherein cytochrome P450 CYP1A1 is induced. Generation of a reactive chemical intermediate(s) then forges DNA adducts but *only* in sensitive tumor types (e.g., mammary, ovarian, and lung tumor cell lines).[9] Phortress, an L-lysylamide prodrug modification of amine **4a**, was equiactive with doxorubicin against a panel of human breast tumor xenografts[10] and is currently in phase 1 clinical studies in the U.K.[11] A related structure, 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole **5** (GW 610, NSC 721648), has an extended spectrum of action compared to **4a**, notably in being active against some colon cell lines ($GI_{50} < 10$ nM) that do not have inducible CYP1A1.[12]

**Chart 1.** Chemical Structures of Bioactive Flavones, Isoflavones, and Benzothiazoles




In this paper we sought to examine whether 3-phenyl-4*H*-1-benzopyran-4-ones (3-phenylchromones), decorated with appropriate fluoro, methoxy, or amino substituents in the A and C rings, possessed biological properties comparable to those of their benzothiazole counterparts **4** and **5**. Previously published work has indicated that palladium(0) coupling chemistry can be applied to forge the link between alkyl- or alkyloxy-substituted phenylboronic acids and unsubstituted 3-halobenzopyranones,[13] and we have explored this route to secure greater structural variety in the A and C rings of 3-benzopyranones (Figure 1).

## Chemistry

**Synthesis of Fluoro- and Alkoxy-Substituted 3-Iodochromones.** Many 2-hydroxyacetophenones (**6**) required as starting

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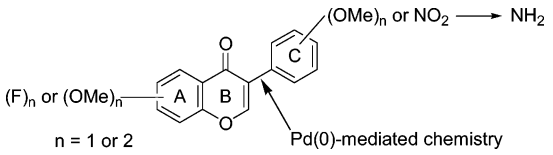
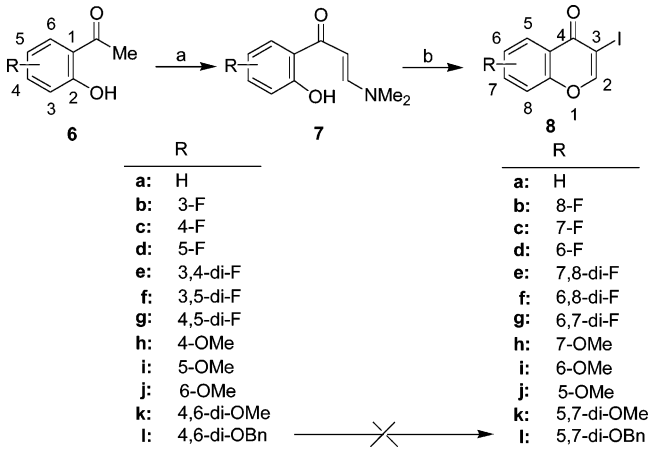



**Figure 1.** Synthetic strategy to substituted 3-aryl-4*H*-1-benzopyran-4-ones.

**Scheme 1.** Synthesis of 3-Iodo-4*H*-1-benzopyran-4-ones **8**[a]




| R | | R | |
|---|---|---|---|
| **a:** | H | **a:** | H |
| **b:** | 3-F | **b:** | 8-F |
| **c:** | 4-F | **c:** | 7-F |
| **d:** | 5-F | **d:** | 6-F |
| **e:** | 3,4-di-F | **e:** | 7,8-di-F |
| **f:** | 3,5-di-F | **f:** | 6,8-di-F |
| **g:** | 4,5-di-F | **g:** | 6,7-di-F |
| **h:** | 4-OMe | **h:** | 7-OMe |
| **i:** | 5-OMe | **i:** | 6-OMe |
| **j:** | 6-OMe | **j:** | 5-OMe |
| **k:** | 4,6-di-OMe | **k:** | 5,7-di-OMe |
| **l:** | 4,6-di-OBn | **l:** | 5,7-di-OBn |

[a] Reagents and conditions: (a) *N*,*N*-dimethylformamide dimethylacetal, 90 °C; (b) $I_2$ in $CHCl_3$ or MeOH.

materials were available commercially, but certain fluoro-substituted precursors (**6b**, **6e**, **6g**) were synthesized via homo-Fries rearrangement of the corresponding acetates, themselves derived from fluoro-substituted phenols.[14] Heating the acetates in 1,2-dichlorobenzene and anhydrous $AlCl_3$ afforded mixtures of isomeric hydroxyacetophenones from which the required isomers were recovered by column chromatography; attempted syntheses of the 3,6- and 4,6-difluoro-2-hydroxyacetophenones by this route were not successful. The di-*O*-benzylated hydroxyacetophenone **6l** was synthesized by reacting 2,4,6-trihydroxyacetophenone with benzyl chloride under basic conditions ($K_2CO_3$) in DMF.[15]

Condensation of 2-hydroxyacetophenones (**6a**−**l**) with dimethylformamide dimethylacetal afforded enamines (**7a**−**l**) that could be cyclized without further purification directly to 3-iodochromones (**8**) (Scheme 1) with iodine in $CHCl_3$ or MeOH, a reaction discovered by Gammill.[16] In some cases it was more efficient to purify crude enamines (eg **7b**−**e**) chromatographically prior to iodinative cyclization. Yields of iodochromones were moderate (Table 1) with a tendency for lower yields in the fluorinated substrates. Attempted conversion of **6l** to 5,7-di-*O*-benzyl-3-iodochromone (**8l**) gave only an inseparable mixture of products.

**Suzuki**−**Miyaura and Stille Couplings.** Initially a Pd(0)-catalyzed borylation of 3-iodochromone (**8a**) was attempted on the basis of Murata's pinacol borane/Pd(dppf)$Cl_2$/base methodology in anhydrous dioxane.[17] In no case was the required boronate ester (**9**) obtained. The major products (from $^1$H NMR) were the dehalogenated chromone (**10**) and the 3,3′-bis-chromone product of bimolecular coupling (**11**) (Scheme 2). Cross-coupling of 3-iodochromones (**8**) with, optimally, 3−4 equiv of substituted phenylboronic acids in the presence of Pd-$(PPh_3)_4$ and $Na_2CO_3$ in benzene gave 3-aryl-4*H*-benzopyran-4-ones (**12a**−**o**) generally in moderate to high yields (Scheme 3, Table 1). Because highly efficient coupling was limited to boronic acids bearing electron-donating methoxyl or electron-neutral groups, a study was conducted to reverse the "polarity" of the coupling partners. Rewardingly, several 3-iodochromones (**8**) were converted efficiently to air-stable and crystallizable 3-(trimethylstannyl)chromones (**13a**−**g**), employing catalytic Pd-$(PPh_3)_4$ and hexamethylditin in dioxane (Scheme 3).

