Antitumor Benzothiazoles. 14.[1] Synthesis and in Vitro Biological Properties of Fluorinated 2-(4-Aminophenyl)benzothiazoles

Ian Hutchinson, Mei-Sze Chua, Helen L. Browne, Valentina Trapani, Tracey D. Bradshaw, Andrew D. Westwell, and Malcolm F. G. Stevens*

Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD, United Kingdom

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Synthetic routes to a series of mono- and difluorinated 2-(4-amino-3-substituted-phenyl)-benzothiazoles have been devised. Whereas mixtures of regioisomeric 5- and 7-fluoro-benzothiazoles were formed from the established Jacobsen cyclization of precursor 3-fluoro-thiobenzanilides, two modifications to this general process have allowed the synthesis of pure samples of these target compounds. Fluorinated 2-(4-aminophenyl)benzothiazoles were potently cytotoxic (GI_{50} < 1 nM) in vitro in sensitive human breast MCF-7 (ER+) and MDA 468 (ER-) cell lines but inactive (GI_{50} > 10 μM) against PC 3 prostate, nonmalignant HBL 100 breast, and HCT 116 colon cells. The biphasic dose−response relationship characteristically shown by the benzothiazole series against sensitive cell lines was exhibited by the 4- and 6-fluoro-benzothiazoles (**10b**,**d**) but not by the 5- and 7-fluoro-benzothiazoles (**10h**,**i**). The most potent broad spectrum agent in the NCI cell panel was 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (**10h**) which, unlike the 6-fluoro isomer (**10d**), produces no exportable metabolites in the presence of sensitive MCF-7 cells. Induction of cytochrome P450 CYP1A1, a crucial event in determining the antitumor specificity of this series of benzothiazoles, was not compromised. **10h** is currently the focus of pharmaceutical and preclinical development.

Introduction

The unusual antitumor activity of 2-(4-aminophenyl)-benzothiazole (**1**) was originally discovered in a program of screening for tyrosine kinase inhibitors.[2] Analogues (**2**−**5**) possessing superior growth inhibitory properties were subsequently synthesized (Figure 1). Their simple structures belie remarkable and intriguing antitumor properties,[3,4] and their biological profile is unlike that of any known investigational anticancer agent. In a consistent pattern, they are active only against certain human cancer cell lines in the National Cancer Institute (NCI) in vitro anticancer drug screen, producing Mean Graph fingerprints which are highly characteristic of this class of compounds only. Sensitive cell lines (e.g., breast MCF-7, MDA 468; renal TK 10; ovarian IGROV 1) efficiently retain and metabolize 2-(4-aminophenyl)-benzothiazoles to acetylated and hydroxylated derivatives;[5,6] these biotransformations dramatically reduce or abolish antitumor potency. Conversely, insensitive cell lines (e.g., breast MDA-MB-435; renal A 498, CAKI-1; prostate PC 3) neither retain nor metabolize these compounds,[6] thereby suggesting a paramount role of metabolism in their selective antitumor properties.

Analysis of structure−activity relationships identified the benzothiazole nucleus as being essential for potent activity, and that substitution at the 3′-position of the phenyl ring (with alkyl or halogen groups) increased the potency and spectrum of activity against tumor cell lines in vitro. Significantly, 2-(4-amino-3-methylphenyl)benzothiazole (**2**) outperformed its 3-halogeno counterparts

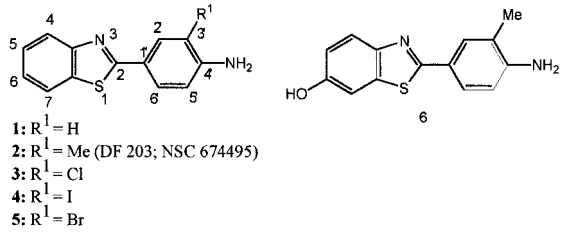

Figure 1. Chemical structures of antitumor 2-(4-aminophenyl)benzothiazoles **1**−**5** with numbering scheme and 6-hydroxy DF 203 metabolite **6**.

3−**5** against in vivo breast[2] and ovarian xenograft models[4] and has been the focus of our research interest to date.

3′-Substituted 2-(4-aminophenyl)benzothiazoles exhibit a unique biphasic dose−response relationship in sensitive cell lines.[3,4] Cell kill occurs at low nanomolar concentrations of the test compounds, followed by a proliferative response at low micromolar concentrations—the second growth phase (SGP). We have postulated that the SGP of this unusual dose−response relationship may be elicited by a metabolite which inactivates the bioactivating enzyme, cytochrome P450 (CYP) 1A1.[7] Indeed, 3′-substituted analogues are extensively ring-hydroxylated by CYP1A1 following its induction by parent compound in sensitive cell lines only. 2-(4-Amino-3-methylphenyl)-6-hydroxybenzothiazole (**6**) has been identified as the main, albeit inactive, metabolite of **2** in MCF-7 cells.[6]

To circumvent deactivating metabolism, we have now synthesized mono- and difluorinated analogues of 2-(4-aminophenyl)benzothiazoles. These substitutions have the potential to block metabolic hydroxylation in the

* To whom correspondence should be addressed. Tel: (115) 951 3414. Fax: (115) 951 3412. E-mail: malcolm.stevens@nottingham.ac.uk.

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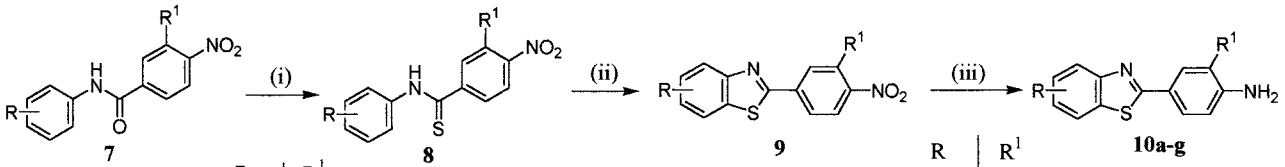
Scheme 1[a]



	R	R^1
a:	2-F	H
b:	2-F	Me
c:	4-F	H
d:	4-F	Me
e:	2,3-di-F	Me
f:	2,4-di-F	Me
g:	3,5-di-F	Me
h:	3-F	Me
i:	3,4-di-F	Me

	R	R^1
a:	4-F	H
b:	4-F	Me
c:	6-F	H
d:	6-F	Me
e:	4,5-di-F	Me
f:	4,6-di-F	Me
g:	5,7-di-F	Me
h:	5-F	Me
i:	7-F	Me
j:	5,6-di-F	Me
k:	6,7-di-F	Me

[a] Reagents: (i) Lawesson's reagent, HMPA, 100 °C; (ii) $K_3Fe(CN)_6$, aq NaOH, 90 °C; (iii) $SnCl_2 \cdot 2H_2O$, EtOH, reflux.

benzothiazole ring, enhance potency, broaden the antitumor spectrum, and, hopefully, optimize the clinical utility of this enigmatic group of compounds.

Chemistry

We have reported recently on the synthesis of the methoxy- and hydroxy-derivatives of 2-(4-aminophenyl)-benzothiazoles;[6] methods devised therein have been adapted to secure the efficient synthesis of ring fluorinated benzothiazoles (**10a**−**g**) as outlined in Scheme 1. The variously fluorinated nitrobenzanilides (**7a**−**i**) were prepared by the interaction of 4-nitrobenzoyl chloride or 3-methyl-4-nitrobenzoyl chloride with the corresponding fluoroanilines (method A). The benzanilides were converted to their thiobenzanilides (**8a**−**i**) with Lawesson's reagent in HMPA (method B) and then cyclized by the Jacobsen synthesis to the fluoro-substituted nitrophenylbenzothiazoles (**9a**−**k**) using potassium ferricyanide in aqueous sodium hydroxide (method C). In the case of cyclization of **8h**, a mixture of the 5- (**9h**) and 7-substituted nitrobenzothiazoles (**9i**) was formed in a ratio 10:1; similarly, from **8i** a mixture of the 5,6-difluoro (**9j**) and 6,7-difluoro-substituted isomers (**9k**) was formed in a ratio of 2:1. Both of these mixtures proved to be too challenging to separate by preparative HPLC, so an alternative route to the synthesis of the pure regioisomers was desirable. In all other cases, only a single nitrobenzothiazole isomer was formed, and the nitrophenylbenzothiazoles (**9a**−**g**) were reduced to their corresponding arylamines (**10a**−**g**) using tin(II) chloride dihydrate in refluxing ethanol (method D). Structures, yields, and physical characteristics of all nitrobenzanilides and nitrothiobenzanilides are given in Table 1; fluorinated 2-(4-nitrophenyl)-benzothiazoles are listed in Table 2.

The 5-fluoro-2-(4-aminophenyl)benzothiazoles (**10h**) and (**10l**) were synthesized by modification of the procedure of Chang et al.[8] (Scheme 2). Hydrolytic cleavage of 5-fluoro-2-aminobenzothiazole (**11**)[9] with aqueous potassium hydroxide at 100 °C, followed by acidification and air oxidation, provided the bis(2-amino-4-fluorophenyl) disulfide (**12**). Interaction of the disulfide with either 3-methyl-4-nitrobenzoyl chloride or 4-nitrobenzoyl chloride gave the corresponding amides (**13a**) and (**13b**), which were cyclized and reduced with tin(II) chloride dihydrate and HCl in ethanol, under reflux (method E), to afford the pure 5-fluoro-2-(4-aminophenyl)benzothiazoles (**10h**,**l**), respectively.

