Antitumor Benzothiazoles. 3.[1] Synthesis of 2-(4-Aminophenyl)benzothiazoles and Evaluation of Their Activities against Breast Cancer Cell Lines *in Vitro* and *in Vivo*

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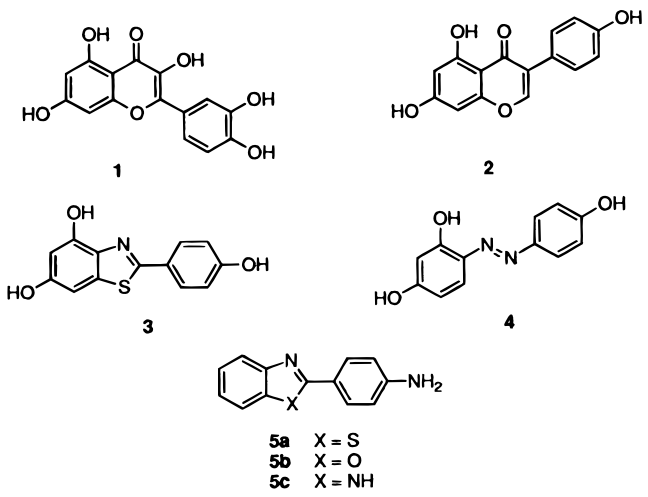
A new series of 2-(4-aminophenyl)benzothiazoles substituted in the phenyl ring and benzothiazole moiety has been synthesized by simple, high-yielding routes. The parent molecule **5a** shows potent inhibitory activity *in vitro* in the nanomolar range against a panel of human breast cancer cell lines, but is inactive (IC_{50} > 30 μM) against other cell types: activity against the sensitive breast lines MCF-7 and MDA 468 is characterized by a biphasic dose−response relationship. Structure−activity relationships derived using these cell types has revealed that activity follows the heterocyclic sequence benzothiazole > benzoxazole ≫ benzimidazole and that 2-(4-aminophenyl)benzothiazoles bearing a 3′-methyl- **9a**, 3′-bromo- **9c**, 3′- iodo- **9f**, and 3′-chloro-substituent **9i** are especially potent and their activity extends to ovarian, lung, and renal cell lines. Four compounds have been evaluated *in vivo* against human mammary carcinoma models in nude mice. Compound **9a** showed the most potent growth inhibition against the ER^+ (MCF-7 and BO) and ER^- (MT-1 and MT-3) tumors. Our efforts to identify a pharmacological mechanism of action for these intriguing compounds have not, as yet, been successful.

Introduction

Previously we have described the cytotoxicities of polyhydroxylated 2-phenylbenzothiazoles against a range of human tumor cell lines[2] and compared their pharmacological properties with those of the flavone quercetin (**1**) and the isoflavone genistein (**2**). These benzothiazoles were designed as potential tyrosine kinase inhibitors modeled on structural comparisons with the natural products which compete at the ATP-binding sites of tyrosine kinases.[3] The most interesting bioactive compounds were 4,6-dihydroxy-2-(4-hydroxyphenyl)benzothiazole (**3**) and the related acyclic structure 2,4,4′-trihydroxyazobenzene (**4**) which have the same overall substitution pattern as genistein. During the course of this work, we prepared 2-(4-aminophenyl)-benzothiazole (**5a**) and some substituted arylamines as synthetic intermediates. These compounds were evaluated against murine fibroblast 3T3 cells and their counterparts transformed by the Abelson murine leukemia virus which express the tyrosine kinase $pp120^{gag-abl}$: the compounds were more potent against the transformed cell lines which lent support to the possibility that they might inhibit the encoded tyrosine kinase. Surprisingly, we now report that compound **5a** elicits pronounced inhibitory effects against certain breast cancer cell lines with an unusual biphasic dose−response relationship against MCF-7 mammary carcinoma cells *in vitro* (see later). Activity was partially retained in the benzoxazole analog **5b** but abolished in the corresponding benzimidazole **5c**. In this paper we describe our extensive studies to explore the SAR in this simple new antitumor benzothiazole pharmacophore[4] and our efforts to identify how the subtle and selective effects might be explained.



