Synthesis and Characterization of 3-Arylquinazolinone and 3-Arylquinazolinethione Derivatives as Selective Estrogen Receptor Beta Modulators

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On the basis of the stucture of genistein, a new series of 3-arylquinazolines was prepared and tested for their estrogen receptor (ER) α and *β* affinities. 5,7-Dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinone (**1aa**) acts as an agonist on both ER subtypes. It has 62-fold higher binding affinity [IC_{50}(ER*β*) = 179 nM] and 38-fold higher functional potency in a transcription assay [EC_{50}(ER*β*) = 76 nM] with ER*β* than with ERα, thus improving upon the selectivity of genistein. All of the analogues showed preferential binding affinity for ER*β*. Many are also more potent in activating transcription by ER*β* than by ERα. Transformation of the C═O functionality at position 4 into a C═S group provided 5,7-dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinethione (**1ba**), which acts as an agonist on both ER subtypes but has 56-fold higher binding affinity for ER*β* over ERα [IC_{50}(ER*β*) = 47 nM] and 215-fold higher potency in the transcription assay [EC_{50}(ER*β*) = 13 nM]. These ER*β*-selective compounds may represent valuable tools in understanding the differences in structure and biological function of ER*β* and ERα.

Introduction

Estrogens are a family of naturally occurring steroid hormones that exert most of their biological effects via interaction with the estrogen receptor (ER), a member of the nuclear hormone receptor superfamily.[1] For the past decade, the physiological effects of estrogens were attributed to a single receptor of the ligand-activated transcription factor family, now known as ERα. The discovery of a second estrogen receptor, ER*β*, in 1996[2,3] resulted in a large research effort to elucidate differences in function and to evaluate the pharmacological potential of selective ligands.[4−6]

ER*β* and ERα share about a 60% identity in the ligand-binding domain (LBD) and only 20% homology in their amino-terminal transactivation domain.[2,3,7] However, the crystal structures of ER*β*[8] and ERα[9] LBDs in the presence of a ligand show little variation in the direct vicinity of the ligand, explaining the high affinity of 17*β*-estradiol to both ER*β* and ERα and emphasizing why attempts to find selective ligands have met with limited success. Several steroidal and nonsteroidal estrogen ligands[10−12] were studied and differences both in potency and response in cell based transcriptional assays were observed, suggesting that it may be possible to develop subtype-selective estrogen receptor modulators. Although functional selectivity of greater than 100-fold for ERα has been observed with certain ligands,[13a,b] potent and selective ligands for ER*β* have remained elusive until recently and the isoflavanoid genistein (Figure 1) remained for a long time the only agonist with modest ER*β* selectivity reported in the literature.[5,14]

A few years ago, arylbenzothiophenes[15a] and 1,3,5-triazine-based compounds[15b] were described as having some selectivity for ER*β*. More recently, several papers describing compounds with >100-fold selectivity for ER*β* relative to ERα were published.[16a−d] Here we report our findings for a series of 3-arylquinazolines (Figure 1, compounds **1a** and **1b**).[17]

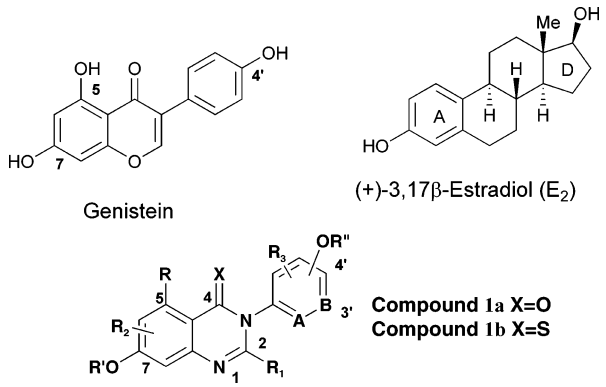

Figure 1.

Chemistry

Compounds of general formula **1a** and **1b** were prepared by different methods depending on the nature and the positions of the substituents. Compounds **1aa**−**al** ($R_1 = R_2 = H$) described in Table 1, were prepared via the general synthetic pathway depicted in Scheme 1.

The commercially available 3,5-dimethoxyaniline **2** was transformed to 4,6-dimethoxyisatin **3** and then to the corresponding 4,6-dimethoxyanthranilic acid **4** using literature procedures.[18] The reaction of triphosgene gave the key intermediate 5,7-dimethoxy isatoic anhydride **5**. The desired anilines **6a**−**i** were then reacted with compound **5** at elevated temperature to yield benzamides **7a**−**i**. Reaction of **7a**−**i** with triethyl orthoformate afforded 3-aryl-5,7-dimethoxyquinazolines **8aa**−**ak.** Cleavage of the methoxy groups with either an excess of BBr_3, NaSEt, or LiCl gave the target compounds **1aa**−**al**.

Compounds where R_2 is not H (**1am**−**ar**; Table 1) were obtained by the sequence described in Scheme 2. Compound **1aa** was iodinated to yield **1am** and **1an**. Iodination of **8aa**, followed by coupling with $MeB(OH)_2$ in the presence of Pd-

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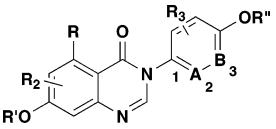
Table 1. Structure and Physical−Chemical Characteristic of Compounds **1aa**−**au** (R_1 = H)[a]

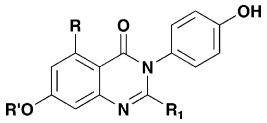


compd no.	R_2	R_3	R	R′	R″	preparation method	formula[c]
1aa	H	H	OH	H	H	Scheme 1	$C_{14}H_{10}N_2O_4$
1ab	H	H	OH	CH_3	H	Scheme 1	$C_{15}H_{12}N_2O_4$
1ac	H	2-CH_3	OH	H	H	Scheme 1	$C_{15}H_{12}N_2O_4$
1ad	H	3-Cl	OH	H	H	Scheme 1	$C_{14}H_9ClN_2O_4$
1ae	H	3-F	OH	H	H	Scheme 1	$C_{14}H_9FN_2O_4$
1af	H	H	OCH_3	H	H	Scheme 1	$C_{15}H_{12}N_2O_4$
1ag	H	2-F	OH	H	H	Scheme 1	$C_{14}H_9FN_2O_4$
1ah	H	2-Cl	OH	H	H	Scheme 1	$C_{14}H_9ClN_2O_4$
1ai	H	3-CH_3	OH	H	H	Scheme 1	$C_{15}H_{12}N_2O_4$
1aj	H	H	OH	CH_3	CH_3	Scheme 1	$C_{16}H_{14}N_2O_4$
1ak[a]	H	H	OH	H	H	Scheme 1	$C_{13}H_9N_3O_4$
1al[a]	H	H	OH	H	H	Scheme 1	$C_{13}H_9N_3O_4$
1am	8-I	H	OH	H	H	Scheme 2	$C_{16}H_{14}N_2O_4$
1an	6,8-di-I	H	OH	H	H	Scheme 2	$C_{14}H_8I_2N_2O_4$
1ao	8-CH_3	H	OH	H	H	Scheme 2	$C_{15}H_{12}N_2O_4$
1ap	6-I	H	OH	H	H	Scheme 2	$C_{14}H_9IN_2O_4$
1aq	6-nPr	H	OH	H	H	Scheme 2	$C_{17}H_{16}N_2O_4$
1ar	6-allyl	H	OH	CH_3	CH_3	Scheme 2	$C_{19}H_{18}N_2O_4$
1as[b]	H	H	H	H	H	Scheme 1	$C_{14}H_{10}N_2O_3$
1at[d]	H	H	OH	H	H[d]	Scheme 1	$C_{14}H_{10}N_2O_4$
1au[e]	H	H	OH	H	e	Scheme 1	$C_{14}H_{10}N_2O_3$

[a] For all compounds A = B = CH, except for **1ak**, where A = N, B = CH, and **1al**, where A = CH, B = N. [b] Compound obtained by the same sequence as described in Scheme 1 starting with the commercial *p*-Me aniline. [c] The purities of the final compounds were assessed by HPLC and were ≥97%, except for **1aq** (93%), **1as** (96%), **1ay** (95%), and **1az** (96%). The formulas were assessed by HRMS and were within ±2 ppm. The NMR spectra are described either in the Experimental Section or in the Supporting Information. [d] The OR″ substituent is in the para position, except for **1at**, where the substitution is on the meta position. [e] Compound **1au** does not contain any OR″ group.

$(dppf)Cl_2$, afforded **8am**. Cleavage of the methoxy groups according to the procedure described above gave **1ao**. The 5-methoxy group of compound **8aa** (A = B=CH, R_3 = H) was selectively cleaved by LiCl. The resulting phenol (**1aj**) was alkylated with allyl bromide to yield **8ao**. Compound **1ar** was obtained by Claisen rearrangement, and **1aq** was obtained by hydrogenation of **1ar** followed by cleavage of the methoxy groups.

Table 2. Structure and Physical−Chemical Characteristic of Compounds **1av**−**aag** (R_1 ≠ H)



compd no.	R_1	R	R′	preparation method	formula[a]
1av	CH_3	OH	H	Scheme 3	$C_{15}H_{12}N_2O_4$
1aw	C_2H_5	OH	H	Scheme 3	$C_{16}H_{14}N_2O_4$
1ax	nPr	OH	H	Scheme 3	$C_{17}H_{16}N_2O_4$
1ay	nBu	OH	H	Scheme 3	$C_{18}H_{18}N_2O_4$
1az	iPr	OH	H	Scheme 3	$C_{17}H_{16}N_2O_4$
1aaa	iBu	OH	H	Scheme 3	$C_{18}H_{18}N_2O_4$
1aab	SH	OH	CH_3	Scheme 4	$C_{15}H_{12}N_2O_4S$
1aac	SH	OH	H	Scheme 4	$C_{14}H_{10}N_2O_4S$
1aad	Cl	OH	H	Scheme 4	$C_{14}H_9ClN_2O_4$
1aae	OH	OH	H	Scheme 4	$C_{14}H_{10}N_2O_5$
1aaf	CH_3	H	H	Scheme 3	$C_{15}H_{12}N_2O_3$
1aag	4-OHPh	H	H	Scheme 3	$C_{20}H_{14}N_2O_4$

[a] The purities of the final compounds were assessed by HPLC and were ≥97%, except for **1ay** and **1aae** (95%), **1aac** (90%), and **1az** (96%). The formulas were assessed by HRMS and were within ±2 ppm. The NMR spectra are described either in the Experimental Section or in the Supporting Information.

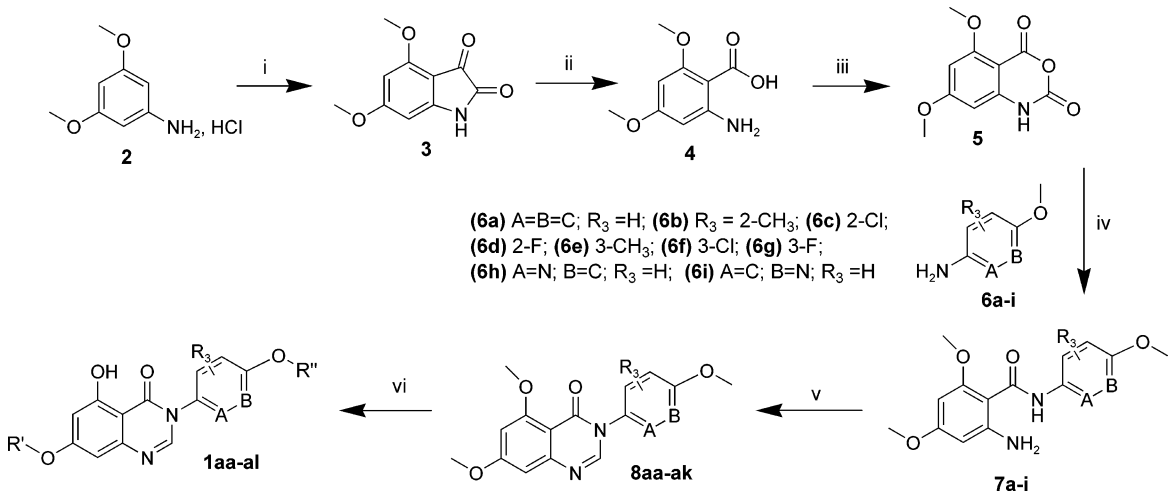
Compounds **1as**−**1au** were similarly prepared in order to complete the SAR (see footnotes of Table 1).

Compounds **1av**−**aaa** (Table 2, R_1 ≠ H) were prepared by the alternative general method depicted in Scheme 3. The 4,6-dimethoxyanthranilic acid **4** was transformed to the key intermediates 5,7-dimethoxy-3,1-benzoxazin-4-ones **9b**−**f** by reaction with the desired alkanoylanhydride (R_1 ≠ H) or alkanoyl chloride. (Compound **9a** was prepared by the reaction of **4** with triethylorthoformate). Further reaction with 4-methoxyaniline **6a** gave the corresponding derivatives **10aa**−**af**. The cleavage of the methoxy groups by BBr_3 or NaSEt yielded the desired derivatives **1av**−**aaa** (Table 2).

Compounds **1aab**−**aae** of Table 2 were prepared according to Scheme 4.

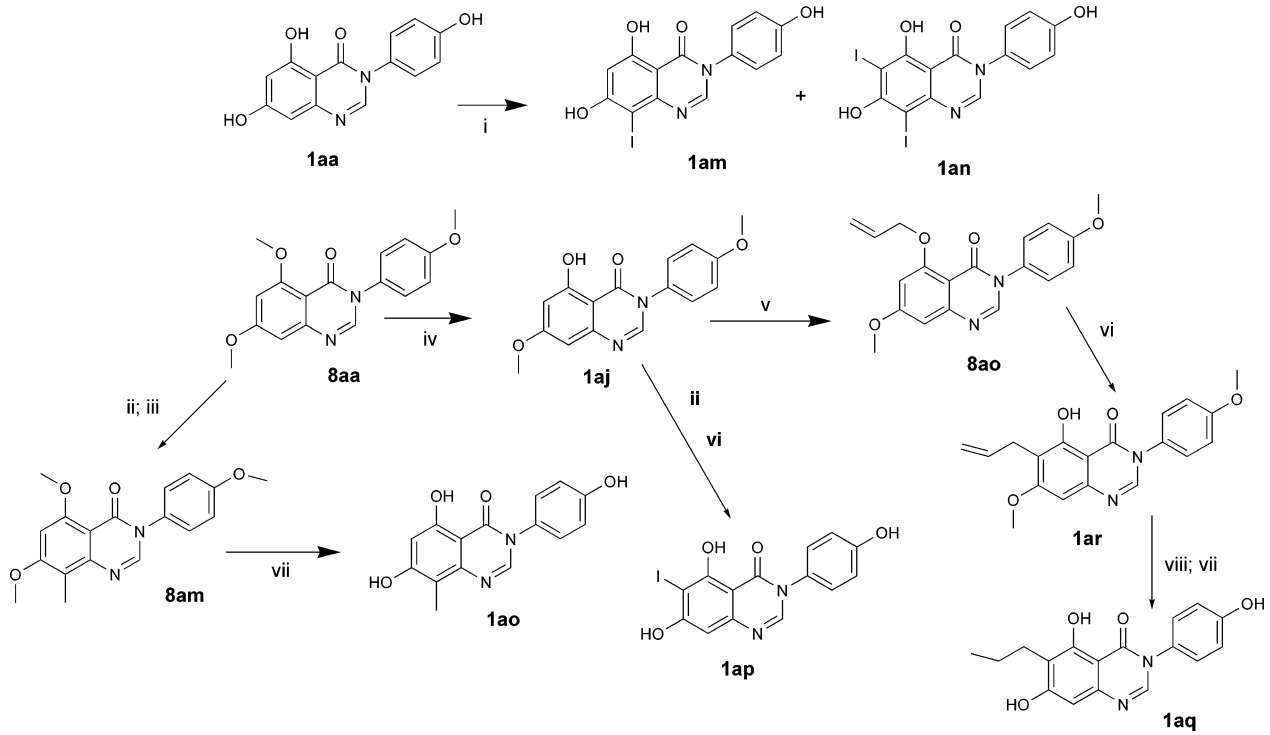
Compounds **4** and **11** were reacted respectively with 1-isocyanato-4-methoxybenzene **12a** (X = O) and 1-isothiocyanato-4-methoxybenzene **12b** (X = S) to yield compounds **10ak** and **10ag**. The corresponding derivatives **1aae** and **1aac** were obtained after ether cleavage as described earlier. Alkylation

Scheme 1[a]



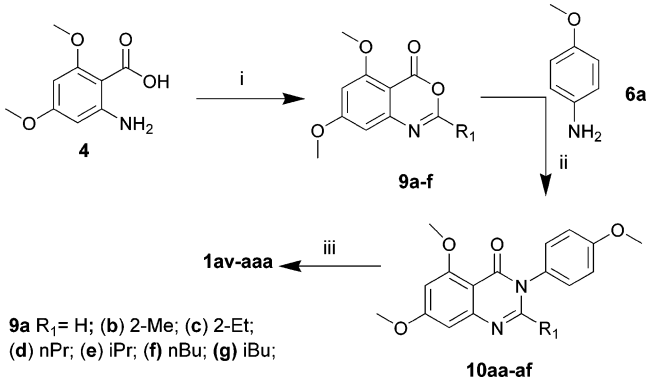
[a] (i) oxalyl chloride, 165 °C 30 min; (ii) 33% NaOH/30% H_2O_2; (iii) triphosgene, 0 °C; (iv) *N*,*N*-(dimethylamino)pyridine, anhydrous dimethylacetamide, 110 °C, 24 h; (v) anhydrous triethylorthoformate, reflux 4 h; (vi) BBr_3, CH_2Cl_2, or EtSNa, DMF, reflux, or LiCl, DMF, 135 °C.