Synthesis of the fluorinated 2-(4-amino-3-halogenophenyl)benzothiazoles is outlined in Scheme 3. Iodination of the fluoro-arylamines (**10a**,**l**,**c**) using iodine monochloride in acetic acid (method F) afforded the 3′-iodo series (**14a**−**c**), respectively; displacement of the iodo function with copper(I) chloride in boiling DMF gave the 3′-chloro derivatives (**15a**−**c**) (method G). Bromination of the fluoro-arylamines (**10a**,**l**,**c**) with bromine in methylene chloride (method H) gave the 3′-bromo series (**16a**−**c**).

The attempted synthesis of 5,6-difluoro-2-(4-amino-3-methylphenyl)benzothiazole (**10j**) using the disulfide

Table 1. Synthetic Methods, Yields, and Physical Characteristics of Fluorinated Nitrobenzanilides and Nitrothiobenzanilides

compd	synthetic method[a]	yield (%)	mp (°C)	formula	MW[b]
7a	A	95	183−185	$C_{13}H_9FN_2O_3$	261.2
7b	A	99	157−158	$C_{14}H_{11}FN_2O_3$	274.9
7c	A	88	164	$C_{13}H_9FN_2O_3$	261.1
7d	A	91	160−162	$C_{14}H_{11}FN_2O_3$	274.9
7e	A	89	130−132	$C_{14}H_{10}F_2N_2O_3$	293.2
7f	A	88	153−154	$C_{14}H_{10}F_2N_2O_3$	293.2
7g	A	92	190−192	$C_{14}H_{10}F_2N_2O_3$	293.2
7h	A	97	127−129	$C_{14}H_{11}FN_2O_3$	275.1
7i	A	58	183−185	$C_{14}H_{10}F_2N_2O_3$	293.2
8a	B	88	122−125	$C_{13}H_9FN_2O_2S$	277.1
8b	B	95	133−136	$C_{14}H_{11}FN_2O_2S$	291.0
8c	B	80	143−145	$C_{13}H_9FN_2O_2S$	276.7
8d	B	64	164−165	$C_{14}H_{11}FN_2O_2S$	291.0
8e	B	76	132−135	$C_{14}H_{10}F_2N_2O_2S$	309.2
8f	B	88	114−116	$C_{14}H_{10}F_2N_2O_2S$	309.2
8g	B	80	130−131	$C_{14}H_{10}F_2N_2O_2S$	309.2
8h	B	83	122−125	$C_{14}H_{11}FN_2O_2S$	291.0
8i	B	83	135−138	$C_{14}H_{10}F_2N_2O_2S$	309.3
18a	A	92	175−176	$C_{14}H_{10}BrFN_2O_3$	352.7/354.9
18b	A	70	168−169	$C_{14}H_{10}BrFN_2O_3$	352.7/355.0
18c	A	65	141−142	$C_{14}H_9BrF_2N_2O_3$	371.5/373.5
19a	B	78	156−158	$C_{14}H_{10}BrFN_2O_2S$	368.9/370.9
19b	B	80	166−167	$C_{14}H_{10}BrFN_2O_2S$	368.8/371.0
19c	B	86	166−168	$C_{14}H_9BrF_2N_2O_2S$	387.6/389.4

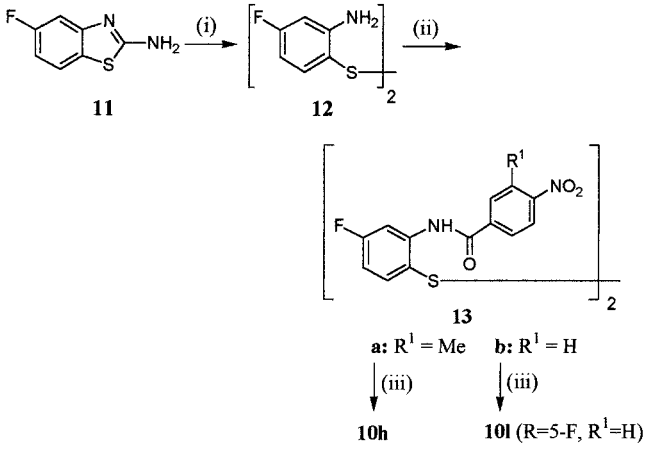
[a] See the Experimental Section. [b] Confirmed by CI-MS. The compounds were also fully characterized by ^{1}H NMR and IR spectroscopy.

Table 2. Synthetic Methods, Yields, and Physical Characteristics of Fluorinated 2-(4-Nitrophenyl)benzothiazoles

compd	synthetic method[a]	yield (%)	mp (°C)	formula	MW[b]
9a	C	43	211−212	$C_{13}H_7FN_2O_2S$	275.1
9b	C	95	197−202	$C_{14}H_9FN_2O_2S$	289.8
9c	C	52	194−195	$C_{13}H_7FN_2O_2S$	275.1
9d	C	60	163−164	$C_{14}H_9FN_2O_2S$	289.4
9e	C	45	150−152	$C_{14}H_8F_2N_2O_2S$	307.3
9f	C	50	202−203	$C_{14}H_8F_2N_2O_2S$	306.9
9g	C	37	221−224	$C_{14}H_8F_2N_2O_2S$	307.3

[a] See the Experimental Section. [b] Confirmed by CI-MS. The compounds were also fully characterized by ^{1}H NMR and IR spectroscopy.

Scheme 2[a]



[a] Reagents: (i) KOH (aq), reflux; (ii) nitrobenzoyl chloride, pyridine, reflux; (iii) $SnCl_2 \cdot 2H_2O$, c.HCl, EtOH, reflux.

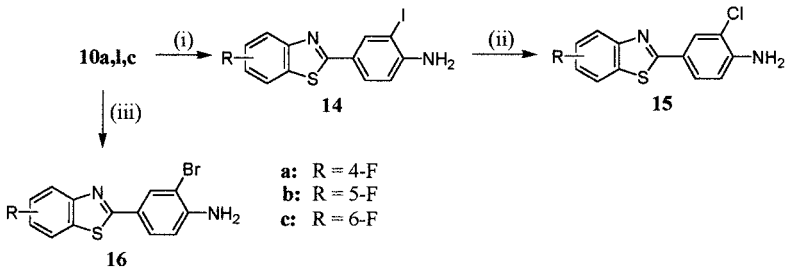
methodology described in Scheme 2 was unsuccessful. In addition, 7-fluoro-2-(4-amino-3-methylphenyl)benzothiazole (**10i**) was not readily accessible by this route, since the required 7-fluoro-2-aminobenzothiazole was recovered as only a minor component during the synthesis of 5-fluoro-2-aminobenzothiazole (**11**)[9] (although separable, with difficulty, by recrystallization). With these observations in mind, alternative regiospecific syntheses of (**10h**−**j**) were developed exploiting a bromo substituent *ortho* to the anilino nitrogen to direct a nucleophilic ring closure reaction, as shown in Scheme 4. A preliminary account of this route has been published.[10] In the case of the synthesis of 5-fluoro-2-(4-amino-3-methylphenyl)benzothiazole (**10h**), the 2-bromo-5-fluorobenzanilide (**18a**) was prepared from 2-bromo-5-fluoroaniline (**17a**)[11] and 3-methyl-4-nitrobenzoyl chloride using method A. Conversion to the corresponding thiobenzanilide (**19a**) with Lawesson's reagent in HMPA (method B) was followed by bromo atom-directed ring closure (method I), using sodium hydride in *N*-methylpyrrolidinone[12] to effect deprotonation of the thioamide (to the corresponding thiolate anion), affording the desired 5-fluoro-2-(4-nitro-3-methyl)benzothiazole (**9h**) as a single compound. It is noteworthy that the use of the standard radical generating conditions of Bu_3SnH-AIBN in refluxing toluene were found not to be compatible with the nitrated thioanilide precursors, although this methodology has been applied to the synthesis of unsubstituted 2-arylbenzothiazoles.[13] Reduction of **9h** using method D gave the desired 5-fluorobenzothiazole (**10h**). An analogous reaction sequence starting from 2-bromo-3-fluoroaniline (**17b**)[14] gave the 7-fluoro-benzothiazole isomer (**10i**). The method described above can also be applied to the synthesis of the 5,6-difluorobenzothiazole (**10j**), starting from the bromodifluoroaniline (**17c**), and it represents an attractive route to those substituted 2-arylbenzothiazoles where the usual Jacobsen cyclization (i.e., Scheme 1) results in a mixture of regioisomers.

Syntheses of the 2-(4-amino-3-fluorophenyl)benzothiazole (**20a**) and 2-(4-amino-3-trifluoromethylphenyl)-benzothiazole (**20b**) were achieved by the condensation, in polyphosphoric acid at 110 °C, of 2-aminothiophenol with 4-amino-3-fluorobenzoic acid[15] and 4-amino-3-trifluoromethylbenzoic acid,[16] respectively (Scheme 5).

