Antitumor Benzothiazoles. 26.[1] 2-(3,4-Dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, NSC 721648), a Simple Fluorinated 2-Arylbenzothiazole, Shows Potent and Selective Inhibitory Activity against Lung, Colon, and Breast Cancer Cell Lines

Catriona G. Mortimer, Geoffrey Wells, Jean-Philippe Crochard, Erica L. Stone, Tracey D. Bradshaw, Malcolm F. G. Stevens, and Andrew D. Westwell*

Cancer Research UK Experimental Cancer Chemotherapy Research Group, Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD U.K.

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A series of new 2-phenylbenzothiazoles has been synthesized on the basis of the discovery of the potent and selective in vitro antitumor properties of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (**8n**; GW 610, NSC 721648). Synthesis of analogues substituted in the benzothiazole ring was achieved via the reaction of *o*-aminothiophenol disulfides with substituted benzaldehydes under reducing conditions. Compounds were evaluated in vitro in four human cancer cell lines, and compound **8n** was found to possess exquisitely potent antiproliferative activity (GI_{50} < 0.1 nM for MCF-7 and MDA 468). Potent and selective activity was also observed in the NCI 60 human cancer cell line panel. Structure−activity relationships established that the compound **8n** stands on a pinnacle of potent activity, with most structural variations having a deactivating in vitro effect. Mechanistically, this new series of agents contrasts with the previously reported 2-(4-aminophenyl)benzothiazoles; compound **8n** is not reliant on induction of CYP1A1 expression for antitumor activity.

Introduction

Taking inspiration from a crystallographic analysis of 5,6-dimethoxy-2-(4-methoxyphenyl)benzothiazole[2] and comparisons with the structures of the bioactive flavone quercetin and isoflavone genistein, we reasoned that planar polyhydroxylated 2-phenylbenzothiazoles might mimic the ATP antagonistic effects of the natural products and have tyrosine kinase-inhibitory properties.[3] Although this aspiration was not realized, unexpected spin-offs from this work revealed structurally related benzothiazoles (Figure 1) with markedly different biological profiles. Thus, the planar arylamine 2-(4-amino-3-methylphenyl)benzothiazole (**1**; DF 203) and the 5-fluoro analogue (**2**; 5F 203) are potent ligands for the arylhydrocarbon receptor (AhR) which translocates with the drug to cell nuclei.[4] Therein the cytochrome P450 CYP1A1 is induced which subsequently leads to the generation of a reactive chemical intermediate (or intermediates) that selectively generates DNA adducts only in sensitive tumor types (e.g. mammary and ovarian tumor cell lines).[5]

A further class of antitumor benzothiazole was obtained originally from the oxidation of 2-(4-hydroxyphenyl)benzothiazole with hypervalent iodine oxidants.[6] The product, a benzothiazole-substituted 4-hydroxycyclohexadieneone (**3**; AW 464), is the prototype of a new series of "quinols" with potent antitumor activity against renal and colon cancer cell lines that affects cell-signaling events downstream of the redox regulatory protein thioredoxin.[7]

As exemplified above, exploiting our "chemistry-driven" approach to anticancer drug discovery,[8] we have struck a rich lode of novel bioactive agents and now report the synthesis and properties of a further new, but different, series of simple 2-phenylbenzothiazoles bearing oxygenated substituents in the phenyl moiety. Remarkably, among an extended library of very close structural analogues, only a compound with a 2-(3,4-dimethoxyphenyl) group and a fluoro substituent in the benzothiazole ring, especially in the 5-position, is associated with exquisite bioactivity. We have studied the biological activity of the most potent compound in the new series (**8n**; GW 610, NSC 721648) and compared its properties with those of the previously investigated 2-(4-aminophenyl)benzothiazoles (**1** and **2**). The arylamine (**2**) is the active moiety derived from the investigational prodrug Phortress (**4**),[9] which has potent activity against human mammary tumor xenografts[10] and is currently in phase I clinical trial in the UK.

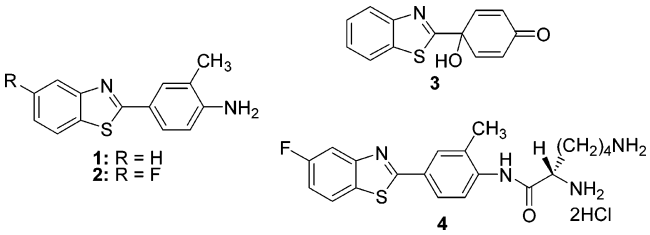

Figure 1. Chemical structures of antitumor benzothiazoles **1**−**4**.

Chemistry

2-Phenylbenzothiazoles unsubstituted in the benzo ring were obtained by condensation of commercially available 2-aminothiophenol (**5**) and benzaldehydes (**6a**−**c**) in refluxing ethanol (method A); the 2,3-dihydrobenzothiazole intermediates (**7a**−**c**) undergo oxidation to the aromatic products (**8a**−**c**) followed by recrystallization of **8a**−**c** from ethanol (Scheme 1). The starting materials for the synthesis of compounds substituted in the benzothiazole moiety (**8d**−**w**) were 2-aminobenzothiazoles (**9**) using a method developed initially by Chang[11] and previously modified by ourselves.[12] For example, to achieve access to 5-halo-substituted 2-arylbenzothiazoles (**8d**−**v**), 2-amino-5-halobenzothiazoles (**9**; R = 5-F, 5-Cl) were converted to the

* To whom correspondence should be addressed. Phone: +44 115 9513419. Fax: +44 115 9513412. E-mail: andrew.westwell@nottingham.ac.uk.

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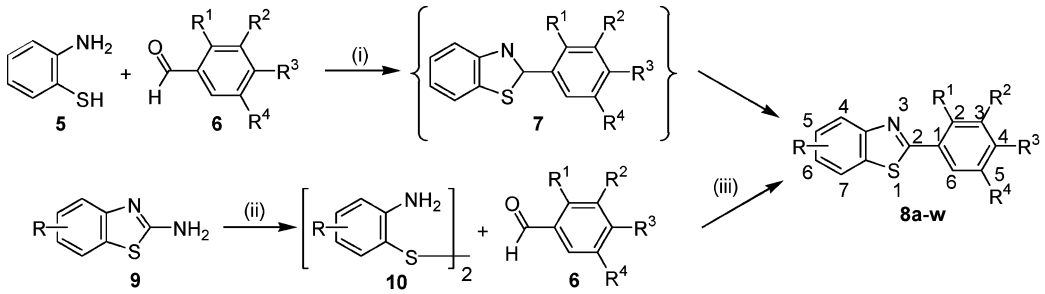
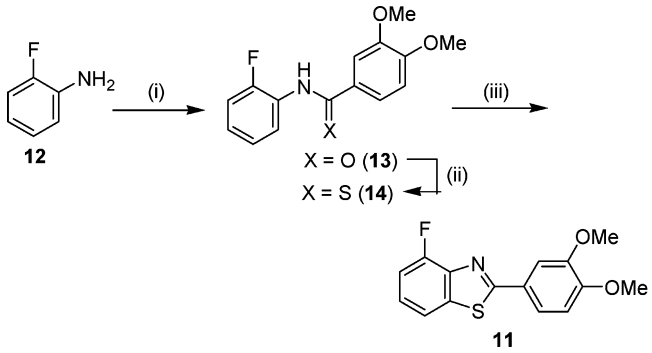
Scheme 1[a]