Scheme 2[a]



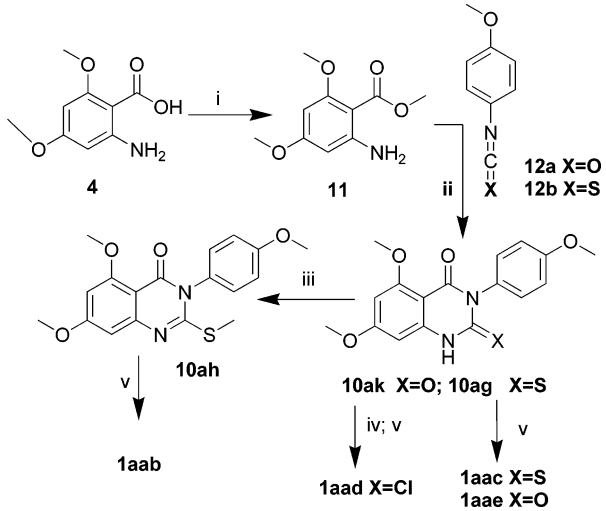
[a] (i) I_2/H_5IO_6, EtOH, rt; (ii) I_2/H_5IO_6, EtOH, 50 °C; (iii) $MeB(OH)_2$, $[PdCl_2(dppf)]CH_2Cl_2$, K_3PO_4, THF, reflux; (iv) LiCl; DMA, 100−120 °C; (v) NaH, allyl bromide, DMF, 0° to rt; (vi) diphenyl ether, 150 °C; (vii) CH_2Cl_2, BBr_3, rt, or EtSNa, DMF, reflux; (viii) H_2, 5% Pd/C, MeOH.

Scheme 3[a]



[a] (i) Triethylorthoformate, 140 °C, 4 h; or alkanoyl chloride or anhydride, TEA, CH_2Cl_2, 40 °C; (ii) xylene/reflux 4 h, or AcOH, 60 °C, 24 h; (iii) CH_2Cl_2, BBr_3, rt, or EtSNa, DMF, reflux.

Scheme 4[a]



[a] (i) Trimethylsilyldiazomethane, THF, MeOH, rt; (ii) toluene, reflux, 15 h; (iii) 1 N NaOH, MeOH; CH_3I; rt, 3 days; (iv) $POCl_3$; (v) CH_2Cl_2, BBr_3 or EtSNa, DMF, reflux.

of the 2-mercapto derivative **10ag** by methyl iodide followed by cleavage of the methoxy groups, gave compound **1aab.** Compound **1aad** was obtained from **10ak** by $POCl_3$ reaction and ether cleavage. Compounds **1aaf** and **1aag** were prepared similarly in order to complete the SAR.

Compounds of formula **1aah** to **1aas** (with the exception of **1aah** and **1aak**) presented in Table 3 were prepared according to Scheme 5. The 5-methoxy group of compound **8aa** was selectively cleaved as previously described (see Scheme 2). The free 5-OH group was triflated with the *N*-phenyltrifluoromethanesulfonimide according to the literature.[19a, b]

Palladium-based coupling chemistry then yielded compounds **8aq**−**ay.** Ether cleavage reaction, according to one of the previously described methods, gave compounds **1aai**−**aas.**

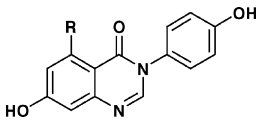
Compounds **1aah** and **1aak** were obtained according to the reaction sequences described in Scheme 6.

Compound **1aah** was obtained by the alkylation of **1aj** followed by the cleavage of the 7- and 4′-methoxy groups using EtSNa. **1aak** was obtained in three steps from **1aa** by benzylation of the 7- and 4′-hydroxy groups and methylation of the 5-hydroxy, followed by hydrogenolysis of the benzyl groups.

Compounds of formula **1ba**−**bj** (X = S) represented in Table 4 were prepared according to the classical thionylation procedure using P_2S_5 or Lawesson's reagent (Scheme 7).

Cleavage of the methoxy groups under the BBr_3 or NaSEt reaction gave the corresponding derivatives **1ba**−**bj**. To complete the SAR, commercially available biochanin A was also thionylated and the 4′-methoxy group cleaved in order to obtain the "thiogenistein" analogue **13**.

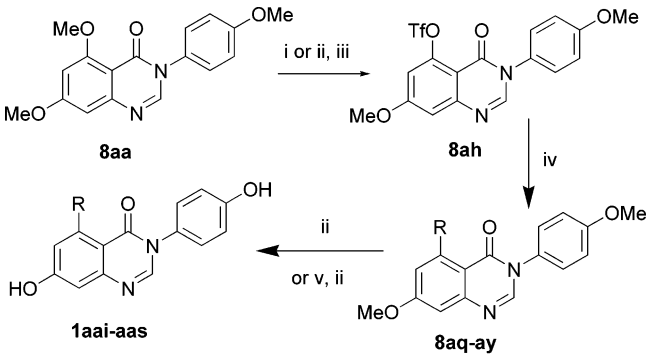
Table 3. Structure and Physical−Chemical Characteristic of Compounds **1aah**−**aas** (R_1 = H)



compd	R	preparation method	formula[a]
1aah[b]	OCH_2CH_3	Scheme 6	$C_{16}H_{14}N_2O_4$
1aai	NH_2	Scheme 5	$C_{14}H_{11}N_3O_3$
1aaj	CH_3	Scheme 5	$C_{15}H_{12}N_2O_3$
1aak	OCH_3	Scheme 6	$C_{15}H_{12}N_2O_4$
1aal	CN	Scheme 5	$C_{15}H_9N_3O_3$
1aam	OTf	Scheme 5	$C_{15}H_9F_3N_2O_6S$
1aan	CH_2CH_3	Scheme 5	$C_{16}H_{14}N_2O_3$
1aao		Scheme 5	$C_{16}H_{12}N_2O_3$
1aap	CO_2CH_3	Scheme 5	$C_{16}H_{12}N_2O_5$
1aaq	Ph	Scheme 5	$C_{20}H_{14}N_2O_3$
1aar	CH_2OH	Scheme 5	$C_{15}H_{12}N_2O_4$
1aas		Scheme 5	$C_{17}H_{14}N_2O_3$

[a] The purities of the final compounds were assessed by HPLC and were ≥97%, except for **1aao** (95%). The formulas were assessed by HRMS and were within ±2 ppm. The NMR spectra are described either in the Experimental Section or in the Supporting Information. [b] Compound **1aah** (see Experimental Section) was obtained by alkylation of **1aj** followed by cleavage of the methoy groups by EtSNa.

Scheme 5[a]



8ap, R=H; **8aq**, R=NH_2; **8ar**, R=CH_3; **8as**, R=CN; **8at**, R=Et; **8au**, R=vinyl; **8av**, R=CO_2CH_3; **8aw**, R= Ph; **8ax**, R= CH_2OH; **8ay**, R=isopropenyl

[a] (i) LiCl, DMF, 100−120 °C; (ii) BBr_3, CH_2Cl_2; (iii) NaH, DMF, *N*-phenyltrifluoromethanesulfonimide; (iv) $Pd(OAc)_2$ BINAP, Cs_2CO_3; (vi) CH_2Cl_2, BBr_3 or EtSNa, DMF, reflux.

Biological Evaluation

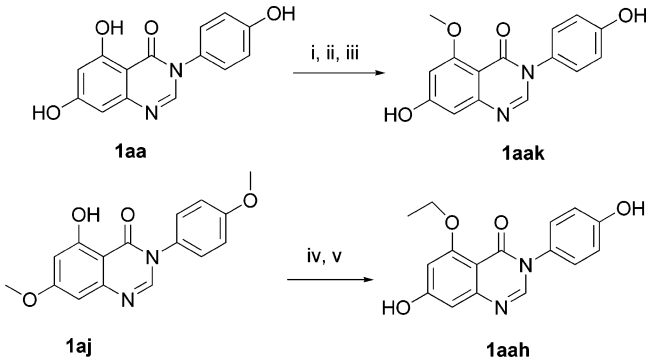
To determine the affinity of compounds and selectivity for ERα versus ER*β*, an in vitro ligand binding assay with [^{3}H]-estrone as radioligand and biotinylated ligand binding domains of ER*β* and ERα were used in a scintillation proximity assay (SPA). For the functional transcription activation assay, HeLa cells were stably transfected with a construct expressing full length human ER*β* or ERα receptor cotransfected with a luciferase reporter gene construct driven by an ERE-TK minimal promoter. The potency of the compounds in the transactivation assay was measured by their ability to activate the estrogen response element (ERE) in a luciferase reporter gene assay.

Molecular Modeling

Molecular modeling was performed using InsightII (Accelrys, Inc., San Diego, CA) for graphical docking, Discover with the CFF98 force field for conformational analysis (Accelrys), ICM (MolSoft, Inc., San Diego, CA) for automated docking, and LOOK (Molecular Applications Group) for homology modeling. An ER*β* homology model was built from the then-known α isozyme complexed with 17-*β*-estradiol.[9]

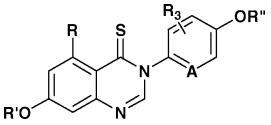
We developed our binding model for the quinazolines by first overlaying the phenol ring of genistein with the A ring of estradiol, found in the ERα crystal structure, and then transfer-

Scheme 6[a]



[a] (i) Anhydrous DMA, NaH, 0 °C to rt; (ii) 0 °C, benzyl bromide 1 h then CH_3I, 1 h at 0 °C, and 1 h at rt; (iii) Pd/C, H_2, EtOAc/EtOH (1:1), 4 h; (iv) DMF, K_2CO_3, C_2H_5I, 120 °C, 3 h; (v) DMF, EtSNa.

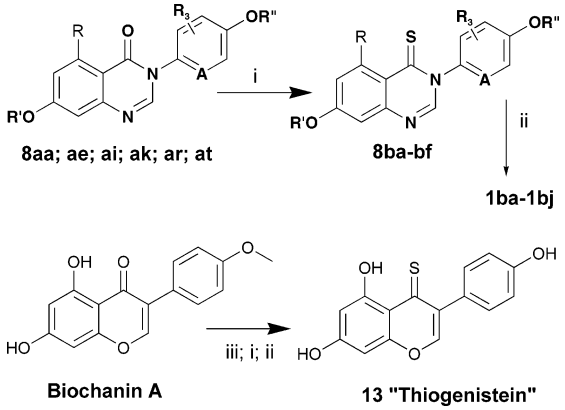
Table 4. Structure and Physical−Chemical Characteristic of Compounds **1ba**−**bj** (X = S) Prepared According to Scheme 7[a]



compd no.	R_3	R	R′	R″	formula[b]
1ba	H	OH	H	H	$C_{14}H_{10}N_2O_3S$
1bb	3-F	OH	H	H	$C_{14}H_9FN_2O_3S$
1bc	3-CH_3	OH	H	H	$C_{15}H_{12}N_2O_3S$
1bd	H	CH_3	H	H	$C_{15}H_{12}N_2O_2S$
1be	H	CH_2CH_3	H	H	$C_{16}H_{14}N_2O_2S$
1bf[a]	H	OH	H	H	$C_{13}H_9N_3O_3S$
1bg	H	$OCOCH_3$	$COCH_3$	$COCH_3$	$C_{20}H_{16}N_2O_6S$
1bh	H	OSO_2CF_3	CH_3	CH_3	$C_{17}H_{13}F_3N_2O_5S_2$
1bi	H	OH	CH_3	CH_3	$C_{16}H_{14}N_2O_3S$
13[c]					

[a] A is CH for all compounds except for **1bf**, where A = N. [b] The purities of the final compounds were assessed by HPLC and were ≥97%, except for **1bf**, **1bh** (93%), and **1bi** (95%). The formulas were assessed by HRMS and were within ±2 ppm. The NMR spectra are described either in the Experimental Section or in the Supporting Information. [c] "Thiogenistein".

Scheme 7[a]



[a] (i) P_2S_5 or Lawesson's reagent, xylene, reflux; (ii) BBr_3, CH_2Cl_2; EtSNa, DMF, reflux or Py/HCl, heat; (iii) Ac_2O, DMAP, Py, rt, 5 h.

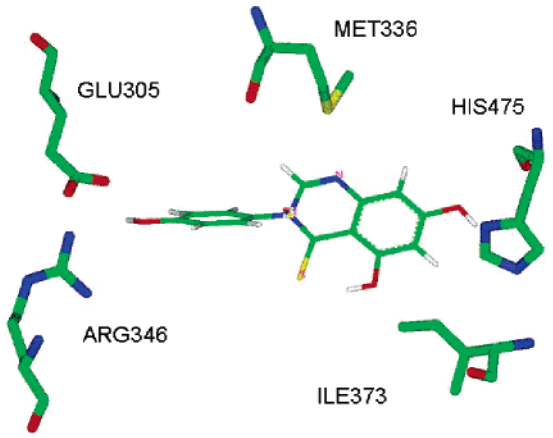


Figure 2. **1ba** docked to the ERβ pocket (ligand and residues colored by atom type). Only the key residues of the ERβ binding site are shown for simplicity (starting from the upper left corner and in a counterclockwise manner: Glu305, Arg346, Ile373, His475, and Met336).

ring the information to our ERβ homology model. Subsequent analysis of the 3-arylquinazoline series proceeded through an overlay onto genistein, and the result is shown in Figure 2. It should be noted that our original genistein docked orientation was subsequently confirmed by crystallographic analysis[8]

Our binding model (Figure 2) indicated that the quinazolines are of an ideal length for forming tight hydrogen bonds between the 4′-hydroxyl and Glu305 and Arg346 at one end of the pocket and between the 7-hydroxyl and His475 at the other end. Conformational analysis (Discover/CFF98) of genistein and the quinazoline series suggest that the ring systems are at lowest energy in an off-planar conformation, with planarity being limited likely by steric repulsion between the ortho substituents. Our model suggests that positions C2 and C5 of the quinazoline are oriented in regions where ERα and ERβ differ in the ligand binding domain. Specifically, Leu384 and Met421 in ERα are Met336 and Ile373 in ERβ, respectively. Modifications of substituents at positions C2 and C5 of the quinazoline may offer the opportunity to improve ERβ/ERα selectivity.

Result and Discussion

On the basis of the structure of genistein, a series of 3-arylquinazolinones was prepared. The structure−activity of compounds **1aa** to **1aas** is shown in Table 5. Quinazolinone **1aa**, the direct analogue of genistein, exhibited a 3-fold loss in ERβ binding [IC_{50}(ERβ) = 179 nM] while maintaining similar activity in ERβ transactivation potency [EC_{50}(ERβ) = 76 nM]. The ERβ versus ERα binding and transactivation selectivity was improved significantly (62-fold in the binding and 215-fold in the transactivation assay).