Biological Results and Discussion

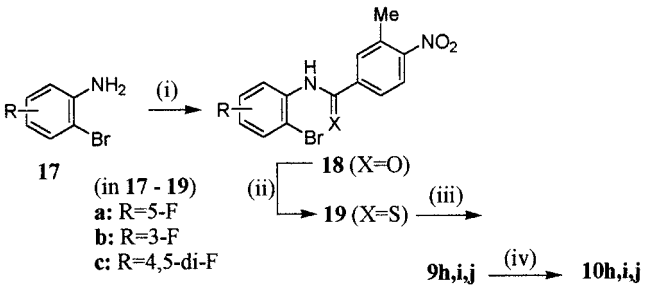
Mitogenic Activity of 2-(4-Amino-3-methylphenyl)-6-hydroxybenzothiazole (6). We have reported previously on the unique profile of antitumor activity of the 2-(4-aminophenyl)benzothiazoles;[6] selectivity is thought to arise following retention and oxidative metabolism of these compounds in sensitive cell lines only.[6,7] However, all 2-(4-aminophenyl)benzothiazoles hydroxylated around the benzothiazole pharmacophore lack antitumor activity.[6] In fact, in culture conditions suboptimal for cell growth (phenol red free medium supplemented with 5% charcoal stripped FCS) between concentrations of 100 nM and 30 μM hydroxy derivatives (e.g., **6**) of **2** evoked a mitogenic response (Figure 2). Even in the presence of growth inhibitory concentrations of **2** (10 nM), growth inhibition was overcome and cellular proliferation stimulated (1−100 μM compound **6**). Compound **2** alone, under these conditions, induced powerful growth inhibition (GI_{50} < 1 nM), but, at concentrations exceeding 1 μM, proliferating cell colonies were observed, as reflected by increased absorbance. Such evidence may explain the unusual biphasic dose response relationship of lead structure **2** in sensitive cell lines in vitro: occurrence of the SGP is consistent with emergence of the inactive 6-hydroxy

Scheme 3[a]



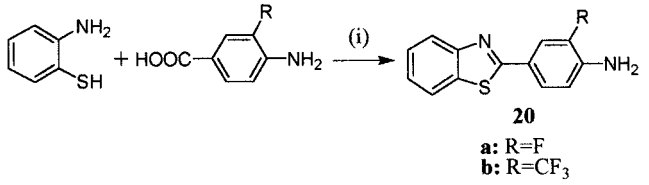
[a] Reagents: (i) ICl, AcOH, 25 °C; (ii) CuCl, DMF, reflux; (iii) Br_2, CH_2Cl_2, 10 °C.

Scheme 4[a]



[a] Reagents: (i) 3-methyl-4-nitrobenzoyl chloride, pyridine, reflux; (ii) Lawesson's reagent, HMPA, 100 °C; (iii) NaH, NMP, 140 °C; (iv) $SnCl_2 \cdot 2H_2O$, EtOH, reflux.

Scheme 5[a]



[a] Reagents: (i) polyphosphoric acid, 110 °C, 0.5 h.

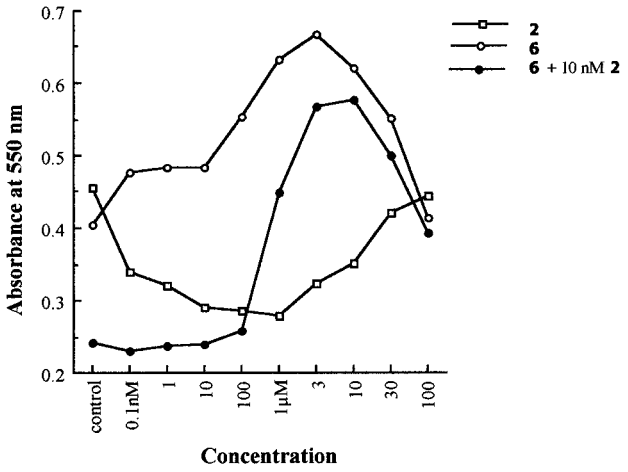


Figure 2. Representative graphs to demonstrate the effects of compounds **2** and **6** on the growth of MCF-7 cells cultured in phenol red free RPMI medium supplemented with 5% charcoal stripped FCS. Cells were seeded at densities of 1×10^4 per well ($n = 8$). Following 72 h exposure, MTT assays were performed to determine cell growth and drug toxicity. Experiments were performed more than three times.

metabolite (**6**) and ablation of induced CYP1A1 activity. The rationale to introduce fluorine atoms into the benzothiazole nucleus is fortified mechanistically: compound **6** inhibits covalent binding between compound **2** and CYP1A1; moreover, the activity of CYP1A1 is significantly inhibited by **6**.[7]

In Vitro Cytotoxicities of Fluorinated 2-(4-Aminophenyl)benzothiazoles. Fluorination is a simple strategy to block undesirable metabolic hydroxylation of bioactive substrates. For example, fluorination in the 2-position of estradiol greatly reduces 2-hydroxylation, thus obliterating nephro-carcinogenicity while retaining estrogen receptor binding affinity.[17,18] In addition, 5,4′-diamino-6,8,3′-trifluoroflavone has enhanced antitumor activity over its precursor 5,4-diaminoflavone, a novel antitumor agent deactivated by ring hydroxylation.[19]

Fluorinated derivatives of compounds **1**−**5** retain exquisite potency and selectivity, yielding GI_{50} values

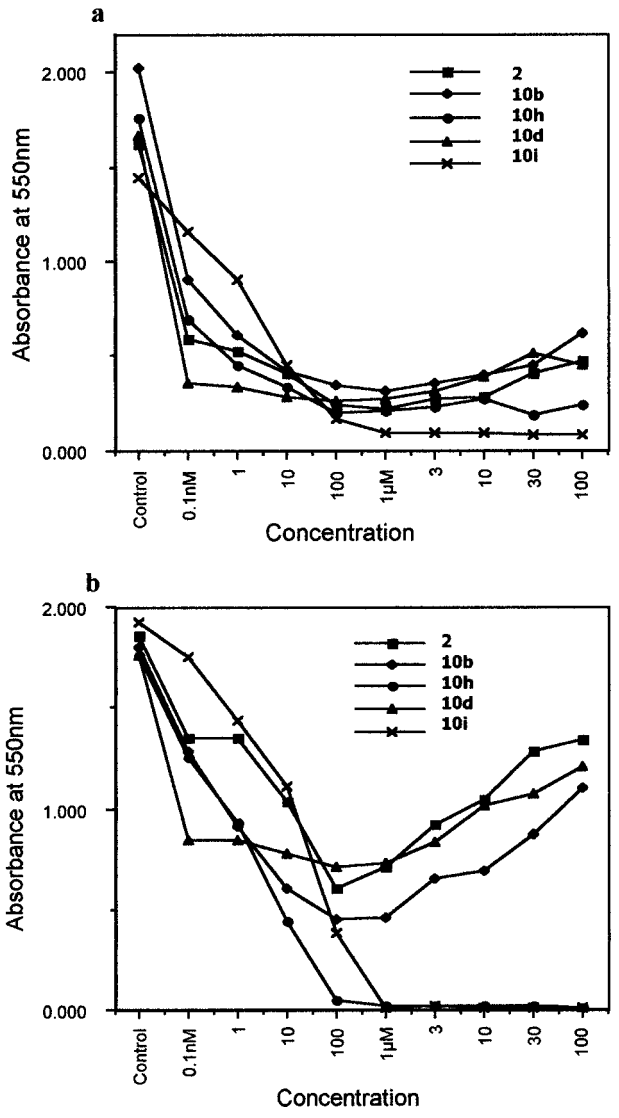
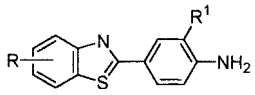


Figure 3. Dose response profiles of **2** and its fluorinated counterparts **10b**,**d**,**h**,**i** against sensitive (a) MCF-7 (ER+) and (b) MDA 468 (ER−) cell lines (MTT assay).

in the nanomolar range against the same cell lines that were growth inhibited by their respective nonfluorinated parent compounds. The dose response profiles of benzothiazole (**2**) and its fluorinated counterparts (**10b**,**d**,**h**,**i**) against sensitive MCF-7 (ER^+) and MDA 468 (ER^-) cell lines are shown in Figure 3. Both 2-(4-amino-3-methylphenyl)-4-fluorobenzothiazole (**10b**) and the 6-fluoro isomer (**10d**) elicited a dose response profile akin to the nonfluorinated parent **2** with a marked SGP especially against the MDA 468 cell line in the 1−100 μM range. In the case of **10d** this was contrary to prediction, since 6-fluorination was designed to block 6-hydroxylation and thus abolish the inactivating and mitogenic effects consequent on the metabolic generation of **6**. Intriguingly, for the 5-F (**10h**) and 7-F (**10i**) analogues, "conventional" growth inhibition curves devoid of the SGP at drug concentrations >1 μM were observed in these two cell lines (Figure 3). In contrast, PC 3 prostate, nonmalignant HBL 100 breast, and HCT 116 colon cells were unresponsive to both parent and fluorinated benzothiazoles with GI_{50} values of >10 μM (data not shown). Similar patterns of activity (GI_{50} values against sensitive cell lines 0.1−10 nM) were

Table 3. Activity of Benzothiazoles in the NCI in Vitro 60-Cell Drug Screen[a]