	R	R^1	R^2	R^3	R^4
a:	H	H	OH	OH	H
b:	H	H	O—CH_2—O		H
c:	H	H	OMe	OMe	H
d:	5-F	H	H	OMe	H
e:	5-F	H	Me	OMe	H
f:	5-F	H	F	OMe	H
g:	5-F	H	Cl	OMe	H
h:	5-F	H	Br	OMe	H
i:	5-F	H	I	OMe	H
j:	5-F	H	OH	OH	H
k:	5-F	H	O—CH_2—O		H
l:	5-F	H	OH	OMe	H
m:	5-F	H	OMe	OH	H
n:	5-F	H	OMe	OMe	H
o:	5-F	H	OMe	H	OMe
p:	5-F	H	OMe	OMe	OMe
q:	5-F	OMe	OMe	OMe	H
r:	5-F	H	OMe	OMe	OH
s:	5-F	H	OMe	OMOM	H
t:	5-F	H	OEt	OMe	H
u:	5-F	H	OEt	OEt	H
v:	5-Cl	H	OMe	OMe	H
w:	6-F	H	OMe	OMe	H

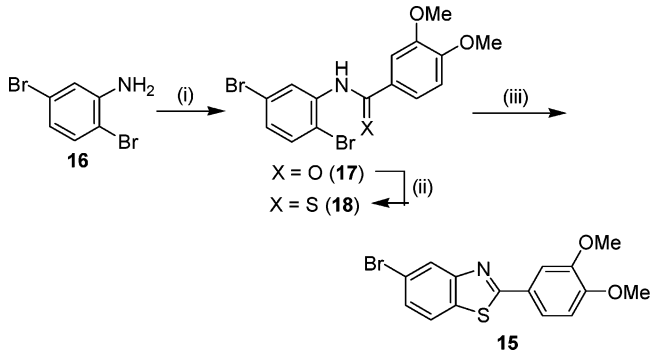
[a] Reagents: (i) EtOH, reflux; (ii) KOH (aq), reflux; (iii) PPh_3, *p*-TsOH, toluene, reflux.

Scheme 2[a]



[a] Reagents: (i) 3,4-dimethoxybenzoyl chloride, pyridine, reflux; (ii) P_4S_{10}, hexamethyldisiloxane (HMDO), toluene, reflux; (iii) $K_3Fe(CN)_6$, NaOH, 95 °C.

Scheme 3[a]



[a] Reagents: (i) 3,4-dimethoxybenzoyl chloride, pyridine, reflux; (ii) P_4S_{10}, hexamethyldisiloxane (HMDO), toluene, reflux; (iii) NaH, NMP 140 °C.

corresponding bis(2-amino-4-halophenyl) disulfides (**10**; R = 4-F, 4-Cl) by treatment with aqueous potassium hydroxide followed by acidification and air oxidation. The disulfides were then reacted with a substituted benzaldehyde and triphenylphosphine in refluxing toluene containing catalytic *p*-toluenesulfonic acid (method B, Scheme 1). Products were purified by column chromatography to remove triphenylphosphine oxide and were obtained in moderate to good yields. The corresponding 6-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (**8w**) was prepared similarly, starting from commercially available 2-amino-6-fluorobenzothiazole (**9**; R = 6-F).

The preparation of 2-(3,4-dimethoxyphenyl)-4-fluorobenzothiazole (**11**) was achieved (Scheme 2) by an alternative methodology through the well-established Jacobson thioanilide radical cyclization chemistry[12] previously used extensively for the synthesis of a range of 2-phenylbenzothiazoles within our group.[13,14] Thus, reaction of 2-fluoroaniline (**12**) with 3,4-dimethoxybenzoyl chloride in pyridine gave the benzanilide (**13**), which was converted to the corresponding thiobenzanilide (**14**) using phosphorus pentasulfide. Cyclization to the 4-fluorobenzothiazole **11** was accomplished using potassium ferricyanide and aqueous sodium hydroxide as previously described.

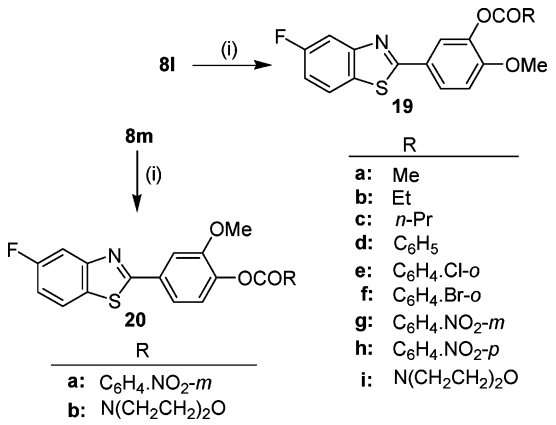
The regiospecific formation of the corresponding 5-bromobenzothiazole (**15**) was effected by a nucleophilic ring closure reaction using a bromo substituent ortho to the anilino nitrogen to direct the cyclization,[15] methodology previously described for the corresponding 2-(4-aminophenyl)benzothiazoles.[13] In this case, the starting 2,5-dibromoaniline (**16**) was reacted with 3,4-dimethoxybenzoyl chloride and the resulting benzanilide (**17**) converted to the corresponding thiobenzanilide (**18**). Regiospecific cyclization using sodium hydride in hot NMP then led to the formation of the required 5-bromobenzothiazole (**15**) (Scheme 3).

Isomeric phenols (**8l** and **8m**) were converted to a series of esters (**19a**−**i** and **20a,b** respectively) by reaction with aliphatic, aromatic, and heteroalicyclic acid chlorides in pyridine at room temperature or in refluxing dichloromethane containing triethylamine−DMAP (Scheme 4).