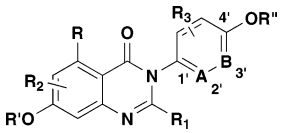
It is known that the hydrogen-bonding network between the A-ring phenol and Glu305/Arg346 and the 17β-hydroxyl and His 475 contributes significantly to the binding of 17β-estradiol. As a result, we investigated the role that the 4′- and 7-hydroxyl groups had on binding affinity in the quinazolinones. Any changes to the hydrogen-bonding network either by removing the 4′-hydroxy (**1au**), shifting the hydroxyl to the 3′-position (**1at**), or protecting the hydroxyl as a methyl ether (**1ab**, **1aj**) led to significant loss in ERβ binding. Next we explored the role of sterics and electronics in the hydrogen bonding of the 4′- and 7-hydroxyl groups. Introduction of a fluoro group (**1ae**) at the 3′-position resulted in a 3-fold loss in ERβ binding compared to **1aa**, but ERβ versus ERα functional selectivity was maintained. Larger groups (**1ad**, **1ai**) at the 3′-position led to a 10-fold loss in ERβ activity, presumably due to a steric effect. A similar steric effect was observed when substituents were introduced at positions 6 and 8 (**1am**−**1ar**). Both electron-withdrawing (**1ag**, **1ah**) and electron-donating groups (**1ac**) at the 2′-position were well-tolerated. Replacing either the 2′-carbon or 3′-carbon with a nitrogen (**1ak**, **1al**) resulted in a loss of activity.

As mentioned in the molecular modeling section, the C2 and C5 positions of the quinazolinone are oriented in the regions were the ERα and ERβ differ in the binding pocket. Modifications of substituents at positions C2 and C5 of the quinazoline may offer the opportunity to improve ERβ/ERα selectivity. We initially focused on the C2-position of the quinazolinone. Introduction of a number of alkyl groups, as well as heteroatoms, at C2 (**1av**−**1aag**) led to a significant loss in activity. It was hypothesized that these substituents at C2 were altering the preferred dihedral angle of the distal phenyl moiety and thus affecting the binding affinity. Nevertheless, it is interesting to note that the binding affinity improved as the length of the alkyl chain increased in size (compare **1ax** with **1ay**). However, this trend was not observed in the transactivation assay.

We turned out attention to the C5-position of the quinazolinone. The 5-hydroxyl presumably participates in an intramolecular hydrogen-bonding interaction with the 4-keto group. Removal of the C5-hydroxyl group (**1as**) resulted in a 48-fold loss in ERβ binding and a 12-fold loss in ERβ transactivation. To evaluate the importance of this 5-hydroxyl, several analogues were prepared (**1aah**−**1aas**). Homologation of the C5-hydroxyl (**1aar**) led to a 7-fold improvement in ERβ binding (IC_{50} = 24 nM), but the selectivity dropped and the transactivation potency remained weak. It is interesting to note that the hydroxymethyl substitution (**1aar**) resulted in a dramatic improvement in efficacy against ERα, possibly aided by hydrogen bonding between the hydroxyl and Met421 sulfur. When the hydroxyl was replaced with an NH_2 (**1aai**), the binding and transactivation potency as well as selectivity dropped considerably. Further loss of activity was observed in the case of methoxy (**1aak**), nitrile (**1aal**), and a bulky group such as phenyl (**1aaq**). Only small alkyl groups such as methyl [**1aaj**, IC_{50}(ERβ) = 317 nM, α/β selectivity = 30] and ethyl [**1aan**, IC_{50}(ERβ) = 265 nM, α/β selectivity = 50] maintained good binding and transactivation potency and selectivity. On the basis of modeling studies, these small groups could make tight contact with the wall of the pocket. Larger substituents have steric conflicts with residues Ile376 and Leu380. Overall, the described structural modifications did not optimize the effect of the lead structure **1aa**.

To further assess the role of the carbonyl function, we prepared the thiocarbonyl analogue of **1aa**. The resulting compound **1ba** (Table 6) had 4-fold improved binding potency with similar selectivity compared to the parent compound **1aa**. Moreover, **1ba** showed a 6-fold improvement in transactivation potency and the best transactivation selectivity observed for the quinazolinone series [transactivation EC_{50}(ERβ) = 13 nM, selectivity α/β ratio = 215). It is postulated that this improvement in binding affinity is due to the increased van der Waals contact between the protein and the sulfur. The most potent and selective compounds from the previous SAR studies were thionylated, and their biological activities are represented in Table 6, together with reference derivatives such as 17β-estradiol, estrone, and genistein. In general, the thiocarbonyl provided an increase in binding affinity that was observed on both receptor subtypes; therefore, no significant improvements of binding selectivity were observed. On the other hand, the transactivation potency increased for every thionylated compound, often with improved selectivity. To our knowledge this

Table 5. Structure and in Vitro Binding of Compounds **1aa**−**aas**[a]



							^{3}H-estrone IC_{50} (nM)[c]			transactivation EC_{50} (nM)[d]		
compd no.	R_1	R_2	R_3	R	R′	R″[b]	ERβ	ERα	select α/β	ERβ	ERα	select α/β
1aa	H	H	H	OH	H	H	179	11097	62	76 ± 8	2918	38
1ab	H	H	H	OH	CH_3	H	9525	25956	3	47%	20%	
1ac	H	H	2′-CH_3	OH	H	H	422	4382	10	**111**	5305	48
1ad	H	H	3′-Cl	OH	H	H	7211	55015	8	**1181 ± 220**	5137	4
1ae	H	H	3′-F	OH	H	H	650	44175	68	**117 ± 26**	3112	27
1af	H	H	H	OH	CH_3	H	39%	10%		8506	36%	
1ag	H	H	2′-F	OH	H	H	256	7485	29	190	1143	6
1ah	H	H	2′-Cl	OH	H	H	263	6247	24	**251 ± 119**	1427	6
1ai	H	H	3′-CH_3	OH	H	H	7487	34626	5	**1254**	4576	4
1aj	H	H	H	OH	CH_3	CH_3	2%	0%		14%	35%	
1ak[a]	H	H	H	OH	H	H	3531	97982	28	**358**	3143	9
1al[a]	H	H	H	OH	H	H	51696	66860	1	**9763**	26%	
1am	H	8-I	H	OH	H	H	4081	40%		**593**	8317	14
1an	H	6,8-di-I	H	OH	H	H	12298	28%		1966	19%	
1ao	H	8-CH_3	H	OH	H	H	1275	15297	12	8870	9344	1
1ap	H	6-I	H	OH	H	H	3970	29%		1638	27%	
1aq	H	6-nPr	H	OH	H	H	7305	10649	1.5	23%	12%	
1ar	H	6-allyl	H	OH	CH_3	CH_3	NT[e]	NT		NT	NT	
1as[b]	H	H	H	H	H	H	8609	53640	6	**961**	39%	
1at[b]	H	H	H	OH	H	H	3842	43814	11	**904**	10082	11
1au[b]	H	H	H	H	H	H	18472	75588	4	**1721**	17%	
1av	CH_3	H	H	OH	H	H	11371	33248	3	**1583**	33%	
1aw	C_2H_5	H	H	OH	H	H	5752	19437	3	**1261**	9317	7.4
1ax	nPr	H	H	OH	H	H	3224	5365	2	25%	43%	
1ay	nBu	H	H	OH	H	H	249	178	0.7	7%	17%	
1az	iPr	H	H	OH	H	H	48086	70399	1.5	24%	10%	
1aaa	iBu	H	H	OH	H	H	NT[d]	NT		NT	NT	
1aab	SH	H	H	OH	CH_3	H	72848	46%		3843	39%	
1aac	SH	H	H	OH	H	H	6994	11111	2	1985	983	0.5
1aad	Cl	H	H	OH	H	H	13426	29%		8776	18%	
1aae	OH	H	H	OH	H	H	9379	70277	8	**1313**	9268	7
1aaf	CH_3	H	H	H	H	H	46%	25%		26%	14%	
1aag	4-OHPh	H	H	H	H	H	440553	48473	0.1	21%	15%	
1aah	H	H	H	OCH_2CH_3	H	H	44265	49%		25%	12%	
1aai	H	H	H	NH_2	H	H	8242	15512	2	**2064**	32%	
1aaj	H	H	H	CH_3	H	H	317	9635	30	**136**	2392	18
1aak	H	H	H	OCH_3	H	H	39%	10%		8506	36%	
1aal	H	H	H	CN	H	H	73677	13%		7579 ± 511	29%	
1aam	H	H	H	OSO_2CF_3	H	H	11979	39%		3804	42%	
1aan	H	H	H	CH_2CH_3	H	H	265	13240	50	**62**	4521	73
1aao	H	H	H		H	H	17004	38%		750	4624	6
1aap	H	H	H	CO_2CH_3	H	H	9536	81324	9	9406	6%	
1aaq	H	H	H	Ph	H	H	30661	101544	3	11685	0%	
1aar	H	H	H	CH_2OH	H	H	24	121	5	4457	16%	
1aas	H	H	H		H	H	2302	43968	19	**729**	4261	6

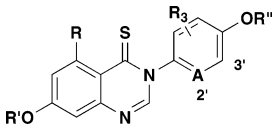
[a] For all compounds A = B = CH, except for **1ak**, where A = N, B = CH, and **1al**, where A = CH, B = N. [b] For all compounds the OR″ substituent is in the para position, except for **1at**, where the substitution is on the meta position, and compound **1au**, which does not contain any OR″ group. [c] For those compounds the binding did not reach 50% inhibition at 100 μM, the percent inhibition at 100 μM is given, unless otherwise indicated. [d] Transactivation values in bold repesent partial agonism, as these compounds "plateaued" at higher concentration and did not reach 100% activity even at 10 μM. For those compounds where the transactivation was low and did not reach a plateau, and the EC_{50} could not be determined, the percent transactivation at 10 μM is given, unless otherwise indicated. The values are an average of two to four experiments with standard deviation, except for few single assays that are the average of duplicate determinations. [e] NT = not tested.

"thio effect" has not been reported in the literature and offers new insight in the SAR of ERβ and ERα modulators.

Conclusion

Several 3-arylquinazolinone analogues of genistein were prepared and their biological activity toward ERα and ERβ receptors evaluated. As expected, all three OH functionalities play an important role, and blocking one of them gave poorer binding (**1ab** or **1af** vs **1aa**). 5,7-Dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinone (**1aa**), an exact analogue of genistein, acting as an agonist on both ER subtypes, exhibited improved ERβ selectivity in binding and transactivation compared to genistein [respectively 62-fold higher binding affinity [IC_{50}(ERβ) = 179 nM] and 38-fold higher potency in the transcription assay [EC_{50}(ERβ) = 76 nM]]. To further investigate the SAR, we prepared a series of analogues in which the substituents in several positions were modified. To varying degrees, all of the analogues showed preferential binding affinity for ERβ.

Table 6. Structure and in Vitro Binding of Compounds **1ba**−**bi** (R_1 = H) and Reference Compounds[a]



compd no.	R_3	R	R′	R″	^{3}H-estrone IC_{50} (nM)[c] ERβ	ERα	select α/β	transactivation EC_{50} (nM)[d] ERβ[b]	ERα	select α/β
1ba	H	OH	H	H	47	2636	56	**13 ± 6**	2793	215
1bb	3′-F	OH	H	H	102	3080	30	**20**	776	39
1bc	3′-CH_3	OH	H	H	658	2854	4	**160**	2406	15
1bd	H	CH_3	H	H	79	1753	22	222	1669	8
1be	H	CH_2CH_3	H	H	156	2260	15	**53**	1462	28
1bf[a]	H	OH	H	H	515	27569	54	173	4859	28
1bg	H	$OCOCH_3$	$COCH_3$	$COCH_3$	26%	11%		11342	18%	
1bh	H	OSO_2CF_3	CH_3	CH_3	26%	18%		23%	21%	
1bi	H	OH	CH_3	CH_3	22%	9%		28%	25%	
(+)-3,17β-estradiol					11 ± 1	10 ± 2	1	0.84 ± 0.22	0.59 ± 0.17	0.7
estrone					31 ± 6	29 ± 7	1	**26 ± 11**	166 ± 13	6
genistein					61 ± 10	1973 ± 444	32	**73 ± 29**	956 ± 8	13
13, "thiogenistein "					1340	55883	42	**14**	1125	80

[a] For all compounds A = CH, except for **1bf**, where A = N. [b] EC_{50} values for compounds in ERβ transactivation given in bold represent partial agonism, as these compounds "plateaued" at higher concentration and did not reach 100% activity even at 10 μM. The values are the average of two to four experiments with standard deviation, except for a few single determinations. [c] For those compounds where the binding did not reach 50% inhibition at 100 μM, the percent inhibition at 100 μM is given, unless otherwise indicated. [d] For those compounds where the transactivation was low and did not reach a plateau and the EC_{50} could not be determined, the percent transactivation at 10 μM is given, unless otherwise indicated.

Many were also more potent in activating transcription through ERβ than through ERα. Substitution of the 5-hydroxyl by an ethyl group (e.g. **1aan**) improved the ERβ selectivity in the transcription assay by 2-fold, despite a 10-fold decrease of selectivity in the binding mode [IC_{50}(ERβ) =265 nM; EC_{50} (ERβ) = 62].

Improvements in the potency and selectivity of these quinazolinone analogues were achieved with the discovery of the "thio effect." Specifically, 5,7-dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinethione (**1ba**) was found to be the most potent and selective derivative both in the binding and transactivation assays [respectively 56-fold higher binding affinity [IC_{50}(ERβ) = 47 nM] and 215-fold higher potency in the transcription assay [EC_{50}(ERβ) = 13 nM]]. This "thio effect," never reported in the literature for ER modulators, was also observed in the thio analogue of genistein itself, compound **13**. These ERβ-selective compounds may represent valuable tools in understanding the differences in structure and biological function of ERβ and ERα receptors.

Experimental Section

Chemistry. All commercial chemicals and solvents are reagent grade and were used without further purification, unless otherwise specified. The following solvents and reagents have been abbreviated: tetrahydrofuran (THF), ethyl ether (Et_2O), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), dichloromethane (DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF), methanol (MeOH), *N*,*N*-(dimethylamino)pyridine (DMAP), triethylamine (TEA), and sodium hexamethyldisilazane (NaHMDS). All reactions except those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. Reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60F-254, E. Merck) and visualized with UV light, iodine vapors, 12-molibdatophosphoric acid hydrate (CAS No. 51429−74−4) or potassium permanganate solution (CAS No. 7722−64−7). Final compounds were typically purified by flash chromatography on silica gel (E. Merck 0.040−0.063 mm) or by preparative reverse-phase high-pressure liquid chromatography. Analytical and preparative HPLC analyses were performed on YMC columns (S-5, 120A ODS, 4.6 × 150 mm; S-10, 120A ODS, 30 × 500 mm) with MeOH:water gradients containing 0.2%H_3PO_4 and 0.1% trifluoroacetic acid, respectively. Solutions were dried with magnesium sulfate unless otherwise noted. Melting points were recorded on a Mel-Temp II capillary apparatus (Laboratory Devices Inc.) and are uncorrected.

Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) spectra were recorded on JEOL EC-400 or EC-500 spectrometers with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. Low-resolution mass spectra (MS) were recorded on a JEOL JMS-AX505HA, JEOL SX-102, or SCIEX-API spectrometer. High-resolution mass spectra were recorded on an AMD-604 (AMD Electra GmbH) high-resolution double-focusing mass spectrometer (Analytical Instrument Group, Raleigh, NC). Mass spectra were acquired in the positive ion mode under electrospray ionization (ESI) or fast atom bombardment (FAB) methods. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA.

General Procedures for the Synthesis of Compounds 1aa−**al, According to Scheme 1. 4,6-Dimethoxyindole-2,3-dione (3).** 3,5-Dimethoxyaniline hydrochloride (**2**) (12 g, 63 mmol) and oxalyl chloride (20 mL, 230 mol) were reacted at 165 °C for 30 min. The excess oxalyl chloride was removed by distillation to give a green-yellow residue. The reaction mixture was cooled and methanol was added. The resulting suspension was heated, filtered, washed with methanol, and dried to yield 13 g of 4,6-dimethoxyindole-2,3-dione **3** (100%): LC/MS (ESI) $(M + H)^+ = 208$; mp 300−304 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.9 (bs, 1H), 6.16 (d, J = 1.76 Hz, 1H), 6.00 (d, J = 1.76 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H).

