Synthesis and Aromatase Inhibitory Activity of Novel Pyridine-Containing Isoflavones

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Aromatase, a cytochrome P450 hemoprotein that is responsible for estrogen biosynthesis by conversion of androgens into estrogens, has been an attractive target in the treatment of hormone-dependent breast cancer. As a result, a number of synthetic steroidal or nonsteroidal aromatase inhibitors have been successfully developed. In addition, there are several classes of natural products that exert potent activities in aromatase inhibition, with the flavonoids being most prominent. Previous studies have exploited flavone and flavanone scaffolds for the development of new aromatase inhibitors. In this paper, we describe the design, synthesis, and biological evaluation of a novel series of 2-(4′-pyridylmethyl)thioisoflavones as the first example of synthetic isoflavone-based aromatase inhibitors.

Introduction

Flavonoids are a diverse group of plant-derived chemicals that are produced by various higher plants,[1] which can therefore be found in numerous food sources such as fruits, vegetables, legumes, and whole grains.[2] Compounds in this class have shown a wide variety of biological activities such as antiviral, antiinflammatory, antibacterial, antifungal, and anticancer activities.[3] In particular, due to their structural and functional similarities to endogenous estrogens, flavonoids have attracted considerable interest as alternative estrogens, termed phytoestrogens, and extensively studied for their potential role in many estrogen-dependent diseases including breast cancer. In fact, numerous flavonoids have shown interesting pharmacological activities in breast cancer biology, including binding affinities for estrogen receptors,[4] antiproliferative activities,[5] and inhibitory activities against aromatase enzyme.[6]

Genistein (GEN, Figure 1) is the most abundant isoflavone in soybeans. Some researchers suggest that GEN may be responsible for the relatively low incidence of hormone-dependent cancers in certain regions with high consumption of soy foods.[7] Indeed, GEN has been one of the most widely studied natural products and has shown a number of important biological activities in breast cancer biology. GEN displays moderate binding affinities to estrogen receptors.[4c] GEN exhibits potent antiproliferative activity in many breast cancer cell lines, and these effects are mediated by several mechanisms of action.[8] Furthermore, it is known that GEN exerts many beneficial effects of endogenous estrogens on several tissues such as bone[9] and cardiovascular system.[10] In addition to estrogenic activities, GEN has demonstrated a variety of other interesting biological activities. It exerts antioxidant activity and is a potent scavenger of hydrogen peroxide.[11] GEN has also shown inhibitory abilities against various enzymes involved in tumor development and growth such as protein tyrosine kinases, DNA topoisomerases, and protein kinase C (PKC). It inhibits angiogenesis and induces apoptosis and cell cycle arrest in the G2-phase.[12]

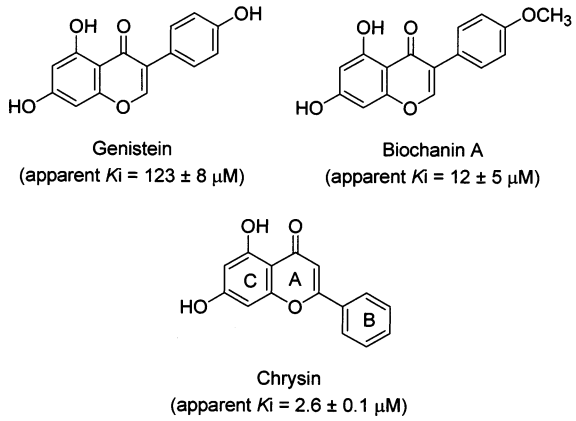

Figure 1. Chemical structures and aromatase inhibitory activities of two isoflavones (genistein, biochanin A) and one flavone (chrysin).[6c]

Inspired by the versatility of genistein, our research group has been investigating exploitation of the isoflavone nucleus as a potential privileged structure[13] for the development of new therapeutic agents for hormone-dependent breast cancer.[14] As part of this effort, we became interested in development of new isoflavone derivatives targeting aromatase (CYP19), a cytochrome P450 hemoprotein that is responsible for estrogen biosynthesis by conversion of androgens into estrogens.[15] Aromatase has been a particularly attractive target for inhibition in the treatment of hormone-dependent breast cancer since the aromatization is the last step in steroid biosynthesis and the rate-limiting step for estrogen synthesis.[16] In general, isoflavones are considered less effective than flavones or flavanones, of which chrysin is a representative (Figure 1), in terms of aromatase inhibition.[6c,17] For this reason, to the best of our knowledge, there has been no medicinal chemistry effort to develop isoflavone-based aromatase inhibitors. Nonetheless, we envisioned that the desired degree of

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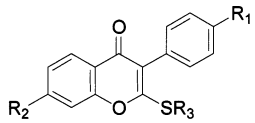
inhibitory activity against aromatase could be achieved by chemical modification of functional groups around the isoflavone nucleus. In the present paper, we wish to report the first examples of synthetic isoflavones as potent aromatase inhibitors.

Inhibitor Design

Most nonsteroidal aromatase inhibitors developed to date are reversible inhibitors that compete with the natural substrates, androstenedione and testosterone. Strong evidence for the binding of flavones to the active site of aromatase was obtained by difference spectral absorption studies by Kellis and Vickery.[18] They reported α-naphthoflavone could displace androstenedione from the aromatase active site and induce a spectrum consistent with the low-spin state of iron. Ibrahim and Abul-Hajj later reported reduction of the flavone 4-keto group was detrimental to aromatase inhibition by these compounds.[19] Based on data obtained from site directed mutagenesis studies and ligand docking into a homology model of the aromatase protein, a binding orientation was predicted in which the A and C rings of the flavone (Figure 1) mimic the C and D rings of the steroid substrate, respectively. Therefore, the 2-phenyl substituent is oriented in a region similar to that occupied by the A ring of the steroid. This analysis places the flavone 4-keto functionality in the same position as the steroid 19-angular methyl group with respect to the heme iron.[6c] Deductions based on the modeling results obtained by other research groups, assuming the isoflavones bind to the aromatase active site in an orientation reminiscent of the flavones, we hypothesize the translation of the phenyl group from the 2 to 3 position of the flavone core may diminish the ability of the 4-keto functionality to adequately coordinate to the heme iron and introduce unfavorable steric interactions within the enzyme active site.

Competitive nonsteroidal aromatase inhibitors usually possess a heteroatom that interferes with hydroxylation of steroids by coordinating with the heme iron of aromatase. Although several heteroatoms, such as sulfur, oxygen, and nitrogen, are known to show abilities to bind to heme iron, the majority of compounds in this class possess a nitrogen-containing heterocyclic moiety such as imidazole, triazole, pyrimidine, or pyridine.[19] We have envisioned that introduction of the appropriate heme-coordinating functional group to the 2-position of the isoflavone could result in a ligand whose binding mode is now biased to mimic the predicted binding mode of the flavones. Such an orientation would place the 3-phenyl functionality in the region occupied by the steroid A-ring, with the 4-keto functionality now pointing away from the heme.

On the basis of this rationale, we designed a library of 2,4′,7-trisubstituted isoflavones as shown in Figure 2. In this study, we have focused on the synthesis of isoflavones containing a (pyridylmethyl)thio moiety at the 2-position, which would be easily prepared via the phase transfer catalysis procedure developed in our laboratory.[14a] We were also interested in the preparation of isoflavones containing a non-nitrogenated moiety at the same position such as benzylthio or allylthio group for comparison. Previous studies have indicated that the presence of hydroxyl group at 4′-position seems to be unfavorable for aromatase inhibitory activity. For example, GEN is 10-fold less potent than its 4′-methoxy analogue, biochanin A (BCA, Figure 1).[6c] Therefore, we were interested in nonpolar alternatives to the 4′-OH such as methoxy, methyl, and hydrogen. In fact, it was proposed that isoflavones without the 4′-hydroxyl group might more efficiently inhibit aromatase.[4c] With regard to the 7-position, a 7-hydroxyl group appears to be the most promising, as its presence is almost ubiquitous among biologically active natural isoflavones. However, 7-methoxy analogues might also be interesting to investigate the consequences of masking the hydroxyl group at the position.