Biological Results and Discussion

A range of compounds were evaluated in MTT assays following 3-day exposure against a panel of two human breast cancer cell lines, MCF-7 (ER+) and MDA 468 (ER−), and two colon cancer cell lines, KM12 and HCC 2998. In general, the breast cell lines were more sensitive to the agents, and the MDA 468 line was the most sensitive of the four lines (Table 1). The benchmark active compound was 5-fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (**8n**) with GI_{50} values < 0.1 nM against the two breast cell lines. Compounds with no substituent in the benzothiazole moiety (**8a**−**c**) retained sub-micromolar activity against MDA 468 but were relatively inactive against the MCF-7, KM12, and HCC 2998 lines (GI_{50} values > 2 μM).

Scheme 4[a]



[a] Reagents: (i) RCOCl, pyridine, 20 °C; or RCOCl, NEt_3, DMAP, CH_2Cl_2, reflux (see Experimental Section for details of method used).

Table 1. Activity of Benzothiazoles against Human Breast and Colon Cancer Cell Lines[a]

	GI_{50} values (μM)[b] in cell lines[c]			
compd	MCF-7	MDA 468	KM 12	HCC 2998
1	<0.0001	<0.0001	>100	>100
2	<0.0001	<0.0001	>100	>100
8a	2.19	0.22	15.58	6.99
8b	57.8	0.80	98.6	68.3
8c	52.7	0.53	74.2	42.5
8d	0.49	0.33	24.6	12.4
8e	0.048	0.058	18.6	0.54
8f	0.87	0.64	20.6	13.7
8g	0.76	0.48	7.05	2.5
8h	0.85	0.42	6.7	3.45
8i	0.82	0.36	6.9	0.94
8j	0.72	0.19	13.00	1.64
8k	20.7	0.57	25.1	22.3
8l	0.5	0.05	18.4	7.6
8m	0.75	0.17	47.33	1.04
8n	<0.0001	<0.0001	0.29	0.00025
8o	1.23	0.31	7.55	5.21
8p	0.0019	0.0021	12.95	21.1
8q	0.023	0.12	14.18	0.037
8r	0.653	0.088	47.47	6.49
8s	0.065	0.048	19.25	53.5
8t	0.080	0.092	5.42	3.32
8u	0.0007	0.055	38.5	NT
8v	27.92	1.00	12.4	4.88
8w	0.062	0.005	24.07	6.21
11	0.005	0.005	0.85	1.06
15	13.6	0.77	65.15	20.0

[a] Determined by MTT assay; see Biology section for details. [b] Compounds tested in triplicate. [c] Cancer cell line origin: MCF-7 (breast), MDA 468 (breast), KM 12 (colon), HCC 2998 (colon). NT = not tested.

Introduction of a 5-fluoro group generally enhanced potency: compounds with a single methoxy group such as 5-fluoro-2-(4-methoxyphenyl)benzothiazole (**8d**), and other analogues with a 4′-methoxyphenyl group (**8e**−**i**) bearing a methyl or halogen replacement for methoxy in the 3′-position were comparable in activity; 5-fluoro-2-(4-methoxy-3-methylphenyl)benzothiazole (**8e**) was the most potent with GI_{50} values 0.048 and 0.058 μM against MCF-7 and MDA 468 cell lines, respectively.

The most interesting series of compounds were those combining a 5-fluoro group with *two* oxygenated substituents in the 3′,4′-positions, where minor, seemingly conservative, structural changes were associated with dramatic variations in activity (when compared to **8n**). Thus, replacement of either (or both) of the methoxy groups by hydroxyl (**8j,l,m**), methylenedioxy (**8k**), methoxymethyleneoxy (MOM; **8s**), or ethoxy substituents (**8t,u**) had a deactivating effect. Similarly, the analogue with a

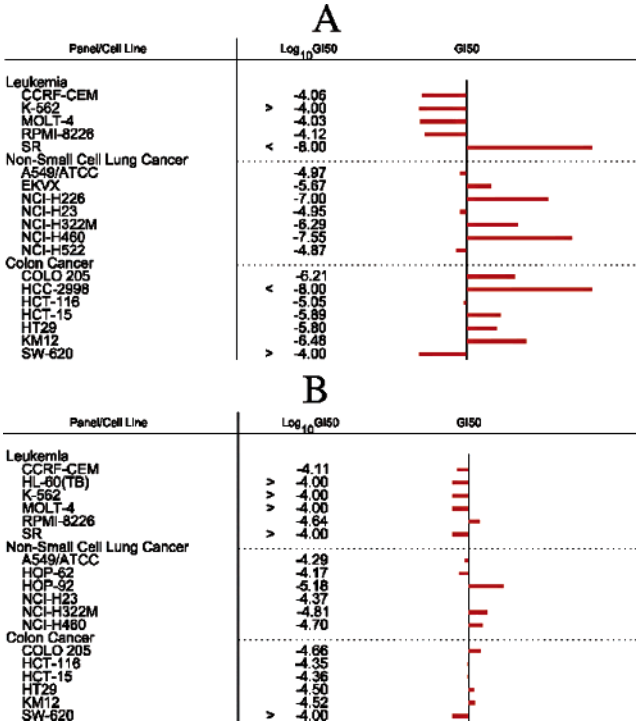


Figure 2. Selected NCI sixty cell screening data highlighting dramatic differences in potency in the non-small-cell lung and colon cancer cell lines for compounds **8n** (A) and its inactive nonfluorinated counterpart **8c** (B). log GI_{50} values for each of the listed cell lines are given; bars to the right of the mean GI_{50} line represent cell lines more sensitive to test compound compared to the mean, whereas bars to the left represent less sensitive cell lines (on a logarithmic scale).

3′,5′-disposition of methoxy groups (**8o**) had only low micromolar inhibitory potency, whereas the 3′,4′,5′-trimethoxy congener (**8p**) retained nanomolar inhibitory potency against the MCF-7 cell and MDA 468 cell lines. The other fluorinated benzothiazoles trisubstituted on the phenyl ring (**8q,r**) were also markedly less active compared to the lead compound **8n**. Of this group of compounds the diethoxy analogue (**8u**) was the most potent against the MCF-7 cell line (GI_{50} value 0.7 nM). Surprisingly, replacement of the 5-fluoro group of **8n** with a 5-chloro (**8v**) or 5-bromo substituent (**15**) essentially abolished inhibitory potency, whereas the 6- (**8w**) and 4-fluoro regioisomer (**11**) retained nanomolar inhibitory activity against one or more of the cell lines. In summary, this in vitro screen led to the identification of the fluorinated 2-(3,4-dimethoxyphenyl)benzothiazole structure as a novel antitumor pharmacophore, with the 5-fluoro analogue (**8n**) being the agent of choice for further pharmacological evaluation.