4,6-Dimethoxyanthranilic Acid (4) To a heated mixture of 4,6-dimethoxyindole-2,3-dione (**3**) (13 g, 63 mmol, in an oversized flask) in 33% NaOH solution was added carefully 20 mL of 30% solution of H_2O_2. A vigorous exothermic reaction occurs. After all the H_2O_2 was added, the reaction mixture was maintained at 100 °C for an additional 10 min. The pH of the solution was brought to 8 with concentrated HCl and acidified to pH 5−6 with acetic acid. The solid was filtered, washed with water, and dried to yield 6.2 g of 4,6-dimethoxyanthranilic acid (**4**) as a pale brown solid (50%): LC/MS (ESI) $(M + H)^+ = 198$; mp 120−125 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.94 (d, J = 1.76 Hz, 1H), 5.79 (d, J = 1.76 Hz, 1H), 3.75 (s, 3H), 3.71 (s, 3H).

1,2-Dihydro-5,7-dimethoxy-3,1-benzoxazine-2,4-dione (4,6-Dimethoxyisatoic Anhydride) (5) To a cooled (0 °C) brown

solution of 4,6-dimethoxyanthranilic acid (**4**) (76.0 g, 0.385 mol) in THF (1.3 L) was added triphosgene (40.0 g, 0.135 mol) in portions over a 20-min period. After 30 min, the reaction was warmed to room temperature and stirred for 1.5 h. The reaction mixture was poured into cold (0 °C) water. Additional water was added to facilitate the stirring of the thick solid formed. After stirring for 30 min, the reaction mixture was filtered to give a beige solid. The solid was washed with water, air-dried, and then dried under high vacuum to give 79.2 g (92%) of the isatoic anhydride **5**: LC/MS (ESI) $(M - H)^- = 221.9$; mp 287−293 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.51 (bs, 1H), 6.36 (d, $J = 1.76$ Hz, 1H), 6.20 (d, $J = 1.76$ Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H).

5,7-Dimethoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (8aa) A mixture of the isatoic anhydride **5** (50 g, 0.224 mol), *p*-anisidine (68.9 g, 0.560 mol), DMAP (2.73 g, 0.022 mol), and 500 mL of anhydrous dimethylacetamide was warmed to 110 °C. A clear brown solution formed at 80−90 °C, and bubbling was observed (CO_2 loss). After 24 h, the reaction was cooled to room temperature, diluted with water (1 L), and extracted with EtOAc (3 × 500 mL). The combined organic layers were washed with brine (1 × 250 mL), dried over $MgSO_4$, filtered, and concentrated to give a brown residue weighing 85.2 g. The brown residue was dissolved in anhydrous triethylorthoformate (720 mL) and warmed to reflux for 4 h. Upon cooling to room temperature an off-white solid precipitated. Filtration, washing with triethyl orthoformate (1 × 1L), air-drying, and drying under high vacuum gave 55.1 g (79%) of 5,7-dimethoxy-3-(4-methoxyphenyl)quinazoline-4-one (**8aa**), as an off white solid: LC/MS (ESI) $(M + H)^+ = 313.1$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.37 (d, 2H, $J = 8.8$ Hz), 7.06 (d, 2H, $J = 8.8$ Hz), 6.73 (d, 1H, $J = 2.2$ Hz), 6.62 (d, 1H, $J = 2.2$ Hz), 3.9 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H).

Ether Cleavage with BBr_3. 5,7-Dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinone (1aa). To an ice cooled mixture of 5,7-dimethoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (**8aa**) (365 mg, 1.17 mmol) in 10 mL of CH_2Cl_2 was added 3 mL (31.7 mmol) of BBr_3. The mixture was stirred at room temperature for 2 days. The solvent was evaporated, and the obtained residue was treated with a cold $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated to yield a solid. Purification by flash chromatography on silica gel (loaded with CH_2Cl_2 and eluted with 66% EtOAc in hexane) gave 165 mg of 5,7-dihydroxy-3- (4-hydroxyphenyl)-4(3*H*)-quinazolinone **1aa** (40%) as a white solid: LC/MS (ESI) $(M + H)^+ = 271.1$; mp 240−245 °C; ^{1}H NMR ($CDCl_3$) δ 6.46 (1H, d, H6), 6.62 (1H, d, H8), 7.0 (2H, d, H3′, 5′), 7.26 (2H, d, H2′, 6′), 8.09 (1H, s, H2).

Ether Cleavage with EtSNa. 5,7-Dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinone (1aa). A mixture of 5,7-dimethoxy-3- (4-methoxyphenyl)-4(3*H*)-quinazolinone (**8aa**) (100 mg, 0.32 mmol) and EtSNa (524 mg, 6.24 mmol) in 2 mL of DMF was refluxed for 3.5 h. The DMF was evaporated and the residue was dissolved in water. Concentrated HCl was added to adjust the pH to 5. The precipitate was filtered, dried, and purified by chromatography on silica gel (loaded with CH_2Cl_2 and a slight amount of MeOH, eluted with 70% EtOAc in hexane) to yield 51 mg of 5,7-dihydroxy-3- (4-hydroxyphenyl)-4(3*H*)-quinazolinone (**1aa**) (59%) as an off-white solid.

Ether Cleavage with LiCl. 5-Hydroxy-7-methoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (1aj). A suspension of the **8aa** (2.40 g, 7.68 mmol) and anhydrous lithium chloride (6.51 g, 153.6 mmol) in anhydrous DMF (51.2 mL) was warmed to 135 °C. After 2.5 h, the reaction was cooled to room temperature, poured into water (100 mL), and acidified with 1.0 N HCl to give a white precipitate. The resulting mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a white suspension in DMF. Filtration provided 1.10 g (48%) of **1aj** as a white solid: LC/MS (ESI) $(M + H)^+ = 299.1$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 (s, 1H), 8.26 (s, 1H), 7.48 (d, $J = 8.4$ Hz, 2H), 7.10 (d, $J = 8.8$ Hz, 2H), 6.72 (d, $J = 2.2$ Hz, 1H), 6.54 (d, $J = 1.3$ Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H); HRMS (ESI, $M + H^+$) calcd for $C_{28}H_{32}N_2O_3$ 445.2481, found 445.2592.

2-Amino-4,6-dimethoxy-*N*-(4-methoxyphenyl)benzamide (7a). A suspension of **5** (3.75 g, 16.8 mmol), *p*-anisidine (5.15 g, 42.0 mmol), and DMAP (0.205 g, 1.68 mmol) in 37.3 mL of *N*,*N*-dimethylacetamide was warmed to 110 °C for 24 h. The dark red-brown solution was cooled to room temperature, diluted with 70 mL of water, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated to yield a dark purple solid. Purification by column chromatography on silica gel (2.5% of MeOH in CH_2Cl_2) yielded 3 g (59%) of **7a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (s, 1H), 7.46 (d, $J = 8.8$ Hz, 2H), 6.89 (d, $J = 8.8$ Hz, 2H), 6.36 (bs, 2H), 5.85 (d, $J = 2.2$ Hz, 1H), 5.82 (d, $J = 2.2$ Hz, 1H), 3.93 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H).

2-Amino-4,6-dimethoxy-*N*-(4-methoxy-2-methylphenyl)benzamide (7b). A mixture of isatoic anhydride **5** (150 mg, 0.673 mmol), 4-methoxy-2-methylaniline (184 mg, 1.34 mmol), and DMAP (5 mg) in *N*,*N*-dimethylacetamide (1.5 mL) was heated at 110 °C overnight. The solvent was removed in vacuo and the residue was dissolved in EtOAc and water. The layers were separated, and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated. Purification via column chromatography on silica gel (35% EtOAc in hexane) gave 155 mg (72%) of **7b** as a pink solid: LC/MS (ESI) $(M + H)^+ = 317$.

2-Amino-*N*-(2-fluoro-4-methoxyphenyl)-4,6-dimethoxybenzamide (7d). To a mixture of 4-amino-3-fluoroanisole (200 mg, 1.42 mmol) and NaHMDS (1 M) in THF (4.39 mmol) were added **5** (320 mg, 1.42 mmol) and 2 mL of *N*,*N*-dimethylacetamide. The resulting suspension was heated at 90 °C for 4 h. The reaction mixture was cooled to room temperature and diluted with EtOAc and 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were washed with water, saturated $NaHCO_3$, and brine; dried over Na_2SO_4; and concentrated. Purification via column chromatography on silica gel (30% EtOAc in hexane) gave 220 mg (48%) of **7d** as a yellow solid: LC/MS (ESI) $(M + H)^+ = 321.1$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.0 (s, 1H), 8.26 (t, 1H), 6.70 (m, 2H), 6.42 (b, 2H), 5.85 (d, 1H), 5.82 (d, 1H), 3.94 (s, 3H), 3.79 (s, 2H).

5,7-Dimethoxy-3-(4-methoxy-2-methylphenyl)-4(3*H*)-quinazolinone (8ab) A suspension of **7b** (150 mg, 0.475 mmol) in triethylorthoformate (3 mL) was heated to reflux for 5 h. The solvent was removed in vacuo to give a pink color solid. Purification via column chromatography on silica gel (70% EtOAc in hexane) provided 140 mg (90%) of 5,7-dimethoxy-3-(4-methoxy-2-methylphenyl)-4(3*H*)-quinazolinone (**8ab**) as an off-white solid: LC/MS (ESI) $(M + H)^+ = 327.1$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.12 (d, $J = 8.8$ Hz, 1H), 6.87 (d, $J = 2.6$ Hz, 1H), 6.83 (dd, $J = 2.6$, 8.8 Hz, 1H), 6.76 (d, $J = 2.2$ Hz, 1H), 6.51 (d, $J = 2.2$ Hz, 1H), 3.94 (s, 6H), 3.84 (s, 3H) 2.1 (s, 3H).

According to the general methods described above the following compounds were obtained.

5-Hydroxy-3-(4-hydroxyphenyl)-7-methoxy-4(3*H*)-quinazolinone (1ab): $C_{15}H_{12}N_2O_4 = 284.27$ g/mol; LC/MS (ESI) $(M + H)^+ = 285.2$, $(M - H)^- = 283.0$; ^{1}H NMR (DMSO-d_6) δ 11.2 (s, 1H, OH), 9.95 (s, 1H, OH), 8.22 (s, 1H, H2), 7.38 (d, 2H, H2′,6′), 6.98 (d, 2H, H3′,5′), 6.72 (d, 1H, H8), 6.55 (d, 1H, H6), 3.90 (s, 3H, OCH_3).

3-(4-Hydroxy-2-methylphenyl)-5,7-dihydroxy-4(3*H*)-quinazolinone (1ac): $C_{15}H_{12}N_2O_4 = 284$ g/mol; LC/MS (ESI) $(M + H)^+ = 285.0$; ^{1}H NMR (DMSO-d_6) δ 11.7 (s, 1H), 10.7 (s, 1H), 9.79 (s, 1H), 8.09 (s, 1H), 7.20 (d, $J = 8.4$ Hz, 1H), 6.78 (d, $J = 2.2$ Hz, 1H), 6.72 (dd, $J = 2.6$ Hz and $J = 8.8$ Hz, 1H), 6.53 (d, $J = 1.8$ Hz, 1H), 6.34 (d, $J = 2.2$ Hz, 1H), 2.01 (s, 3H).

3-(3-Chloro-4-hydroxyphenyl)-5,7-dihydroxy-4(3*H*)-quinazolinone (1ad): $C_{14}H_9ClN_2O_4 = 304$ g/mol; ESI/MS $(M + H)^+ = 304.8$; ^{1}H NMR (DMSO-d_6) δ 11.7 (s, 1H), 10.7 (s, 1H), 8.18 (s, 1H), 7.62 (d, 1H), 7.30 (d, 1H), 7.08 (d, 1H), 6.52 (d, 1H), 6.34 (d, 1H).

5,7-Dihydroxy-3-(3-Fluoro-4-hydroxyphenyl)-4(3*H*)-quinazolinone (1ae): $C_{14}H_9FN_2O_4 = 288$ g/mol; LC/MS (ESI) $(M + H)^+$

= 288.9; ^{1}H NMR (DMSO-d_6) δ 11.7 (s, 1H), 10.6 (b), 8.18 (s, 1H), 7.47 (dd, 1H), 7.18 (d, 1H), 7.07 (t, 1H), 6.52 (d, 1H), 6.34 (d, 1H).

7-Hydroxy-3-(4-hydroxyphenyl)-5-methoxy-4(3*H*)-quinazolinone (1af): $C_{15}H_{12}N_2O_4$ = 284.27 g/mol; LC/MS (ESI) $(M + H)^+$ = 285.2 and $(M - H)^-$ = 283.0; ^{1}H NMR (DMSO-d_6) δ 8.05 (s, 1H), 7.19 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 6.52 (s, 1H), 6.46 (s, 1H), 3.78 (s, 3H).

5,7-Dihydroxy-3-(2-fluoro-4-hydroxyphenyl)-4(3*H*)-quinazolinone (1ag): $C_{14}H_9FN_2O_4$ = 288 g/mol; LC/MS (ESI) $(M + H)^+$ = 288.8; ^{1}H NMR (DMSO-d_6) δ 11.5 (s, 1H), 10.6 (b), 8.19 (s, 1H), 7.44 (t, 1H), 6.77 (dd, 1H), 6.77 (dd, 1H), 6.54 (d, 1H), 6.36 (s, 1H).

3-(2-Chloro-4-hydroxyphenyl)-5,7-dihydroxy-4(3*H*)-quinazolinone (1ah): $C_{14}H_9ClN_2O_4$ = 304 g/mol; LC/MS (ESI) $(M + H)^+$ = 304.8; ^{1}H NMR (DMSO-d_6) δ 11.5 (s, 1H), 10.6 (b), 8.14 (s, 1H), 7.49 (d, 1H), 7.05 (d, 1H), 6.90 (dd, 1H), 6.55 (d, 1H), 6.36 (d, 1H).

5,7-Dihydroxy-3-(4-hydroxy-3-methylphenyl)-4(3*H*)-quinazolinone (1ai): $C_{15}H_{12}N_2O_4$ = 284.27 g/mol; LC/MS (ESI) $(M + H)^+$ = 284.8; LC/MS (ESI) $(M - H)^-$ = 282.9; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H), 9.85 (s, 1H), 8.15 (s, 1H), 7.22 (s, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 6.52 (s, 1H), 6.34 (s, 1H), 2.16 (s, 3H).

5,7-Dihydroxy-3-(5-hydroxy-2-pyridyl)-4(3*H*)-quinazolinone (1ak): $C_{13}H_9N_3O_4$ = 271.06 g/mol; LC/MS (ESI) $(M + H)^+$= 271.8 and $(M - H)^-$ = 269.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 10.65 (s, 1H), 8.3 (s, 1H), 8.13 (d, J = 2.6 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.4 (q, J = 2.6 and 8.4 Hz, 1H), 6.56 (d, J = 1.8 Hz, 1H), 6.36 (d, J = 1.8 Hz, 1H).

5,7-Dihydroxy-3-(6-hydroxy-3-pyridinyl)-4(3*H*)-quinazolinone (1al): $C_{13}H_9N_3O_4$; LC/MS (ESI) $(M + H)^+$ = 272; mp >360 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.6 (s, 1H, OH), 10.7 (s, 1H, OH) 8.18 (s, 1H, H2), 7.77 (s, 1H, H6′), 7.59 (d, 1H, H4′), 6.51 (d, 1H, H8), 6.61 (d, 1H, H3′) 6.34 (d, 1H, H6).

7-Hydroxy-3-(4-hydroxyphenyl)-3H-quinazolin-4-one (1as): $C_{14}H_{10}N_2O_3$ = 254.24 g/mol; LC/MS (ESI) $(M - H)^-$ = 255.1; ^{1}H NMR (CD_3OD) δ 6.93 (2H, d, J = 8.8 Hz), 7.03 (1H, d, J = 2.6 Hz), 7.07 (1H, q, J = 2.6 and 8.8 Hz), 7.28 (2H, d, J = 8.8 Hz).

5,7-Dihydroxy-3-(3-hydroxyphenyl)-3*H*-quinazolin-4-one (1at): $C_{14}H_{10}N_2O_4$; LC/MS (ESI) $(M + H)^+$ = 271.06; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.73 (s, 1H), 10.71 (bs, 1H), 9.92 (bs, 1H), 8.19 (s, 1H), 7.32 (m, 1H), 6.92 (m, 3H), 6.53 (s, 1H), 6.35 (s, 1H).