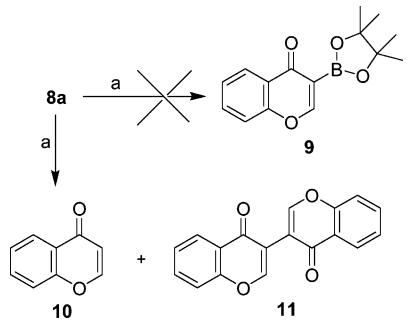
Stille couplings, despite their unpredictable outcomes,[18] seemed a logical methodology to adapt to synthesize 3-(4-nitrophenyl)chromones. Attempted Stille coupling between 3-iodochromone (**8a**) and (4-nitrophenyl)trimethylstannane did not lead to the formation of compound **14a**. We therefore examined the interaction of 3-(trimethylstannyl)chromone (**13a**) and 4-iodonitrobenzene as a reverse process for the formation of isoflavone **14a**. On the basis of similarities between the coupling partners, reaction conditions favorable for coupling iodoarenes and vinylstannanes were employed.[19] A range of catalysts (Pd/C, $PdCl_2$, $Pd_2(dba)_3$, $Pd(PPh_3)_4$, $Pd(MeCN)_2Cl_2$, $Pd(PhCN)_2Cl_2$, $Pd(PPh_3)_2Cl_2$, and Pd(dppf)$Cl_2$·$CH_2Cl_2$), ligands ($Ph_3As$, LiCl, LiI), and solvents (dioxane, THF, and NMP) were reacted in a parallel mode, but in all cases $^1$H NMR analysis of

**Table 1.** Yields and Melting Points of 3-Iodo-4*H*-1-benzopyran-4-ones **8** and 3-Aryl-4*H*-1-benzopyran-4-ones **12**

| compd | starting material | general method[a] | yield (%) | mp (°C) | molecular formula[b] |
|---|---|---|---|---|---|
| **8b** | **7b** | C[d] | 78 | 102−103 | $C_9H_4FIO_2$ |
| **8c** | **7c** | C[d] | 66 | 107−109 | $C_9H_4FIO_2$ |
| **8d** | **7d** | C[d] | 85 | 120−122 | $C_9H_4FIO_2$ |
| **8e** | **7e** | C[d] | 63 | 135−137 | $C_9H_3F_2IO_2$ |
| **8f** | **7f**[c] | C[d] | 41 | 93−95 | $C_9H_3F_2IO_2$ |
| **8g** | **7g**[c] | C[d] | 27 | 118−120 | $C_9H_3F_2IO_2$ |
| **8h** | **7h**[c] | C[e] | 59 | 103−105 | $C_{10}H_7IO_3$ |
| **8i** | **7i**[c] | C[e] | 64 | 112−113 | $C_{10}H_7IO_3$ |
| **8j** | **7j**[c] | C[e] | 43 | 143−145 | $C_{10}H_7IO_3$ |
| **8k** | **7k**[c] | C[e] | 29 | 157−159 | $C_{11}H_8I_2O_4$ |
| **12b** | **8a** | D | 20 | 182−184 | $C_{15}H_9BrO_2$ |
| **12c** | **8a** | D | 50 | 194−196 | $C_{15}H_9NO_4$ |
| **12d** | **8a** | D | 95 | 78−79 | $C_{16}H_{12}O_3$ |
| **12e** | **8a** | D | 99 | 138−139 | $C_{16}H_{12}O_3$ |
| **12f** | **8a** | D | 76 | 136−138 | $C_{17}H_{14}O_4$ |
| **12g** | **8d** | D | 93 | 181−183 | $C_{16}H_{11}FO_3$ |
| **12h** | **8d** | D | 79 | 152−154 | $C_{17}H_{13}FO_4$ |
| **12i** | **8d** | D | 36 | 221−223 | $C_{16}H_9FO_4$ |
| **12j** | **8c** | D | 96 | 171−173 | $C_{16}H_{11}FO_3$ |
| **12k** | **8c** | D | 67 | 165−167 | $C_{17}H_{13}FO_4$ |
| **12l** | **8b** | D | 42 | 146−148 | $C_{17}H_{13}FO_4$ |
| **12m** | **8g** | D | 27 | 169−171 | $C_{17}H_{12}F_2O_4$ |
| **12n** | **8f** | D | 72 | 178−180 | $C_{17}H_{12}F_2O_4$ |
| **12o** | **8e** | D | 52 | 181−183 | $C_{17}H_{12}F_2O_4$ |

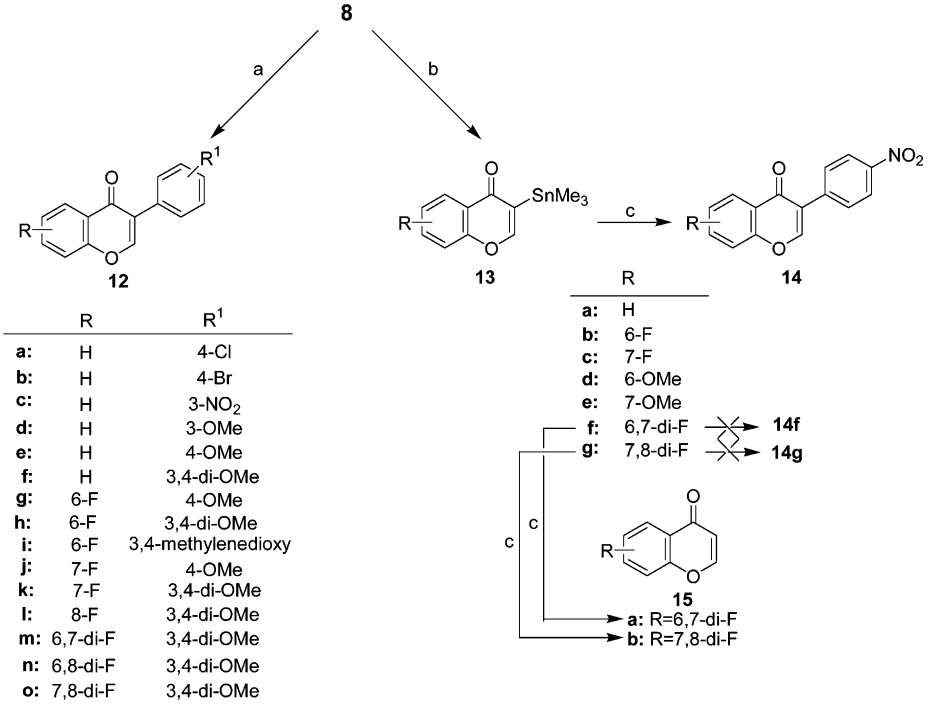
[a] See Experimental Section. [b] Microanalytical and spectroscopic data for new compounds provided in Supporting Information. [c] Prepared in situ from the corresponding 2-hydroxyacetophenone (**6**) and used without further purification. [d] $CHCl_3$ as solvent. [e] MeOH as solvent.

**Scheme 2.** Attempted Borylation of 3-Iodo-4*H*-1-benzopyran-4-one **8**[a]




[a] Reagents and conditions: (a) bis(diphenylphosphino)ferrocene palladium(0), bis(pinacolato)diboron, $NEt_3$, dioxane, 25 °C.

**Scheme 3.** Suzuki−Miyaura and Stille Couplings[a]




| | R | $R^1$ |
|---|---|---|
| **a:** | H | 4-Cl |
| **b:** | H | 4-Br |
| **c:** | H | 3-$NO_2$ |
| **d:** | H | 3-OMe |
| **e:** | H | 4-OMe |
| **f:** | H | 3,4-di-OMe |
| **g:** | 6-F | 4-OMe |
| **h:** | 6-F | 3,4-di-OMe |
| **i:** | 6-F | 3,4-methylenedioxy |
| **j:** | 7-F | 4-OMe |
| **k:** | 7-F | 3,4-di-OMe |
| **l:** | 8-F | 3,4-di-OMe |
| **m:** | 6,7-di-F | 3,4-di-OMe |
| **n:** | 6,8-di-F | 3,4-di-OMe |
| **o:** | 7,8-di-F | 3,4-di-OMe |

| R | |
|---|---|
| **a:** | H |
| **b:** | 6-F |
| **c:** | 7-F |
| **d:** | 6-OMe |
| **e:** | 7-OMe |
| **f:** | 6,7-di-F → **14f** |
| **g:** | 7,8-di-F → **14g** |

[a] Reagents and conditions: (a) substituted arylboronic acid, $Pd(PPh_3)_4$, $Na_2CO_3$, benzene reflux; (b) $Me_6Sn_2$, $Pd(PPh_3)_4$, dioxane, reflux; (c) 4-iodonitrobenzene, $Pd_2(dba)_3$, CuI, LiCl, $Ph_3As$, NMP, 80 °C.