All of the ester analogues **19** and **20** were found to be markedly less active in the cancer cell lines tested when compared to the lead compound **8n**. Although low to submicromolar GI_{50} values were observed for some analogues against the MCF-7 cell line, activity in the other cell lines tested was found to be weak, with $GI_{50} > 10$ μM in many cases (data not shown).

Results from the more extensive NCI in vitro 60 human cancer cell panel[16] corroborated the results described above. The mean GI_{50} values across the range of compounds, including the two series of esters (**19** and **20**) are an unremarkable 10−100 μM (data not shown); however, these values disguise significant cell selectivity. For example, compound **8n** has exquisite activity in the colon and NSCLC subpanels (Figure 2A). GI_{50} values <10 nM for **8n** are reached in some cell lines, such as the HCC 2998 colon and leukemic SR lines with similar activity in the

NSCLC subpanel: the essential requirement for a fluoro group was confirmed in the attenuated pattern of activity of 2-(3,4-dimethoxyphenyl)benzothiazole (**8c**) (Figure 2B). This result contrasts with our earlier experience in the related 2-(4-aminophenyl)benzothiazole series,[4,5,13−15] where the characteristic cell line selectivity of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (**2**) is also displayed by its nonfluorinated counterpart (**1**). The mean GI_{50} graphs of the 4-fluoro- (**11**) and 6-fluoro-2-(3,4-dimethoxyphenyl)benzothiazoles (**8w**) showed potent (but reduced compared to **8n**) activity in the colon and NSCL subpanels. Notably, NCI mean graphs for the corresponding 5-chloro- and 5-bromobenzothiazole analogues (**8v** and **15** respectively) show poor selectivity and potency, with GI_{50} values in the micromolar region (data not shown). Full NCI mean GI_{50} graphs for compounds **1**, **2**, **8c**, **8n**, **8w**, and **11** are included in the Supporting Information.

At present, a definitive molecular target underpinning the antitumor activity of this intriguing series has not been identified, and explanation of the structure−activity relationships described above is not therefore possible. In this sense the identification of compound **8n** and analogues represents a drug discovery project in the best traditions of our chemistry-driven (as opposed to target-driven) appoach.[8]

In pursuit of (a) possible mechanism(s) of action of novel lead benzothiazole **8n**, the ability of this agent, and inactive or less active congeners, to induce CYP1A1 in sensitive and inherently resistant cell lines has been examined. It became evident that, like (aminophenyl)benzothiazole analogues,[17] **8n** is able to induce (in a dose-dependent manner) CYP1A1 protein expression in cell lines with inducible CYP1A1 (MCF-7 and MDA 468). However, the CYP1A1 inhibitor resveratrol was found not to affect the potency of **8n** in sensitive cell lines. Moreover, in sensitive colon cell lines (KM12 and HCC2998) CYP1A1 protein was neither constituitively expressed nor could it be induced by **8n**. Our observations have led us to conclude that while induced CYP1A1 may metabolize intracellular **8n**, the mechanism of action of this molecule is unlikely to be exclusively dependent upon CYP1A1-catalyzed bioactivation. An additional intriguing observation was that CYP1A1 protein was also expressed in MCF-7 and MDA 468 cells exposed to inactive analogues such as **8c** and **8k**.

In an attempt to understand the mechanistic relationship between (aminophenyl)benzothiazoles and (dimethoxyphenyl)-benzothiazole **8n**, cell lines were generated with acquired resistance to **1** and **8n**, respectively. Intriguingly, while breast cell lines with acquired resistance to **1** (MCF-7, MDA 468; $GI_{50} > 10\ \mu M$) retained sensitivity to **8n** ($GI_{50} < 1$ nM), breast cell lines initially sensitive to **1** (MCF-7, MDA 468; $GI_{50} <$ 1 nM) generated to acquire resistance to **8n** ($GI_{50} > 10\ \mu M$) were additionally cross resistant to **1** ($GI_{50} > 10\ \mu M$). Such an observation suggests that during acquirement of resistance to **8n**, mechanisms rendering cells sensitive to **1** are also disabled; in contrast, biochemical mechanisms of action or molecular target(s) of **8n** remain intact in breast cell lines with acquired resistance to **1**. Full details of our investigations into mechanisms of action of this intriguing molecule (**8n**) will be reported elsewhere.

Experimental Section

Chemistry. Melting points were measured on a Galenkamp apparatus and are uncorrected. IR spectra (as KBr disks) were recorded on a Perkin-Elmer Series 1 FT-IR spectrometer. Mass spectra were recorded on either a Micromass Platform spectrometer, an AEI MS-902 (nominal mass), or a VG Micromass 7070E or a Finigan MAT900XLT spectrometer (accurate mass). NMR spectra were recorded on either a Bruker AVANCE 400 MHz or Bruker ARX 250 instrument; coupling constants are in hertz. Merck silica gel 60 (40−60 μm) was used for column chromatography. All commercially available starting materials were used without further purification.

General Method (A) for the Synthesis of 2-Arylbenzothiazoles Unsubstituted on the Benzothiazole Ring. A mixture of 2-aminothiophenol (**5**; 100 mmol) and 3,4-disubstituted benzaldehyde (**6**; 100 mmol) in ethanol (150 mL) was heated at reflux for 2 h. After cooling to room temperature, the solution was concentrated in vacuo. The residue was partitioned between water and ethyl acetate (500 mL), and the aqueous layer was extracted using further ethyl acetate (2 × 500 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give the crude product, which was recrystallized from ethanol. The following compounds were prepared.

2-(3,4-Dihydroxyphenyl)benzothiazole (8a) was formed from 3,4-dihydroxybenzaldehyde (38% yield): mp 217 °C (lit.[18] mp 222 °C); IR ν_{max} 3491, 2702, 1601, 1439, 1277, 1182, 905, 756 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.63 (2H, brs, OH), 8.08 (1H, m, H-4 or H-7), 7.98 (1H, m, H-4 or H-7), 7.46 (4H, m, ArH), 6.91 (1H, d, $J = 8.2$ Hz, H-5′).

2-(3,4-Methylenedioxyphenyl)benzothiazole (8b)[19] was formed from 3,4-methylenedioxybenzaldehyde (37% yield): mp 133−135 °C; ^1H NMR ($CDCl_3$) δ 8.02 (1H, d, $J = 8.0$ Hz, H-7), 7.88 (1H, d, $J = 8.0$ Hz, H-4), 7.61 (2H, m, H-2′, H-6′), 7.48 (1H, dt, $J =$ 1.0, 8.7 Hz, ArH), 7.37 (1H, dt, $J = 0.8$, 8.7 Hz, ArH), 6.91 (1H, d, $J = 8.0$ Hz, H-5′), 6.06 (2H, s, OCH_2O). Anal. ($C_{14}H_9NO_2S$) C, H, N.