5,7-Dihydroxy-3-phenyl-3*H*-quinazolin-4-one (1au): $C_{14}H_{10}N_2O_3$; LC/MS (ESI) $(M + H)^+$ = 255.07; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.75 (s, 1H), 10.77 (s, 1H), 8.28 (s, 1H), 7.61 (s, 5H), 6.6 (s, 1H), 6.41 (s, 1H).

Preparation of Compounds 1am−1ar According to Scheme 2. 5,7-Dihydroxy-3-(4-hydroxyphenyl)-8-iodo-3*H*-quinazolin-4-one (1am) and 5,7-Dihydroxy-3-(4-hydroxyphenyl)-6,8-diiodo-3*H*-quinazolin-4-one (1an). To a solution of **1aa** (0.0603 g, 0.223 mmol) in ethanol (2 mL) was added a solution of iodine (0.0295 g, 0.116 mmol) and periodic acid (0.0173 g, 0.076 mmol) in ethanol (2 mL). The reaction was stirred at room temperature overnight. Concentration gave 0.103 g of the crude material. Purification by preparative HPLC gave the 8-iodo derivative **1am** and the 6,8-diiodo derivative **1an**. **1am**: ^{1}H NMR (400 MHz, $MeOD_4$) δ 8.12 (s, 1H), 7.28 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.50 (s, 1H). **1an**: ^{1}H NMR (400 MHz, $MeOD_4$) 8.19 (s, 1H), 7.29 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-8-methyl-3*H*-quinazolin-4-one (1ao). To a solution of **8aa** (2.0 g, 6.41 mmol) in ethanol (80 mL) was added a solution of iodine (1.62 g, 6.38 mmol) and periodic acid (0.45 g, 1.974 mmol) in ethanol (80 mL). The reaction was stirred at 50 °C overnight and then cooled to room temperature and concentrated to give a solid residue. The residue was dissolved in dichloromethane, washed with saturated sodium sulfite and brine, dried over $MgSO_4$, filtered, and concentrated to give 2.73 g (97%) of the 8-iodo derivative **8al** as a yellow solid, which was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H), 6.55 (s, 1H), 4.05 (s, 3H), 4.01 (s, 3H), 3.86 (s, 3H).

A flame-dried flask containing **8al** (0.299 g, 0.682 mmol), methaneboronic acid (0.164 g, 2.74 mmol), K_3PO_4 (0.5796 g, 2.73 mmol), and 1,1′-Bis(Diphenylphosphino)Ferrocene Palladium (II) Chloride, Complex with Dichloromethane (1:1) [$PdCl_2$(dppf)]-CH_2Cl_2 (0.112 g, 0.137 mmol) was purged with argon. Next, degassed THF (6.8 mL) was added and the reaction was placed in a preheated oil bath (70 °C) for 10 h. The resulting yellow solution was cooled to room temperature and diluted with dichloromethane (50 mL). The organic layer was washed with water (25 mL) and brine (25 mL) and filtered through a plug of Celite. The filtrate was dried over $MgSO_4$, filtered, and concentrated to give an orange-yellow solid weighing 0.479 g. Column chromatography on silica gel (50% EtOAc/hexane) afforded 18.9 mg (8%) of **8am**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.20 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 6.49 (s, 1H), 3.91 (s, 6H), 3.78 (s, 3H), 2.32 (s, 3H).

To a cooled (0 °C) solution of **8am** (0.0193 g, 0.058 mmol) in dichloromethane (0.12 mL) was added boron tribromide (0.083 mL, 0.88 mmol). The reaction was allowed to warm to room temperature. After 3 days, the reaction was added dropwise to a cooled (0 °C), rapidly stirred biphasic mixture of saturated $NaHCO_3$ and ethyl acetate. The layers were separated, and the organic layer was dried over $MgSO_4$, filtered, and concentrated to give 17.7 mg of crude material. Column chromatography on silica gel (10% methanol/dichloromethane) gave 9.6 mg (58%) of **1ao** as an off-white solid: $C_{15}H_{12}N_2O_4$; LC/MS (ESI) $(M + H)^+$ = 285.03; mp 266−268 °C (dec); ^{1}H NMR (400 MHz, $MeOD_4$) δ 8.04 (s, 1H), 7.26 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.39 (s, 1H), 2.28 (s, 3H).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-6-iodo-3*H*-quinazolin-4-one (1ap). To a solution of 5-hydroxy-7,4′-dimethoxy derivative **1aj** (0.454 g, 1.52 mmol) in ethanol (7.6 mL) was added a solution of iodine (0.300 g, 1.18 mmol) and periodic acid (0.064 g, 0.281 mmol) in ethanol (5.7 mL). The resulting orange suspension was stirred at room temperature. After 24 h, additional periodic acid (0.060 g, 0.263 mmol) was added to the reaction. After 18 h, the reaction was concentrated, redissolved in dichloromethane (100 mL), washed with saturated Na_2SO_3 (2 × 50 mL) and brine, dried over $MgSO_4$, filtered, and concentrated to give 0.593 g of the crude material. Column chromatography on silica gel (20% EtOAc/hexane) gave 0.0232 g (3.6%) of 5-hydroxy-6-iodo-7-methoxy-3-(4-methoxyphenyl)-3*H*-quinazolin-4-one: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.5 (s, 1H), 7.98 (s, 1H), 7.26 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 8.8 Hz, 2H), 6.66 (s, 1H), 3.95 (s, 3H), 3.80 (s, 3H).

To a cooled (0 °C) solution of the above compound (0.0232 g, 0.055 mmol) in dichloromethane (0.10 mL) was added boron tribromide (0.051 mL, 0.54 mmol). The reaction was allowed to warm to room temperature. After 24 h, the reaction was added dropwise to a cooled (0 °C), rapidly stirred biphasic mixture of saturated $NaHCO_3$ and ethyl acetate. The layers were separated, and the organic layer was dried over $MgSO_4$, filtered, and concentrated to give 0.0202 g. Column chromatography on silica gel (10% methanol/dichloromethane) gave 0.0010 g (4.6%) of **1ap** as a white solid: $C_{14}H_9IN_2O_4$; LC/MS (ESI) $(M + H)^+$ = 396.98; mp 225−227 °C (dec); ^{1}H NMR (400 MHz, $MeOD_4$) δ 8.15 (s, 1H), 7.28 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.68 (s, 1H).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-6-propyl-3*H*-quinazolin-4-one (1aq). To a cooled (0 °C) solution of 5-hydroxy-7,4′-dimethoxyquinazolinone (**1aj**) (1.45 g, 4.85 mmol) in DMF (16 mL) was added NaH (0.250 g, 10.4 mmol). The reaction was allowed to warm to room temperature. After 2 h, the reaction was cooled to 0 °C and allyl bromide (0.46 mL, 5.3 mmol) was added. The reaction was allowed to warm to room temperature and stir for 18 h. The reaction was poured into water (200 mL) and extracted with ethyl acetate (200 mL). The organic layer was washed with water (3 × 100 mL) and brine (1 × 100 mL), dried over $MgSO_4$, filtered, and concentrated to give 2.04 g. Column chromatography

on silica gel (50% EtOAc/hexane) gave 1.15 g (70%) of **8ao**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H), 6.75 (d, J = 2.6 Hz, 1H), 6.49 (d, J = 2.2 Hz, 1H), 6.10 (tdd, J = 17.6, 10.1. 4.8 Hz, 1H), 5.64 (dq, J = 17.6, 1.8 Hz, 1H), 5.31 (dq, J = 10.1, 1.4 Hz, 1H), 4.64 (dt, J = 4.8, 1.3 Hz, 2H), 3.92 (s, 3H), 3.85 (s, 3H).

A mixture of **8ao** (0.654 g, 1.91 mmol) in diphenyl ether (1.9 mL) was heated to 150 °C for 24 h. The reaction was cooled and purified directly. Column chromatography on silica gel (50% EtOAc/hexane) gave 0.552 g (86%) of the 6-allyl derivative **1ar** as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.77 (s, 1H), 7.98 (s, 1H), 7.32 (d, J = 8.8 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 6.75 (s, 1H), 5.98 (tdd, J = 17.2, 9.9, 6.2 Hz, 1H), 5.03 (dq, J = 17.2, 1.8 Hz, 1H), 4.98 (dq, J = 9.9, 1.8 Hz, 1H), 3.95 (s, 3H), 3.87 (s, 3H), 3.48 (dt, J = 6.2, 1.4 Hz, 2H).

A suspension of 6-allyl derivative **1ar** (0.075 g, 0.222 mmol) and 10% Pd/C (0.0118 g) in a mixture of methanol (4.5 mL)/ethyl acetate (2 mL) was purged with hydrogen and then stirred under a hydrogen atmosphere. After 2 h, the reaction was filtered through Celite and the filtrate was concentrated to give 0.065 g (91%) of 5-hydroxy-7-methoxy-3-(4-methoxyphenyl)-6-propyl-3*H*-quinazolin-4-one as a gray solid, which was used without further purification: $C_{19}H_{20}N_2O_4$; LC/MS (ESI) $(M + H)^+ = 341.08$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.72 (s, 1H), 7.97 (s, 1H), 7.32 (d, J = 8.8 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 6.73 (s, 1H), 3.94 (s, 3H), 3.87 (s, 3H), 2.71−2.67 (m, 2H), 1.60−1.52 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H).

To a cooled (0 °C) solution of 5-hydroxy-7-methoxy-3-(4-methoxyphenyl)-6-propyl-3*H*-quinazolin-4-one (0.0684 g, 0.201 mmol) in dichloromethane (0.40 mL) was added boron tribromide (0.190 mL, 2.0 mmol). The reaction was allowed to warm to room temperature. Additional boron tribromide (0.76 mL, 8.0 mmol) was added over the course of several days. After 7 days, the reaction was added dropwise to a cooled (0 °C), rapidly stirred biphasic mixture of saturated $NaHCO_3$ and ethyl acetate. The layers were separated, and the organic layer was dried over $MgSO_4$, filtered, and concentrated to give a yellow solid. Column chromatography on silica gel (5% methanol/dichloromethane) gave 0.0010 g (1.5%) of **1aq** as a white solid: $C_{17}H_{16}N_2O_4$; LC/MS (ESI) $(M + H)^+ = 313.09$; ^{1}H NMR (400 MHz, $MeOD_4$) δ 8.04 (s, 1H), 7.26 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.59 (s, 1H), 2.67−2.63 (m, 2H), 1.58−1.53 (m, 2H), 0.94 (t, J = 7.2 Hz, 3H).

General Procedure for the Synthesis of Compounds 1av−1aaa according to Scheme 3. 5,7-Dimethoxy-3,1-benzoxazine-4-one (9a). 4,6-Dimethoxyanthranilic acid (**4**) (1.0 g, 5.07 mmol) and 15 mL of triethylorthoformate (90.2 mmol) were heated to 140 °C for 4 h. The reaction mixture was concentrated to give 0.8 g of 5,7-dimethoxy-3,1-benzoxazine-4-one (**9a**) as a yellow solid (76% yield), which was used without any further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H), 6.66 (d, J = 2.2 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 3.98 (s, 3H), 3.93 (s, 3H).

5,7-Dimethoxy-2-methyl-3,1-benzoxazine-4-one (9b). To anthranilic acid **4** (388 mg, 1.97 mmol) in methylene chloride (10 mL) were added sequentially TEA (2.36 mmol) and acetic anhydride (2.36 mmol). The reaction mixture was heated to 40 °C. After 18 h, the reaction was poured into water and extracted with methylene chloride. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo to afford 5,7-dimethoxy-2-methyl-3,1-benzoxazine-4-one (**9b**) (51%): LC/MS (ESI) $(M + H)^+ = 222.07$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.58 (d, J = 1.76 Hz, 1H), 6.46 (d, J = 1.76 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 2.24 (s, 3H).

5,7-Dimethoxy-2-ethyl-3,1-benzoxazine-4-one (9c). To a cooled (0 °C) mixture of anthranilic acid **4** (207 mg, 1.05 mmol) and TEA (2.73 mmol) in methylene chloride (3 mL) was added dropwise propionyl chloride (2.52 mmol). The resulting solution was allowed to warm to ambient temperature over 2 h. The reaction mixture was quenched with water and extracted methylene chloride (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to afford 5,7-dimethoxy-2-ethyl-3,1-benzoxazine-4-one (**5c**) (83%): LC/MS (ESI) $(M + H)^+ = 236.09$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33 (3H, t, J = 7.5 Hz), 2.66 (2H, q, J = 7.5 Hz), 3.91 (3H, s), 3.97 (3H, s), 6.46 (1H, d, J = 2.2 Hz), 6.61 (1H, d, J = 2.2 Hz).

5,7-Dimethoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (10aa). A mixture of 200 mg (0.97 mmol) of the benzoxazinone **9a**, 119 mg (0.97 mmol) of *p*-anisidine, and 5 mL of xylene was heated to reflux for 4 h. The reaction was concentrated. Column chromatography on silica gel (40% EtOAc/hexane) gave 120 mg of 5,7-dimethoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (**10aa**), which is identical to **8aa** (40%), as a white solid: LC/MS (ESI) $(M + H)^+ = 313$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H, H2), 7.36 (d, 1H, H2′), 7.06 (d, 1H, H3′), 6.73 (d, 1H, H8), 6.61 (d, 1H, H6), 3.9 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H).

5,7-Dimethoxy-2-methyl-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (10ab). A mixture of 135.5 mg (0.612 mmol) of benzoxazinone **9b** and *p*-anisidine (0.735 mmol) in glacial acetic acid (2 mL) was heated to 60 °C. After 24 h, the reaction mixture was concentrated in vacuo and then dissolved in ethyl acetate. The organic layer was washed with saturated $NaHCO_3$, water, and brine; dried over $MgSO_4$; filtered; and concentrated to give 23.7 mg of a yellow oil (13.7%): LC/MS (ESI) $(M + H)^+ = 327.0$; ^{1}H NMR ($CDCl_3$) δ 2.19 (s, 3H, methyl), 3.86 (s, 3H, methoxy), 3.90 (s, 3H, methoxy), 3.91 (s, 3H, methoxy), 6.43 (d, 1H, H8), 6.68 (d, 1H, H6), 7.01 (d, 2H, H3′,5′), 7.13 (d, 2H, H2′,6′).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-2-methyl-3*H*-quinazolin-4-one (1av): $C_{15}H_{12}N_2O_4 = 284.27$ g/mol; LC/MS (ESI) $(M + H)^+ = 285.04$; LC/MS (ESI) $(M - H)^- = 283.02$; ^{1}H NMR (CD_3-OD) δ 2.17 (s, 3H), 6.29 (d, J = 2.2 Hz, 1H), 6.47 (d, J = 2.2 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H).

5,7-Dihydroxy-2-ethyl-3-(4-hydroxyphenyl)-3*H*-quinazolin-4-one (1aw): $C_{16}H_{14}N_2O_4 = 298.30$ g/mol; LC/MS (ESI) $(M - H)^- = 297.17$; ^{1}H NMR (CD_3OD) δ 1.07 (t, J = 7.5 Hz, 3H), 2.35 (q, J = 7.5 Hz, 2H), 6.20 (d, J = 2.2 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-2-propyl-3*H*-quinazolin-4-one (1ax): $C_{17}H_{16}N_2O_4 = 312.g/mol$; LC/MS (ESI) $(M + H)^+ = 313.1$; LC/MS (ESI) $(M - H)^- = 311.0$; ^{1}H NMR (CD_3OD) δ 0.77 (t, J = 7.46 Hz, 3H), 1.57 (m, 2H), 2.37 (t, J = 7.7 Hz, 2H), 6.24 (d, J = 2 Hz, 1H), 6.44 (d, J = 2 Hz, 1H), 6.86 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H).