**Table 2.** Yield and Melting Points of 3-(Trimethylstannyl)-4*H*-1-benzopyran-4-ones **13** and 3-(4-Nitrophenyl)-4*H*-1-benzopyran-4-ones **14**

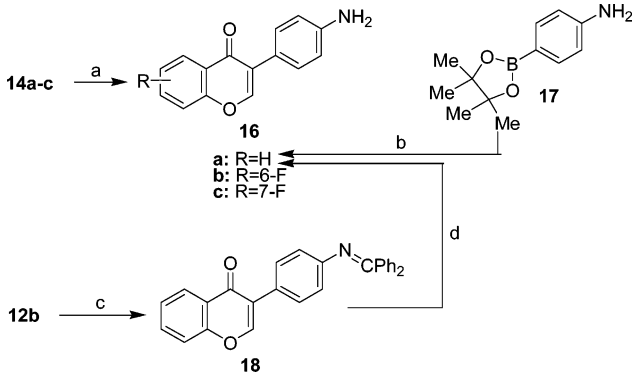
| compd | starting material | general method[a] | yield (%) | mp (°C) | molecular formula[b] |
|---|---|---|---|---|---|
| **13b** | **8d** | E | 90 | 118−120 | $C_{12}H_{13}FO_2Sn$ |
| **13c** | **8c** | E | 80 | 106−108 | $C_{12}H_{13}FO_2Sn$ |
| **13d** | **8i** | E | 73 | 83−85 | $C_{13}H_{16}O_3Sn$ |
| **13e** | **8h** | E | 38 | 87−89 | $C_{13}H_{16}O_3Sn$ |
| **13f** | **8g** | E | 77 | 171−173 | $C_{12}H_{12}F_2O_2Sn$ |
| **13g** | **8e** | E | 66 | 176−178 | $C_{12}H_{12}F_2O_2Sn$ |
| **14b** | **13b** | F | 53 | 235−237 | $C_{15}H_8FNO_4$ |
| **14c** | **13c** | F | 46 | 222−225 | $C_{15}H_8FNO_4$ |
| **14d** | **13d** | F | 26 | 229−231 | $C_{16}H_{11}NO_3$ |
| **14e** | **13e** | F | 23 | 241−243 | $C_{16}H_{11}NO_3$ |

[a] See Experimental Section. [b] Microanalytical and spectroscopic data for new compounds provided in Supporting Information.

the reactions revealed a complex mixture of products, including low yields of the required nitrophenylchromone (**14a**) contaminated with the 3,3′-bis-chromone (**11**) and starting material. Eventually, optimal conditions employing the stannylated substrate (**13**), $Pd_2(dba)_3$ catalyst (1 mol %), $Ph_3As$ (8 mol %), copper(I) iodide (10 mol %), and LiCl (3 mol equiv) in dry NMP at 80 °C were adopted. Products **14a**−**e** were isolated in moderate yields, whereas only traces of the difluorinated products **14f** and **14g** were detected in the reaction mixtures ($^1$H NMR) from **13f** and **13g**, and products (∼25−30%) were assigned as the 6,7-difluoro- and 7,8-difluoro-4*H*-1-benzopyran-4-ones (**15a** and **15b**), respectively, formed following catalytic reductive destannylation. Yields and physical properties of 3-(trimethylstannyl)chromones (**13**) and substituted 3-(4-nitrophenyl)chromones (**14**) are recorded in Table 2.

**Synthesis of 3-(4-Aminophenyl)-4*H*-benzopyran-4-ones.** High yields of amines **16a**−**c** were obtained by tin(II) chloride reduction of precursor nitro compounds **14a**−**c**. Less efficient were direct Pd(0)-mediated routes to these amines. For example,

**Scheme 4.** Synthesis of 3-(4-Aminophenyl)-4*H*-benzopyran-4-ones (**16**)[a]




[a] Reagents and conditions: (a) $SnCl_2 \cdot 2H_2O$, EtOH, reflux, then excess NaOH; (b) **8a**, diphenylphosphinoferrocene palladium(0), $K_2CO_3$, dioxane−ethanol, reflux; (c) benzophenone imine, $Pd_2(dba)_3$, NaO$^t$Bu, rac-BINAP, toluene, 80 °C; (d) 2 M HCl in THF, 25 °C.

**16a** was formed in only 14% yield from 3-iodochromone (**8a**) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**17**). No reaction occurred between chlorophenylchromone (**12a**) and the surrogate amine (benzophenone imine) using racemic BINAP as the ligand, but coupling of 4-bromophenylchromone **12b** afforded a crude imine **18**, which was successfully hydrolyzed using 2 M HCl in dioxane to give amine **16a** in 61% overall yield (Scheme 4).

As expected, the nature of the substituent at C(3) of the 4*H*-benzopyran-4-one moiety has a significant bearing on the chemical shift of the proton H-2. In the $^1$H NMR spectrum of the unsubstituted 3-iodobenzopyranone (**8a**), this proton resonates at $\delta$ 8.32 in $CDCl_3$. Fluorination in the phenyl ring has a minimal effect on H-2, which resonates in the range $\delta$ 8.29−8.34 for **8b**−**g**. An additional aryl residue at C(3) imparts a small shielding effect (relative to iodo) with chemical shifts in

**Table 3.** Growth Inhibitory Properties[a] of 3-Aryl-4*H*-benzopyran-4-ones and Reference Compounds against Human Cancer Cell Lines in Vitro[b]

| | $GI_{50}$ ($\mu$M)[c] | | | |
|---|---|---|---|---|
| compd | MDA-MB-468 | MCF-7 | HT29 | HCT-116 |
| **4a**[d] | <0.0001 | <0.0001 | 63 | 16 |
| **5**[e] | <0.0001 | 0.008 | 2.6 | 5.4 |
| **12d** | 29.0 | >100 | 88.0 | >100 |
| **12e** | 27.2 | 81.4 | 74.0 | 64.0 |
| **12f** | 66.5 | 88.1 | >100 | >100 |
| **12h** | 0.9 | 93.1 | 88.2 | 65.3 |
| **12i** | 1.5 | 60.0 | | |
| **12j** | 9.9 | 43.8 | 17.6 | 8.5 |
| **12k** | 58.9 | 90.6 | 87.0 | 80.2 |
| **12l** | 41.9 | 75.5 | 88.0 | 67.8 |
| **12m** | 17.2 | 41.5 | 22.6 | 39.7 |
| **12n** | 6.3 | 46.7 | 7.2 | 8.1 |
| **12o** | 21.6 | 47.2 | 31.7 | 26.0 |
| **16a** | 6.0 | 64.9 | 8.1 | 17.3 |
| **16b** | 4.8 | 8.8 | 6.1 | 8.5 |
| **16c** | 6.4 | 7.9 | 8.6 | 7.2 |

[a] MTT assays: 72 h of drug exposure. [b] Cancer cell line origin: MDA-MB-468 (breast); MCF-7 (breast); HT29 (colon); HCT-116 (colon). [c] Mean of two experiments. [d] Ref 22. [e] Ref 12.

tight ranges of $\delta$ 7.98−8.07 for series **12a**,**b**,**d**−**o** and $\delta$ 8.11−8.16 for the nitrophenyl analogues **12c** and **14a**−**e**. The 3-(trimethylstannyl) group exerts a stronger shielding influence with H-2 resonating in the range $\delta$ 7.50−7.61 for compounds **13a**−**g**.