2-(3,4-Dimethoxyphenyl)benzothiazole (8c)[20] was formed from 3,4-dimethoxybenzaldehyde (50% yield): mp 141−143 °C; ^1H NMR ($CDCl_3$) δ 8.05 (1H, d, $J = 7.5$ Hz, H-7), 7.88 (1H, d, $J =$ 7.5 Hz, H-4), 7.72 (1H, d, $J = 2.3$ Hz, H-2′), 7.61 (1H, dd, $J =$ 2.3, 8.0 Hz, H-6′), 7.48 (1H, dt, $J = 2.5$, 7.5 Hz, ArH), 7.38 (1H, dt, $J = 2.7$, 7.5, ArH), 6.96 (1H, d, $J = 8.0$ Hz, H-5′), 4.03 (3H, s, OMe), 3.96 (3H, s, OMe). Anal. ($C_{15}H_{13}NO_2S$) C, H, N.

General Method (B) for the Synthesis of 2-Arylbenzothiazoles Substituted on the Benzothiazole Ring. Disubstituted benzaldehyde (**6**; 3.5 mmol), *p*-toluenesulfonic acid (0.35 mmol), and triphenylphosphine (1.75 mmol) were added to a solution of bis-(2-amino-4-fluorophenyl) disulfide (**10**; 1.75 mmol)[11,13] in toluene (20 mL). The reaction mixture was heated at reflux for 24 h, allowed to cool, and concentrated in vacuo. The crude product was purified by column chromatography (2% MeOH/DCM) to give the required substituted 2-arylbenzothiazole in yields 13−98%. The following compounds were prepared.

5-Fluoro-2-(4-methoxyphenyl)benzothiazole (8d) was formed from bis(2-amino-4-fluorophenyl) disulfide and 4-methoxybenzaldehyde (43% yield): mp 123−124 °C; ^1H NMR ($CDCl_3$) δ 8.03 (2H, d, $J = 8.9$ Hz, H-2′, H-6′), 7.80 (1H, dd, $J = 5.1$, 8.8 Hz, H-6), 7.72 (1H, dd, $J = 2.5$, 9.6 Hz, H-4), 7.14 (1H, dt, $J = 2.5$, 8.8 Hz, H-5), 7.01 (2H, d, $J = 8.9$ Hz, H-3′, H-5′), 3.90 (3H, s, OMe); *m*/*z* (CI) 261 ($M^+ + 1$). Anal. ($C_{14}H_{10}FNOS$) C, H, N.

5-Fluoro-2-(4-methoxy-3-methylphenyl)benzothiazole (8e) was formed from bis(2-amino-4-fluorophenyl) disulfide and 4-methoxy-3-methylbenzaldehyde (50% yield): mp 120 °C; IR ν_{max} 2851 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (2H, m, ArH), 7.76 (1H, dd, $J = 5.2$, 8.8 Hz, H-7), 7.71 (1H, dd, $J = 2.4$, 9.7 Hz, H-6′), 7.13 (1H, dt, $J =$ 2.5, 8.8 Hz, H-6), 6.88 (1H, d, $J = 9.2$ Hz, H-5′), 3.89 (3H, s, OCH_3), 2.29 (3H, s, CH_3); *m*/*z* (CI) 274 ($M^+ + 1$). Anal. ($C_{15}H_{12}$-FNOS) C, H, N.

5-Fluoro-2-(3-fluoro-4-methoxyphenyl)benzothiazole (8f) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3-fluoro-4-methoxybenzaldehyde (65% yield): mp 157−159 °C; ^1H NMR ($CDCl_3$) δ 7.84 (3H, m, H-2′, H-6′, H-7), 7.73 (1H, dd, $J = 2.5$, 9.1 Hz, H-4), 7.17 (1H, dt, $J = 2.5$, 9.1 Hz, H-6), 7.07 (1H, t, $J =$ 8.5 Hz, H-5′), 3.99 (3H, s, OMe); *m*/*z* (CI) 278 ($M^+ + 1$). Anal. ($C_{14}H_9F_2NOS$) C, H, N.

2-(3-Chloro-4-methoxyphenyl)-5-fluorobenzothiazole (8g) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3-chloro-4-methoxybenzaldehyde (64% yield): mp 160−161 °C; ^1H NMR

($CDCl_3$) δ 8.13 (1H, d, J = 2.2 Hz, H-2′), 7.94 (1H, dd, J = 2.2, 8.6 Hz, H-6′), 7.81 (1H, dd, J = 5.1, 8.8 Hz, H-7), 7.72 (1H, dd, J = 2.5, 9.5 Hz, H-4), 7.16 (1H, dt, J = 2.5, 8.8 Hz, H-6), 7.02 (1H, d, J = 8.6 Hz, H-5′), 3.99 (3H, s, OMe); *m/z* (CI) 294 ($M^+ + 1$). Anal. ($C_{14}H_9ClFNOS$) C, H, N.

2-(3-Bromo-4-methoxyphenyl)-5-fluorobenzothiazole (8h) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3-bromo-4-methoxybenzaldehyde (46% yield): mp 173−175 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (1H, d, J = 2.2 Hz, H-2′), 7.99 (1H, dd, J = 2.2, 8.6 Hz, H-6′), 7.81 (1H, dd, J = 5.1, 8.8 Hz, H-7), 7.72 (1H, dd, J = 2.5, 9.5 Hz, H-4), 7.16 (1H, dt, J = 2.5, 8.8 Hz, H-6), 7.00 (1H, d, J = 8.6 Hz, H-5′), 3.99 (3H, s, OMe); *m/z* (CI) 338 ($M^+ + 1$). Anal. ($C_{14}H_9BrFNOS$) C, H, N.

5-Fluoro-2-(3-iodo-4-methoxyphenyl)benzothiazole (8i) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3-iodo-4-methoxybenzaldehyde (56% yield): mp 167 °C; ^{1}H NMR ($CDCl_3$) δ 8.52 (1H, d, J = 2.2 Hz, H-2′), 8.03 (1H, dd, J = 2.2, 8.6 Hz, H-6′), 7.82 (1H, dd, J = 5.1, 8.8 Hz, H-7), 7.72 (1H, dd, J = 2.5, 9.5 Hz, H-4), 7.16 (1H, dt, J = 2.5, 8.8 Hz, H-6), 6.92 (1H, d, J = 8.6 Hz, H-5′), 3.98 (3H, s, OMe); *m/z* (CI) 386 ($M^+ + 1$). Anal. ($C_{14}H_9FINOS$) C, H, N.