2-Butyl-5,7-dihydroxy-3-(4-hydroxyphenyl)-3*H*-quinazolin-4-one (1ay): $C_{18}H_{18}N_2O_4 = 326$ g/mol; LC/MS (ESI) $(M + H)^+ = 327.13$; LC/MS (ESI) $(M - H)^- = 325.09$; ^{1}H NMR (CD_3OD) δ 0.71 (t, J = 7.5 Hz, 3H), 1.14 (m, 2H), 1.50 (m, 2H), 2.32 (t, J = 7.9 Hz, 2H), 6.19 (d, J = 2.2 Hz, 1H), 6.41 (d, J = 2.2 Hz, 1H), 6.85 (d, J = 8.6 Hz, 2H), 7.05 (d, J = 8.6 Hz, 2H).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-2-isopropyl-3*H*-quinazolin-4-one (1az): $C_{17}H_{16}N_2O_4 = 312.33$ g/mol; LC/MS (ESI) $(M + H)^+ = 313.0$; LC/MS (ESI) $(M - H)^- = 311.0$; ^{1}H NMR (CD_3OD) δ 1.08 (d, J = 6.6 Hz, 6H), 2.61 (m, 1H), 6.08 (d, J = 2.2 Hz, 1H), 6.34 (d, J = 2.2 Hz, 1H), 6.83 (d, J = 8.8 Hz, 2H), 7.01 (d, J = 8.8 Hz, 2H).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-2-isobutyl-3*H*-quinazolin-4-one (1aaa): $C_{18}H_{18}N_2O_4 = 326$ g/mol; LC/MS (ESI) $(M + H)^+ = 327.17$; LC/MS (ESI) $(M - H)^- = 325.12$; ^{1}H NMR (CD_3OD) δ 0.76 (d, J = 6.6 Hz, 6H), 1.94 (m, 1H), 2.24 (d, J = 7 Hz, 2H), 6.21 (d, J = 2.2 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 8.8 Hz, 2H).

General Procedure for the Synthesis of Compounds 1aab−1aae According to Scheme 4. 5,7-Dimethoxy-3-(4-methoxyphenyl)-2,4-(1*H*,3*H*)-quinazolinedione (10ak). A mixture of anthranilic acid **4** (627 mg, 3.18 mmol) and trimethylsilyldiazomethane (8 mL) in MeOH (7 mL) and THF (7 mL) was stirred at room temperature overnight. The reaction mixture was concentrated to yield 0.9 g of methyl 2-amino-4,6-dimethoxybenzoate (**11**), which was used in the next step without further purification.

Compound **11** (489 mg, 2.48 mmol) was dissolved in anhydrous ethyl acetate and stirred over 4 Å molecular sieves. 4-Methoxybenzoic isocyanate (2.48 mmol) was added and the reaction mixture was heated to reflux for 1 h. The reaction mixture was cooled to ambient temperature. The reaction mixture was filtered to remove

the molecular sieves. The solvent was removed in vacuo to afford a mixture of uncyclized product and cyclized product. The crude mixture was taken up in ethanol and saturated with hydrochloric acid. The reaction mixture was heated to reflux for 2 h and then allowed to cool to ambient temperature and concentrated. Column chromatography on silica gel (50% EtOAc/hexane) provided 479.9 mg (59%) of **10ak**: LC/MS (ESI) $(M + H)^+ = 329.13$; LC/MS (ESI) $(M - H)^- = 327.07$; ^{1}H NMR (DMSO-d_6) δ 3.78 (3H, s, methoxy), 3.79 (3H, s, methoxy), 3.83 (3H, s, methoxy), 6.28 (1H, d, H8), 6.31 (1H, d, H6), 6.97 (2H, d, H3′,5′), 7.12 (2H, d, H2′,6′).

2-Mercapto-5,7-dimethoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (10ag). A mixture of compound **11** (0.675 g, 3.19 mmol) and 4-methoxyphenylisothiocyanate (0.525 g, 3.2 mmol) in toluene (20 mL) was heated to reflux for 15 h. The solvent was evaporated and the residue triturated with MeOH/CH_2Cl_2 to yield 0.537 g (49%) of **10ag** as an off-white solid: LC/MS (ESI) $(M + H)^+ = 345.2$; mp 322−326 °C; ^{1}H NMR ($CDCl_3$) δ 3.85 (3H, s, OCH_3), 3.9 (3H, s, OCH_3), 3.91 (3H, s, OCH_3) 6.06 (1H, d, H5), 6.08 (1H, d, H7), 7.02 (1H, d, H3′,5′) 7.16 (2H, d, H2′,6′), 9.38 (1H, s, SH).

5,7-Dimethoxy-3-(4-methoxyphenyl)-2-(methylthio)-4(3*H*)-quinazolinone (10ah). A mixture of 171 mg (0.497 mmol) of **10ag**, 15 mL of MeOH, and 1 mL of 1 N NaOH was heated gently to complete the dissolution. Next, 295 mg (2 mmol) of iodomethane was then added and the reaction was stirred at room temperature for 3 days. The precipitate was filtered to yield 89.9 mg (50%) of 5,7-dimethoxy-2-methylthio-3-(4-methoxyphenyl)quinazolin-4-one as an off-white solid: LC/MS (ESI) $(M + H)^+ = 358.9$; mp 212−215 °C; ^{1}H NMR ($CDCl_3$) δ 2.49 (3H, s, SCH_3), 3.86 (3H, s, OCH_3), 3.9 (3H, s, OCH_3), 3.92 (3H, s, OCH_3) 6.38 (1H, d, H5), 6.64 (1H, d, H7), 7.00 (1H, d, H3′,5′) 7.17 (2H, d, H2′,6′).

5-Hydroxy-3-(4-hydroxyphenyl)-2-mercapto-7-methoxy-3*H*-quinazolin-4-one (1aab): LC/MS (ESI) $(M + H)^+ = 316.8$; LC/MS (ESI) $(M - H)^- = 314.8$; mp 325−328 °C; ^{1}H NMR (CD_3OD) δ 7.75 (1H, s), 7.03 (2H, d, $J = 9.1$ Hz), 6.91 (2H, d, $J = 9.1$ Hz), 6.28 (2H, s), 3.88 (3H, s).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-2-mercapto-3*H*-quinazolin-4-one (1aac): LC/MS (ESI) $(M + H)^+ = 303$; mp 320−326 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.01 (2H, d, $J = 8.8$ Hz), 6.86 (2H, d, $J = 8.8$ Hz), 6.16 (1H, d, $J = 1.9$ Hz), 6.11 (1H, d, $J = 1.9$ Hz).

2-Chloro-5,7-dihydroxy-3-(4-hydroxyphenyl)-3*H*-quinazolin-4-one (1aad): LC/MS (ESI) $(M + H)^+ = 304.9$; LC/MS (ESI) $(M - H)^- = 302.96$; ^{1}H NMR (CD_3OD) δ 7.16 (2H, m), 6.91 (2H, m), 6.47 (1H, d, $J = 2.2$ Hz), 6.35 (1H, d, $J = 2.2$ Hz).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-1*H*-quinazoline-2,4-dione (1aae): LC/MS (ESI) $(M + H)^+ = 287.02$; LC/MS (ESI) $(M - H)^- = 284.99$; ^{1}H NMR (CD_3OD) δ 5.98 (1H, d, $J = 2.2$ Hz), 6.09 (1H, d, $J = 2.2$ Hz), 6.68 (2H, d, $J = 8.8$ Hz), 7.33 (2H, d, $J = 8.8$ Hz).

General Procedure for the Synthesis of Compounds 1aah−1aas According to Schemes 5 and 6. 7-Hydroxy-5-methoxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinone (1aak). To a cooled (0 °C), clear, colorless solution of 5,7-dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinone (**1aa**) (0.330 g, 1.22 mmol) in anhydrous dimethylacetamide (18.5 mL) was added sodium hydride (60% dispersion, 0.274 g, 6.85 mmol) in five portions over a 25 min period. Following the final addition, the reaction mixture was stirred for an additional 30 min at 0 °C and then warmed to room temperature for 1.5 h. Upon recooling to 0 °C, benzyl bromide (0.29 mL, 2.44 mmol) was added. The reaction stirred at 0 °C for 1 h, and over this time, the reaction became a clear, orange solution. Next, iodomethane (0.14 mL, 2.22 mmol) was added. After 1 h at 0 °C, the reaction was allowed to warm to room temperature and stir for 1 h. The reaction was diluted with water (45 mL) and extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to give an orange residue weighing 0.67 g. Column chromatography on silica gel (1:1 hexane/EtOAc) provided 0.25 g (44%) 5-methoxydibenzyloxy compound, as a white foam: LC/MS (ESI) $(M + H)^+ = 465.2$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.48−7.32 (m, 10H), 7.29 (d, $J = 9.2$ Hz, 2H), 7.07 (d, $J = 8.8$ Hz, 2H) 6.83 (d, $J = 2.2$ Hz, 1H), 6.59 (d, $J = 2.2$ Hz, 1H), 5.18 (s, 2H), 5.12 (s, 2H) 3.93 (s, 3H).

To a solution of 5-methoxydibenzyloxy compound (0.200 g, 0.430 mmol) in 1:1 EtOAc/ethanol (12.9 mL) was added 10% palladium on carbon (0.100 g). Hydrogen (balloon) was bubbled through the reaction for 5 min to purge the vessel and then the reaction was stirred vigorously under a hydrogen atmosphere for 4 h. The reaction was filtered through Celite and the filtrate was concentrated to give a white solid weighing 0.122 g. The crude material was absorbed on silica gel (600 mg). Column chromatography (7.5% MeOH in CH_2Cl_2) gave 0.015 g (12%) of **1aak** as a white solid: LC/MS (ESI) $(M + H)^+ = 285$; mp 240−360 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.19 (d, $J = 8.8$ Hz, 2H), 6.85 (d, $J = 8.4$ Hz, 2H), 6.52 (s, 1H), 6.46 (s, 1H), 3.78 (s, 3H).

5-Ethoxy-7-hydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinone (1aah). To a suspension of 5-hydroxy-7-methoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (**1aj**) (0.0.15 g, 0.0503 mmol) in dimethylformamide (0.26 mL) were added potassium carbonate (0.069 g, 0.503 mmol) and iodoethane (0.040 mL, 0.503 mmol). The reaction vessel was placed in a preheated oil bath (120 °C). After 3 h, the reaction was cooled to room temperature and excess ethyl iodide was removed via rotary evaporator. The reaction mixture was diluted with water (0.75 mL) and extracted with EtOAc (3×). The combined organic layers were washed with brine (1×), dried over $MgSO_4$, filtered, and concentrated to give a yellow solid weighing 0.010 g. Absorption onto silica gel (60 mg) and column chromatography (1:1 hexane/EtOAc) provided 0.0080 g (49%) of 5-ethoxy-7-methoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone as a flaky white solid: LC/MS (ESI) $(M + H)^+ = 327.2$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.0 (s, 1H), 7.29 (d, $J = 8.8$ Hz, 2H), 7.00 (d, $J = 8.8$ Hz, 2H), 6.74 (d, $J = 2.6$ Hz, 1H), 6.48 (d, $J = 2.6$, 1H),4.12 (q, $J = 7.0$, 2H), 3.92 (s, 3H), 3.85 (s, 3H), 1.52 (t, $J = 7.0$ Hz, 3H).

To a solution of 5-ethoxy-7-methoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinone (0.0080 g, 0.0245 mmol) in dimethylformamide (0.25 mL) was added sodium ethanethiolate (0.0047 g, 0.0563 mmol). The resulting suspension was warmed to 120 °C, at which time a solution formed. After each hour (for 7 h) additional sodium ethanethiolate (0.0047, 0.0563 mmol) was added. The cloudy orange reaction was cooled to room temperature and diluted with water (0.75 mL). The resulting yellow solution was acidified with 1.0 N HCl (pH 3) and the reaction became cloudy. The reaction was extracted with EtOAc (3×) and dichloromethane (3×). The combined organic layers were washed with brine (1×), dried over $MgSO_4$, filtered, and concentrated. Column chromatography on silica gel (5% MeOH in CH_2Cl_2) gave 0.0017 g (23%) of 5-ethoxy-7-hydroxy-3-(4-hydroxyphenyl)quinazolin-4-one (**1aah**), as a white solid: LC/MS (ESI) $(M + H)^+ = 299.1$; ^{1}H NMR (400 MHz, CD_3OD) δ 8.09 (s, 1H), 7.22 (d, $J = 8.8$ Hz, 2H), 6.90 (d, $J = 8.2$ Hz, 2H), 6.61 (d, $J = 2.2$ Hz, 1H), 6.52 (d, $J = 2.2$ Hz, 1H), 4.13 (q, $J = 7.0$ Hz, 2H), 1.44 (t, $J = 7.0$ Hz, 3H).

Trifluoromethanesulfonic Acid 7-Hydroxy-3-(4-hydroxyphenyl)-4-oxo-3,4-dihydroquinazolin-5-yl Ester (1aam). To a cooled (0 °C) solution of **1aj** (5.83 g, 19.54 mmol) in DMF (65 mL) was added in portions 60% sodium hydride in mineral oil (1.01 g, 25.41 mmol). Gas evolution was observed and after 30 min at 0 °C, the clear, slightly yellow solution was warmed to room temperature. After 30 min, the reaction was cooled to 0 °C and *N*-phenyltrifluoromethanesulfonimide (7.33 g, 20.52 mmol) was added. The reaction was stirred at 0 °C for 30 min and then warmed to room temperature. After 30 min, the reaction was quenched with saturated NH_4Cl and diluted with water (150 mL). The reaction was extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an off-white solid. Column chromatography on silica gel (1:1 hexane/EtOAc) provided 7.13 g (85%) of **8ah** as a white solid: LC/MS (ESI) $(M + H)^+ = 430.9$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.32 (d, $J = 8.8$ Hz, 2H), 7.18 (d, $J = 2.2$ Hz, 1H), 7.03 (d, $J = 8.8$ Hz, 2H), 6.92 (d, $J = 2.2$ Hz, 1H), 3.96 (s, 3H), 3.85 (s, 3H).

To a cooled (0 °C), clear, yellow solution of **8ah** (1.88 g, 4.36 mmol) in CH_2Cl_2 (43.6 mL) was added dropwise over 5 min boron tribromide (6.20 mL, 65.5 mmol). The reaction was stirred at 0 °C for 1 h and then warmed to room temperature. After 7 days, the reaction was added dropwise to a vigorously stirred, cold (ice bath) mixture of ethyl acetate (150 mL) and saturated $NaHCO_3$ (100 mL). The mixture was stirred for 10 min, and then the layers were separated. The aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an orange foam weighing 2.47 g. Column chromatography on silica gel (5% MeOH in CH_2Cl_2) gave 1.63 g (93%) of **1aam** as a white foam: LC/MS (ESI) $(M + H)^+ = 402.9$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.39 (s, 1H), 9.86 (s, 1H), 8.29 (s, 1H), 7.28 (d, $J = 8.6$ Hz, 2H), 7.08 (bs, 1H), 6.95 (bs, 1H), 6.90 (d, $J = 8.6$ Hz, 2H).

7-Hydroxy-3-(4-hydroxyphenyl)-5-methyl-3*H*-quinazolin-4-one (1aaj). A flame-dried flask containing **8ah** (0.300 g, 0.697 mmol), $[PdCl_2(dppf)]CH_2Cl_2$ (0.114 g, 0.139 mmol), potassium phosphate tribasic (0.592 g, 2.78 mmol), and methylboronic acid (0.166 g, 2.78 mmol) was purged with argon for 10 min, and then degassed THF (4.65 mL) was added. The resulting dark red-purple mixture was placed in a preheated oil bath (73 °C). After 5 h, the reaction was cooled to room temperature, diluted with methylene chloride (20 mL), washed with water (5 mL) and brine (5 mL), and filtered through a plug of Celite. The filtrate was dried over $MgSO_4$, filtered, and concentrated to give an off-white solid weighing 0.282 g. Column chromatography on silica gel (1:1 hexane/EtOAc) gave 0.174 g (84%) of **8ar** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.31 (d, $J = 8.8$ Hz, 2H), 7.03 (d, $J = 8.8$ Hz, 2H), 7.00 (d, $J = 2.5$ Hz, 1H), 6.86 (d, $J = 2.5$ Hz, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 2.82 (s, 3H).

Compound **8ar** was converted to **1aaj** according to the deprotection procedure described for **1aam**: LC/MS (ESI) $(M + H)^+ = 269.0$; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.44 (s, 1H), 9.79 (s, 1H), 8.10 (s, 1H), 7.23 (d, $J = 8.8$ Hz, 2H), 6.87 (d, $J = 8.8$ Hz, 2H), 6.81 (d, $J = 2.2$ Hz, 1H), 6.76 (d, $J = 2.2$ Hz, 1H), 2.67 (s, 3H).