R_1 = H, Me, OMe, OH
R_2 = OH, OMe, OBn
R_3 = allyl, benzyl, pyridylmethyl

Figure 2. Chemical structures of the target 2,4′,7-trisubstituted isoflavones.

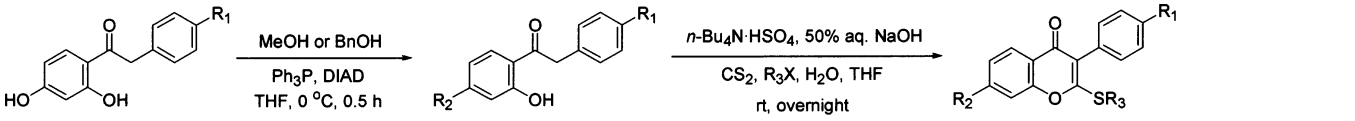
Chemistry

The synthesis of the target compounds was carried out as outlined in Scheme 1 and Scheme 2. The starting deoxybenzoins **1a**−**c** are commercially available or can be easily prepared by published procedure.[21] The 4-hydroxyl groups of compounds **1** were selectively protected with a methyl or benzyl group under Mitsunobu reaction conditions to give monoalkyl ethers **2a**−**e** in excellent yields (Scheme 1). The treatment of each deoxybenzoin **2** was treated with carbon disulfide and an appropriate alkyl halide in a THF−aqueous NaOH solution in the presence of 10 mol % of tetrabutylammonium hydrogensulfate (n-$Bu_4N{\cdot}HSO_4$) gave the corresponding 2-(alkylthio)isoflavone **3** in good to excellent yields. Dealkylation reactions of selected compounds **3** were performed with boron tribromide in dichloromethane yielding hydroxy compounds **4a**−**f** (Scheme 2). All attempts to prepare the 7-hydroxy-4′-methoxy analogue **4g** from **3k** using typical debenzylation procedures (i.e. catalytic hydrogenation reactions with various hydrogen sources in the presence of palladium on carbon) failed presumably due to catalyst poisoning of the sulfide group of **3k**. The use of 1 equiv of boron tribromide at a low temperature also proved to be inefficient, only providing a mixture of **3k**, **4f**, and **4g**. However, we found that $BF_3{\cdot}OEt_2$−Me_2S reagent is mild enough to achieve the selective removal of benzyl group, leaving the 4′-methoxy group intact to give **4g** in a good yield. This reaction condition was originally reported as a mild alternative debenzylation method in order to avoid undesirable 1,4-conjugate addition of ethanethiol to substrates containing a Michael acceptor.[22]

Biological Evaluation

The aromatase assay was performed according to the modified method of the procedure previously reported by our laboratory, in which human placental microsomes were used as the aromatase source.[23] IC_{50} values of the compounds were determined from dose−response curves (Figure 3 for compounds **3j**, **4f**, and **4g**)

Scheme 1. Synthesis of Isoflavones **3a**−**k**

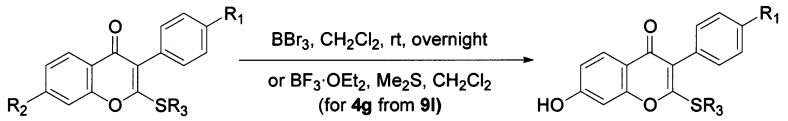


1a: R_1 = H
1b: R_1 = Me
1c: R_1 = OMe

2a: R_1 = H, R_2 = OMe (88%)
2b: R_1 = Me, R_2 = OMe (93%)
2c: R_1 = OMe, R_2 = OMe (81%)
2d: R_1 = H, R_2 = OBn (87%)
2e: R_1 = OMe, R_2 = OBn (95%)

3a: R_1 = OMe, R_2 = OBn, R_3 = allyl (92%)
3b: R_1 = OMe, R_2 = OBn, R_3 = Bn (89%)
3c: R_1 = H, R_2 = OMe, R_3 = allyl (96%)
3d: R_1 = H, R_2 = OMe, R_3 = Bn (97%)
3e: R_1 = H, R_2 = OMe, R_3 = (4-pyridyl)methanethio (81%)
3f: R_1 = H, R_2 = OMe, R_3 = (3-pyridyl)methanethio (89%)
3g: R_1 = H, R_2 = OMe, R_3 = (2-pyridyl)methanethio (92%)
3h: R_1 = Me, R_2 = OMe, R_3 = (4-pyridyl)methanethio (86%)
3i: R_1 = OMe, R_2 = OMe, R_3 = (4-pyridyl)methanethio (82%)
3j: R_1 = H, R_2 = OBn, R_3 = (4-pyridyl)methanethio (86%)
3k: R_1 = OMe, R_2 = OBn, R_3 = (4-pyridyl)methanethio (82%)

Scheme 2. Synthesis of Isoflavones **4a**−**g**



3a: R_1 = OMe, R_2 = OBn, R_3 = allyl
3b: R_1 = OMe, R_2 = OBn, R_3 = Bn
3d: R_1 = H, R_2 = OMe, R_3 = Bn
3e: R_1 = H, R_2 = OMe, R_3 = (4-pyridyl)methanethio
3f: R_1 = H, R_2 = OMe, R_3 = (3-pyridyl)methanethio
3k: R_1 = OMe, R_2 = OBn, R_3 = (4-pyridyl)methanethio

4a: R_1 = OH, R_3 = allyl (91%)
4b: R_1 = OH, R_3 = Bn (92%)
4c: R_1 = H, R_3 = Bn (88%)
4d: R_1 = H, R_3 = (4-pyridyl)methanethio (62%)
4e: R_1 = H, R_3 = (3-pyridyl)methanethio (47%)
4f: R_1 = OH, R_3 = (4-pyridyl)methanethio (86%)
4g: R_1 = OMe, R_3 = (4-pyridyl)methanethio (84%)

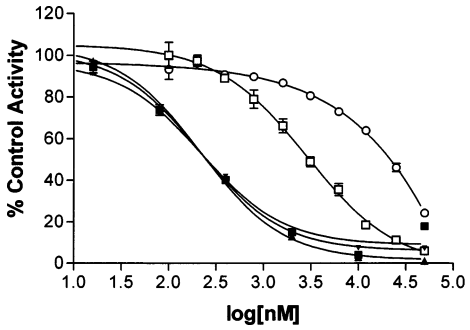


Figure 3. Aromatase inhibitory activities of compounds **3j** (▼), **4f** (■), and **4g** (▲). Aminoglutethimide (□) and biochanin A (○) were used as references. Error bars represent standard error (n = 3), and the data were statistically analyzed by a nonlinear regression analysis method.

and are listed in Table 1. The IC_{50} values of (±)-aminoglutethimide (AG) and BCA were also determined in our assay system for comparison. To examine the mode of aromatase inhibition and more accurately distinguish relative potencies, kinetic studies for selected compounds (**3e**, **3h**−**j**, **4f**, and **4g**) were also performed, and their apparent K_i values are listed in Table 2 with apparent K_m values and K_i/K_m ratios. Lineweaver−Burk plot of compound **3j**, the most potent analogue in this series, is shown in Figure 4.