Chemistry

The most versatile route to 2-(4-aminophenyl)benzothiazoles bearing substituents in both phenyl and benzothiazolyl rings started with nitrobenzanilides **6**, prepared by interaction of nitrobenzoyl chlorides and arylamines in pyridine (method A). The benzanilides were converted to thiobenzanilides **7** with Lawesson's reagent in hexamethylphosphoramide (HMPA) (method B) or chlorobenzene (method C) and cyclized to benzothiazoles **8** by the Jacobson synthesis[5] with alkaline

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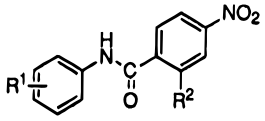
Table 1. Structure, Yields, and Physical Characteristics of Nitro-Substituted Benzanilides **6**, Nitro-Substituted Thiobenzanilides **7**, and 2-(4-Nitrophenyl)benzothiazoles **8**



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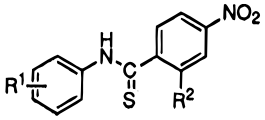

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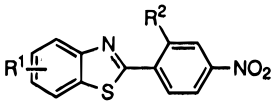

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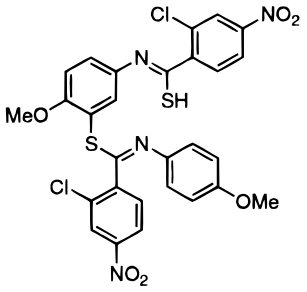
compd	R^1	R^2	method[j]	yield (%)	mp (°C)	formula[a]
6a	4-OMe	H	A	85	199−202[b]	−
6b	2,4-di-OMe	H	A	85	173−175[c]	$C_{15}H_{14}N_2O_5$
6c	3,4-di-OMe	H	A	83	180−182[d]	$C_{15}H_{14}N_2O_5$
6d	3,5-di-OMe	H	A	88	211−213[c]	$C_{15}H_{14}N_2O_5$
6e	2-OMe	Cl	A	83	144−146[b]	$C_{14}H_{11}ClN_2O_4$
6f	3-OMe	Cl	A	82	138−140[b]	$C_{14}H_{11}ClN_2O_4$
6g	4-OMe	Cl	A	90	181−183[b]	$C_{14}H_{11}ClN_2O_4$
6h	3-benzyloxy	Cl	A	91	190−191[b]	$C_{20}H_{15}ClN_2O_4$
6i	4-benzyloxy	Cl	A	97	208−210[b]	$C_{20}H_{15}ClN_2O_4$
7a	4-OMe	H	B	84	173−175[b]	−
7b	2,4-di-OMe	H	B	89	184−185[e]	$C_{15}H_{14}N_2O_4S$
7c	3,4-di-OMe	H	B	85	171−172[b]	$C_{15}H_{14}N_2O_4S$
7d	3,5-di-OMe	H	B	90	138−140[e]	$C_{15}H_{14}N_2O_4S$
7e	2-OMe	Cl	C	57	145−147[b]	$C_{14}H_{11}ClN_2O_3S$
7f	3-OMe	Cl	C	71	119−121[b]	$C_{14}H_{11}ClN_2O_3S$
7g	4-OMe	Cl	C	70	177−179[b]	$C_{14}H_{11}ClN_2O_3S$
7h	3-benzyloxy	Cl	C	63	111−113[b]	$C_{20}H_{15}ClN_2O_3S$
7i	4-benzyloxy	Cl	B	83	205−207[e]	$C_{20}H_{15}ClN_2O_3S$
8a	H	H	B[f]	85	229−231[b]	−
8b	6-OMe	H	D	62	216−217[g]	$C_{14}H_{10}N_2O_3S$
8c	4,6-di-OMe	H	D	29	215−217[g]	$C_{15}H_{12}N_2O_4S$
8d	5,6-di-OMe	H	D	38	235−236[g]	$C_{15}H_{12}N_2O_4S$
8e	5,7-di-OMe	H	D	60	238−239[h]	$C_{15}H_{12}N_2O_4S$
8f	4-OMe	Cl	D	46	187−188[b]	$C_{14}H_9ClN_2O_3S$
8g	5-OMe	Cl	D	12	225−227[g]	$C_{14}H_9ClN_2O_3S$
8h	6-OMe	Cl	D	24[i]	177−178[g]	$C_{14}H_9ClN_2O_3S$
8i	7-OMe	Cl	D	58	180−182[g]	$C_{14}H_9ClN_2O_3S$
8j	5-benzyloxy	Cl	D	32	156−159[g]	$C_{20}H_{13}ClN_2O_3S$
8k	7-benzyloxy	Cl	D	46	216−217[g]	$C_{20}H_{13}ClN_2O_3S$