5-Fluoro-2-(3,4-dihydroxyphenyl)benzothiazole (8j) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3,4-dihydroxybenzaldehyde (13% yield): mp 237−239 °C; IR 3300 (O−H) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.66 (2H, br s, 3′-OH, 4′-OH), 8.11 (1H, dd, J = 5.4, 8.9 Hz, H-7), 7.82 (1H, dd, J = 2.5, 9.9 Hz, H-4), 7.54 (1H, d, J = 2.2 Hz, H-2′), 7.41 (1H, dd, J = 2.2, 8.2 Hz, H-6′), 7.31 (1H, dt, J = 2.5, 8.9 Hz, H-6), 6.91 (1H, d, J = 8.2 Hz, H-5′); *m/z* (CI) 262 ($M^+ + 1$). Anal. ($C_{13}H_8FNO_2S \cdot 2H_2O$) C, H, N.

5-Fluoro-2-(3,4-methylenedioxyphenyl)benzothiazole (8k) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3,4-methylenedioxybenzaldehyde (78% yield): mp 161−164 °C; ^{1}H NMR ($CDCl_3$) δ 7.78 (1H, dd, J = 5.2, 8.8 Hz, H-7), 7.69 (1H, dd, J = 2.0, 9.6 Hz, H-6′), 7.57 (2H, m, H-2′, H-4), 7.13 (1H, dt, J = 2.4, 8.8 Hz, H-6), 6.90 (1H, d, J = 7.6 Hz, H-5′), 6.06 (2H, s, OCH_2O); *m/z* (CI) 274 ($M^+ + 1$). Anal. ($C_{14}H_8FNO_2S$) C, H, N.

5-Fluoro-2-(3-hydroxy-4-methoxyphenyl)benzothiazole (8l) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3-hydroxy-4-methoxybenzaldehyde (92% yield): mp 169−172 °C; IR ν_{max} 2577, 1601, 1568, 1481, 1252, 1148, 963, 851 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.57 (1H, brs, OH), 8.15 (1H, dd, J = 5.4, 8.8 Hz, H-4), 7.86 (1H, dd, J = 2.2, 9.7 Hz, H-7), 7.54 (2H, m, H-2′, H-6′), 7.34 (1H, td, J = 2.5, 9.1 Hz, H-6), 7.10 (1H, d, J = 8.0 Hz, H-5′), 3.88 (3H, s, OMe); *m/z* (CI) 276 ($M^+ + 1$). Anal. ($C_{14}H_{10}NO_2SF \cdot {}^1/_2H_2O$) C, H, N.

5-Fluoro-2-(4-hydroxy-3-methoxyphenyl)benzothiazole (8m) was formed from bis(2-amino-4-fluorophenyl) disulfide and 4-hydroxy-3-methoxybenzaldehyde (88% yield): mp 156 °C; IR ν_{max} 1609, 1591, 1476, 1451, 1252, 1182, 963, 860 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.94 (1H, brs, OH), 8.14 (1H, dd, J = 5.3, 8.8 Hz, H-4), 7.86 (1H, dd, J = 2.4, 10.0 Hz, H-7), 7.63 (1H, d, J = 2.0 Hz, H-2′), 7.52 (1H, dd, J = 2.0, 8.2 Hz, H-6′), 7.33 (1H, td, J = 2.5, 9.1 Hz, H-6), 6.96 (1H, d, J = 8.2 Hz, H-5′), 3.91 (3H, s, OMe); *m/z* (CI) 276 ($M^+ + 1$). Anal. ($C_{14}H_{10}NO_2SF \cdot {}^1/_4H_2O$) C, H, N.

5-Fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (8n) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3,4-dimethoxybenzaldehyde (88% yield): mp 110 °C; IR ν_{max} 1597, 1485, 1443, 1269, 1140, 1121, 959, 843 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.17 (1H, dd, J = 5.4, 8.8 Hz, H-4), 7.89 (1H, dd, J = 2.5, 10.0 Hz, H-7), 7.64 (2H, m, H-2′,6′), 7.37 (1H, td, J = 2.5, 9.1 Hz, H-6), 7.15 (1H, d, J = 9.0 Hz, H-5′), 3.91 (3H, s, OMe), 3.88 (3H, s, OMe); *m/z* (CI) 290 ($M^+ + 1$). Anal. ($C_{15}H_{12}NO_2SF$) C, H, N.

5-Fluoro-2-(3,5-dimethoxyphenyl)benzothiazole (8o) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3,5-dimethoxybenzaldehyde (75% yield): mp 115 °C; ^{1}H NMR ($CDCl_3$) δ 7.82 (1H, dd, J = 5.1, 8.8 Hz, H-7), 7.75 (1H, dd, J = 2.4, 9.5 Hz, H-4), 7.24 (2H, d, J = 2.3 Hz, H-2′, H-6′), 7.17 (1H, dt, J = 2.5, 8.8 Hz, H-6), 6.61 (1H, t, J 2.3 Hz, H-4′), 3.90 (6H, s, 3′-OMe, 5′-OMe); IR ν_{max} 1601, 1458, 1350, 1273, 1126, 1065, 966, 820 cm^{-1}; *m/z* (CI) 290 ($M^+ + 1$). Anal. ($C_{15}H_{12}NO_2SF \cdot H_2O$) C, H, N.

5-Fluoro-2-(3,4,5-trimethoxyphenyl)benzothiazole (8p) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3,4,5-trimethoxybenzaldehyde (77% yield): mp 120−122 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (1H, dd, J = 5.1, 8.8 Hz, H-7), 7.75 (1H, dd, J = 2.5, 9.6 Hz, H-4), 7.32 (2H, s, H-2′, H-6′), 7.17 (1H, dt, J = 2.5, 8.8 Hz, H-6), 4.00 (6H, s, 3′-OMe, 5′-OMe), 3.94 (3H, s, 4′-OMe); *m/z* (CI) 320 ($M^+ + 1$). Anal. ($C_{16}H_{14}FNO_3S$) C, H, N.

5-Fluoro-2-(2,3,4-trimethoxyphenyl)benzothiazole (8q) was formed from bis(2-amino-4-fluorophenyl) disulfide and 2,3,4-trimethoxybenzaldehyde (91% yield): mp 109−110 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (2H, m, H-7, H-6′), 7.86 (1H, dd, J = 2.5, 10.0 Hz, H-4), 7.34 (1H, dt, J = 2.5, 9.0 Hz, H-6), 7.08 (1H, d, J = 9.1 Hz, H-5′), 4.04 (3H, s, OMe), 3.95 (3H, s, OMe), 3.86 (3H, s, OMe); *m/z* (CI) 320 ($M^+ + 1$). Anal. ($C_{16}H_{14}FNO_3S$) C, H, N.