## Biological Results and Discussion

Evaluation of the series of 3-aryl-4*H*-1-benzopyran-4-ones **12** and **16** in an in vitro panel of human cancer cell lines (breast MDA-MB-468, breast MCF-7, colon HCT-116 and colon HT29 carcinoma cells) allowed profiles of activity to be compared with those of (aminophenyl)benzothiazole (**4a**,**b**) of defined mechanism[8,9,11] and the (dimethoxyphenyl)benzothiazole (**5**) of unknown mechanism.[12] Overall, the MDA-MB-468 ($ER^-$) cell line was the most chemosensitive line (Table 3) and benzothiazoles **4a** and **5** were the most potent compounds, particularly against the breast models. Within the series **12** bearing at least one methoxy group in ring C, the presence and position of fluorine atoms in ring A have a significant impact on in vitro activity. Higher potency against MDA-MB-468 cells is seen in the 6-fluorobenzopyranones (**12h**,**i**) ($GI_{50} \geq 1.5$ $\mu$M) and the 6,8-difluoro analogue (**12n**) ($GI_{50} = 6.3$ $\mu$M) than in the 7-fluoro- (**12j**), 6,7-difluoro- (**12m**) and 7,8-difluorobenzopyranones (**12o**) ($GI_{50} < 25$ $\mu$M) and in unfluorinated agents (**12d**−**f**) ($GI_{50} > 25$ $\mu$M). Evidently, in the monofluoro series, a fluoro group in the 6-position in the 3-arylbenzopyranone series has an enhancing growth inhibitory role, as is the case for the 5-fluoro group in the two benzothiazole compounds **4a** and **5**.

The aminoisoflavonoid **16a** yielded $GI_{50}$ values less than 10 $\mu$M in MDA-MB-468 and HT29 carcinoma cells and $GI_{50}$ values of 65 and 17 $\mu$M in MCF-7 and HCT 116 cell lines, respectively. Fluorinated congeners **16b** and **16c** lead to enhanced potency in all cell lines ($GI_{50} < 10$ $\mu$M). However, these agents did not match the exquisite potency of the benzothiazoles (**4a** or **5**) in the breast cell lines (Table 3), and in contrast to aminophenylbenzothiazole analogues, no selectivity between CYP1A1 inducible and noninducible phenotypes could be determined.

Representative compounds in series **12** and **16** were also evaluated in the NCI 60-cell panel in vitro.[20] Mean $GI_{50}$ values were in the 50−100 $\mu$M range with no cell line selectivity observed (data not shown). Compounds were not COMPARE positive with the exemplar (4-aminophenyl)benzothiazole (**4a**) with Pearson correlation coefficients less than 0.6.[21] Unlike the benzothiazole compound whose selective antitumor activity within the NCI panel correlated with inducible CYP1A1 activity,[22] a comparable mechanism involving CYP1A1-mediated DNA damage could be argued as unlikely.[8,9] However, Western blot analyses performed using lysates of MDA-MB-468 cells treated for 24 h with **12h** (1, 10, 100 $\mu$M) revealed weak induction of CYP1A1 protein following exposure of cells to 10 or 100 $\mu$M **12h** (data not shown).

Paucity of growth inhibition by, for example, **12h** in MCF-7 ($GI_{50} = 93$ $\mu$M) contrasting with MDA-MB-468 ($GI_{50} = 0.9$ $\mu$M) cells also appears at first glance to be inconsistent with a mode of action involving CYP1A1-mediated DNA damage. However, multiple mechanisms may be engaged and could include an estrogenic effect that would mask growth inhibitory effects in ER+ (e.g., MCF-7) cell lines. Indeed, the 6-hydroxylated product **4c** of CYP1A1-metabolized metabolism of 2-(4-amino-3-methylphenyl)benzothiazole (**4b**)[22,23] and apigenin possess estrogenic mitogenic properties, impeding activity of **4b** and 5,4′-diaminoflavone,[24] respectively, in MCF-7 cells. In addition, **4c** and **4b** have been shown to inhibit induced CYP1A1 activity, crucial for **4b**-evoked growth inhibition. It is believed that these mechanisms combine to generate the observed biphasic dose response relationship for **4b**. Indeed, the dose response observed following treatment of MDA-MB-468 cells with **12h** is also biphasic, inferring a role for metabolic processes (Figure 2). Moreover, the CYP1A1 antagonist **4c**[22] was able to completely abrogate growth inhibitory activity of **12h**, increasing the $GI_{50}$ value to greater than 100-fold. It has been established that of the isoflavone analogues examined (**12h**, **12k**, **12e**, and **12g**) all inhibit deethylation of ethoxyresorufin[25] (CYP1A1 activity, EROD assay) in a dose-dependent manner ($IC_{50} < 100$ $\mu$M), as shown in Figure 3. One explanation for this is that these compounds act as substrates for CYP1A1 metabolism but do not themselves, or only weakly, induce cyp transcription. As noted, **12h** did confer meagre induction of the CYP1A1 protein.

Intriguingly, when MDA-MB-468 cells were co-incubated with 2,3,7,8-tetrabromodioxin (TBDD, 10 nM) and isoflavone analogues (**12d**, **12f**, **12h**, **12i**, **12k**, **12l**, **12o**), growth inhibition was significantly potentiated, giving $GI_{50}$ values less than 1 $\mu$M (Figure 3). Such observations corroborate a role for CYP1A1 activity in antitumor activity, since TBDD is a potent AhR ligand and powerful inducer of CYP1A1 activity. Furthermore, analogues that alone cause negligible growth inhibition (e.g., **12l** and **12k** where $GI_{50} > 25$ $\mu$M) evoke a cytotoxic response at 3 $\mu$M in combination with TBDD and an overall biphasic profile. TBDD is not, however, a CYP substrate, being resistant to metabolism, and alone demonstrates no growth inhibitory properties against MDA-MB-468 cells. The notable exception within series **12** contains a methylenedioxy bridge (**12i**). $GI_{50}$ values of 1.5 and 2.0 $\mu$M in the absence and presence of 10 nM TBDD were not potentiated by TBDD-induced CYP1A1. The methylene bridge of **12i** may render this analogue resistant to metabolic modification. Thus, CYP-mediated production of (DNA-reactive) putative oxonium ion intermediates of isoflavones **12d**, **12f**, **12h**, **12k**, **12l**, and **12o** is suggested.

A final piece of evidence to support mediation of activity via the AhR/CYP1A1 pathway has emerged following treatment of MDA-MB-435 cells with **12h**, **12j**, **12m**, and **12n**. MDA-MB-435 cells express a splice variant of ARNT that traps the AhR constitutively in the nucleus.[26] Therefore, cytoplasmic AhR/ligand bound complexes cannot be formed. No growth