[a] Analyses of all new compounds for C, H and N agree within ± 0.4% of calculated values for indicated atoms. [b] Crystallized from methanol. [c] Crystallized from ethanol. [d] Crystallized from aqueous methanol. [e] Crystallized from aqueous ethanol. [f] Also prepared (74%) from 2-aminothiophenol and 4-nitrobenzoyl chloride in pyridine. [g] Purified by flash chromatography (ethyl acetate-hexane). [h] Crystallized from ethanol-ethyl acetate. [i] Also formed (72%) was the product **10**. [j] Synthetic methods (see Experimental Section for details): A, from the aniline and nitrobenzoyl chloride in pyridine; B, from the nitro-substituted benzanilides and Lawesson's reagent in HMPA; C, from the nitro-substituted benzanilides and Lawesson's reagent in chlorobenzene: D, from the thiobenzanilides and potassium ferricyanide in aqueous sodium hydroxide.

potassium ferricyanide (method D). In the case of the 3-methoxythiobenzanilide (**7f**) a mixture of 5- and 7-substituted benzothiazoles **8g** and **8i**, respectively, was isolated from the Jacobson cyclization in the ratio 1:5. Similarly, the 3-(benzyloxy)thiobenzanilide **7h** gave a mixture of isomers **8j** and **8k** with cyclization preferred at the sterically crowded, but more electron-rich center *ortho* to the alkoxy group. In one case an unexpected product was isolated. Jacobson cyclization of the 4-methoxythiobenzanilide **7g** gave the expected 6-methoxybenzothiazole **8h** in only 24% yield but the major product (72%) had MW 643 (FAB MS) corresponding to a "dimer−2H" of the starting material. Originally a disulfide structure was assigned to this product, logically formed by oxidation of the thiol tautomer of **7g** in the alkaline ferricyanide:[2] however, this product was surprisingly stable with no cyclization to the benzothiazole **8h** being observed at 160 °C in diphenyl ether, behavior incompatible with a disulfide structure. Also, the ^1H and ^{13}C NMR spectra were more complex than those expected for a "dimeric" dithiol. Thus there were two distinct OMe absorptions at δ 3.15 and 3.31 in the ^1H spectrum. We propose, tentatively, structure **10** for this product, formed by a substitution *ortho* to the methoxy group in the Π-excessive ring of the starting substrate by its oxidatively derived thiol radical.[2] We prefer the imidoylthiol tautomer as shown in **10** rather than the thioamide tautomer since the ^1H