Results and Discussion

In screening assay studies, analogues lacking a pyridyl group (**3a**−**d** and **4a**−**c**) showed no significant inhibition in enzyme assays containing up to 100 μM of the compound. Therefore, dose−response studies of these compounds were not pursued. On the other hand, most of the pyridine-containing analogues showed inhibitory activities, suggesting that the functionality might play a role in inhibiting aromatase. It is obvious that the position of the nitrogen atom in the pyridyl moiety is important for inhibitory activity. 4′-Pyridyl analogue **3e** is ~6-fold more potent that the 3′-pyridyl analogue **3f**, whereas the 2′-pyridyl analogue **3g** has no observable inhibitory activity in our assay system. This trend in activity is echoed in the 7-hydroxy analogues of this series; the 4′-pyridyl analogue **4d** is also ~6-fold more potent than 3′-pyridyl analogue **4e**. These results suggest that the position of the pyridyl nitrogen influences inhibitory activity. One possible explanation is that the pyridyl nitrogen may coordinate the active site

Table 1. Aromatase Inhibitory Activities of Isoflavones **3a**−**k** and **4a**−**g**

compd	R_1	R_2	R_3	IC_{50} (μM)	log IC_{50} (nM) (±SE)[a]
3a	OMe	OBn	allyl	>100	-
3b	OMe	OBn	benzyl	>100	-
3c	H	OMe	allyl	>100	-
3d	H	OMe	benzyl	>100	-
3e	H	OMe	(4′-pyridyl)methyl	1.6	3.21 ± 0.11
3f	H	OMe	(3′-pyridyl)methyl	9.2	3.96 ± 0.16
3g	H	OMe	(2′-pyridyl)methyl	>100	-
3h	Me	OMe	(4′-pyridyl)methyl	3.0	3.48 ± 0.05
3i	OMe	OMe	(4′-pyridyl)methyl	3.1	3.49 ± 0.02
3j	H	OBn	(4′-pyridyl)methyl	0.21	2.33 ± 0.03
3k	OMe	OBn	(4′-pyridyl)methyl	0.53	2.72 ± 0.11
4a	OH	OH	allyl	ND[b]	-
4b	OH	OH	benzyl	ND[b]	-
4c	H	OH	benzyl	ND[b]	-
4d	H	OH	(4′-pyridyl)methyl	0.61	2.79 ± 0.11
4e	H	OH	(3′-pyridyl)methyl	3.6	3.56 ± 0.06
4f	OH	OH	(4′-pyridyl)methyl	0.28	2.44 ± 0.07
4g	OMe	OH	(4′-pyridyl)methyl	0.22	2.34 ± 0.04
AG				2.8	3.45 ± 0.05
BCA				34	4.53 ± 0.06

[a] IC_{50} values were calculated by a nonlinear regression analysis (GraphPad Prism). Each dose−response curve contained 10 concentrations, each in triplicate. [b] Not determined.

Table 2. Enzyme Kinetic Parameters for Selected Isoflavones and Reference Compounds

compd	apparent K_i (μM) (±SE)[a]	apparent K_m (μM) (±SE)[a]	K_i/K_m
3e	0.90 ± 0.04	0.11 ± 0.06	8.18
3h	1.16 ± 0.07	0.12 ± 0.01	9.67
3i	1.69 ± 0.18	0.11 ± 0.01	15.4
3j	0.22 ± 0.02	0.13 ± 0.01	1.69
4f	0.31 ± 0.02	0.11 ± 0.07	2.82
4g	0.26 ± 0.02	0.10 ± 0.02	2.60
AG	1.41 ± 0.10	0.09 ± 0.01	15.7
BCA	12.0 ± 5[b]	-	-

[a] Apparent K_m, apparent K_i, and SE values were calculated by weighted regression analysis.[24] [b] See ref 6c.

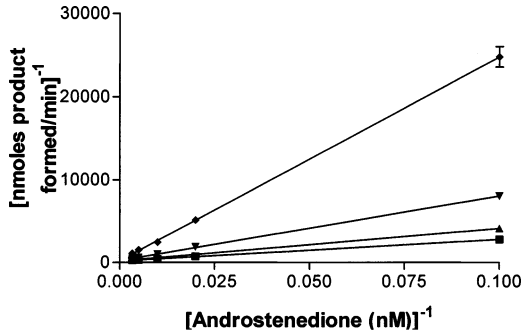


Figure 4. Lineweaver−Burk plot of aromatase inhibition by compound **3j**. Various concentrations of androstenedione (10−300 nM) were incubated with microsomal enzyme preparations at inhibitor concentrations of 0 nM (■), 100 nM (▲), 500 nM (▼), or 2000 nM (◆). Each point represents the average of three determinations with less than 10% variation from the mean.

heme iron. A method to probe this potential interaction is to obtain difference spectra with the active compounds and the enzyme preparation. Interestingly, we are unable to obtain spectra supportive of Type II binding with human placental microsomes or with *CYP19* baculovirus-transfected insect cell microsomes (B−D Biosciences, Woburn, MA).

The 7-hydroxy analogues **4d** and **4e** exhibit greater inhibitory activity than the corresponding 7-methoxy analogues **3e** and **3f**, respectively. This result suggests the presence of a hydrogen bond donor at the C-7 position may elicit a favorable interaction with the enzyme. With regard to the 4′ substituent, the presence of a hydrogen atom, as in **3e**, at this position is ∼2-fold more favorable in achieving aromatase enzyme activity compared to the 4′-methyl- and 4′-methoxy-substituted analogues **3h** and **3i**. The IC_{50} values of **3h** and **3i** are quite similar, and it is difficult to cast judgment about the superiority of one compound over another based on dose−response data alone. Toward this goal, we conducted enzyme kinetic studies (Table 2) of compounds **3e**, **3h**, and **3i** to verify the IC_{50} results and to more accurately discern the relative activities of these analogues. These compounds demonstrated typical competitive-type inhibition with androstenedione in Lineweaver−Burk plots (data not shown). As computed from the relative potencies (K_i/K_m), 4′-methyl analogue **3h** is 37% more active than the 4′methoxy analogue, **3i**. Interestingly, this structure−activity relationship does not translate to the 7-hydroxy analogues. In this case, the 4′-methoxy analogue is more potent than the 4′-hydrogen analogue **4d**. In addition, the potency of 4′,7-dihydroxy analogue **4f** was comparable to the 7-hydroxy-4′-methoxy analogue **4g**, but ∼2-fold greater than that of the 7-hydroxy-4′-hydrogen analogue **4d**. This result suggests the presence of a proton acceptor in the 4′ position in the hydroxy analogues may be beneficial for aromatase activity.

To our surprise, 7-benzyloxy analogue **3k**, originally prepared as a precursor of **4g**, displayed promising inhibitory activity. This observation was unexpected because the compound was assumed to be too bulky to fit within the enzyme active site. This led us to synthesize the 7-benzyloxy-4′-hydrogen analogue **3j** with the hypothesis that 7-benzyloxy analogues would display similar structure−activity relationships at the 4′ position as observed in the 7-methoxy series. Indeed, the elimination of the 4′-methoxy resulting in **3j** proved effective in producing a compound with enhanced aromatase inhibitory activity. In addition, **3j** exhibits ∼8-fold improvement compared to the corresponding 7-methoxy analogue **3e**. Finally, we obtained IC_{50} values in our assay system for the isoflavone lead compound, BCA, and the extensively studied aromatase inhibitor AG. The most potent compound in this series in terms of data obtained from dose−response studies, **3j**, demonstrated 162- and 13-fold improvements in aromatase inhibition over these compounds, respectively.

Enzyme kinetic studies were also conducted with the most potent compounds of this series identified in the dose−response studies. Compounds **3j**, **4f**, and **4g** demonstrated competititive-type inhibition with the androstenedione substrate (Figure 3, plots for **4f** and **4g** not shown). The computed relative inhibitory potency ratios reflected the same trend (**3j** > **4g** > **4f**) determined in the dose−response studies. As reflected by the relative potency, **3j** is the most potent compound within this series and is more potent than AG. This result is in agreement within the estimated experimental uncertainty of the result obtained from the dose−response studies. Compound **3j** also demonstrates 50-fold enhancement in potency compared to the natural product lead, BCA. The present enzyme kinetic results emphasize the importance of these structural modifications for optimization of aromatase inhibition by isoflavones.