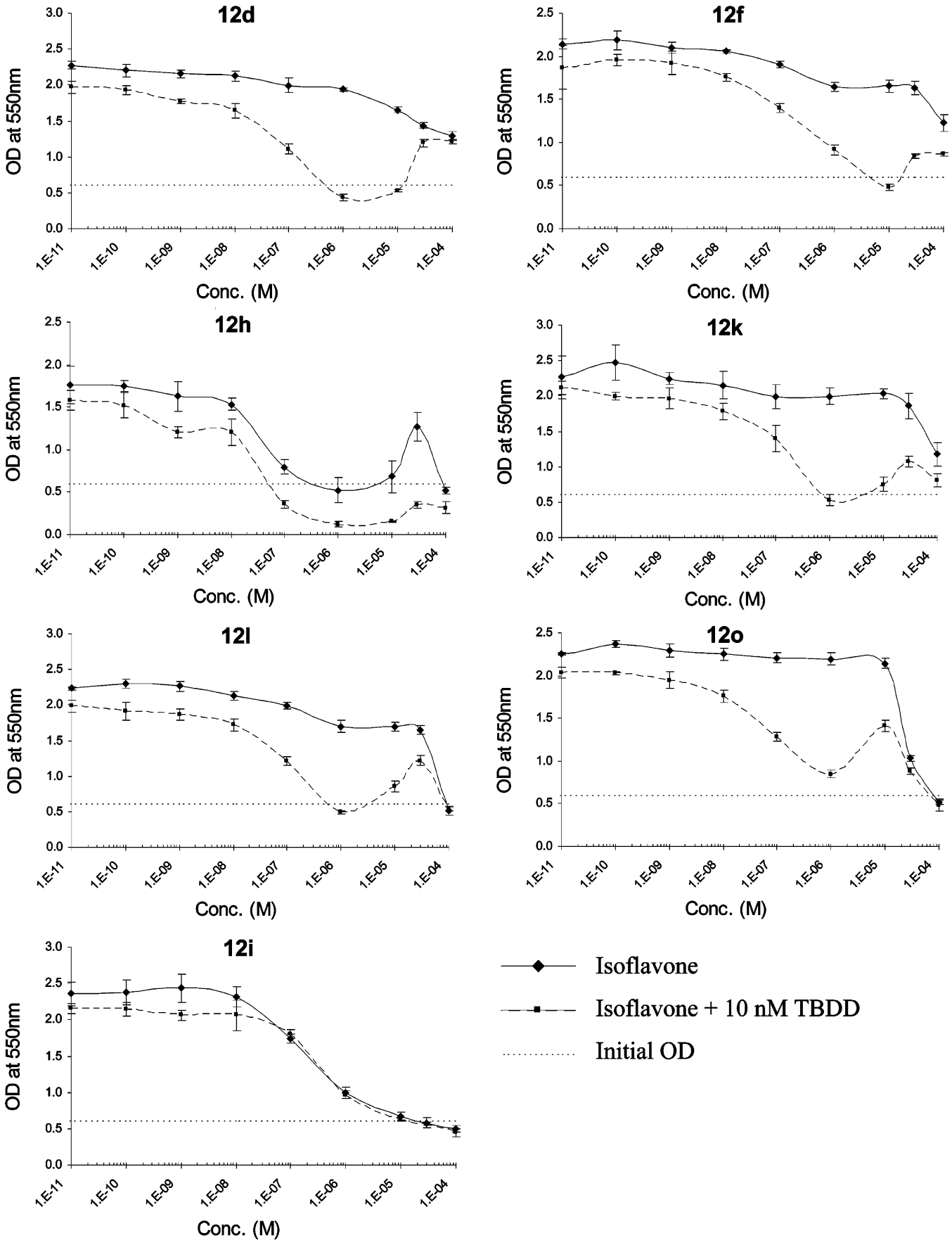



**Figure 2.** Effect of **12d**, **12f**, **12h**, **12k**, **12l**, **12o**, and **12i** on the growth of MDA-MB-435 cells. MTT assays determined cellular viability following 72 h of exposure to isoflavone analogues in the absence and presence of 10 nM TBDD.

inhibitory activity was encountered, $GI_{50}$ values greater than 30 $\mu$M being consistently observed (data not shown).

In conclusion, the evidence presented here indicates that novel substituted isoflavones such as **12h**, while being only moderately

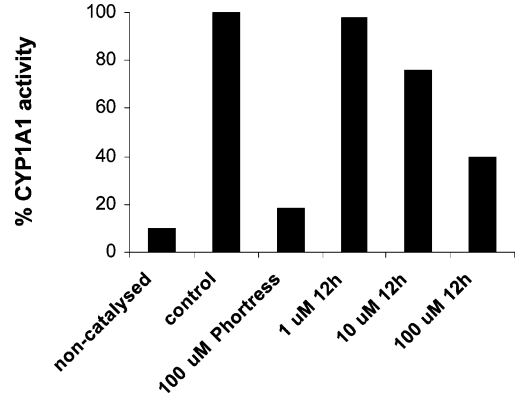



**Figure 3.** Inhibition of CYP1A1 activity by **12h** (EROD assay). The ability of **12h** (1, 10, and 100 $\mu$M) to inhibit deethylation of ethoxyresorufin, catalyzed by CYP1A1 microsomes, was determined. Microsomes were excluded from negative control incubates to ascertain noncatalyzed production of resorufin. Control incubates contained DMSO vehicle only. Phortress (100 $\mu$M)[11] was used as a positive control for inhibition of catalysis.

active in their own right, have potent in vitro antitumor activity when coadministered with a CYP1A1 inducer such as TBDD. The isoflavones examined appear to inhibit CYP1A1 activity (EROD assay) and are therefore likely CYP1A1 substrates, despite their apparent weak ability to induce CYP1A1. The development of other CYP-inducing agents that will uncover the latent antitumor activity associated with these novel isoflavones may add further interest to potential therapeutic application; for example, the selective AhR modulator 6-alkyl-1,3,8-trichlorodibenzofuran has been shown to exhibit agonist activity with respect to induction of CYP1A1 (Fretland et al.[27]).

## Experimental Section

All new compounds were characterized by elemental analysis (% C, H, and N values within 0.4% of theoretical values). Melting points were measured on a Gallenkamp apparatus and are reported uncorrected. IR spectra were recorded on a Mattson 2020 Galaxy series or Perkin-Elmer Spectrum One FT-IR spectrometer. Mass spectra were recorded on a Micromass Platform spectrometer, an AEI MS-902 (nominal mass), a VG Micromass 7070E, or a Finigan MAT900XLT spectrometer (accurate mass). NMR spectra were recorded on a Bruker ARX 250 instrument. Coupling constants are in Hz. TLC systems for routine monitoring of reaction mixtures and for confirming the homogeneity of analytical samples used Kieselgel 60$F_{254}$ (0.25 mm) silica gel TLC aluminum backed sheets. Sorbsil silica gel C 60-H (40−60 $\mu$m) was used for flash chromatographic separations. All commercially available starting materials were used without further purification.

**General Method A for the Synthesis of Substituted 2-Hydroxyacetophenones.** Typically, to prepare hydroxyacetophenones **6b**, **6e**, **6g**, which were not available commercially, substituted phenyl acetates (1.50 mmol) were added to a mixture of anhydrous aluminum(III) chloride (1.65 mmol, 1.1 mol equiv) in 1,2-dichlorobenzene (25 mL) and heated to 100 °C for 24 h. To the cooled mixtures was added dichloromethane (25 mL), and the solutions were poured into iced 2 M HCl (40 mL). The aqueous layers were extracted with further portions of DCM (2 × 25 mL), and the combined organic fractions were dried ($MgSO_4$) and concentrated under vacuum. Products were purified by flash column chromatography using cyclohexanes−EtOAc as eluent. The following new 2-hydroxyacetophenone was synthesized by general method A.

**3,4-Difluoro-2-hydroxyacetophenone (6e).** From 2,3-difluorophenyl acetate, the difluoroacetophenone **6e** was formed (28%) as a white solid, mp 40−42 °C; IR (KBr) 1659, 1616, 1512, 1447, 1358, 1323, 1282 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 12.59 (1H, s, OH), 7.53 (1H, m, ArH), 6.73 (1H, m, ArH), 2.64 (3H, s, $CH_3$); MS-CI (*m*/*z*) 173 ($M^+ + 1$). Anal. ($C_8H_6F_2O_2$) C, H.

The di-*O*-benzylated hydroxyacetophenone (**6l**) was synthesized by reacting 2,4,6-trihydroxyacetophenone with benzyl chloride.

**General Method B for the Synthesis of Substituted 3-Dimethylamino-1-(2-hydroxyphenyl)propenones.**[16] A mixture of a substituted 2-hydroxyacetophenone (1.2 mmol) and *N*,*N*-dimethylformamide dimethylacetal (2.4 mmol, 2 mol equiv) was heated at 90 °C overnight and allowed to cool. The residual solvent was removed by vacuum evaporation, and the product was purified by column chromatography using hexanes−EtOAc as eluent.