			GI_{50} (μM) in cell lines[b]									mean GI_{50}[c] (μM)	activity rating (AR)[d]
compd	R	R^1	NCI-H266	NCI-H460	HCC-2998	IGROV1	OVCAR-4	OVCAR-5	TH-10	MCF-7	T47-D		
2	H	Me	18.6	29.5	NT	<0.01	1.1	1.9	<0.01	<0.01	0.02	12.9	4/8
6	6-OH	Me	60.3	38.9	>100	47.9	37.2	83.2	77.6	56.2	22.4	41.7	0/9
10b	4-F	Me	5.5	1.0	>100	22.9	20.9	1.7	NT	<0.01	<0.01	34.6	2/8
10c	6-F	H	46.8	24.5	17.8	21.3	22.9	26.9	26.3	<0.01	NT	30.2	1/8
10d	6-F	Me	60.3	0.1	34.7	1.3	0.01	>100	<0.01	<0.01	NT	47.9	4/8
10h	5-F	Me	1.3	0.05	NT	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	4.4	7/8
10i	7-F	Me	NT	4.2	13.8	0.07	7.4	1.3	0.04	<0.01	0.02	20.0	4/8
10j	5,6-di-F	Me	NT	>100	>100	<0.01	60.3	>100	<0.01	<0.01	<0.01	67.6	4/8
10l	5-F	H	<0.01	0.87	NT	11.5	13.5	29.5	11.5	<0.01	0.26	23.4	2/8
14b	5-F	I	10.2	NT	<0.01	<0.01	<0.01	0.28	0.07	<0.01	0.13	7.6	5/8
14c	6-F	I	0.07	>100	0.32	<0.01	0.03	0.10	<0.01	<0.01	<0.01	33.9	8/9
15b	5-F	Cl	0.03	0.04	NT	<0.01	0.32	<0.01	?	<0.01	0.04	5.8	7/8
15c	6-F	Cl	14.1	0.1	0.09	<0.01	0.02	0.07	<0.01	<0.01	NT	22.3	7/8
16b	5-F	Br	0.13	0.11	<0.01	<0.01	24.0	0.05	0.05	<0.01	0.02	5.1	6/9
20a	H	F	<0.01	<0.01	0.1	<0.01	18.6	<0.01	<0.01	<0.01	<0.01	14.5	8/9
20b	H	CF_3	15.1	14.5	10.7	2.6	15.8	17.4	13.2	1.5	5.4	14.1	0/9

[a] For details of methodology, see ref 4. [b] Origin of cell lines: NCI-H266 and NCI-H460 (nonsmall cell lung cancer); HCC-2998 (colon cancer); IGROV1, OVCAR-4, and OVCAR-5 (ovarian cancer); MCF-7 and T-47D (ER^+ breast cancer). [c] Mean value over the NCI 60-cell panel. [d] AR defined as the number of cell lines in the eight or nine cell panel with sensitivity to the drug >100-fold higher than the mean GI_{50} value for the full 60-cell panel.

displayed by the fluorinated derivatives of the related benzothiazoles (**1**,**3**−**5**) (data not shown).

We have reported previously on the activity of benzothiazole (**2**) and its main (inactive) metabolite (**6**) in the NCI 60-cell line in vitro anticancer drug screen.[6] It is informative to compare these published results with those of the new fluorinated compounds (Table 3). Mean GI_{50} values are recorded together with GI_{50} values in a subpanel of nine cell lines which we have shown to be consistently hypersensitive to this benzothiazole class. To give a measure of the comparative spectrum of action of the compounds, an activity rating (AR) has been devised which records the number of cell lines in the subpanel with GI_{50} values that are >2 logs lower (i.e., >100-fold more sensitive) than the mean GI_{50} value for the 60-cell panel as a whole.

The AR is an imperfect measure to assess structure−activity relationships since, in the responses of some of the cell lines to some compounds, a GI_{50} value had to be estimated because the dose response profile reached a plateau or was biphasic; also, not all the compounds described in the Chemistry section were selected for screening. However, within these limitations, the data in Table 3 show that, although the mean GI_{50} values in the full 60-cell panel only span a 1 log range, compounds of the 5-fluoro series with a 3′-methyl or halogen substituent (**10h**, **14b**, **15b**, and **16b**) are overall more cytotoxic than their 4-, 6-, and 7-fluoro regioisomers. In the series of fluorobenzothiazoles with a 3′-methyl substituent in the aryl group, the ARs were in the following order: 5-F (**10h**) > 6-F (**10d**) > 5,6-di-F (**10j**) and 7-F (**10i**) > 4-F (**10b**). Two new 3′-fluoro-substituted compounds without additional substituents in the benzothiazole nucleus (**20a**,**b**) showed an interesting divergence in spectrum of action. Although both compounds gave near identical mean GI_{50} values, seven of the lines in the nine-cell panel were ultrasensitive to the 3′-fluoro compound (**20a**) at GI_{50} values of <0.01 μM, whereas the 3′-trifluoromethyl-derivative (**20b**) was only weakly active (GI_{50} values 1.5−17.4 μM) across the subpanel of cell lines. Significantly, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (**10h**) was the most potent agent overall (lowest mean GI_{50} value) with a broader spectrum of action than the original lead compound **2**. It is approximately equiactive on the activity rating measure to its 3′-halogeno congeners (**14b**, **15b**, **16b**). In exposure time course assays, **10h** displayed prominent growth inhibitory and cytocidal activity in three breast cell lines under scrutiny (MCF-7, T47D, and ZR75; Dr. M. Alley, personal communication). GI_{50} values in the nanomolar range were obtained following exposure times of <1 h.

Metabolism of Fluorinated 2-(4-Amino-3-methylphenyl)benzothiazoles. Both 2-(4-amino-3-methylphenyl)-6-fluorobenzothiazole (**10d**) and the 5-fluoro isomer (**10h**) were stable in tissue culture media in the absence of cells. However, in the presence of sensitive MCF-7 cells, **10d** was partly biotransformed to unidentified metabolites (Figure 4) which were exported into the medium: this supports the hypothesis that a metabolite may be responsible for the distinctive biphasic dose−response relationship of this compound and the existence of the SGP phenomenon. Surprisingly, the 5-fluoro isomer (**10h**) was metabolically stable in terms of having no exportable metabolites for >7 d in the presence of these cells, confirming that oxidative metabolism of **2** in the 6-position is essentially completely blocked by 5-fluorination. This unexpected observation may explain the in vitro biological superiority of compound **10h** over **10d** and has mechanistic implications. Those metabolically labile fluoro-benzothiazoles (e.g. **10d**) could undergo a range of transformations including (i) direct hydroxylation in the benzothiazole ring; (ii) hydroxylation at the 3′-methyl group or exocyclic amino group; (iii) epoxidation/hydroxylation in the benzothiazole ring accompanied by an NIH shift ("fluorine walk"), a phenomenon described for other fluoro-benzene containing compounds;[20,21] or (iv) oxidative defluorination with 6-hydroxylation accompanied by loss of fluoride

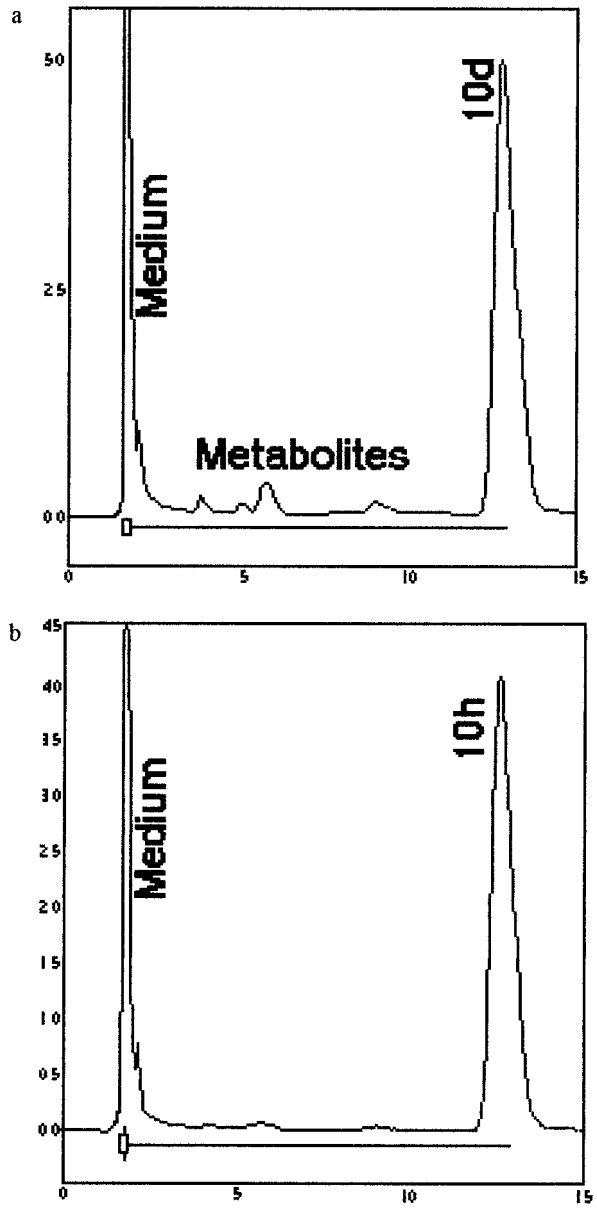


Figure 4. HPLC analyses of media supporting MCF-7 culture, following exposure (3 d, 30 μM) of cells to compounds **10d** and **10h** (for conditions, see Experimental Section metabolism studies).

anion. Conceivably, different fluoro regioisomers might yield the same metabolites. We are currently exploring the metabolism of further examples of the fluorobenzothiazole series (**10**) in the presence of sensitive and resistant cell lines, and this work will be published separately.

Mechanism of Action Studies. Neither activity nor expression of the P450 isoform CYP1A1 is constitutive in MCF-7 breast carcinoma cells.[7] The fluorobenzothiazoles (**10b**,**d**,**h**,**i**,**j**) all induced CYP1A1 protein expression in lysates of breast MCF-7 cells exposed to 1 μM doses of drug for 24 h (Figure 5). In contrast, induction of CYP1A1 protein expression could not be detected in unresponsive HCT 116 colon cell lysates following exposure to the same agents (data not shown). These observations confirm that induction of CYP1A1 is not compromised by isosteric fluorination around the benzothiazole nucleus of compound **2**.