Conclusions

To date, the isoflavone ring system has been considered an inappropriate scaffold for development of aromatase inhibitors. This study has shown that aromatase inhibitory activity can be achieved in the isoflavone nucleus by introducing functional groups with the potential to coordinate the heme iron. The structure−activity relationships indicate that the binding modes of 7-protected analogues **3e**−**k** might be different from those of 7-hydroxy analogues **4d**−**g**. Hydrogen-bonding potential appears to be important in the 7-hydroxy analogues **4d**−**g** for their aromatase inhibitory activity, whereas hydrophobic interactions appear to play a role in 7-protected analogues **3e**−**k**. Among the tested isoflavones, compounds **3j**, **4f**, and **4g** show more potent aromatase inhibitory activities than the others. Based on the kinetic studies, it is clear that these compounds compete at the active site of aromatase with the natural substrate, androstenedione. While investigations are currently underway to evaluate the exact nature of the enzyme−ligand interactions, these compounds could be

new leads for the development of more potent inhibitors in this series. Especially of great interest as a new lead is compound **3j**, containing a benzyl group that seems to be a key feature for the inhibitory activity. Therefore, one can envision that introduction of a proper functionality on the phenyl ring of the 7-benzyloxy group of **3j** may confer more potent aromatase inhibitory activity. Based on this hypothesis, synthetic efforts for structural optimization of **3j** are also currently underway.

Experimental Section

Unless otherwise noted, chemicals were commercially available and used as received without further purification. Moisture sensitive reactions were carried out under a dry argon atmosphere in flame-dried glassware. Solvents were distilled before use under argon. Tetrahydrofuran was distilled from sodium metal in the presence of benzophenone; dichloromethane was distilled from calcium hydride. Thin-layer chromatography was performed on precoated silica gel F254 plates (Whatman). Silica gel column chromatography was performed using silica gel 60A (Merck, 230−400 Mesh). Melting points were determined in open glass capillaries using a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet Protégé 460 spectrometer using KBr pellets. High-resolution electrospray ionization mass spectra were obtained on the Micromass QTOF Electrospray mass spectrometer at The Ohio State Chemical Instrumentation Center. All the NMR spectra were recorded on a Bruker DPX 250, or Bruker DRX 400 model spectrometer in either DMSO-d_6 or $CDCl_3$. Chemical shifts (δ) for ^{1}H NMR spectra are reported in parts per million to residual solvent protons. Chemical shifts (δ) for ^{13}C NMR spectra are reported in parts per million relative to residual solvent carbons.

General Procedure for the Preparation of 4-Alkoxydeoxybenzoins (2a−e). To a solution of 2-aryl-1-(2,4-dihydroxyphenyl)ethanone (31.0 mmol) and alcohol (32.55 mmol) in THF (150 mL) was added triphenylphosphine (8.538 g, 32.55 mmol), followed by diisopropyl azodicarboxylate (6.41 mL, 32.55 mmol) at 0 °C, and the resulting yellow solution was stirred at 0 °C for 10 min. The solvent was removed under reduced pressure, and the oily residue was directly purified by silica gel column chromatography (eluting with EtOAc: hexane, 1:4) and recrystallization (EtOAc and hexane) to yield desired product.

1-(2-Hydroxy-4-methoxyphenyl)-2-phenylethanone (2a). Using the previous procedure and starting from 1-(2,4-dihydroxyphenyl)-2-phenylethanone (7.08 g, 31.0 mmol) and methanol (1.32 mL, 32.55 mmol), 6.60 g (88%) of the title compound was obtained as a white solid: mp 87−88 °C (lit. 92 °C);[24a] IR (KBr) 1635, 1620, 1589, 1437, 1350, 1291, 1230, 1206, 1127, 1021, 958, 802, 739, 729, 551 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.71 (br s, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.31−7.34 (m, 2H), 7.23−7.26 (m, 3H), 6.40−6.44 (m, 2H), 4.20 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.38, 166.61, 166.30, 134.82, 132.47, 129.76, 129.16, 127.50, 113.58, 108.27, 101.44, 56.00, 45.27.

1-(2-Hydroxy-4-methoxyphenyl)-2-(4-methylphenyl)-ethanone (2b). Using the previous procedure and starting from 1-(2,4-dihydroxyphenyl)-2-(4-methylphenyl)ethanone (7.51 g, 31.0 mmol) and methanol (1.32 mL, 32.55 mmol), 7.38 g (93%) of the title compound was obtained as a white solid: mp (EtOAc/hexane) 71−72 °C; IR (KBr) 1639, 1623, 1590, 1516, 1508, 1439, 1388, 1355, 1268, 1231, 1205, 1131, 1033, 1010, 957, 800, 780, 571, 504, 491 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.72 (br s, 1H), 7.73 (d, J = 8.6 Hz, 1H), 7.24−7.14 (m, 4H), 6.43−6.40 (m, 2H), 4.15 (s, 2H), 3.81 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.63, 166.56, 166.28, 137.14, 132.47, 131.71, 129.87, 129.61, 113.60, 108.20, 101.43, 55.98, 44.89, 21.48; HRMS calculated for $C_{16}H_{16}NaO_3$ $(M + Na)^+$ 279.0997, found 279.0989.

1-(2-Hydroxy-4-methoxyphenyl)-2-(4-methoxylphenyl)-ethanone (2c). Using the previous procedure and starting from 1-(2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethanone (8.0 g, 31.0 mmol) and methanol (1.32 mL, 32.55 mmol), 6.85 g (81%) of the title compound was obtained as a white solid: mp 101−102 °C (lit. 104 °C);[24b] IR (KBr) 1637, 1611, 1513, 1459, 1346, 1301, 1237, 1174, 1148, 1026, 797, 787, 626, 522 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.72 (br s, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.17 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 6.40−6.44 (m, 2H), 4.13 (s, 2H), 3.81 (s, 3H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ202.73, 166.55, 166.27, 159.07, 132.42, 130.78, 126.73, 114.61, 113.53, 108.21, 101.42, 55.99, 55.67, 44.37.

1-[2-Hydroxy-4-(phenylmethoxy)phenyl]-2-phenylethanone (2d). Using the previous procedure and starting from 1-(2,4-dihydroxyphenyl)-2-phenylethanone (7.08 g, 31.0 mmol) and benzyl alcohol (3.37 mL, 32.55 mmol), 8.62 g (87%) of the title compound was obtained as a white solid: mp 106−108 °C (lit. 104−105 °C);[24c] IR (KBr) 1620, 1572, 1500, 1389, 1352, 1270, 1230, 1192, 1134, 1000, 974, 830, 760, 729, 697, 628, 561 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.69 (br s, 1H), 7.48 (d, J = 8.7 Hz, 1H), 7.25−7.39 (m, 10H), 6.49−6.51(m, 2H), 5.07 (s, 2H), 4.20 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.41, 166.20, 165.68, 136.22, 134.79, 132.53, 129.76, 129.17, 129.14, 128.76, 127.95, 127.51, 113.77, 108.75, 102.47, 70.66, 45.28; HRMS calculated for $C_{21}H_{18}NaO_3$ $(M + Na)^+$ 341.1154, found 341.1136.