5-Ethyl-7-hydroxy-3-(4-hydroxyphenyl)-3*H*-quinazolin-4-one (1aan). A flame-dried flask containing compound **8ah** (0.300 g, 0.697 mmol), $[PdCl_2(dppf)]Cl_2CH_2Cl_2$ (0.114 g, 0.139 mmol), and potassium phosphate tribasic (0.592 g, 2.78 mmol) was purged with argon for 10 min, and then degassed THF (6.97 mL) and 1.0 M triethylborane in THF (2.78 mL, 2.78 mmol) were added. The resulting rust-colored mixture was placed in a preheated oil bath (75 °C). After 5.5 h, the black reaction was cooled to room temperature, diluted with methylene chloride (15 mL), washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give a black solid. Column chromatography on silica gel (1:1 hexane/EtOAc) gave 0.168 g (78%) of **8at** as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.32 (d, $J = 9.0$ Hz, 2H), 7.04 (d, $J = 9.0$ Hz, 2H), 7.01 (d, $J = 2.4$ Hz, 1H), 6.90 (d, $J = 2.4$ Hz, 1H), 3.93 (s, 3H), 3.86 (s, 3H), 3.27 (q, $J = 7.5$ Hz, 2H), 1.26 (t, $J = 7.5$ Hz, 3H).

Compound **8at** was converted to **1aan** according to the deprotection procedure described for **1aam**: LC/MS (ESI) $(M + H)^+ = 283.0$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.24 (d, $J = 8.8$ Hz, 2H), 6.87 (d, $J = 8.8$ Hz, 2H), 6.83 (d, $J = 2.4$ Hz, 1H), 6.78 (d, $J = 2.4$ Hz, 1H), 3.13 (q. $J = 7.3$ Hz, 2H), 1.14 (q, $J = 7.3$ Hz, 3H).

7-Hydroxy-5-hydroxymethyl-3-(4-hydroxyphenyl)-3*H*-quinazolin-4-one (1aar). Into a flame-dried flask were placed compound **8ah** (0.250 g, 0.581 mmol), palladium acetate (0.0130 g, 0.058 mmol), and diphenylphosphinopropane (0.0240 g, 0.058 mmol). Next, dimethyl sulfoxide (1.81 mL), methanol (1.16 mL), and triethylamine (0.17 mL) were added. Carbon monoxide (balloon) was bubbled through the cloudy, yellow mixture for 5 min, and the vessel was maintained under a carbon monoxide atmosphere. The reaction was placed in a preheated oil bath (70 °C) and a clear, slightly yellow solution formed. After 15 h, additional palladium acetate (0.0130 g, 0.058 mmol), diphenylphosphinopropane (0.0240 g, 0.058 mmol), methanol (1.16 mL), and triethylamine (0.17 mL) were added. The reaction was purged with carbon monoxide (balloon) as described above. The reaction was placed in a preheated oil bath (70 °C). After 2 h, the dark orange reaction was cooled to room temperature. The reaction was diluted with water (6 mL) and extracted with methylene chloride (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an orange solid. Column chromatography on silica gel (1:1 hexane/EtOAc) provided 0.166 g (84%) of **8av** as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.32 (d, $J = 9.0$ Hz, 2H), 7.19 (d, $J = 2.2$ Hz, 1H), 7.05 (d, $J = 2.2$ Hz, 1H), 7.02 (d, $J = 9.0$ Hz, 2H), 3.96 (s, 3H), 3.95 (s, 3H), 3.85 (s, 3H).

To a cooled (0 °C) suspension of compound **8av** (0.083 g, 0.243 mmol) in THF (1.21 mL) was added dropwise 1.0 M lithium aluminum hydride in THF (0.24 mL). The resulting clear, yellow-brown solution was stirred at 0 °C. Over the course of the reaction a precipitate formed. After 4 h, the reaction was quenched with saturated ammonium chloride and diluted with ethyl acetate. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a yellow residue weighing 0.063 g. The crude material was dissolved in ethanol (1.0 mL) to give a clear, yellow solution. Next, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.048 g, 0.213 mmol) was added. The resulting clear, orange solution was stirred at room temperature. A precipitate formed and additional ethanol (2 mL) was added to facilitate stirring. After 4 h, the excess solvent was removed and the reaction was purified directly. Column chromatography on silica gel (5% MeOH in CH_2Cl_2) gave 0.070 g (92%) of **8ax** as an off-white solid: LC/MS (ESI) $(M + H)^+ = 312.9$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.33 (d, $J = 9.2$ Hz, 2H), 7.12 (d, $J = 2.6$ Hz, 1H), 7.10−7.04 (m, 3H), 4.88 (d, $J = 7.9$ Hz, 2H), 4.72 (t, $J = 7.9$ Hz, 1H), 3.95 (s, 3H), 3.88 (s, 3H).

To a cooled (0 °C) suspension of compound **8ax** (0.040 g, 0.128 mmol) in methylene chloride (1.28 mL) was added dropwise boron tribromide (0.24 mL, 2.56 mmol). The suspension was stirred at 0 °C for 30 min and then warmed to room temperature to give a clear, orange solution. After 24 h, the reaction was then added dropwise to a vigorously stirred cold (ice bath) mixture of ethyl acetate/saturated $NaHCO_3$. The mixture was stirred for 10 min, and then the layers were separated. The aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give 5-bromomethyl-7-hydroxy-3-(4-hydroxyphenyl)-3*H*-quinazolin-4-one, as an off-white solid weighing 0.0525 g.

To a clear, slightly brown solution of the crude 5-bromomethyl compound in acetonitrile (1.5 mL) was added potassium acetate (0.0444 g, 0.453 mmol) and 18-crown-6 (0.120 g, 0.453 mmol). The reaction was warmed to 50 °C. After 15 h, the clear, colorless reaction was cooled to room temperature and 1.0 M sodium hydroxide (0.60 mL, 0.604 mmol) was added. The biphasic mixture was stirred vigorously for 5 h. The reaction was acidified to pH 4 with 1.0 M hydrogen chloride. The reaction was extracted with ethyl acetate (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to a slightly purple solid weighing 0.153 g. Column chromatography on silica gel (5% MeOH in CH_2Cl_2) gave 0.017 g as an off-white solid, which was 93% pure. Purification by preparative HPLC (method 1: 30−90% B, 10 min gradient) (method 1: YMC S5 ODS 30 × 100 mm column, solvent A = 10% MeOH/H_2O containing 0.1% TFA, solvent B = 90% MeOH/H_2O containing 0.1% TFA, flow rate 40 mL/min, UV detection at 220 nm) gave 0.0123 g (34%) of **Iaar** as white solid: LC/MS (ESI) $(M + H)^+ = 284.9$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.80 (s, 1H), 8.11 (s, 1H), 7.29 (d, $J = 2.5$ Hz, 1H), 7.23 (dd, $J = 8.8$, 2.2 Hz, 2H), 6.87 (dd, $J = 8.8$, 2.2 Hz, 2H), 6.83 (d, $J = 2.5$ Hz, 1H), 5.26 (t, $J = 5.6$ Hz, 1H), 4.98 (d, $J = 5.6$ Hz, 2H).

7-Hydroxy-3-(4-hydroxyphenyl)-5-isopropenyl-3*H*-quinazolin-4-one (1aas). Isopropenylmagnesium bromide (0.5 M in tetrahydrofuran, 0.93 mL, 0.464 mmol) was added dropwise to a 0.5 M solution of zinc chloride in THF (0.93 mL, 0.464 mmol). A

mild exotherm was observed and a white precipitate formed. The resulting suspension was stirred at room temperature for 1.5 h. In a separate flamed-dried flask was placed compound **8ah** (0.100 g, 0.232 mmol) and tetrakis(triphenylphosphine)palladium (0.0404 g, 0.035 mmol). The vessel was purged with argon for 10 min, and then degassed THF (1.16 mL) was added. To the clear, yellow solution was added the isopropenyl zinc halide suspension prepared above. The resulting bright yellow suspension was placed in a preheated oil bath (75 °C) for 2 h. The reaction was cooled to room temperature, quenched with saturated ammonium chloride, and extracted with EtOAc (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a yellow foam weighing 0.120 g. Column chromatography on silica gel (1:1 hexane/EtOAc) provided 0.052 g (69%) of **8ay** as a pale yellow foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.32 (d, J = 9.0 Hz, 2H), 7.08 (d, J = 2.6 Hz, 1H), 7.01 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 2.6 Hz, 1H), 5.09 (d, J = 1.3 Hz, 1H), 4.85 (d, J = 1.3 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 2.10 (s, 3H).

Compound **8ay** was converted to **1aas** according to the deprotection procedure described for **1aam**: LC/MS (ESI) $(M + H)^+$ = 295.0; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H); 7.23 (d, J = 8.8 Hz, 2H); 6.96 (d, J = 2.8 Hz, 1H), 6.92 (d, J = 8.8 Hz, 2H), 6.75 (d, J = 2.8 Hz, 1H), 5.01 (d, J = 1.4 Hz, 1H), 4.77 (d, J = 1.4 Hz, 1H), 2.05 (s, 3H).

5-Amino-7-hydroxy-3-(4-hydroxyphenyl)-3*H*-quinazolin-4-one (1aai). A flame-dried vessel containing compound **8ah** (0.933 g, 2.17 mmol), palladium acetate (0.0487 g, 0.217 mmol), (*S*)-BINAP (0.203 g, 0.326 mmol), and cesium carbonate (0.990 g, 3.04 mmol) was purged with argon for 10 min. Next, degassed THF (10.8 mL) was added, followed by benzophenone imine (0.44 mL, 2.60 mmol). The resulting dark orange solution was placed in a preheated oil bath (75 °C). After 24 h, the cloudy dark orange mixture was cooled to room temperature, diluted with diethyl ether (10 mL), and filtered through Celite. Concentration gave a burgundy foam. The burgundy foam was dissolved in THF (10.8 mL) and 1 N hydrochloric acid (4.33 mL) was added. The reaction was stirred at room temperature for 3 h. The resulting clear orange solution was diluted with methylene chloride (50 mL) and quenched with saturated $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with methylene chloride (15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a yellow solid weighing 1.34 g. Column chromatography (1:1 hexane/EtOAc) gave 0.471 g (73%) of **8aq** as an off-white solid: LC/MS (ESI) $(M + H)^+$ = 298.1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 8.8 Hz, 2H), 6.49 (d, J = 2.2 Hz, 1H), 6.22 (bs, 2H), 6.15 (d, J = 2.2 Hz, 1H), 3.86 (s, 3H).

A clear, slightly yellow solution of **8aq** (0.0245 g, 0.0.082 mmol) and sodium ethanethiolate (0.0693 g, 0.82 mmol) in dimethylformamide (0.82 mL) was warmed to 120 °C. After 5 h, the reaction was cooled to 0 °C and diluted with water (2 mL). The reaction was acidified to pH 5 with the dropwise addition of 1 N hydrochloric acid. The reaction was extracted with methylene chloride (3×) and ethyl acetate (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a brown solid weighing 0.026 g. The crude material was absorbed onto silica gel (0.250 g). Column chromatography (5% MeOH in CH_2Cl_2) gave 0.0086 g (39%) of **1aai** as an off-white solid: LC/MS (ESI) $(M + H)^+$ = 270.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.0 (bs, 1H), 9.80 (s, 1H), 7.94 (s, 1H), 7.21 (d, J = 8.8 Hz, 2H), 7.17 (bs, 2H), 6.86 (d, J = 8.8 Hz, 2H), 6.12 (d, J = 2.2 Hz, 1H), 6.08 (d, J = 2.2 Hz, 1H).

7-Hydroxy-3-(4-hydroxyphenyl)-4-oxo-3,4-dihydroquinazoline-5-carbonitrile (1aal). A flame-dried test tube containing compound **8ah** (0.250 g, 0.581 mmol), tris(dibenzylideneacetone)dipalladium (0.0531 g, 0.058 mmol), and 1,1′-bis(diphenylphosphino)ferrocene (0.128 g, 0.232 mmol) was purged with argon for 10 min. Next, degassed dimethylformamide (1.9 mL) was added and the reaction was placed in a preheated oil bath (90 °C) to give a clear, orange-brown solution. A suspension of zinc cyanide (0.0818 g, 0.697 mmol) in dimethylformamide (1.4 mL) was added in portions (0.2 mL every 15 min). Following the addition, the reaction was continued for 3 h. The reaction was cooled to room temperature and diluted with water (6 mL), giving an orangish precipitate, which was extracted with methylene chloride (4 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an orange solid. Column chromatography on silica gel (2:1 EtOAc/hexane) provided 0.148 g (83%) of **8as** as a faint orange solid: LC/MS (ESI) $(M + H)^+$ = 308.1; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.48 (d, J = 2.6 Hz, 1H), 7.35−7.33 (m, 3H), 7.04 (d, J = 8.8 Hz, 2H), 3.98 (s, 3H), 3.87 (s, 3H).

A suspension of compound **8as** (0.120 g, 0.393 mmol) and sodium ethanethiolate (0.662 g, 7.87 mmol) in dimethylformamide (4.0 mL) was warmed to 120 °C to give a clear, yellow solution. After 6.5 h, the reaction was cooled to 0 °C and diluted with water (12 mL). The reaction was acidified to pH 5 with the dropwise addition of 1 N hydrochloric acid. The reaction was extracted with methylene chloride (3 × 10 mL). The combined organic layers were washed with brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to give a yellow solid weighing 0.264 g. The crude material was adsorbed onto silica gel (1.0 g). Column chromatography (7.5% MeOH in CH_2Cl_2) gave 0.027 g as yellow solid, which was further purified by preparative HPLC (method 2: 0−100% B, 30 min gradient). The fractions were combined and neutralized with saturated $NaHCO_3$, and the volume was reduced by approximately 80%. A few drops of 1 N hydrochloric acid were added and the volumne was concentrated to give a residue. Extraction with methanol and concentration gave 0.0047 g (4%) of **1aal** as a faint yellow solid: LC/MS (ESI) $(M + H)^+$ = 277.8 ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.34 (bs, 1H), 7.29 (d, J = 8.8 Hz, 2H), 7.10 (bs, 1H), 6.88 (d, J = 8.8 Hz, 2H).

7-Hydroxy-3-(4-hydroxyphenyl)-5-vinyl-3*H*-quinazolin-4-one (1aao). A flame-dried flask containing compound **1aam** (0.200 g, 0.497 mmol), bis(triphenylphosphine)palladium dichloride (0.035 g, 0.049 mmol), triphenylphosphine (0.052 g, 0.199 mmol), lithium chloride (0.084 g, 1.99 mmol), and 2,6-di-*tert*-butyl-4-methylphenol (a crystal) was purged with argon for 10 min. Next, dimethylformamide (degassed, 4.97 mmol) and tributylvinylstannane (0.29 mL, 0.99 mmol) were added. The reaction was placed in a preheated oil bath (120 °C). After 4 h, the cloudy, yellow reaction was cooled to room temperature, diluted with water (15 mL), and extracted with ethyl acetate (3×) and methylene chloride (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a viscous oil weighing 0.447 g. Column chromatography on silica gel (5% MeOH in CH_2Cl_2) gave 0.069 g (50%) of **1aao** as an off-white solid: LC/MS (ESI) $(M + H)^+$ = 278.8 ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.60 (s, 1H), 9.81 (s, 1H), 8.14 (s, 1H), 7.91 (dd, J = 17.4, 11.0 Hz, 1H), 7.24 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 2.5 Hz, 1H), 6.93 (d, J = 2.5 Hz, 1H), 6.87 (d, J = 8.5 Hz, 2H), 5.58 (dd, J = 17.4, 1.6 Hz, 1H), 5.28 (d, J = 11.0 Hz, 1H).

7-Hydroxy-3-(4-hydroxyphenyl)-4-oxo-3,4-dihydroquinazoline-5-carboxylic Acid Methyl Ester (1aap). Into a flame-dried flask were placed compound **1aam** (0.300 g, 0.746 mmol), palladium acetate (0.0168 g, 0.075 mmol), and diphenylphosphinopropane (0.0309 g, 0.075 mmol). Next, dimethyl sulfoxide (2.3 mL), methanol (3.0 mL), and triethylamine (0.44 mL) were added. Carbon monoxide (balloon) was bubbled through the clear, yellow solution for 10 min, and the vessel was maintained under a carbon monoxide atmosphere. The reaction was placed in a preheated oil bath (73 °C). After 5 h, the dark brown reaction was cooled to room temperature. The reaction was diluted with water (10 mL), acidified (pH 3) with 1.0 M hydrochloric acid, and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an orange residue weighing 0.400 g. Column chromatography on silica gel (5% MeOH in CH_2Cl_2) provided 0.0580 g (25%) of **1aap** as a white solid: LC/MS (ESI) $(M + H)^+$ = 312.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H), 9.85 (s, 1H), 8.23 (s, 1H), 7.26 (d, J = 8.8 Hz, 2H), 7.04 (d, J = 2.2 Hz, 1H), 6.92 (d, J = 2.2 Hz, 1H), 6.88 (d, J = 8.8 Hz, 2H), 3.76 (s, 3H).