**3-Dimethylamino-1-(2-hydroxyphenyl)propenone (7a). 7a** was obtained from 2-hydroxyacetophenone (**6a**) as a brown solid (82%), mp 127−128 °C (lit.,[16] 132−134 °C); $^1$H NMR $\delta_H$ ($CDCl_3$) 7.88 (1H, d, $J$ = 12.5, olefinic CH), 6.60−6.75 (4H, m, ArH), 5.75 (1H, d, $J$ = 12.5, olefinic CH), 3.11 (3H, s, $NCH_3$), 2.89 (3H, s, $NCH_3$).

The following new propenones were synthesized.

**3-Dimethylamino-1-(3-fluoro-2-hydroxyphenyl)propenone (7b). 7b** was obtained from 3-fluoro-2-hydroxyacetophenone (**6b**) as a yellow solid (46%), mp 110−112 °C; IR (KBr) 1640, 1614, 1587, 1557, 1518, 1494, 1441, 1363, 1272, 1219 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 14.26 (1H, s, OH), 7.93 (1H, d, $J$ = 12.0, olefinic CH), 7.47 (1H, d, $J$ = 8.0, H-6), 7.17 (1H, m, ArH), 6.73 (1H, m, ArH), 5.75 (1H, d, $J$ = 12.0, olefinic CH), 3.23 (3H, s, $NCH_3$), 3.00 (3H, s, $NCH_3$). HRMS-EI (*m*/*z*): calcd for $C_{11}H_{11}FNO_2$, 208.0774 ($M^+ - 1$); found, 208.079 65.

**3-Dimethylamino-1-(4-fluoro-2-hydroxyphenyl)propenone (7c). 7c** was obtained from 4-fluoro-2-hydroxyacetophenone (**6c**) as light-brown crystals (65%), mp 113−115 °C; IR (KBr) 1635, 1614, 1545, 1511, 1425, 1373, 1279, 1238 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 14.43 (1H, s, OH), 7.90 (1H, d, $J$ = 12.0, olefinic CH), 7.69 (1H, m, ArH), 6.57 (2H, m, ArH), 5.69 (1H, d, $J$ = 12.0, olefinic CH), 3.21 (3H, s, $NCH_3$), 2.99 (3H, s, $NCH_3$); MS-CI (*m*/*z*) 210 ($M^+ + 1$). Anal. ($C_{11}H_{12}FNO_2$) C, H, N.

**3-Dimethylamino-1-(5-fluoro-2-hydroxyphenyl)propenone (7d). 7d** was obtained from 5-fluoro-2-hydroxyacetophenone (**6d**) as yellow crystals (60%), mp 106−107 °C; IR (KBr) 1634, 1592, 1540, 1489, 1433, 1422, 1400, 1342, 1287, 1272, 1243, 1226 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 13.62 (1H, s, OH), 7.92 (1H, d, $J$ = 12.5, olefinic CH), 7.36 (1H, dd, $J$ = 2.5, 10.0, ArH), 7.10 (1H, td, $J$ = 2.5, 10.0, ArH), 6.90 (1H, m, ArH), 5.68 (1H, d, $J$ = 12.5, olefinic CH), 3.23 (3H, s, $NCH_3$), 3.03 (3H, s, $NCH_3$); MS-CI (*m*/*z*) 210 ($M^+ + 1$). Anal. ($C_{11}H_{12}FNO_2$) C, H, N.

**3-Dimethylamino-1-(3,4-difluoro-2-hydroxyphenyl)propenone (7e). 7e** was obtained from 3,4-difluoro-2-hydroxyacetophenone (**6e**) as yellow-orange crystals (37%), mp 171−173 °C; IR (KBr) 1631, 1553, 1509, 1492, 1459, 1436, 1420, 1370, 1321, 1258 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 14.76 (1H, s, OH), 7.92 (1H, d, $J$ = 12.0, olefinic CH), 7.43 (1H, m, ArH), 6.60 (1H, m, ArH), 5.65 (1H, d, $J$ = 12.0, olefinic CH), 3.23 (3H, s, $NCH_3$), 3.00 (3H, s, $NCH_3$); MS-CI (*m*/*z*) 228 ($M^+ + 1$). Anal. ($C_{11}H_{11}F_2NO_2$) C, H, N.

**3-Dimethylamino-1-(4,6-dibenzyloxy-2-hydroxyphenyl)propenone (7l). 7l** was obtained from 4,6-dibenzyloxy-2-hydroxyacetophenone (**6l**) as a yellow solid (83%), mp 125−127 °C; IR (KBr) 1608, 1542, 1478, 1427, 1399, 1357, 1280, 1235, 1208 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 15.97 (1H, s, OH), 7.83 (1H, d, $J$ = 12.5, olefinic CH), 7.41 (10H, m, ArH), 6.11−6.25 (3H, m, ArH and olefinic CH), 5.07 (2H, s, $OCH_2$), 5.00 (2H, s, $OCH_2$), 3.05 (3H, br s, $NCH_3$), 2.32 (3H, br s, $NCH_3$). HRMS-EI (*m*/*z*): calcd for $C_{25}H_{25}NO_4$, 403.1783; found, 403.1765.

**General Method C for the Synthesis of Substituted 3-Iodo-4*H*-1-benzopyran-4-ones (8).**[16] To a solution of a substituted 3-(dimethylamino)-1-(2-hydroxyphenyl)propenone (**7**, 0.6 mmol) in $CHCl_3$ (15 mL) or MeOH (15 mL) was added iodine (1.2 mmol, 1.5 mol equiv), and the mixture was stirred at 25 °C overnight. The solution (reduced under vacuum if MeOH used) was washed with saturated $Na_2S_2O_3$ (15 mL), and the aqueous layer was extracted with $CHCl_3$ (20 mL). The combined organic fractions were purified by column chromatography using hexanes−EtOAc as eluent.

**3-Iodo-4*H*-1-benzopyran-4-one (8a). 8a** was prepared according to method C, in chloroform from 3-(dimethylamino)-1-(2-hydroxyphenyl)propenone (**7a**), as a white solid (78%), mp 102−103 °C; $^1$H NMR $\delta_H$ ($CDCl_3$) 8.32 (1H, s, H-2), 8.25 (1H, dd, $J$ = 2.0, 8.0, ArH), 7.72 (1H, m, ArH), 7.45 (2H, m, ArH); $^{13}$C NMR $\delta_C$ ($CDCl_3$) 173.0 (C=O), 157.5 (CH), 155.8 (C), 133.9 (CH), 126.2 (CH), 125.7 (CH), 121.4 (CH), 117.8 (C), 86.1 (C−I); MS-CI (*m*/*z*) 273 ($M^+ + 1$). Anal. ($C_9H_5IO_2$) C, H.

Yields and melting points of 3-iodopyranones (**8b**−**k**) prepared by general method C are listed in Table 1. Analytical data and spectroscopic properties of new compounds are provided in Supporting Information.

**Attempted Pd(0)-Mediated Borylations of 3-Iodo-4*H*-1-benzopyran-4-one (8a).** A mixture of bis(diphenylphosphino)ferrocene palladium(0) (0.026 g, 0.03 mmol), triethylamine (0.43 mL), bis-(pinacolato)diboron (0.46 mL, 3.20 mmol), **8a** (0.2272 g, 1.0 mmol), and dry dioxan (4.0 mL) were stirred in a sealable tube at 25 °C until hydrogen liberation ceased. The tube and contents were flushed with nitrogen, and the sealed tube was heated at 80 °C for 24 h. Chromatographic fractionation of the reaction products afforded small samples of 4*H*-1-benzopyran-4-one (**10**) ($^1$H NMR $\delta_H$ ($CDCl_3$) 8.22 (1H, m, ArH), 7.86 (1H, d, $J$ = 6.0, C=CH), 7.67 (1H, m, ArH), 7.45 (2H, m, ArH), 6.35 (1H, d, $J$ = 6.0, C=CH); MS-CI (*m*/*z*) 147 ($M^+ + 1$)) and the bis-chromone (**11**) (mp 251−254 °C; IR (KBr) 1644, 1615, 1463, 1369, 1317, 1260 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 8.91 (2H, s, H-2,22), 8.30 (2H, dd, $J$ = 1.8, 8.0, ArH), 7.70 (2H, td, $J$ = 1.8, 8.0, ArH), 7.54 (2H, dd, $J$ = 1.8, 8.0, ArH), 7.46 (2H, td, $J$ = 1.8, 8.0, ArH); MS-CI (*m*/*z*) 291 ($M^+ + 1$)]. $^1$H NMR analysis of reaction mixtures employing a range of different bases ($K_2CO_3$, $Cs_2CO_3$, $KH_2PO_4$, $BaOH_2$) similarly showed the presence of **10** and **11**.