1-[2-Hydroxy-4-(phenylmethoxy)phenyl]-2-(4-methoxyphenyl)ethanone (2e). Using the previous procedure and starting from 1-(2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)-ethanone (8.0 g, 31.0 mmol) and benzyl alcohol (3.37 mL, 32.55 mmol), 10.23 g (95%) of the title compound was obtained as a white solid: mp 97−98 °C (lit. 93−95 °C);[24d] IR (KBr) 1635, 1611, 1512, 1496, 1387, 1351, 1289, 1227, 1173, 1131, 1029, 994, 948, 842, 830, 791, 745, 725, 695, 535 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 12.73 (s, 1H), 7.75 (d, J = 9.6 Hz, 1H), 7.33−7.40 (m, 5H), 7.18 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 6.53−6.49 (m, 2H), 5.07 (s, 2H), 4.14 (s, 2H), 3.78 (s, 3H); ^{13}C NMR (69.3 MHz, $CDCl_3$) δ 202.77, 166.19, 165.63, 159.10, 136.26, 132.51, 130.82, 129.15, 128.77, 127.97, 126.71, 114.63, 113.73, 108.71, 102.48, 70.65, 55.69, 44.38; HRMS calculated for $C_{22}H_{20}NaO_4$ $(M + Na)^+$ 371.1259, found 371.1265.

General procedure for the preparation of 2-alkylthioisoflavones (3a−k) from 2′-hydroxydeoxybenzoins. To a stirred mixture of a deoxybenzoin (1 mmol), carbon disulfide (0.6 mL, 10 mmol), alkyl halide (2.2 mmol), and n-$Bu_4N{\cdot}HSO_4$ (34 mg, 0.1 mmol) in THF (3 mL) and water (1 mL) was slowly added 10 M solution of NaOH in water (1.2 mL, 12 mmol) at room temperature. A slight exothermic reaction and a color change of the mixture were observed. The resulting mixture was vigorously stirred at room temperature for several hours, and the product was extracted with EtOAc (2 × 10 mL). The combined organic layer was washed with water (10 mL) and then with brine (10 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (eluting with MeOH/$CHCl_3$ or EtOAc/hexane) and recrystallization (EtOAc/hexane) to yield desired product.

3-(4-Methoxyphenyl)-7-(phenylmethoxy)-2-[(propen-2-yl)thio]-4*H*-1-benzopyran-4-one (3a). Using 1-[2-hydroxy-4-(phenylmethoxy)phenyl]-2-(4-methoxyphenyl)ethanone (0.348 g, 1.0 mmol) as a starting deoxybenzoin and allyl bromide (0.208 mL, 2.4 mmol) as an alkyl halide, 0.396 g (92%) of the title compound was obtained as a white solid: mp 101−102 °C; IR (KBr) 1618, 1509, 1438, 1363, 1342, 1291, 1247, 1176, 1152, 1101, 1030, 944, 821, 740, 696 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.13 (d, J = 8.8 Hz, 1H), 7.33−7.46 (m, 5H), 7.25 (d, J = 8.7 Hz, 2H), 7.02 (dd, J = 8.9, 2.3 Hz, 1H), 6.95 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 2.3 Hz, 1H), 5.87 (ddt, J = 16.9, 10.0, 6.9 Hz, 1H), 5.25 (dd, J = 16.9, 1.2 Hz, 1H), 5.11−5.06 (m, 3H), 3.82 (s, 3H), 3.69 (d, J = 6.9 Hz, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.34, 163.40, 163.10, 159.93, 158.38, 136.17, 133.30, 132.30, 129.20, 128.84, 128.48, 127.95, 124.64, 122.88, 119.22, 117.86, 114.96, 114.36, 101.29, 71.01, 55.65, 34.59; HRMS calculated for $C_{26}H_{22}NaO_4S$ $(M + Na)^+$ 453.1137, found 453.1123. Anal. ($C_{26}H_{22}O_4S{\cdot}0.3H_2O$) C, H.

3-(4-Methoxyphenyl)-7-(phenylmethoxy)-2-[(phenylmethyl)thio]-4*H*-1-benzopyran-4-one (3b). Using 1-[2-hydroxy-4-(phenylmethoxy)phenyl]-2-(4-methoxyphenyl)ethanone (0.348 g, 1.0 mmol) as a starting deoxybenzoin and benzyl bromide (0.274 mL, 2.3 mmol) as an alkyl halide, 0.427 g (89%) of the title compound was obtained as a white solid: mp 131−132 °C; IR (KBr) 1618, 1508, 1438, 1364, 1247, 1176, 1029, 822, 697 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 8.14 (d, J = 8.9 Hz, 1H), 7.22−7.48 (m, 10H), 7.21 (d, J = 8.7 Hz, 2H), 7.03 (dd, J = 8.9, 2.3 Hz, 1H), 6.93 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 2.3 Hz, 1H), 5.17 (s, 2H), 4.28 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.33, 163.61, 163.09, 159.91, 158.38, 136.61, 136.17, 132.25, 129.33, 129.25, 129.18, 128.88, 128.49, 128.15, 127.96, 124.52, 122.42, 117.82, 115.03, 114.36, 101.25, 71.01, 55.65, 36.17; HRMS calculated for $C_{30}H_{24}NaO_4S$ $(M + Na)^+$ 503.1293, found 503.1258. Anal. ($C_{30}H_{24}O_4S$) C, H.

7-Methoxy-3-phenyl-2-[(propen-2-yl)thio]-4*H*-1-benzopyran-4-one (3c). Using 1-(2-hydroxy-4-methoxyphenyl)-2-phenylethanone (0.242 g, 1.0 mmol) as a starting deoxybenzoin and allyl bromide (0.190 mL, 2.2 mmol) as an alkyl halide, 0.314 g (96%) of the title compound was obtained as a white solid (Method B): mp 117−118 °C; IR (KBr) 1635, 1615, 1585, 1546, 1503, 1435, 1373, 1345, 1252, 1197, 1108, 1017, 942, 922, 831, 753, 701, 662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.9 Hz, 1H), 7.30−7.44 (m, 5H), 6.96 (dd, J = 8.9, 2.4 Hz, 1H), 6.81 (d, J = 2.3 Hz, 1H), 5.83−5.94 (m, 1H), 5.27 (dd, J = 16.9, 1.2 Hz, 1H), 5.14 (dd, J = 10.1, 0.8 Hz, 1H), 3.91 (s, 3H), 3.70 (d, J = 6.9 Hz, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.21, 164.12, 163.40, 158.50, 133.20, 132.61, 131.08, 128.82, 128.68, 128.39, 123.35, 119.27, 117.69, 114.51, 100.13, 56.31, 34.54; HRMS calculated for $C_{19}H_{16}NaO_3S$ $(M + Na)^+$ 347.0718, found 347.0705. Anal. ($C_{19}H_{16}O_3S \cdot 0.2H_2O$) C, H.

7-Methoxy-3-phenyl-2-[(phenylmethyl)thio]-4*H*-1-benzopyran-4-one (3d). Using 1-(2-hydroxy-4-methoxyphenyl)-2-phenylethanone (0.242 g, 1.0 mmol) as a starting deoxybenzoin and benzyl bromide (0.262 mL, 2.2 mmol) as an alkyl halide, 0.365 g (97%) of the title compound was obtained as a white solid: mp 153−154 °C; IR (KBr) 1636, 1617, 1586, 1546, 1502, 1438, 1373, 1341, 1252, 1205, 1106, 1016, 942, 831, 699, 661 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 8.9 Hz, 1H), 7.22−7.41 (m, 10H), 6.95 (dd, J = 8.9, 2.4 Hz, 1H), 6.81 (d, J = 2.3 Hz, 1H), 4.30 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.22, 164.10, 163.54, 158.48, 136.51, 132.52, 131.04, 129.32, 129.17, 128.81, 128.66, 128.39, 128.17, 122.97, 117.70, 114.49, 100.19, 56.31, 36.17; HRMS calculated for $C_{23}H_{18}NaO_3S$ $(M + Na)^+$ 397.0874, found 397.0856. Anal. ($C_{23}H_{18}O_3S$) C, H.