Paradoxically, it has been argued that at concentrations greater than 10 μM, compound **2** (or, more likely, a reactive intermediate derived therefrom) may be an irreversible inhibitor of CYP1A1 activity.[7] In a concentration range spanning 1 nM to 100 μM, the CYP1A1 activity profile was biphasic, with maximum induction of CYP1A1 activity by **2** observed following exposure of cells to 1 μM. Moreover, in EthoxyResorufin O-Diethylase (EROD) assays, coincubation of microsomes expressing recombinant CYP1A1 with concentrations of compound **2** exceeding 10 μM resulted in decreased amounts of resorufin reaction product, implying inhibition of CYP1A1 activity.[7] Similarly, inhibition of ethoxyresorufin O-deethylation was encountered when CYP1A1 microsomes were coincubated with 30 μM of compound **2** and its fluorinated analogues (**10b**,**d**,**h**,**i**,**j**). A 57−84% inhibition of EROD activity was observed (untreated microsomes used to determine 100% activity), indicating that these compounds also inhibit the catalytic activity of CYP1A1 at high concentrations.

Figure 5. Induction of CYP1A1 protein expression in lysates of MCF-7 cells exposed to fluorinated analogues of compound **2** (1 μM) for 24 h. Protein (50 μg) was loaded into each well. Positive control comprised 50 μg of microsomes expressing recombinant CYP1A1. In lysates of benzothiazole-unresponsive HCT116 colon carcinoma cells pretreated with these agents, expression of CYP1A1 protein was not detected. Left to right: positive control, negative control (no drug), **2**, **10b**, **10h**, **10d**, **10i**, **10j**.

Conclusions

We have successfully prevented undesirable metabolic hydroxylation of 2-(4-aminophenyl)-benzothiazoles by fluorine substitution in the benzothiazole ring, with synthesis accomplished via novel routes. Fluorinated analogues of 2-(4-aminophenyl)benzothiazoles possess the selective, potent, and unique in vitro antitumor fingerprint of their nonfluorinated counterparts. The position of fluorination produces subtle yet distinct differences in the dose−response profiles: the highly potent 5-fluoro analogues (regardless of 3′-substituent) consistently failed to elicit the undesirable second growth phase which is characteristic of this group of novel compounds. This may be due to blockade of 6-hydroxylation, which is suspected to underlie this biphasic dose−response phenomenon.[7] In contrast, the 6-fluoro analogues still elicit the biphasic profile although they were expected to prevent 6-hydroxylation to a greater extent than the 5- or 7-fluoro analogues. Thus, the growth inhibiting (at low doses) and growth stimulating (at high doses) properties of 2-(4-aminophenyl)benzothiazoles appear to be potentially separable events which can be dissected apart by appropriate structural modifications.

CYP1A1 is strongly implicated as the primary target for mediation of the antitumor activity of 2-(4-aminophenyl)benzothiazoles.[7] CYP1A1 inducibility by fluorinated benzothiazoles is retained and parallels that of the nonfluorinated agents. The biological sequelae of this inducibility are the metabolic generation of a family of structurally related reactive chemical intermediates—as yet unidentified—which are sequestered within cells in the form of DNA adducts. Importantly, massive DNA

damage occurs only in the sensitive cell lines and is absent in the insensitive cell lines.[22] The results of these studies and a survey of the in vivo activities of fluorinated benzothiazoles will be reported in forthcoming papers in this series.

Because of its resistance to metabolic *C*-hydroxylation, its potency, and its broad spectrum of action in vitro, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (**10h**) has emerged as the most potent of the new generation of antitumor benzothiazoles and is currently the favored clinical candidate. However, because of its lipophilicity and weakly basic nature the drug substance per se is not suitable for development in a parenteral dosage form. We have synthesized the L-alanine and L-lysine amino acid prodrugs of this agent[23] which form pharmaceutically robust, water-soluble hydrochloride salts. These prodrugs have in vivo activity in human xenograft systems and have the potential to be converted to elegant formulations to instigate phase 1 trials.

Experimental Section

All new fluorinated benzothiazoles were characterized by elemental microanalysis (C, H, and N values within 0.4% of theoretical values). Melting points were determined with a Gallenkamp melting point apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX250 spectrometer. IR spectra (as KBr disks) were determined on a Mattson 2020 GALAXY series FT-IR spectrometer. Mass spectra were recorded on an AEI MS-902 or a VG Micromass 7070E spectrometer. TLC systems for routine monitoring of reaction samples and confirming the homogeneity of analytical samples, used Kieselgel 60F254 (0.25 mm) silica gel TLC aluminum sheets. Sorbsil silica gel C 60-H (40−60 μm) was used for flash chromatographic separations.

General Method for the Synthesis of Fluoro Substituted Benzanilides 7a−i. Method A. 3-Methyl-4-nitrobenzoyl chloride or 4-nitrobenzoyl chloride (0.2 mol) was added slowly to a solution of the appropriately substituted fluoroaniline (0.2 mol) in pyridine (100 mL). The resulting solution was heated under reflux for 1 h, then poured into water (300 mL). The precipitate was collected and washed with water (100 mL), followed by methanol, to afford the benzanilides as white solids (see Table 1 for yields and physical characteristics).

General Method for the Synthesis of Fluoro Substituted Thiobenzanilides 8a−i. Method B. Lawesson's reagent (0.07 mol) was added to a solution of the appropriate fluoro-benzanilide (0.1 mol) in HMPA (50 mL). The resulting solution was heated at 100 °C for 15 h, then poured into water (300 mL). The product was extracted into diethyl ether (3 × 300 mL), and the ethereal layer was washed with water (3 × 200 mL). Evaporation of the solvent followed by recrystallization from methanol gave the thiobenzanilides as bright orange solids (see Table 1 for yields and physical characteristics).

General Method for the Synthesis of Fluoro Substituted 2-(4-Nitrophenyl)benzothiazoles 9a−k. Method C. A solution of the fluoro-substituted thiobenzanilide (0.2 mol) in aqueous sodium hydroxide (1.8 mol) in water (50 mL) containing ethanol (5 mL) was added dropwise to a solution of potassium ferricyanide (0.8 mol) in water (20 mL) at 90 °C over a period of 60 min. The resulting solution was stirred at 90 °C for a further 2 h and then cooled in ice. The precipitate was collected and washed with water (100 mL). Products were purified by column chromatography (30% hexane/chloroform) to furnish the nitrophenyl-benzothiazoles as bright yellow solids (see Table 2 for yields and physical characteristics).

General Method for the Reduction of Fluoro Substituted 2-(4-Nitrophenyl)benzothiazoles 9a−g to 10a−g. Method D. Fluoro substituted 2-(4-nitrophenyl)benzothiazoles (0.03 mol) and tin(II) chloride dihydrate (0.15 mol) were suspended in ethanol (150 mL) and heated under reflux for 2 h. The solvent was removed under vacuum and the resulting oil taken up in ethyl acetate (700 mL). The organic layer was washed with 2 M sodium hydroxide (2 × 200 mL), water (100 mL), and salt brine (30 mL). Removal of the solvent under vacuum, followed by recrystallization from methanol, gave the 2-(4-aminophenyl)benzothiazoles as a pale yellow solids.

Yields and physical characteristics of aminophenyl-benzothiazoles prepared by reduction of nitrophenyl-benzothiazoles by method D are given (below).

2-(4-Aminophenyl)-4-fluorobenzothiazole (10a): from **9a**, yield 90%; mp 219−221 °C; IR 3456, 3350 (NH_2), 1604 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.88 (1H, dd, J = 1.5, 8.75 Hz, H-7), 7.78 (2H, d, J = 8.5 Hz, H-2′, H-6′), 7.41−7.28 (2H, m, H-5, H-6), 6.71 (2H, d, J = 8.5 Hz, H-3′, H-5′), 3.56 (2H, brs, NH_2); MS (CI) m/z 245.1 (M + 1). Anal. ($C_{13}H_9FN_2S$) C, H, N.

2-(4-Amino-3-methylphenyl)-4-fluorobenzothiazole (10b): from **9b**, yield 64%; mp 203−205 °C; IR 3491, 3369 (NH_2), 1624 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.86 (1H, dd, J = 1.5, 8.5 Hz, H-7), 7.71 (1H, d, J = 2 Hz, H-2′), 7.66 (1H, dd, J = 2, 8.25 Hz, H-6′), 7.37−7.30 (2H, m, H-5, H-6), 6.73 (1H, d, J = 8.25, H-5′), 5.78 (2H, brs, NH_2), 2.17 (3H, s, CH_3); MS (CI) m/z 259.5 (M + 1). Anal. ($C_{14}H_{11}FN_2S$) C, H, N.

2-(4-Aminophenyl)-6-fluorobenzothiazole (10c): from **9c**, yield 88%; mp 152−155 °C; IR 3333, 3219 (NH_2), 1604 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.98−7.88 (2H, m, H-4, H-7), 7.74 (2H, d, J 8.5 Hz, H-2′, H-6′), 7.31 (1H, dt, J = 2.25, 9 Hz, H-5) 6.69 (2H, d, J = 8.5 Hz, H-3′, H-5′), 5.95 (2H, brs, NH_2); MS (CI) m/z 245.1 (M + 1). Anal. ($C_{13}H_9FN_2S$) C, H, N.

2-(4-Amino-3-methylphenyl)-6-fluorobenzothiazole (10d): from **9d**, yield 92%; mp 203−205 °C; IR 3467, 3306 (NH_2), 1604 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.88 (1H, dd, J = 5, 9 Hz, H-4), 7.78 (1H, d, J = 1.25 Hz, H-2′), 7.70 (1H, dd, J = 1.25, 8.25 Hz, H-6′), 7.51 (1H, dd, J = 2.5, 8.25 Hz, H-7), 7.16 (1H, dd, J = 2.5, 9 Hz, H-5), 6.71 (1H, d, J = 8.25 Hz, H-5′), 3.95 (2H, brs, NH_2), 2.34 (3H, s, CH_3); MS (CI) m/z 259.5 (M + 1). Anal. ($C_{14}H_{11}FN_2S$) C, H, N.