2-(3,4-Dimethoxy-5-hydroxyphenyl)-5-fluorobenzothiazole (8r) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3,4-dimethoxy-5-hydroxybenzaldehyde (52% yield): mp 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 9.83 (1H, brs, OH), 8.18 (1H, dd, J = 5.3, 8.8 Hz, H-7), 7.91 (1H, dd, J = 2.5, 9.9 Hz, H-4), 7.38 (1H, dt, J = 2.6, 9.1 Hz, H-6), 7.22 (2H, m, H-2′, H-6′), 3.91 (3H, s, OMe), 3.78 (3H, s, OMe); *m/z* (CI) 306 ($M^+ + 1$). Anal. ($C_{15}H_{12}FNO_3S$) C, H, N.

5-Fluoro-2-(3-methoxy-4-methoxymethyloxyphenyl)benzothiazole (8s) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3-methoxy-4-methoxymethyloxybenzaldehyde (35% yield): mp 101−102 °C; ^{1}H NMR (DMSO-d_6) δ 8.18 (1H, dd, J = 5.4, 8.8 Hz, H-7), 7.91 (1H, dd, J = 2.5, 9.9 Hz, H-4), 7.69 (1H, d, J = 2.1 Hz, H-2′), 7.62 (1H, dd, J = 2.1, 8.4 Hz, H-6′), 7.37 (1H, dt, J = 2.5, 9.0 Hz, H-6), 7.26 (1H, d, J = 8.4 Hz, H-5′), 5.30 (2H, s, OCH_2OCH_3), 3.93 (3H, s, OCH_3), 3.43 (3H, s, OCH_2OCH_3); *m/z* (CI) 320 ($M^+ + 1$). Anal. ($C_{16}H_{14}FNO_3S$) C, H, N.

2-(3-Ethoxy-4-methoxyphenyl)-5-fluorobenzothiazole (8t) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3-ethoxy-4-methoxybenzaldehyde (41% yield): mp 136−139 °C; ^{1}H NMR ($CDCl_3$) δ 7.82 (1H, dd, J = 5.1, 8.8 Hz, H-7), 7.73 (2H, m, H-2′, H-4), 7.59 (1H, dd, J = 2.1, 8.4 Hz, H-6′), 7.16 (1H, dt, J = 2.5, 8.8 Hz, H-6), 6.97 (1H, d, J = 8.4 Hz, H-5′), 4.21 (2H, q, J = 7.0 Hz, OCH_2CH_3), 4.04 (3H, s, OCH_3), 1.55 (3H, t, J = 7.0 Hz, OCH_2CH_3); *m/z* (CI) 304 ($M^+ + 1$). Anal. ($C_{16}H_{14}FNO_2S$) C, H, N.

2-(3,4-Diethoxyphenyl)-5-fluorobenzothiazole (8u) was formed from bis(2-amino-4-fluorophenyl) disulfide and 3,4-diethoxybenzaldehyde (59% yield): mp 109−113 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, dd, J = 5.1, 8.7 Hz, H-7), 7.71 (2H, m, H-2′, H-4), 7.57 (1H, dd, J = 2.1, 8.4 Hz, H-6′), 7.14 (1H, dt, J = 2.5, 8.8 Hz, H-6), 6.95 (1H, d, J = 8.4 Hz, H-5′), 4.22 (4H, m, 3′-OCH_2CH_3, 4′-OCH_2CH_3), 1.53 (6H, m, 3′-OCH_2CH_3, 4′-OCH_2CH_3); *m/z* (CI) 318 ($M^+ + 1$). Anal. ($C_{17}H_{16}FNO_2S$) C, H, N.

5-Chloro-2-(3,4-dimethoxyphenyl)benzothiazole (8v) was formed from bis(2-amino-4-chlorophenyl) disulfide[11,21] and 3,4 dimethoxybenzaldehyde (65% yield): mp 154−155 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (1H, d, J = 2.1 Hz, H-4), 7.71 (1H, d, J = 8.3 Hz, H-7), 7.68 (1H, d, J = 2.3 Hz, H-2′), 7.59 (1H, dd, J = 2.3, 8.4 Hz, H-6′), 7.39 (1H, dd, J = 8.3, 2.1 Hz, H-6), 6.97 (1H, d, J = 8.4 Hz, H-5′), 4.01 (3H, s, OMe), 3.98 (3H, s, OMe); *m/z* (CI) 307 ($M^+ + 1$). Anal. ($C_{15}H_{12}ClNO_2S$) C, H, N.

6-Fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (8w) was formed from bis(2-amino-5-fluorophenyl) disulfide and 3,4-dimethoxybenzaldehyde (98% yield): mp 153−155 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (1H, dd, J = 8.8, 4.8 Hz, H-4), 7.64 (1H, d, J = 2.3 Hz, H-2′), 7.51 (1H, dd, J = 10.5, 2.3 Hz, H-6′), 7.51 (1H, d, J = 3.5 Hz, H-7), 7.18 (1H, dt, J = 9.0, 2.5 Hz, H-5), 6.89 (1H, d, J = 10.5 Hz, H-5′), 4.02 (3H, s, OMe), 3.96 (3H, s, OMe); *m/z* (CI) 290 ($M^+ + 1$). Anal. ($C_{15}H_{12}FNO_2S$) C, H, N.

4-Fluoro-2-(3,4-dimethoxyphenyl)benzothiazole (11). A solution of *N*-(2-fluorophenyl)-3,4-dimethoxythiobenzamide (0.850 g, 2.92 mmol)[13] and sodium hydroxide (0.93 g, 23.3 mmol) in water (10 mL) and ethanol (0.5 mL) was added dropwise to a solution of potassium ferricyanide (3.84 g, 11.7 mmol) in water (5 mL) at 95

°C. The resulting solution was stirred at 95 °C for a further 2 h and then cooled in an ice bath. The precipitate was collected by vacuum filtration, washed with water, and dissolved in ethyl acetate (10 mL), and insoluble material was removed by filtration. The filtrate was concentrated in vacuo and the crude product purified by column chromatography (dichloromethane) to give the required product as a pale yellow powder (0.46 g, 55% yield): mp 129 °C; ^{1}H NMR (DMSO-d_6) δ 7.95 (1H, m, ArH), 7.65 (2H, m, ArH), 7.42 (2H, m, ArH), 7.15 (1H, m, ArH), 3.90 (3H, s, OMe), 3.88 (3H, s, OMe); *m*/*z* (CI) 290 ($M^+ + 1$). Anal. ($C_{15}H_{12}FNO_2S$) C, H, N.