7-Methoxy-3-phenyl-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (3e). Using 1-(2-hydroxy-4-methoxyphenyl)-2-phenylethanone (0.242 g, 1.0 mmol) as a starting deoxybenzoin and 4-(bromomethyl)pyridine hydrobromide (0.557 g, 2.2 mmol) as an alkyl halide, 0.305 g (81%) of the title compound was obtained as a white solid: mp 136−137 °C; IR (KBr) 1634, 1622, 1600, 1549, 1497, 1433, 1369, 1257, 1200, 1098, 1067, 1013, 943, 831, 775, 756, 703, 658, 570 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.53 (dd, J = 4.5, 1.5 Hz, 2H), 8.09 (d, J = 8.9 Hz, 1H), 7.33−7.42 (m, 3H), 7.24−7.27 (m, 4H), 6.93 (dd, J = 8.9, 2.4 Hz, 1H), 6.70 (d, J = 2.3 Hz, 1H), 4.21 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.09, 164.21, 161.95, 158.33, 150.45, 146.38, 132.22, 130.95, 128.89, 128.86, 128.46, 124.04, 123.58, 117.59, 114.50, 100.16, 56.31, 34.79; HRMS calculated for $C_{22}H_{17}NNaO_3S$ $(M + Na)^+$ 398.0827, found 398.0818. Anal. ($C_{22}H_{17}NO_3S \cdot 0.2H_2O$) C, H, N.

7-Methoxy-3-phenyl-2-[(3-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (3f). Using 1-(2-hydroxy-4-methoxyphenyl)-2-phenylethanone (0.242 g, 1.0 mmol) as a starting deoxybenzoin and 3-(bromomethyl)pyridine hydrobromide (0.557 g, 2.2 mmol) as an alkyl halide, 0.334 g (89%) of the title compound was obtained as a white solid: mp 151.5−152 °C; IR (KBr) 1635, 1617, 1585, 1547, 1503, 1438, 1427, 1373, 1344, 1253, 1203, 1108, 1017, 943, 831, 754, 701, 662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.62 (br s, 1H), 8.49 (d, J = 4.3 Hz, 1H), 8.10 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 7.9 Hz, 1H), 7.35−7.41 (m, 3H), 7.23−7.28 (m, 3H), 6.95 (dd, J = 8.9, 2.1 Hz, 1H), 6.79 (d, J = 2.1 Hz, 1H), 4.27 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.11, 164.24, 162.30, 158.42, 150.24, 149.35, 136.81, 132.93, 132.29, 130.96, 128.86, 128.80, 128.41, 124.11, 123.46, 117.60, 114.66, 100.10, 56.34, 33.21; HRMS calculated for $C_{22}H_{17}NNaO_3S$ $(M + Na)^+$ 398.0827, found 398.0840. Anal. ($C_{22}H_{17}NO_3S$) C, H, N.

7-Methoxy-3-phenyl-2-[(2-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (3g). Using 1-(2-hydroxy-4-methoxyphenyl)-2-phenylethanone (0.242 g, 1.0 mmol) as a starting deoxybenzoin and 2-(bromomethyl)pyridine hydrobromide (0.557 g, 2.2 mmol) as an alkyl halide, 0.345 g (92%) of the title compound was obtained as a white solid: mp 168.5−169 °C; IR (KBr) 1634, 1617, 1586, 1546, 1502, 1431, 1373, 1344, 1252, 1202, 1153, 1106, 1016, 943, 831, 782, 752, 698, 661 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.47 (ddd, J = 4.9, 1.7, 0.9 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.74 (dt, J = 7.7, 1.8 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.30−7.39 (m, 3H), 7.34 (ddd, J = 7.6, 4.9, 0.9 Hz, 1H), 7.17−7.19 (m, 3H), 7.03 (dd, J = 8.8, 2.4 Hz, 1H), 4.54 (s, 2H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.33, 164.34, 163.86, 158.54, 157.30, 150.20, 137.88, 133.14, 131.46, 129.03, 128.86, 127.75, 124.04, 123.43, 122.40, 117.23, 115.46, 101.23, 57.02, 37.39; HRMS calculated for $C_{22}H_{17}NNaO_3S$ $(M + Na)^+$ 398.0827, found 398.0819. Anal. ($C_{22}H_{17}NO_3S$) C, H, N.

7-Methoxy-3-(4-methylphenyl)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (3h). Using 1-(2-hydroxy-4-methoxyphenyl)-2-(4-methylphenyl)ethanone (0.256 g, 1.0 mmol) as a starting deoxybenzoin and 4-(bromomethyl)pyridine hydrobromide (0.557 g, 2.2 mmol) as an alkyl halide, 0.335 g (86%) of the title compound was obtained as a white solid: mp 157−160 °C; IR (KBr) 1628, 1598, 1585, 1543, 1497, 1434, 1373, 1343, 1254, 1198, 1182, 1099, 1016, 936, 837, 814 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, J = 5.6 Hz, 2H), 8.08 (d, J = 8.9 Hz, 1H), 7.26 (d, J = 5.5 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 6.92 (dd, J = 8.9, 2.1 Hz, 1H), 6.69 (d, J = 2.0 Hz, 1H), 4.20 (s, 2H), 3.87 (s, 3H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.20, 164.15, 161.79, 158.32, 150.46, 146.37, 138.72, 130.75, 129.67, 129.17, 128.46, 124.05, 123.46, 117.58, 114.44, 100.14, 56.29, 34.79, 21.85; HRMS calculated for $C_{23}H_{19}NNaO_3S$ $(M + Na)^+$ 412.0983, found 398.1004. Anal. ($C_{23}H_{19}NO_3S$) C, H, N.

7-Methoxy-3-(4-methoxyphenyl)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (3i). Using 1-(2-hydroxy-4-methoxyphenyl)-2-(4-methoxyphenyl)ethanone (0.272 g, 1.0 mmol) as a starting deoxybenzoin and 4-(bromomethyl)pyridine hydrobromide (0.557 g, 2.2 mmol) as an alkyl halide, 0.332 g (82%) of the title compound was obtained as a white solid: mp 140−141 °C; IR (KBr) 1622, 1609, 1549, 1510, 1434, 1369, 1343, 1288, 1250, 1199, 1180, 1099, 1024, 961, 945, 835, 821, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, J = 5.8 Hz, 2H), 8.08 (d, J = 8.9 Hz, 1H), 7.27 (d, J = 5.7 Hz, 2H), 7.18 (d, J = 8.6 Hz, 2H), 6.92−6.94 (m, 3H), 6.69 (d, J = 2.1 Hz, 1H), 4.21 (s, 2H), 3.88 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.29, 164.14, 161.82, 160.05, 158.32, 150.42, 146.46, 132.16, 128.48, 124.24, 124.06, 123.11, 117.57, 114.42, 114.40, 100.13, 56.29, 55.65, 34.82; HRMS calculated for $C_{23}H_{19}NNaO_4S$ $(M + Na)^+$ 428.0932, found 428.0949. Anal. ($C_{23}H_{19}NO_4S$) C, H, N.

3-Phenyl-7-(phenylmethoxy)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (3j). Using 1-[2-hydroxy-4-(phenylmethoxy)phenyl]-2-phenylethanone (0.318 g, 1.0 mmol) as a starting deoxybenzoin and 4-(bromomethyl)pyridine hydrobromide (0.557 g, 2.2 mmol) as an alkyl halide, 0.386 g (86%) of the title compound was obtained as a pale yellow solid: mp 169−170 °C; IR (KBr) 1619, 1599, 1584, 1540, 1491, 1440, 1372, 1344, 1259, 1196, 1157, 1099, 991, 943, 836, 819, 781, 747, 695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.51 (dd, J = 4.5, 1.6 Hz, 2H), 8.11 (d, J = 8.9 Hz, 1H), 7.34−7.45 (m, 8H), 7.22−7.26 (m, 4H), 7.03 (dd, J = 8.9, 2.3 Hz, 1H), 6.78 (d, J = 2.3 Hz, 1H), 5.15 (s, 2H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.03, 163.22, 161.99, 158.23, 150.61, 146.23, 136.06, 132.19, 130.94, 129.25, 128.90, 128.88, 128.57, 127.88, 123.94, 123.59, 117.80, 115.11, 101.25, 71.03, 34.78; HRMS calculated

for $C_{28}H_{21}NNaO_3S$ $(M + Na)^+$ 474.1140, found 474.1136. Anal. ($C_{28}H_{21}NO_3S \cdot 0.1H_2O$) C, H, N.