2-(4-Amino-3-methylphenyl)-4,5-difluorobenzothiazole (10e): from **9e**, yield 86%; mp 204−205 °C; IR 3466, 3387 (NH_2), 1616 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.89 (1H, m, H-6), 7.70 (1H, d, J = 2 Hz, H-2′), 7.67 (1H, dd, J = 2, 8.25 Hz, H-6′), 7.46 (1H, m, H-7), 6.72 (1H, d, J = 8.25 Hz, H-5′), 5.86 (2H, brs, NH_2), 2.16 (3H, s, CH_3); MS (CI) m/z 277.2 (M + 1). Anal. ($C_{14}H_{10}F_2N_2S$) C, H, N.

2-(4-Amino-3-methylphenyl)-4,6-difluorobenzothiazole (10f): from **9f**, yield 74%; mp 197−199 °C; IR 3475, 3385 (NH_2), 1622 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.86 (1H, dd, J = 2.5, 8.5 Hz, H-7), 7.67 (1H, d, J = 2.25 Hz, H-2′), 7.65 (1H, dd, J = 2.25, 8.25 Hz, H-6′), 7.43 (1H, dt, J = 2.5, 8.5 Hz, H-5), 6.72 (1H, d, J = 8.25 Hz, H-5′), 5.79 (2H, brs, NH_2), 2.16 (3H, s, CH_3); MS (CI) m/z 277.2 (M + 1). Anal. ($C_{14}H_{10}F_2N_2S$) C, H, N.

2-(4-Amino-3-methylphenyl)-5,7-difluorobenzothiazole (10g): from **9g**, yield 67%; mp 201−203 °C; IR 3483, 3323 (NH_2), 1616 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.72−7.66 (3H, m, H-6′, H-2′, H-4), 7.37 (1H, dt, J = 2.25, 9.75 Hz, H-6), 6.72 (1H, d, J = 8 Hz, H-5′), 5.88 (2H, brs, NH_2), 2.18 (3H, s, CH_3); MS (CI) m/z 277.2 (M + 1). Anal. ($C_{14}H_{10}F_2N_2S$) C, H, N.

Synthesis of Bis(aminofluorophenyl) Disulfides. Bis-(2-amino-4-fluorophenyl) Disulfide (12). 2-Amino-5-fluorobenzothiazole (**11**)[9] (5 g, 0.03 mol) was added to a solution of potassium hydroxide (25 g) in water (50 mL). The resulting mixture was heated under reflux for 5 h, after which complete solution had occurred. After cooling, the reaction mixture was acidified (to pH 6) by the addition of acetic acid. Water (50 mL) was added, and the resulting mixture was stirred overnight. The solid precipitate was collected and recrystallized from ethanol/water to give the disulfide (3.5 g, 86%) as a pale yellow solid, mp 75−76 °C; IR 3427, 3302 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.93 (1H, dd, J = 8, 8.5 Hz, H-6), 6.46 (1H, dd, J = 2.75, 8.5 Hz, H-3), 6.26 (1H, dt, J = 2.75, 8.5 Hz, H-5), 5.81 (2H, brs, NH_2); ^{13}C (DMSO-d_6) 165, 153, 139, 112, 105, 102; MS (CI) m/z 285.1 (M + 1). Anal. ($C_{12}H_{10}F_2N_2S_2$) C, H, N.

Bis[4-fluoro-2-(3-methyl-4-nitrobenzoyl)aminophenyl] Disulfide (13a). 3-Methyl-4-nitrobenzoyl chloride (1.45 g, 7.3 mmol) was added to a solution of bis(2-amino-4-fluorophenyl) disulfide (**12**) (1 g, 3.65 mmol) in pyridine (10 mL). The resulting mixture was heated under reflux for 30 min and then poured into water (50 mL). The precipitate was collected and washed with water (50 mL) to leave a pale yellow solid (1.65 g, 77%), mp 223−225 °C; IR 3342 (NH), 1680 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.4 (1H, brs, NH), 8.10 (1H, d, J = 7.5 Hz, H-5′), 7.95 (1H, d, J = 2.5 Hz, H-2′), 7.90 (1H, dd, J = 8.5, 2.5 Hz, H-6′), 7.67 (1H, dd, J = 8, 10 Hz, H-6), 7.43 (1H, dd, J 2.5, 10 Hz, H-3), 7.13 (1H, dt, J = 2.5, 10 Hz, H-5), 2.58 (3H, s, CH_3); ^{13}C (DMSO-d_6) 164, 160, 151, 138, 137, 134, 133, 132, 127, 126, 124, 114, 112, 20; MS (CI) m/z 611.0 (M + 1). Anal. ($C_{28}H_{20}F_2N_4O_6S_2$) C, H, N.

Bis[4-fluoro-2-(4-nitrobenzoyl)aminophenyl] Disulfide (13b). Similarly prepared, from **12** and 4-nitrobenzoyl chloride, was the disufide (96%) mp 205−207 °C; IR 3354 (NH), 1678 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.5 (1H, bs, NH), 8.38 (2H, d, J = 7.5 Hz, H-3′, H-5′), 8.09 (2H, d, J = 7.5 Hz, H-6′, H-2′), 7.68 (1H, dd, J = 8.5, 5.5 Hz, H-6), 7.44 (1H, dd, J = 2, 9.5 Hz, H-3), 7.18 (1H, dt, J = 2, 8.5 Hz, H-5); ^{13}C 164, 160, 149, 139, 138, 133, 129, 127, 123, 114, 112; MS (CI) m/z 582.8 (M + 1). Anal. ($C_{26}H_{16}F_2N_4O_6S_2$) C, H, N.

Reductive Cyclization of Disulfides 13a,b. Method E. To a solution of 10 M HCl (10 mL), ethanol (20 mL), and water (2 mL) was added the disulfide (1.6 mmol) and tin(II) chloride dihydrate (9.8 mmol). The reaction mixture was heated under reflux for 15 h, cooled to 25 °C, and poured into water (75 mL). Sodium hydroxide (2 g) was added slowly, and the mixture was stirred for 1 h. The precipitate was collected and washed with water (10 mL) to leave a yellow solid which was purified by column chromatography (dichloromethane) followed by recrystallization from ethanol to give colorless needles. The following fluoro substituted 2-(4-aminophenyl)benzothiazoles were prepared by method E.

2-(4-Amino-3-methylphenyl)-5-fluorobenzothiazole (10h): from **13a**, yield 71%, mp 195−196 °C; IR 3433, 3302 (NH_2), 1622 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.07 (1H, dd, J = 5.5, 8.8 Hz, H-7), 7.8 (1H, dd, J = 2.5, 10 Hz, H-4), 7.69 (1H, d, J = 1.5 Hz, H-2′), 7.65 (1H, dd, J = 1.5, 8.3 Hz, H-6′), 7.26 (1H, dt, J = 2.5, 8.8 Hz, H-6), 6.70 (1H, d, J = 8.3 Hz, H-5′), 5.77 (2H, brs, NH_2), 2.10 (3H, s, CH_3); MS (CI) m/z 259.8 (M + 1). Anal. ($C_{14}H_{11}FN_2S$) C, H, N.

2-(4-Aminophenyl)-5-fluorobenzothiazole (10l): from **13b**, yield 66%, mp 153−155 °C; IR 3460, 3290 (NH_2), 1637 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ_8.06 (1H, dd, J = 8.8, 5.3 Hz, H-7), 7.80 (2H, d, J = 8.8 Hz, H-2′, H-6′), 7.77 (1H, dd, J = 2.5, 9 Hz, H-4), 7.25 (1H, dt, J = 2.5, 9 Hz, H-6), 6.68 (2H, d, J = 8.8 Hz, H-3′, H-5′), 6.00 (2H, brs, NH_2); MS (CI) m/z 245.1 (M + 1). Anal. ($C_{13}H_9FN_2S$) C, H, N.

General Method for the Synthesis of Fluoro Substituted 2-(4-Amino-3-iodophenyl)-benzothiazoles 14a−c. Method F. A solution of the fluoro substituted 2-(4-aminophenyl)benzothiazole (4.5 mmol) in acetic acid (20 mL) was added dropwise to a solution of iodine monochloride (5.8 mmol) in acetic acid (20 mL) at 25 °C. The resulting solution was stirred for 2 h, and then the solvent was removed under vacuum. The residue was dissolved in chloroform (100 mL) and washed successively with aqueous sodium carbonate (50 mL), aqueous sodium thiosulfate (50 mL), and water (50 mL). Evaporation of the solvent, followed by column chromatography (chloroform) and recrystallization from methanol, gave pale cream needles. The following iodophenyl-benzothiazoles were prepared.

2-(4-Amino-3-iodophenyl)-4-fluorobenzothiazole (14a): from **10a**, (65%); mp 210−211 °C; IR 3474, 3377 (NH_2), 1610 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.29 (1H, d, J = 2 Hz, H-2′), 7.90 (1H, dd, J = 1.75, 8.5 Hz, H-7), 7.80 (1H, dd, J = 2, 8.5 Hz, H-6′), 7.44−7.31 (2H, m, H-5, H-6), 6.85 (1H, d, J = 8.5 Hz, H-5′), 6.01 (2H, brs, NH_2); MS (CI) m/z 370.9 (M + 1). Anal. ($C_{13}H_8FIN_2S$) C, H, N.