**General Method D for the Synthesis of 3-Aryl-4*H*-1-benzopyran-4-ones (12) by Suzuki−Miyaura Coupling.** To a solution of a substituted 3-iodo-4*H*-1-benzopyran-4-one (**8**, 0.35 mmol), Pd-($PPh_3$)$_4$ (0.035 mmol, 0.1 mol equiv), and 2M $Na_2CO_3$ (0.7 mmol, 2 mol equiv) in benzene (15 mL) was added phenylboronic acid (1.4 mmol, 4 mol equiv) in EtOH (15 mL). The mixture was heated under reflux under nitrogen (20 h). After addition of water (20 mL), the aqueous phase was extracted with dichloromethane (2 × 30 mL) and the combined organic fractions were dried ($MgSO_4$) and concentrated under vacuum. Products were purified by column chromatography using hexanes−EtOAc as eluent.

**3-(4-Chlorophenyl)-4*H*-1-benzopyran-4-one (12a). 12a** was prepared (57%), from **8a** and 4-chlorophenylboronic acid by general method D, as a white solid, mp 186−188 °C (lit. 182 °C[27]); IR (KBr) 1638, 1617, 1605, 1597, 1574, 1566, 1492, 1466, 1373, 1356, 1289, 1229 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 8.22 (1H, dd, $J$ = 2.0, 8.0, ArH), 7.93 (1H, s, H-2), 7.58 (2H, m, ArH), 7.41 (4H, m, ArH), 7.32 (1H, m, ArH); MS-CI (*m*/*z*) 257/259 ($M^+ + 1$).

Yields and melting points of 3-aryl-4*H*-1-benzopyran-4-ones (**12b**−**o**) prepared by general method D are listed in Table 1. Analytical data and spectroscopic properties of new compounds are provided in Supporting Information.

**General Method E for the Palladium(0)-Mediated Stannylation of 3-Iodo-4*H*-1-benzopyran-4-ones (8).** A substituted 3-iodo-4*H*-1-benzopyran-4-one (0.4 mmol) and Pd($PPh_3$)$_4$ (0.008 mmol, 0.02 mol equiv) were placed in a two-necked flask fitted with a reflux condenser, and the system was evacuated and filled with nitrogen. Dioxane (20 mL), followed by hexamethylditin (4.0 mmol, 10 mol equiv), was added, and the mixture was refluxed overnight. Products were partitioned between EtOAc (25 mL) and water (25 mL), and the pooled organic fractions were dried ($MgSO_4$) and concentrated in vacuo. Products were purified by column chromatography using hexanes−EtOAc as eluent.

**3-(Trimethylstannyl)-4*H*-1-benzopyran-4-one (13a). 13a** was prepared (1.87 g, 80%) from **8a** (2.05 g, 7.60 mmol), Pd($PPh_3$)$_4$ (0.174 g, 0.152 mmol), and hexamethylditin (5.0 g, 15.2 mmol) in dioxane (30 mL) by general method E as above. The trimethylstannylbenzopyranone formed white crystals (from EtOH), mp 85−86 °C; IR (KBr) 1609, 1556, 1463, 1378, 1340, 1333, 1313, 1251, 1209 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 8.15 (1H, dd, $J$ = 1.8, 8.0, H-5), 7.64 (1H, td, $J$ = 1.8, 8.0, H-7), 7.61 (1H, s, H-2), 7.41 (2H, m, H-6, H-8), 0.35 (9H, s, $SnMe_3$); $^{13}$C NMR $\delta_C$ ($CDCl_3$) 181.6 (C=O), 157.6 (CH), 157.2 (C), 133.8 (CH), 126.3 (CH), 125.4 (CH), 124.2 (C), 123.3 (C), 118.4 (CH), −8.9 ($CH_3$); MS-CI (*m*/*z*) 310 ($M^+ + 1$), 295 ($-CH_2$). Anal. ($C_{12}H_{14}O_2Sn$) C, H.

Yields and melting points of 3-stannylpyranones (**13b**−**g**) prepared by general method E are listed in Table 2. Analytical data and spectroscopic properties of new compounds are provided in Supporting Information.

**General Method F for the Stille Coupling of 3-(Trimethylstannyl)-4*H*-1-benzopyran-4-ones (13) and 4-Iodonitrobenzene.** A Schlenk tube was charged with 4-iodonitrobenzene (0.3 mmol), a substituted 3-(trimethylstannyl)-4*H*-1-benzopyran-4-one (0.33 mmol, 1.1 mol equiv), triphenylarsine (0.024 mmol, 8 mol %), $Pd_2$-(dba)$_3$(0) (0.003 mmol, 1 mol %), copper(I) iodide (0.03 mmol, 10 mol %), and LiCl (0.9 mmol, 3 mol equiv). Dry NMP (15 mL) was added to give a solution of 0.13 M with respect to the iodide. The tube was evacuated, sealed under nitrogen, and heated at 80 °C for 48 h. Products were partitioned between EtOAc (20 mL) and saturated brine (20 mL), and the dried ($MgSO_4$) organic layer was concentrated in vacuo. Products were purified by column chromatography using hexanes−EtOAc as eluent.

**3-(4-Nitrophenyl)-4*H*-1-benzopyran-4-one (14a). 14a** was prepared (68%) from 4-iodonitrobenzene (0.11 g, 0.44 mmol), **13a** (0.15 g, 0.49 mmol), triphenylarsine (0.011 g, 0.035 mmol), $Pd_2$-(dba)$_3$(0) (0.004 g, 0.0044 mmol), copper(I) iodide (0.0084 g, 0.044 mmol), and LiCl (0.056 g, 1.32 mmol) in NMP (3.4 mL). The nitrophenylbenzopyranone was isolated as an orange solid, mp 203−204 °C (lit., 202−204 °C[28]); IR (KBr) 1632, 1594, 1514, 1463, 1369, 1317, 1289, 1260 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 8.13 (1H, s, H-2), 7.38−8.46 (8H, m, ArH); MS-CI (*m*/*z*) 268 ($M^+ + 1$).

Yields and melting points of 3-(4-nitrophenyl)-4*H*-1-benzopyran-4-ones **(14b**−**e)** prepared by general method F are listed in Table 2. Analytical data and spectroscopic properties of new compounds are provided in Supporting Information.

**3-(4-Aminophenyl)-4*H*-1-benzopyran-4-one (16a).** (i) A suspension of 3-(4-nitrophenyl)-4*H*-1-benzopyran-4-one (**14a**) (0.117 g, 0.43 mmol) and tin(II) chloride dehydrate (0.395 g, 1.75 mmol) in EtOH (3.0 mL) was heated under reflux (20 h), cooled, and concentrated in vacuo, and the products were partitioned between EtOAc and excess 1 M NaOH (to pH 11). The organic layer was washed with brine and water, dried ($MgSO_4$), and concentrated in vacuo to yield the aminophenylpyranone (0.07 g, 67%), mp 194−196 °C; IR (KBr) 3461, 3352, 1626, 1609, 1566, 1516, 1464, 1379 cm$^{-1}$; $^1$H NMR $\delta_H$ ($CDCl_3$) 8.33 (1H, dd, $J$ = 2.0, 8.0, ArH), 8.00 (1H, s, H-2), 7.68 (1H, m, ArH), 7.45 (4H, m, ArH), 6.77 (2H, m, ArH), 3.77 (2H, br s, $NH_2$); MS-CI (*m*/*z*) 238 ($M^+ + 1$). Anal. ($C_{15}H_{11}NO_2$) C, H, N.