7-Hydroxy-3-(4-hydroxyphenyl)-5-phenyl-3H-quinazolin-4-one (1aaq). A flame-dried flask containing compound **8ah** (0.300 g, 0.697 mmol), $[PdCl_2(dppf)]CH_2Cl_2$ (0.0853 g, 0.104 mmol), potassium phosphate tribasic (0.592 g, 2.78 mmol), and phenylboronic acid (0.340 g, 2.78 mmol) was purged with argon for 10 min. Next, degassed THF (7.0 mL) was added. The resulting dark orange suspension was placed in a preheated oil bath (73 °C). After 14 h, the dark red reaction was cooled to room temperature and diluted with ethyl acetate (20 mL). The reaction was washed with water (5 mL), saturated $NaHCO_3$ (5 mL), and brine (5 mL); dried over $MgSO_4$; filtered; and concentrated to give a burgundy residue weighing 0.667 g. Column chromatography on silica gel (1:1 hexane/EtOAc) gave 0.216 g (86%) of **8aw** as a white solid: LC/MS (ESI) $(M + H)^+ = 359.15$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.35−7.29 (m, 5H), 7.24 (d, $J = 8.9$ Hz, 2H), 7.18 (d, $J = 2.6$ Hz, 1H), 6.94 (d, $J = 8.9$ Hz, 2H), 6.91 (d, $J = 2.6$ Hz, 1H), 3.96 (s, 3H), 3.80 (s, 3H).

To a cooled (0 °C), clear, colorless solution of compound **8aw** (0.095 g, 0.265 mmol) in methylene chloride (2.60 mL) was added dropwise boron tribromide (0.50 mL, 5.30 mmol). The resulting bright yellow solution was stirred at 0 °C for 30 min and then warmed to room temperature. After 72 h, the reaction was added dropwise to a vigorously stirred cold (ice bath) mixture of ethyl acetate/saturated $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an off-white solid. Trituration with ethyl acetate and filtration gave 0.0547 g (62%) of **1aaq** as a white solid: LC/MS (ESI) $(M + H)^+ = 331.2$; ^1H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H), 9.76 (s, 1H), 8.22 (s, 1H), 7.33−7.22 (m, 5H), 7.18 (d, $J = 8.8$ Hz, 2H), 6.99 (d, $J = 2.6$ Hz, 1H), 6.82 (d, $J = 8.8$ Hz, 2H), 6.70 (d, $J = 2.6$ Hz, 1H).

General Procedure for the Synthesis of "Thio" Derivatives 1ba−1bi According to Scheme 7. 5,7-Dimethoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinethione (8ba): The mixture of 5,7-dimethoxy-3-(4-methoxyphenyl)quinazolin-4-one (**8aa**) (240 mg, 0.76 mmol) and Lawesson's reagent (310 mg, 0.76 mmol) in toluene (4 mL) was refluxed overnight. The mixture was evaporated to dryness. EtOAc was added and the mixture was washed with water and brine, dried over $MgSO_4$, and concentrated. Column chromatography on silica gel (45% EtOAc in hexane) gave 180 mg (72%) of **8ba** as a yellow foam: LC/MS (ESI) $(M + H)^+ = 329$; ^1H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1H, H2), 7.20 (d, 1H, H2′), 7.00 (d, 1H, H3′), 6.71 (d, 1H, H8), 6.52 (d, 1H, H6), 3.9 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3).

Ether Cleavage. 5,7-Dihydroxy-3-(4-hydroxyphenyl)-4(3*H*)-quinazolinethione (1ba). 5,7-Dimethoxy-3-(4-methoxyphenyl)-4(3*H*)-quinazolinethione (**8ba**) (100 mg, 0.30 mmol) was mixed with pyridine hydrochloride salt (701 mg, 6.1 mmol). The mixture was heated to 189 °C for 2.5 h. The reaction was cooled to room temperature. The resulting residue was purified by preparative HPLC to give 35 mg (40%) of **1ba** as a yellow solid: LC/MS (ESI) $(M + H)^+ = 287$; mp 275−280 °C; ^1H NMR (400 MHz, CD_3OD) δ 8.28 (s, 1H, H2), 7.18 (d, 2H, H2′,6′), 6.93 (d, 2H, H3′,5′), 6.52 (d, 1H, H8), 6.42 (d, 1H, H6).

5,7-Dihydroxy-3-(3-fluoro-4-hydroxyphenyl)-4(3*H*)-quinazolinethione (1bb): $C_{14}H_9FN_2O_3S = 304.3$ g/mol; LC/MS (ESI) $(M + H)^+ = 305.8$; ^1H NMR (400 MHz, DMSO-d_6) δ 13.4 (1H, s), 10.51 (1H, bs), 8.35 (1H, s), 7.42 (1H, dd, $J = 11.9$ and 2.2 Hz), 7.11 (2H, m), 6.56 (1H, d, $J = 2.2$ Hz), 6.45 (1H, d, $J = 2.2$ Hz).

5,7-Dihydroxy-3-(4-hydroxy-3-methylphenyl)-4(3*H*)-quinazolinethione (1bc): $C_{15}H_{12}N_2O_3S = 300.8$ g/mol; LC/MS (ESI) $(M + H)^+ = 300.8$; LC/MS (ESI) $(M - H)^- = 298.8$; ^1H NMR (400 MHz, DMSO-d_6) δ 13.52 (1H, s), 11.03 (1H, s), 9.86 (1H, s), 8.34 (1H, s), 7.15 (1H, s), 7.07 (1H, d, $J = 8.8$ Hz), 6.90 (1H, d, $J = 8.8$ Hz), 6.58 (1H, d, $J = 1.8$ Hz), 6.46 (1H, d, $J = 1.8$ Hz), 2.15 (3H, s, Me).

7-Hydroxy-3-(4-hydroxyphenyl)-5-methyl-3*H*-quinazoline-4-thione (1bd). A yellow suspension of **8ar** (0.124 g, 0.42 mmol) and Lawesson's reagent (1.18 g, 2.93 mmol) in *m*-xylene (2.0 mL) was warmed to 145 °C. After 44 h, additional Lawesson's reagent (0.590 g, 1.46 mmol) was added and the reaction was stirred at 145 °C for an additional 26 h. The reaction was cooled to room temperature, diluted with methylene chloride, filtered, and concentrated to give an orange solid weighing 0.865 g. Column chromatography on silica gel (1.5:1 hexane/EtOAc) gave 0.0599 g (46%) of **8bd** as a yellow solid: ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.23 (d, $J = 8.8$ Hz, 2H), 7.04 (d, $J = 8.8$ Hz, 2H), 7.02 (d, $J = 2.2$ Hz, 1H), 6.97 (d, $J = 2.2$ Hz, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.07 (s, 3H).

To a cooled (0 °C), clear, yellow solution of **8bd** compound (0.0599 g, 0.191 mmol) in methylene chloride (1.91 mL) was added dropwise boron tribromide (0.09 mL, 0.958 mmol). The resulting clear, dark orange solution was stirred at 0 °C for 30 min and then warmed to room temperature. After 9 h, the reaction was then added dropwise to a vigorously stirred cold (ice bath) mixture of ethyl acetate/saturated $NaHCO_3$. The mixture was stirred for 10 min, and then the layers were separated. The aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give a orange solid weighing 0.063 g. The crude material was adsorbed onto silica gel (0.200 g). Column chromatography (5% MeOH in CH_2Cl_2) gave 0.0252 g (46%) of **1bd** as a yellow solid: LC/MS (ESI) $(M + H)^+ = 284.9$; ^1H NMR (500 MHz, $MeOD_4$) δ 8.20 (s, 1H); 7.11 (d, $J = 8.8$ Hz, 2H); 6.91−6.87 (m, 4H), 2.99 (s, 3H); HRMS *m*/*z* calcd for $C_{15}H_{13}N_2O_2S$ $(M + H)^+$ 285.0698, found 285.0696.

5-Ethyl-7-hydroxy-3-(4-hydroxyphenyl)-3*H*-quinazoline-4-thione (1be). A yellow suspension of **8at** (0.116 g, 0.373 mmol) and Lawesson's reagent (0.755 g, 1.87 mmol) in *m*-xylene (2.0 mL) was warmed to 145 °C. After 72 h, additional Lawesson's reagent (1.51 g, 3.74 mmol) was added and the reaction was stirred at 145 °C for an additional 24 h. The reaction was cooled to room temperature, diluted with methylene chloride (40 mL), filtered, and concentrated to give a dark brown solid weighing 0.773 g. Column chromatography on silica gel (2:1 hexane/EtOAc) gave 0.0211 g (17%) of **8be** as a yellow solid: LC/MS (ESI) $(M + H)^+ = 327.1$; ^1H NMR (500 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.23 (d, $J = 8.8$ Hz, 2H), 7.04−6.95 (m, 4H), 3.94 (s, 3H), 3.88 (s, 3H), 3.65 (q, $J = 7.4$ Hz, 2H), 1.29 (t, $J = 7.4$ Hz, 3H).

To a cooled (0 °C), clear, yellow solution of **8be** (0.0211 g, 0.0646 mmol) in methylene chloride (0.65 mL) was added dropwise boron tribromide (0.09 mL, 0.97 mmol). The resulting orange solution was stirred at 0 °C for 30 min and then warmed to room temperature. After 8 h, the reaction was then added dropwise to a vigorously stirred cold (ice bath) mixture of ethyl acetate/saturated $NaHCO_3$. The mixture was stirred for 10 min, and then the layers were separated. The aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to give an orange solid weighing 0.0192 g. Column chromatography (5% MeOH in CH_2Cl_2) gave 0.0117 g (60%) of **1be** as a yellow foam: LC/MS (ESI) $(M + H)^+ = 298.9$; ^1H NMR (500 MHz, $MeOD_4$) δ 8.22 (s, 1H); 7.12 (d, $J = 8.2$ Hz, 2H); 6.92−6.88 (m, 4H), 3.61 (q, $J = 7.2$ Hz, 2H), 1.25 (t, $J = 7.2$ Hz, 3H).

5,7-Dimethoxy-3-(5-methoxypyridin-2-yl)quinazoline-4(3*H*)-thione (8bf): LC/MS (ESI) $(M + H)^+ = 330.1$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.23 (d, $J = 8.8$ Hz, 2H), 7.04−6.95 (m, 4H), 3.94 (s, 3H), 3.88 (s, 3H), 3.65 (q, $J = 7.4$ Hz, 2H), 1.29 (t, $J = 7.4$ Hz, 3H).

5,7-Dihydroxy-3-(5-hydroxypyridin-2-yl)quinazoline-4(3*H*)-thione (1bf): LC/MS (ESI) $(M + H)^+ = 287.04$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.23 (d, $J = 8.8$ Hz, 2H), 7.04−6.95 (m, 4H), 3.94 (s, 3H), 3.88 (s, 3H), 3.65 (q, $J = 7.4$ Hz, 2H), 1.29 (t, $J = 7.4$ Hz, 3H).

5,7-Dihydroxy-3-(4-hydroxyphenyl)-4*H*-chromene-4-thione (13). To a solution of biocanin A (500 mg, 1.89 mmol) in 5 mL of pyridine were added 2 mg (0.016 mmol) of DMAP and 357 μL of Ac_2O (3.78 mmol). The mixture was stirred at room temperature for 5 h. The volatiles were evaporated, the residue was dissolved in 5 mL of EtOAc, and the solution was washed with water (2 ×

3 mL) and then with 1 N HCl (3 mL), dried, and evaporated to yield 650 mg (93%) of the acetic acid 7-acetoxy-3-(4-methoxyphenyl)-4-oxo-4*H*-chromen-5-yl ester, which was used in the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.4 (d, J = 8.8 Hz, 2H), 7.24 (d, J = 2.2 Hz, 1H) 6.96 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 2.2 Hz, 1H), 3.83 (s, 3H), 2.42 (s, 3H), 2.35 (s, 3H).

A mixture of 370 mg (1.09 mmol) of acetic acid 7-acetoxy-3-(4-methoxyphenyl)-4-oxo-4*H*-chromen-5-yl ester and 370 mg (1.66 mmol) of P_2S_5 in 5 mL of toluene was refluxed for 16 h and then concentrated in vacuo. The residue was purified by flash chromatography on silica gel (60% EtOAc/hexane) to give 650 mg (93%) of a yellow solid.

To a cooled solution (0 °C) of the above compound (50 mg, 0.166 mmol) in 1 mL of CH_2Cl_2 was added 0.5 mL of BBr_3. The reaction mixture was stirred at room temperature overnight, cooled to −70 °C, and quenched with 1 mL of MeOH. The mixture was then evaporated to dryness, 2 mL of AcOEt was added, and the solution was washed with saturated $NaHCO_3$ solution and brine, dried, and evaporated. Further purification by preparative HPLC yielded a yellow solid: $C_{15}H_{10}O_4S$; LC/MS (ESI) $(M + H)^+$ = 286.03; mp 245−248 °C; ^{1}H NMR (500 MHz, $MeOD_4$) δ 7.75 (s, 1H), 7.17 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.8 Hz, 2H), 6.36 (d, J = 2.6 Hz, 1H), 6.29 (d, J = 2.6 Hz, 1H).

Biological Assays. Estrogen Receptor Binding Assay. A fusion protein, expressed in *Escherichia coli*, consisted of maltose binding protein (MBP), a specific biotinylation sequence (BioP) that is a substrate for biotin ligase, an enterokinase cleavage site (EK), and the ligand binding domain of ERβ (ERβ-LBD) or ERα (ERα-LBD). ERβ-LBD and ERα-LBD were purified by affinity chromatography on an amylose column and biotinylated with biotin ligase. For competition binding studies, purified biotinylated ERα-LBD (biotin−ERα-LBD) and ERβ-LBD (biotin−ERβ-LBD) were used. The assay was performed in 100 μL of 25 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl, 1 mM EDTA, and 1 mM dithiotheitol in a 96-well white OptiPlate (Packard).

Biotin−ERβ-LBD (40 nM) was incubated with 22 nM [^{3}H]-estrone and 75 μg of streptavidin SPA bead (Amersham), or biotin−ERα-LBD (25 nM) was incubated with 11 nM [^{3}H]estrone and 50 μg of streptavidin SPA bead (Amersham), and to both were added increasing concentrations of compounds for 2 h at room temperature with gentle shaking. Total binding in the absence of compounds and nonspecific binding in the presence of 1 μM estradiol were determined. After 2 h, the plate was counted in a TopCount (Perkin-Elmer). All the compounds tested were dissolved in DMSO to 10 mM concentration, diluted in 100% DMSO, with 10× intermediate dilution in assay buffer. The final concentration of DMSO in the reaction was 1%. The binding IC_{50} value (the concentration of compound required for 50% inhibition of [^{3}H]estrone binding to ERβ-LBD or ERα-LBD) was calculated using XL-fit one-site dose response.

Transactivation Assay. To determine the agonist activity of the compounds on ERα and ERβ receptors, the full length receptors were stably expressed under ERE promoter with luciferase as reporter in Hela cells. The clones were screened and the best responders with estradiol were selected. Cells were cultured in DMEM [medium with high glucose containing nonessential amino acids, sodium pyruvate, antibiotic antimycotic solution, 5% heat-inactivated FBS, 0.3 μg/mL of puromycin, and 1 mg/mL geneticin (GIBCO)] to 75% confluency. Cells (20 000 cells/well) were plated into tissue culture (TC) treated 96-well white plates (Perkin-Elmer) in phenol-red-free medium, without antibiotics, and returned to the TC incubator. After overnight incubation, the medium was aspirated, and increasing concentrations of compounds were added in PBS supplemented with 0.5% BSA and 10 mM glucose to the cells, which were incubated at 37 °C in the TC incubator overnight. The medium was aspirated and the luciferase induced was measured using LucScreen assay kit (Tropix). The signal was read in a TopCount (Perkin-Elmer). The transactivation EC_{50} (the concentration of compound required to achieve 50% of transactivation caused by 10 nM estradiol) was calculated using XL-fit one-site dose response.

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Supporting Information Available: Analytical and spectral data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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