***N*-(2,5-Dibromophenyl)-3,4-dimethoxybenzamide (17).** 2,5-Dibromoaniline (5.16 g, 20.5 mmol) was dissolved in pyridine (35 mL), and 3,4-dimethoxybenzoyl chloride (4.53 g, 22.6 mmol) was added slowly with stirring. The mixture was heated under reflux for 1 h and then poured into water (150 mL). The resulting precipitate was collected by vacuum filtation and then recrystallized from methanol to give the required product as a white solid (8.51 g, 79% yield): mp 152−153 °C; ^{1}H NMR (DMSO-d_6) δ 9.98 (1H, s, NH), 7.83 (1H, d, J = 2.4 Hz, H-5), 7.70 (1H, d, J = 8.6 Hz, H-3), 7.66 (1H, dd, J = 8.5, 2.1 Hz, H-6′), 7.58 (1H, d, J = 2.1 Hz, H-2′), 7.44 (1H, dd, J = 8.6, 2.4 Hz, H-4), 7.12 (1H, d, J = 8.5 Hz, H-5′), 3.86 (6H, 2 × s, 3′-OMe, 4′-OMe); *m*/*z* (CI) 414 ($M^+ + 1$). Anal. ($C_{15}H_{13}Br_2NO_3$) C, H, N.

***N*-(2,5-Dibromophenyl)-3,4-dimethoxythiobenzamide (18).** *N*-(2,5-Dibromophenyl)-3,4-dimethoxybenzamide (6.32 g, 15.2 mmol) was dispersed in toluene (75 mL), and HMDO (4.56 g, 28.1 mmol) was added with stirring. The mixture was heated to 60 °C, phosphorus pentasulfide (2.2 g, 4.95 mmol) was added with further toluene (25 mL), and then the mixture was heated under reflux for 5 h. After cooling, toluene was evaporated under reduced pressure. The product was purified by column chromatography (hexane:ethyl acetate 3:2) to give the required product as a yellow-orange solid (4.29 g, 65% yield): mp 158−161 °C; ^{1}H NMR (DMSO-d_6) δ 11.51 (1H, s, NH), 7.74 (2H, m, H-3, H-6), 7.67 (2H, m, H-2′, H-6′), 7.54 (1H, dd, J = 8.6, 2.3 Hz, H-4), 7.09 (1H, d, J = 8.3 Hz, H-5′), 3.85 (6H, 2 × s, 3′-OMe, 4′-OMe); *m*/*z* (CI) 430 ($M^+ + 1$). Anal. ($C_{15}H_{13}Br_2NO_2S$) C, H, N.

5-Bromo-2-(3,4-dimethoxyphenyl)benzothiazole (15). NaH (0.438 g of 60% in oil, 0.0109 mol) was added slowly to a solution of *N*-(2,5-dibromophenyl)-3,4-dimethoxythiobenzamide (4.29 g, 9.95 mmol) in dry NMP (9.55 mL, 10 equiv) under nitrogen, and the mixture was heated to 140 °C for 1 h with stirring. The mixture was allowed to cool, water (100 mL) was added, and the resulting brown precipitate was collected by vacuum filtration and washed with water. Purification by column chromatography (hexane:ethyl acetate 1:1) followed by recrystallization from ethanol/water gave the required product as a white solid (1.78 g, 51% yield): mp 157−158 °C; ^{1}H NMR ($CDCl_3$) δ 8.19 (1H, d, J = 1.8 Hz, H-4), 7.74 (1H, d, J = 8.5 Hz, H-7), 7.70 (1H, d, J = 2.0 Hz, H-2′), 7.60 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 7.48 (1H, dd, J = 8.5, 1.8 Hz, H-6), 6.96 (1H, d, J = 8.4 Hz, H-5′), 4.04 (3H, s, OMe), 3.98 (3H, s, OMe); *m*/*z* (CI) 350 ($M^+ + 1$). Anal. ($C_{15}H_{12}BrNO_2S$) C, H, N.

Biology. In Vitro Assays. Compounds were prepared as 10 mM top stock solutions, dissolved in DMSO, and stored at 4 °C, protected from light for a maximum period of 4 weeks. Human-derived cell lines [HCC 2998, KM12 colon carcinoma; MCF-7 (ER+), MDA 468 (ER−) breast carcinoma] were routinely cultivated at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal calf serum and subcultured twice weekly to maintain continuous logarithmic growth. Cells were seeded into 96-well microtiter plates at a density of 5×10^3 per well and allowed 24 h to adhere before drugs were introduced (final concentration 0.1 nM to 100 μM, n = 8). Serial drug dilutions were prepared in medium immediately prior to each assay. At the time of drug addition and following 72 h exposure, MTT was added to each well (final concentration 400 μg/mL). Incubation at 37 °C for 4 h allowed reduction of MTT by viable cells to an insoluble formazan product. Well contents were aspirated and formazan solubilized by addition of DMSO:glycine buffer (pH 10.5) (4:1). Absorbance was read on an Anthos Labtec systems plate reader at 550 nm as a measure of cell viability; thus, cell growth or drug toxicity was determined.

NCI Growth Inhibitory Determination. Cell culture and drug application procedures have been described previously.[16] Briefly, cell lines were inoculated into a series of 96-well microtiter plates, with varied seeding densities depending on the growth characteristics of each cell line. Following a 24-h drug-free incubation, test agents were added at five 10-fold dilutions with a maximum concentration of 100 μM. Cellular protein levels were determined after 48 h of drug exposure by sulforhodamine B colorimetry.

Western Blot Protocol. Whole cell lysates were prepared for examination of CYP1A1 protein expression from untreated MCF-7, MDA 468, HCC 2998, and KM12 cultures and following exposure of cells (10 nM to 10 μM, 24 h) to test compounds. Following protein determination[21] and addition of sample buffer, samples were heated at 95 °C for 5 min and solubilized proteins separated by SDS polyacrylamide gel (10%) electrophoresis. Proteins were electroblotted to PVDF membranes and probed for CYP1A1 protein with polyclonal antiserum specific for human CYP1A1/1A2 (Gentest Corp). Secondary antibody was conjugated to alkaline phosphatase, and CYP1A1 was detected following brief (<10 min) incubation with bromochloroindolyl phosphate and nitro-blue tetrazolium in alkaline phosphatase buffer. Molecular weight markers and a positive control of recombinant CYP1A1 (Gentest Corp), included in all blots, confirmed detection of 52 kDa CYP1A1 protein.

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Supporting Information Available: Full NCI mean GI_{50} graphs for compounds **1**, **2**, **8c**, **8n**, **8w**, and **11**; spectroscopic data for ester analogues **19a**−**i** and **20a,b**; microanalytical data (C, H, N) for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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