3-(4-Methoxyphenyl)-7-(phenylmethoxy)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (3k). Using 1-[2-hydroxy-4-(phenylmethoxy)phenyl]-2-(4-methoxyphenyl)ethanone (0.348 g, 1.0 mmol) as a starting deoxybenzoin and 4-(bromomethyl)pyridine hydrobromide (0.557 g, 2.2 mmol) as an alkyl halide, 0.402 g (84%) of the title compound was obtained as a white solid: mp 169.5−170.5 °C; IR (KBr) 1616, 1539, 1509, 1440, 1414, 1371, 1342, 1294, 1251, 1197, 1173, 1156, 1100, 1029, 991, 943, 824, 735, 697, 665 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, J = 5.8 Hz, 2H), 7.87 (d, J = 8.8 Hz, 1H), 7.35−7.49 (m, 7H), 7.30 (d, J = 2.3 Hz, 1H), 7.08−7.12 (m, 3H), 6.93 (d, J = 8.7 Hz, 2H), 5.26 (s, 2H), 4.39 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.39, 163.30, 162.91, 159.90, 158.31, 150.68, 147.69, 136.95, 132.64, 129.46, 129.08, 128.82, 127.86, 124.79, 124.70, 122.37, 117.32, 115.95, 114.55, 102.25, 70.95, 55.96, 34.03; HRMS calculated for $C_{29}H_{23}NNaO_4S$ $(M + Na)^+$ 504.1245, found 504.1238. Anal. ($C_{29}H_{23}NO_4S \cdot 0.2H_2O$) C, H, N.

General Procedure for Dealkylation Using Boron Tribromide (4a−g). To a stirred solution of 2-substituted 7-alkoxy-3-aryl-4*H*-1-benzopyran-4-one (0.5 mmol) in CH_2Cl_2 (10 mL) was slowly added a 1.0 M solution of BBr_3 (2.0 mL, 2 mmol) at 0 °C, and the resulting suspension was allowed to warm to room temperature and stirred overnight. After being cooled to 0 °C, the reaction mixture was quenched with water and concentrated under reduced pressure. The residue was suspended in a mixture of water and EtOAc, and the insoluble product was collected by filtration. The filtrate was extracted with EtOAc twice (2 × 20 mL), and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give additional product. The combined solid was purified by silica gel column chromatography (eluting with MeOH/$CHCl_3$) and/or directly applied to recrystallization.

7-Hydroxy-3-(4-hydroxyphenyl)-2-[(propen-2-yl)thio]-4*H*-1-benzopyran-4-one (4a). Using the previous procedure and starting from 3-(4-methoxyphenyl)-7-(phenylmethoxy)-2-[(propen-2-yl)thio]-4*H*-1-benzopyran-4-one (0.179 g, 0.416 mmol), 0.123 g (91%) of the title compound was obtained as a pale yellow solid: mp 230−232 °C (decomposed); IR (KBr) 3231, 1610, 1561, 1539, 1512, 1497, 1439, 1376, 1245, 1220, 1194, 1174, 1103, 972, 949, 928, 846, 827, 809 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.72 (br s, 1H), 9.50 (br s, 1H), 7.81 (d, J = 9.3 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H), 6.85−6.88 (m, 2H), 6.74 (d, J = 8.6 Hz, 2H), 5.84 (ddt, J = 16.9, 9.9, 6.9 Hz, 1H), 5.27 (dd, J = 16.9, 1.4 Hz, 1H), 5.09 (dd, J = 9.9, 1.4 Hz, 1H), 3.75 (d, J = 6.9 Hz, 2H),; ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.61, 163.10, 163.07, 158.51, 158.01, 134.75, 132.67, 128.07, 123.49, 122.50, 119.27, 116.24, 115.83, 115.80, 102.70, 34.24; HRMS calculated for $C_{18}H_{14}NaO_4S$ $(M + Na)^+$ 349.0511, found 349.0529. Anal. ($C_{18}H_{14}O_4S \cdot 0.1H_2O$) C, H.

7-Hydroxy-3-(4-hydroxyphenyl)-2-[(phenylmethyl)thio]-4*H*-1-benzopyran-4-one (4b). Using the previous procedure and starting from 3-(4-methoxyphenyl)-7-(phenylmethoxy)-2-[(phenylmethyl)thio]-4*H*-1-benzopyran-4-one (0.147 g, 0.305 mmol), 0.106 g (92%) of the title compound was obtained as a white solid: mp 261−264 °C (decomposed); IR (KBr) 3280, 1625, 1605, 1592, 1508, 1459, 1375, 1246, 1228, 1189, 1174, 1111, 966, 948, 843, 821, 694 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.75 (br s, 1H), 9.49 (br s, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.35−7.37 (m, 2H), 7.18−7.29 (m, 3H), 6.94 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 2.2 Hz, 1H), 6.86 (dd, J = 8.7, 2.2 Hz, 1H), 6.72 (d, J = 8.5 Hz, 2H), 4.36 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.51, 163.25, 163.06, 158.48, 158.01, 138.18, 132.61, 129.75, 129.40, 128.20, 128.06, 123.38, 121.96, 116.23, 115.86, 115.81, 102.73, 35.38; HRMS calculated for $C_{22}H_{16}NaO_4S$ $(M + Na)^+$ 399.0667, found 399.0656. Anal. ($C_{22}H_{16}O_4S \cdot 0.5H_2O$) C, H.

7-Hydroxy-3-phenyl-2-[(phenylmethyl)thio]-4*H*-1-benzopyran-4-one (4c). Using the previous procedure and starting from 7-methoxy-3-phenyl-2-[(phenylmethyl)thio]-4*H*-1-benzopyran-4-one (0.223 g, 0.596 mmol), 0.189 g (88%) of the title compound was obtained as a white solid: mp 215−217 °C; IR (KBr) 3413, 1610, 1559, 1486, 1453, 1375, 1269, 1250, 1219, 1194, 1105, 970, 947, 848, 699 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.80 (br s, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.20−7.37 (m, 8H), 7.13−7.19 (m, 2H), 6.94 (d, J = 2.2 Hz, 1H), 6.87 (dd, J = 8.7, 2.2 Hz, 1H), 4.38 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.31, 163.47, 163.20, 158.54, 138.11, 133.26, 131.46, 129.76, 129.41, 128.99, 128.77, 128.24, 128.08, 122.21, 116.20, 115.97, 102.79, 35.39; HRMS calculated for $C_{22}H_{16}NaO_3S$ $(M + Na)^+$ 383.0718, found 383.0710. Anal. ($C_{22}H_{16}O_3S \cdot 0.4H_2O$) C, H.

7-Hydroxy-3-phenyl-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (4d). Using the previous procedure and starting from 7-methoxy-3-phenyl-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (0.165 g, 0.439 mmol), 0.098 g (62%) of the title compound was obtained as a pale yellow solid: mp 229−230 °C (decomposed); IR (KBr) 3427, 1617, 1584, 1544, 1504, 1417, 1366, 1260, 1191, 1102, 1015, 969, 945, 843, 701 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.80 (br s, 1H), 8.48 (d, J = 4.0 Hz, 2H), 7.80 (d, J = 8.4 Hz, 1H), 7.33−7.40 (m, 5H), 7.16 (d, J = 7.0 Hz, 2H), 6.86−6.88 (m, 2H), 4.38 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.28, 163.19, 162.72, 158.48, 150.65, 147.82, 133.12, 131.45, 129.06, 128.87, 128.09, 124.61, 122.50, 116.14, 116.01, 102.71, 34.05; HRMS calculated for $C_{21}H_{15}NNaO_3S$ $(M + Na)^+$ 384.0670, found 384.0667. Anal. ($C_{21}H_{15}NO_3S \cdot 0.2H_2O$) C, H, N.