(ii) 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**17**) (0.29 g, 1.0 mmol) in dioxan (5.0 mL) was mixed with diphenylphosphinoferrocene palladium(0) (0.026 g, 0.03 mmol) and EtOH (2.0 mL). To the solution was added 3-iodo-4*H*-1-benzopyran-4-one (**8a**) and $K_2CO_3$, and the mixture was heated under reflux (72 h) under nitrogen. The products were partitioned between EtOAc and water, and the organic fraction was concentrated in vacuo. Chromatographic purification (EtOAc−hexane as eluent) gave the same (IR and $^1$H NMR) aminophenylpyranone (**16a**) (14%).

(iii) Into an oven-dried Schlenk tube were combined 3-(4-bromophenyl)-4*H*-1-benzopyran-4-one (**12b**) (0.25 g, 0.83 mmol), $Pd_2$(dba)$_3$(0) (0.0092 g, 0.001 mmol), (±)-BINAP (0.0019 g, 1.0 mmol), benzophenone oxime (1.0 g, 1.162 mmol), and sodium *tert*-butoxide (0.11 g, 1.16 mmol) in toluene (5.0 mL). The tube was flushed with nitrogen and heated at 80 °C for 16 h. Diethyl ether (50 mL) was added. The mixture was filtered, and the solvents were removed in vacuo. Chromatographic separation of the products (EtOAc−hexane as eluent) afforded crude imine **18**, which was hydrolyzed with 2 M HCl in THF at 25 °C to give the same (IR and $^1$H NMR) aminophenylpyranone **16a** (61%). No reaction occurred between 3-(4-chloro-phenyl)-4*H*-1-benzopyran-4-one (**12a**)

and the same reagents utilizing sodium *tert*-butoxide or potassium phosphate as bases.

**3-(4-Aminophenyl)-6-fluoro-4*H*-1-benzopyran-4-one (16b). 16b** was prepared (94%) by reduction of 6-fluoro-3-(4-nitrophenyl)-4*H*-1-benzopyranone (**14b**) with tin(II) chloride dihydrate in EtOH. The amine had mp 136−138 °C; IR (KBr) 3358, 1638, 1579, 1518, 1483, 1449, 1367 $cm^{-1}$; $^1H$ NMR $\delta_H$ (DMSO-$d_6$) 8.43 (1H, s, H-2), 8.20 (1H, m, ArH), 7.66 (1H, m, ArH), 7.40 (1H, m, ArH), 7.30 (2H, d, $J$ = 8.5, H-2′, H-6′), 6.65 (2H, d, $J$ = 8.5, H-3′, H-5′), 3.85 (2H, br m, $NH_2$); MS-EI (*m*/*z*) 255 ($M^+$). HRMS-EI (*m*/*z*): calcd for $C_{15}H_{10}FNO_2$, 255.0696 ($M^+$); found, 255.0703.

**3-(4-Aminophenyl)-7-fluoro-4*H*-1-benzopyran-4-one (16c). 16c** was prepared (75%) by reduction of 7-fluoro-3-(4-nitrophenyl)-4*H*-1-benzopyranone (**14c**) with tin(II) chloride dihydrate in EtOH. The amine had mp 153−155 °C; $^1H$ NMR $\delta_H$ ($CDCl_3$) 7.99 (1H, s, H-2, ArH), 7.37−7.52 (5H, m, ArH), 6.77 (2H, d, $J$ = 8.5, H-3′, H-5′), 3.75 (2H, br m, $NH_2$); MS-EI (*m*/*z*) 255 ($M^+$). HRMS-EI (*m*/*z*): calcd for $C_{15}H_{10}FNO_2$, 255.0696 ($M^+$); found, 255.0686.

**In Vitro Assays.** Compounds were prepared as 10 mM top stocks, dissolved in DMSO, and stored at 4 °C, protected from light for a maximum period of 4 weeks. Human-derived cell lines (MCF-7 (ER+), MDA 468 (ER-), MDA-MB-435 breast carcinoma; HCT 116, HT29 colon carcinoma) were routinely cultivated at 37 °C in an atmosphere of 5% $CO_2$ in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal calf serum and subcultured twice weekly to maintain continuous logarithmic growth. Cells were seeded into 96-well microtiter plates at a density of $5 \times 10^3$ per well and allowed 24 h to adhere before drugs were introduced (isoflavone final concentrations of 0.1 nM to 100 $\mu$M, $n$ = 8; TBDD final concentration of 10 nM, $n$ = 4). Serial drug dilutions were prepared in medium immediately prior to each assay. At the time of drug addition and following 72 h of exposure, MTT was added to each well (final concentration of 400 $\mu$g/mL). Incubation at 37 °C for 4 h allowed reduction of MTT by viable cells to an insoluble formazan product. Well contents were aspirated, and formazan was solubilized by addition of DMSO/glycine buffer (pH 10.5) (4:1). Absorbance was read on an Anthos Labtec systems plate reader at 550 nm as a measure of cell viability; thus, cell growth or drug toxicity was determined.

**Western Blot.** Whole-cell lysates were prepared for examination of protein expression from untreated cultures and following exposure of MDA-MB-468 cells to isoflavone (**12h**) (1, 10, 100 $\mu$M) and 2-(4-amino-3-methylphenyl)benzothiazole (**4b**) (positive control for CYP1A1 induction). Following protein determination ($n$ = 3, Bradford[30]) and addition of sample buffer, samples were heated to 95 °C for 5 min and solubilized proteins (50 $\mu$g) were separated by SDS−PAGE (10%). Proteins were electroblotted to PVDF membranes and probed for CYP1A1 protein with polyclonal antiserum specific for human CYP1A1/1A2 (Gentest Corporation). Secondary antibody was conjugated to alkaline phosphatase, and CYP1A1 was detected following brief (<10 min) incubation with bromochloroindolyl phosphate and nitro-blue tetrazolium in alkaline phosphatase buffer. Molecular weight markers and a positive control of recombinant CYP1A1 (Gentest Corporation), included in all blots, confirmed detection of 52 kDa CYP1A1 protein.

**Determination of EROD Activity.** A sensitive and rapid fluorometric assay was used to measure EROD activity.[27] Incubates (total 1 mL) consisted of 100 mM Tris-HCl (pH 7.4), 50 $\mu$M $MgCl_2$, 100 $\mu$M 7-ethoxyresorufin, and microsomes expressing recombinant CYP1A1 (0.1 mg/mL) in the presence or absence of 1, 10, or 100 $\mu$M (**12e**, **12g**, **12h**, **12k**) in order to determine inhibition of CYP1A1 activity by these isoflavone analogues. Reaction mixtures were preincubated for 5 min at 37 °C before initiation of reaction by addition of NADPH (500 $\mu$M). Following further incubation at 37 °C (15 min), reactions were terminated by addition of 3 mL of ice-cold acetonitrile. Reaction mixtures were centrifuged at 1400 rpm, 10 min before analyses of supernatants. Fluorescence was read on a Perkin-Elmer LS-5 luminescence spectrometer (excitation 530 nm; emission 585 nm). Estimation of resorufin reaction product (nM/mg protein), as a measure of CYP1A1 activity, was determined following performance of a resorufin standard curve.

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**Supporting Information Available:** Full compound NMR characterization data for compounds **8b**−**k**, **12b**−**o**, **13b**−**g**, and **14b**−**e**; additional references; microanalytical data for new compounds; and details of the attempted synthesis of compounds **14f**−**g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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