2-(4-Amino-3-iodophenyl)-5-fluorobenzothiazole (14b): from **10l**, (63%); mp 187−188 °C; IR 3447, 3317 (NH_2), 1612 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.28 (1H, d, J = 2 Hz, H-2′), 8.10 (1H, dd, J = 5.5, 8.75 Hz, H-7), 7.80 (1H, dd, J = 2, 8.5 Hz, H-6′), 7.79 (1H, dd, J = 2.5, 9 Hz, H-4), 7.30 (1H, dt, J = 2.5, 9 Hz, H-6), 6.89 (1H, d, J = 8.5 Hz, H-5′), 6.20 (2H, brs, NH_2); MS (CI) m/z 370.9 (M + 1). Anal. ($C_{13}H_8FIN_2S$) C, H, N.

2-(4-Amino-3-iodophenyl)-6-fluorobenzothiazole (14c): from **10c**, (77%); mp 198−200 °C; IR 3445, 3290 (NH_2), 1620 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.26 (1H, d, J = 2.25 Hz, H-2′), 8.00−7.93 (2H, m, H-4, H-7), 7.77 (1H, dd, J = 2.25, 8.5 Hz, H-6′), 7.35 (1H, dt, J = 2.75, 9 Hz, H-5), 6.86 (1H, d, J = 8.5 Hz, H-5′), 6.02 (2H, brs, NH_2); MS (CI) m/z 370.9 (M + 1). Anal. ($C_{13}H_8FIN_2S$) C, H, N.

General Method for the Synthesis of Fluoro Substituted 2-(4-Amino-3-chlorophenyl)-benzothiazoles 15a−c. Method G. A solution of the fluoro substituted 2-(4-amino-3-iodophenyl)benzothiazole (1.35 mmol) and copper(I) chloride (6.75 mol) in DMF (5 mL) was heated under reflux overnight. After cooling, the reaction mixture was poured into ethyl acetate (100 mL); the precipitated solids were filtered off and the resulting solution was evaporated to dryness. The product was purified by column chromatography (dichloromethane) followed by recrystallization from methanol to give a pale green solid. The following chlorophenyl-benzothiazoles were prepared.

2-(4-Amino-3-chlorophenyl)-4-fluorobenzothiazole (15a): from **14a**, (80%); mp 181−183 °C; IR 3477, 3381 (NH_2), 1620 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.93 (1H, d, J = 2 Hz, H-2′), 7.89 (1H, dd, J = 2.25, 8.5 Hz, H-7), 7.77 (1H, dd, J = 2, 8.5 Hz, H-6′), 7.45−7.31 (2H, m, H-5, H-6), 6.90 (1H, d, J = 8.5 Hz, H-5′), 6.26 (2H, brs, NH_2); MS (CI) m/z 278.9/ 280.8 (M + 1). Anal. ($C_{13}H_8ClFN_2S$) C, H, N.

2-(4-Amino-3-chlorophenyl)-5-fluorobenzothiazole (15b): from **14b**, (80%); mp 177−178 °C; IR 3481, 3369 (NH_2), 1631 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.12 (1H, dd, J = 5.25, 8.75 Hz, H-7), 7.92 (1H, d, J = 2.5 Hz, H-2′), 7.70 (1H, dd, J = 2.5, 8.5 Hz, H-6′), 7.73(1H, dd, J = 2.25, 8.5 Hz, H-4), 7.33 (1H, dt, J = 2.5, 9 Hz, H-6), 6.92 (1H, d, J = 8.5 Hz, H-5′), 6.24 (2H, brs, NH_2); MS (CI) m/z 278.9/280.8 (M + 1). Anal. ($C_{13}H_8ClFN_2S$) C, H, N.

2-(4-Amino-3-chlorophenyl)-6-fluorobenzothiazole (15c): from **14c**, (68%); mp 194−195 °C; IR 3472, 3310 (NH_2), 1628 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.02 (1H, dd, J = 2.5, 8.75 Hz, H-7), 7.97 (1H, dd, J = 5, 9 Hz, H-4), 7.90 (1H, d, J = 2.25 Hz, H-2′), 7.73(1H, dd, J = 2.25, 8.5 Hz, H-6′), 7.37 (1H, dt, J = 2.75, 9 Hz, H-5), 6.90 (1H, d, J = 8.5 Hz, H-5′), 6.19 (2H, brs, NH_2); MS (CI) m/z 278.9/280.8 (M + 1). Anal. ($C_{13}H_8ClFN_2S$) C, H, N.

General Method for the Synthesis of Fluoro Substituted 2-(4-Amino-3-bromophenyl)-benzothiazoles 16a−c. Method H. Bromine (0.8 mmol) was added to a solution of the fluoro substituted 2-(4-aminophenyl)benzothiazole (0.8 mmol) in dichloromethane (20 mL) at 10 °C. The resulting solution was stirred for 10 min, then poured into water/ice (10 mL). The organic layer was removed, washed with 10% sodium thiosulfate (10 mL) and water (10 mL), and evaporated. The product was purified by column chromatography (dichloromethane) to leave a white solid. The following bromophenyl-benzothiazoles were prepared.

2-(4-Amino-3-bromophenyl)-4-fluorobenzothiazole (16a): from **10a**, (83%); mp 211−213 °C; IR 3416, 3379 (NH_2), 1618 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.08 (1H, d, J = 2 Hz, H-2′), 7.91 (1H, dd, J = 1.25, 7.25 Hz, H-7), 7.80 (1H, dd, J = 2, 8.5 Hz, H-6′), 7.45−7.31 (2H, m, H-5, H-6), 6.93 (1H, d, J = 8.5 Hz, H-5′), 6.21 (2H, brs, NH_2); MS (CI) m/z 322.9/324.9 (M + 1). Anal. ($C_{13}H_8BrFN_2S$) C, H, N.

2-(4-Amino-3-bromophenyl)-5-fluorobenzothiazole (16b): from **10l**, (79%); mp 181−183 °C; IR 3464, 3311 (NH_2), 1612 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.12 (1H, dd, J = 5.25, 8.75 Hz, H-7), 8.08 (1H, d, J = 2 Hz, H-2′), 7.80 (1H, dd, J = 2, 8.5 Hz, H-6′), 7.79(1H, dd, J = 2.75, 9 Hz, H-4), 7.30 (1H, dt, J = 2.75, 9 Hz, H-6), 6.90 (1H, d, J = 8.5 Hz, H-5′), 6.18 (2H, brs, NH_2); MS (CI) m/z 323/325 (M + 1). Anal. ($C_{13}H_8BrFN_2S$) C, H, N.

2-(4-Amino-3-bromophenyl)-6-fluorobenzothiazole (16c): from **10c**, (85%); mp 209−211 °C; IR 3462, 3300 (NH_2), 1626 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.05 (1H, d, J = 2 Hz, H-2′), 8.01 (1H, dd, J = 2.75, 8.75 Hz, H-7), 7.97 (1H, dd, J = 5, 9 Hz, H-4), 7.76(1H, dd, J = 2, 8.5 Hz, H-6′), 7.36 (1H, dt, J = 2.75, 9 Hz, H-5), 6.90 (1H, d, J = 8.5 Hz, H-5′), 6.14 (2H, brs, NH_2); MS (CI) m/z 322.9/324.9 (M + 1). Anal. ($C_{13}H_8BrFN_2S$) C, H, N.

Synthesis of Fluoro Substituted *ortho*-Bromobenzanilides 18a−c. These benzanilides were prepared as white solids from fluoro substituted 2-bromoanilines according to general method A (see Table 1 for yields and physical characteristics).

Synthesis of Fluoro Substituted *o*-Bromothiobenzanilides 19a−c. These thiobenzanilides were prepared as yellow-orange solids from fluoro substituted *ortho*-bromobenzanilides according to general method B (see Table 1 for yields and physical characteristics).

General Method for the Synthesis of Fluoro Substituted 2-(3-Methyl-4-nitrophenyl)benzothiazoles 9h−j. Method I. Sodium hydride (3.1 mmol) was slowly added to a solution of the appropriate fluoro substituted *o*-bromothiobenzanilide (2.8 mmol) in anhydrous *N*-methyl-2-pyrrolidinone (28 mmol) at 140 °C with stirring. The mixture was heated at 140 °C for 1 h then allowed to cool. Water (50 mL) was then added, and the precipitate was collected by filtration and dried in vacuo to give the solid product. The following fluoro substituted 2-(3-methyl-4-nitrophenyl)benzothiazoles were prepared.

5-Fluoro-2-(3-methyl-4-nitrophenyl)benzothiazole (9h): from **19a**, (73%); mp 190−192 °C; IR 1613, 1518 (NO_2), 1339 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (1H, d, J = 8.5 Hz, H-5′), 8.08 (1H, d, J 2.5 Hz, *H*-2′), 8.02 (1H, dd, J = 2.5, 8.5 Hz, H-6′), 7.88 (1H, ddd, J = 0.4, 5.3, 8.5 Hz, H-7), 7.79 (1H, ddd, J = 0.3, 2.5, 9.3 Hz, H-4), 7.21 (1H, dd, J 2.5, 8.5 Hz, H-6), 2.72 (3H, s, CH_3); MS (CI) m/z 289 (M + 1), 259. Anal. ($C_{14}H_9FN_2O_2S$) C, H, N.

7-Fluoro-2-(3-methyl-4-nitrophenyl)benzothiazole (9i): from **19b**, (78%); mp 203−205 °C; IR 1605 (C═N), 1520 (NO_2), 1460 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.20 (3H, m, H-2′, H-5′, H-6′), 8.04 (1H, dd, J = 1.75, 8.25 Hz, H-4), 7.69 (1H, td, J = 5.75, 8.25 Hz, H-5), 7.48 (1H, dd, J = 1.75, 8.25 Hz, H-6), 2.66 (3H, s, CH_3); MS (CI) m/z 289 (M + 1), 259. Anal. ($C_{14}H_9FN_2O_2S$) C, H, N.