7-Hydroxy-3-phenyl-2-[(3-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (4e). Using the previous procedure and starting from 7-methoxy-3-phenyl-2-[(3-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (0.181 g, 0.482 mmol), 0.082 g (47%) of the title compound was obtained as a yellow solid: mp 194−197 °C (decomposed); IR (KBr) 3053, 1618, 1584, 1542, 1502, 1462, 1366, 1268, 1219, 1187, 1102, 970, 946, 843, 782, 754, 701 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.82 (br s, 1H), 8.73 (br s, 1H), 8.53 (d, J = 4.4 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.53 (dd, J = 7.8, 5.1 Hz, 1H), 7.30−7.39 (m, 3H), 7.13−7.15 (m, 2H), 6.95 (d, J = 2.1 Hz, 1H), 6.89 (dd, J = 8.7, 2.1 Hz, 1H), 4.46 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.51, 163.12, 162.83, 158.55, 148.07, 147.00, 140.30, 136.09, 132.92, 131.36, 129.15, 128.99, 128.13, 125.64, 122.61, 116.10, 116.04, 102.79, 32.23; HRMS calculated for $C_{21}H_{15}NNaO_3S$ $(M + Na)^+$ 384.0670, found 384.0674. Anal. ($C_{21}H_{15}NO_3S \cdot 0.3H_2O$) C, H, N.

7-Hydroxy-3-(4-hydroxyphenyl)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (4f). Using the previous procedure and starting from 3-(4-methoxyphenyl)-7-(phenylmethoxy)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (0.173 g, 0.36 mmol), 0.117 g (86%) of the title compound was obtained as a pale yellow solid: mp > 240 °C (decomposed); IR (KBr) 3430, 1621, 1607, 1560, 1513, 1499, 1369, 1263, 1234, 1194, 1171, 1107, 950, 807 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.74 (br s, 1H), 9.52 (br s, 1H), 8.76 (d, J = 6.4 Hz, 2H), 7.94 (d, J = 6.4 Hz, 2H), 7.78 (d, J = 9.0 Hz, 1H), 6.97 (d, J = 8.5 Hz, 2H), 6.86−6.88 (m, 2H), 6.76 (d, J = 8.5 Hz, 2H), 4.58 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.49, 163.15, 161.71, 158.42, 158.17, 157.23, 144.66, 132.61, 128.07, 126.94, 123.07, 122.47, 116.14, 115.91, 115.89, 102.83, 34.15; HRMS calculated for $C_{21}H_{15}NNaO_4S$ $(M + Na)^+$ 400.0619, found 400.0627. Anal. ($C_{21}H_{15}NO_4S$) C, H, N.

7-Hydroxy-3-(4-methoxyphenyl)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (4g). To a stirred suspension of 3-(4-methoxyphenyl)-7-(phenylmethoxy)-2-[(4′-pyridylmethyl)thio]-4*H*-1-benzopyran-4-one (0.200 g, 0.415 mmol) in Me_2S (3.0 mL) and CH_2Cl_2 (3.0 mL) was slowly added $BF_3 \cdot OEt_2$ (1.52 mL, 12 mmol) at room temperature. The resulting yellow solution was vigorously stirred at room-temperature overnight. After cooling to 0 °C, the reaction mixture was quenched with water and concentrated under reduced pressure. The residue was suspended in a mixture of water and EtOAc, and the insoluble product was collected by filtration. The filtrate was extracted with EtOAc twice (2 × 25 mL), and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give additional product. The combined solid was purified by silica

gel column chromatography (eluting with MeOH/$CHCl_3$) and then recrystallized from ethanol to give a white solid (0.136 g, 84%): mp 197−200 °C; IR (KBr) 3400, 1623, 1606, 1561, 1512, 1455, 1363, 1293, 1247, 1219, 1180, 1106, 1076, 1023, 1003, 968, 856, 822 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.76 (br s, 1H), 8.79 (d, J = 6.4 Hz, 2H), 8.01 (d, J = 6.5 Hz, 2H), 7.80 (d, J = 8.5 Hz, 1H), 7.11 (d, J = 8.7 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 6.86−6.89 (m, 2H), 4.56 (s, 2H), 3.73 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.45, 163.22, 161.74, 159.96, 158.48, 158.09, 144.07, 132.68, 128.10, 127.18, 124.80, 122.28, 116.13, 115.98, 114.57, 102.87, 56.00, 34.12; HRMS calculated for $C_{22}H_{18}NO_4S$ $(M + H)^+$ 392.0956, found 392.0962. Anal. ($C_{22}H_{17}NO_4S$) C, H, N.

Preparation of Human Placental Microsomes. Human term placentas were processed immediately after delivery from The Ohio State University Hospitals at 4 °C. The placenta was washed with normal saline, and connective and vascular tissue was removed. Microsomes were prepared from the remaining tissue using the method described by Kellis and Vickery.[18] Microsomal suspensions were stored at −80 °C until required.

Inhibition Study. Inhibition of human placental aromatase was determined by monitoring the amount of ^3H_2O released as the enzyme converts [1β-^{3}H]androst-4-ene-3,17-dione to estrone. Ten inhibitor concentrations ranging from 100 nM to 50 μM were evaluated. Aromatase activity assays were carried in 0.1 M potassium phosphate buffer (pH 7.0) with 5% propylene glycol. All samples contained a NADPH regenerating system consisting of 2.85 mM glucose-6-phosphate, 1.8 mM $NADP^+$ and 1.5 units of glucose-6-phosphate dehydrogenase (Sigma, St. Louis, MO). Samples contained 100 nM androst-4-ene-3,17-dione (400 000−450 000 dpm). Reactions were initiated with the addition of 50 μg of microsomal protein. The total incubation volume was 2.0 mL. Incubations were allowed to proceed for 15 min in a shaking water bath at 37 °C. Reactions were quenched by the addition of 2.0 mL of chloroform. Samples were then vortexed and centrifuged for 5 min and the aqueous layer was removed. The aqueous layer was subsequently extracted twice in the same manner with 2.0 mL chloroform. A 0.5 mL aliquot of the final aqueous layer was combined with 5 mL 3a70B scintillation cocktail (Research Products International Corp., Mt. Prospect, IL) and the amount of radioactivity determined. Each sample was run in triplicate, and background values were determined with microsomal protein inactivated by boiling. Samples containing 50 μM (±) aminoglutethimide (Sigma, St. Louis, MO) were used a positive control. IC_{50} sigmoidal dose−response data were analyzed with the Graphpad Prism (Version 3.0) program.

Kinetic Study. Enzyme kinetic studies of compounds **3e**, **3h-j**, **4f**, and **4g** were conducted to investigate the nature of aromatase inhibition. Michaelis−Menten enzyme kinetic parameters were determined by varying the concentration of androst-4-ene-3,17-dione from 10 to 300 nM in the prescence of a fixed concentration of 0, 100, 500, and 2000 nM inhibitor. Assay conditions were the same as those described in the IC_{50} studies except reactions were initiated by the addition of 15 μg microsomal protein. Analysis of the enzyme kinetic data was performed with the weighted linear regression analysis previously described by Cleland.[25]

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Supporting Information Available: Elemental analyses data. This material is available free of charge via the Internet at http://pubs.acs.org.

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