5,6-Difluoro-2-(3-methyl-4-nitrophenyl)benzothiazole (9j): from **19c**, (87%); mp 229−232 °C; IR 1518 (NO_2), 1464, 882 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.11 (1H, d, J = 8.5 Hz, H-5′), 8.06 (1H, d, J = 1.6 Hz, H-2′), 7.99 (1H, dd, J = 1.6, 8.5 Hz, H-6′), 7.90 (1H, dd, J = 7.5, 9.8, ArH), 7.73 (1H, dd, J = 7.8, 8.8 Hz, ArH), 2.72 (3H, s, CH_3); MS (CI) m/z 307 (M + 1), 261. Anal. ($C_{14}H_8F_2N_2O_2S$) C, H, N.

Synthesis of Fluoro Substituted 2-(4-Amino-3-methylphenyl)benzothiazoles 10h−j. The following compounds were prepared from fluoro substituted nitrophenyl-benzothiazoles **9h−j** by method D.

2-(4-Amino-3-methylphenyl)-5-fluorobenzothiazole (10h): from **9h**, (45%); identical to the sample prepared by method E.

2-(4-Amino-3-methylphenyl)-7-fluorobenzothiazole (10i): from **9i**, (82%); mp 175−177 °C; IR 3021 (NH_2), 1621 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.80 (1H, dd, J = 0.75, 8.0 Hz, H-4), 7.77 (1H, d, J = 2 Hz, H-2′), 7.68 (1H, dd, J = 2, 8.25 Hz, H-6′), 7.51 (1H, td, J = 5.75, 8 Hz, H-5), 7.27 (1H, dd, J = 0.75, 8 Hz, H-6), 6.72 (1H, d, J = 8.25 Hz, H-5′), 5.82 (2H, brs, NH_2), 2.15 (3H, s, CH_3); MS (CI) m/z 259.0 (M + 1). Anal. ($C_{14}H_{11}FN_2S$) C, H, N.

5,6-Difluoro-2-(4-amino-3-methylphenyl)benzothiazole (10j): from **9j**, (54%); mp 226−228 °C; IR 3497, 3333 (NH_2), 1632 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.22 (1H, dd, J = 8.0, 10.3 Hz, H-7), 7.98 (1H, dd, J = 7.4, 11.4 Hz, H-4), 7.63 (2H, m, H-2′, H-6′), 6.71 (1H, d, J = 8.3 Hz, H-5′), 5.91 (2H, brs, NH_2), 2.15 (3H, s, CH_3); MS (CI) m/z 277 (M + 1). Anal. ($C_{14}H_{10}F_2N_2S$) C, H, N.

2-(4-Amino-3-fluorophenyl)benzothiazole (20a). 4-Amino-3-fluorobenzoic acid (2.0 mmol) was dissolved in polyphosphoric acid (10 g) at 110 °C. 2-Aminothiophenol (2.0 mmol) was added and the resulting solution stirred at 110 °C for 30 min. After cooling, the reaction mixture was poured into aqueous ammonia (10 mL). The precipitate was collected and washed with water (50 mL). The product was purified by column chromatography ($CHCl_3$) to give the fluorophenyl-benzothiazole as a white solid (0.33 g, 68%), mp 160−161 °C; IR 3230, 3180 (NH_2), 1596 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.04 (1H, d, J = 7.25 Hz, H-7), 7.93 (1H, d, J = 7.25 Hz, H-4), 7.70 (1H, dd, J = 2, 11 Hz, H-2′), 7.63 (1H, dd, J = 2, 8.25 Hz, H-6′), 7.48 (1H, t, J = 7.25 Hz, H-6), 7.46 (1H, t, J = 7.25 Hz, H-5), 6.87 (1H, t, J = 8.75 Hz, H-5′), 5.98 (2H, brs, NH_2); MS (CI) m/z 245.2 (M + 1). Anal. ($C_{13}H_9FN_2S$) C, H, N.

2-[4-Amino-3-(trifluoromethyl)phenyl]benzothiazole (20b). Similarly prepared (37%), from 4-amino-3-(trifluoromethyl)benzoic acid and 2-aminothiophenol in polyphosphoric acid, mp 144−145 °C; IR 3317, 3190 (NH_2), 1649 (C═N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.08 (4H, m, H-4, H-7, H-2′, H-6′), 7.49 (2H, m, H-5, H-6), 6.98 (1H, d, J = 8.75 Hz, H-5′), 6.42 (2H, brs, NH_2); ^{13}C NMR 167, 154, 149, 135, 133, 127, 126 (q, J = 5 Hz), 125, 125 (q, J = 272 Hz), 123, 122, 120, 118, 111 (q, J = 30 Hz); MS (CI) m/z 295.1 (M + 1). Anal. ($C_{14}H_9N_2F_3S$) C, H, N.

Biological Experimental. Growth Inhibitory Assays. Fluorinated benzothiazole analogues were prepared as 10 mM top stocks, dissolved in DMSO, and stored at 4 °C, protected from light for a maximum period of 4 weeks. MCF-7 (ER+) and MDA 468 (ER−) human derived breast carcinoma cells, cultivated at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% fetal calf serum, 100 IU/mL penicillin, and 100 g/mL streptomycin, were routinely subcultured twice weekly to maintain in continuous logarithmic growth. Cells were seeded into 96-well microtiter plates at a density of 5×10^3 (1×10^4 in phenol red free RPMI medium supplemented with 5% charcoal stripped FCS) 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 mitochondrial dehydrogenase 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.

Metabolism Studies. MCF-7 cells were seeded into 25 mL flasks at appropriate densities (2×10^5, 10^6). After 24 h (to allow cells to adhere and begin mitoses) medium was changed and drug introduced at a final concentration of 30 μM. Media samples, collected from flasks at time zero and thereafter following 24 h intervals, were mixed with 3-fold volumes of HPLC-grade acetonitrile to precipitate protein and centrifuged at 14 000 rpm for 10 min. Supernatants were analyzed by HPLC. The analytical system consisted of a Hewlett-Packard 1050 series module (solvent delivery pump, autosampler, and multiple wavelength detector) and a Hewlett-Packard 1046A fluorescence detector. Separation of parent compounds and biotransformation products was effected at room temperature on a C18 reversed-phase column (150 × 4.6 mm i.d.) using a mobile phase of 65% methanol and 35% H_2O, delivered at a flow rate of 1 mL/min. Compounds were detected at 338 nm with UV detection and with fluorescence detection (excitation 344 nm; emission 434 nm). Identities of major biotransformation products were confirmed by chromatographic analyses of authentic samples.

Western Blot Protocol. Whole cell lysates were prepared for examination of CYP1A1 protein expression from untreated MCF-7 and HCT 116 cultures and following exposure of cells (1 μM, 24 h) to compounds **2**, **10b**, **10h**, **10d**, **10i**, and **10j**. Following protein determination[24] and addition of sample buffer, samples were boiled at 95 °C for 5 min and solubilized proteins separated by SDS polyacrylamide gel (10%) electro-

phoresis. Proteins were electroblotted to PVDF membranes and probed for CYP1A1 protein with polyclonal antiserum specific for human CYP1A1/1A2 (Gentest Corporation). Secondary antibody was conjugated to alkaline phosphatase, and CYP1A1 was detected following brief (<10 min) incubation with bromochloroindolyl phosphate and nitro-blue tetrazolium in alkaline phosphatase buffer. Molecular weight markers and a positive control of recombinant CYP1A1 (Gentest Corporation), included in all blots, confirmed detection of 52 kDa CYP1A1 protein.

Determination of EROD Activity. A sensitive and rapid fluorometric assay was used to measure EROD activity.[25] Incubation mixtures (total 1 mL) consisted of 100 mM Tris-HCl (pH 7.4), 50 μM $MgCl_2$, 100 μM 7-ethoxyresorufin, and 100 μL of MCF-7 cell homogenate. Homogenates were prepared following treatment of MCF-7 cells for 24 h with 1 μM compound **2**, **10b**, **10h**, **10d**, **10i**, and **10j** or vehicle alone; protein content was determined by the method of Bradford.[24] Thus induction of ethoxyresorufin O-deethylation by agents under study, catalyzed by CYP1A1 activity, could be determined. Alternatively, microsomes expressing recombinant CYP1A1 (0.1 mg/mL) provided the engine of EROD catalysis, in the presence or absence of 30 μM fluorinated 2-(4-aminophenyl)benzothiazole analogue, to determine inhibition of CYP1A1 activity by compounds **2**, **10b**, **10h**, **10d**, **10i**, and **10j**. Incubation mixtures were preincubated for 5 min at 37 °C before initiation of reaction by addition of NADPH (500 μM). Following further incubations at 37 °C (30 min for MCF-7 homogenates, 15 min for CYP1A1 microsomes), reactions were terminated by addition of 3 mL of ice-cold acetonitrile. Reaction mixtures were centrifuged at 1400 rpm, 10 min before analyses of supernatants. Fluorescence was read on a Perkin-Elmer LS-5 luminescence spectrometer (excitation 530 nm; emission 585 nm). Estimation of resorufin reaction product (nM/mg protein), as a measure of CYP1A1 activity, was determined following performance of the resorufin standard curve.

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Supporting Information Available: Microanalytical data and a table containing percent inhibition values for compounds **2**, **10b**, **10h**, **10d**, **10i**, and **10j**. This material is available free of charge via the Internet at http://pubs.acs.org.

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