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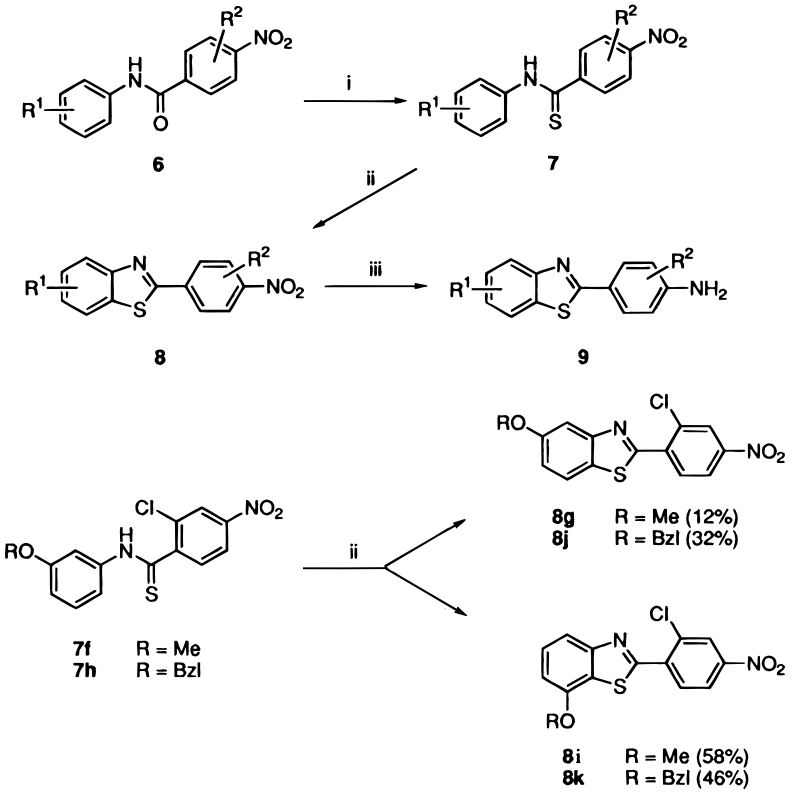
NMR spectrum in $CHCl_3$ showed an exchangeable SH proton at δ 3.29 whereas other thiobenzanilides **7** typically show (exchangeable) NH absorptions at δ 9.5−12.5. This anomalous behavior is restricted to thiobenzanilides derived from 4-methoxyanilines and explains the low yields of benzothiazoles from these starting materials by Jacobson cyclization. (Structures, yields and physical properties of all nitrobenzanilides, nitrothiobenzanilides and nitrophenylbenzothiazoles are listed in Table 1). In consistent manner, no cyclized-benzothiazole was isolated from the attempted cyclization of the 4-(benzyloxy)thiobenzanilide **7i**.

Scheme 1[a]

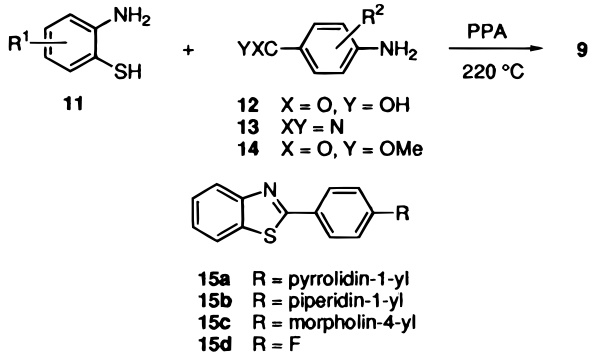


[a] Reagents: (i) Lawesson's reagent, HMPA or chlorobenzene; (ii) $K_3Fe(CN)_6$, aqueous NaOH; (iii) $SnCl_2 \cdot H_2O$, EtOH.

Reduction of (nitrophenyl)benzothiazoles with tin(II) chloride dihydrate in ethanol[6] (method E) gave the required (aminophenyl)benzothiazoles **9** (Scheme 1). Structures, yields, and physical characteristics of all aminophenylbenzothiazoles prepared by this, and other, routes are listed in Table 2.

Certain 2-(4-aminophenyl)benzothiazoles with no substituents in the benzothiazolyl moiety can be prepared by interaction of 2-aminothiophenol **11** (R^1 = H) and a 4-aminobenzoic acid **12** (method F)[7] or benzonitrile **13** (method G) in polyphosphoric acid (PPA) at 220 °C. Thus, treatment of 2-aminothiophenol with the corresponding substituted 4-aminobenzoic acids (**12**, R^2 = Me; 3,5-di-Cl; OH) in PPA gave compounds **9a**, **9k**, and **9w**, respectively. This route also afforded the 2-(2-aminopyridin-5-yl)benzothiazole **19a** from the reaction of 6-aminonicotinic acid and 2-aminothiophenol in PPA. Similarly, the monochloroamine **9i** and chlorotoluidine **9j** can be prepared from condensation of 4-amino-3-chlorobenzonitrile (**13**; R^2 = 3-Cl) and 4-amino-3-chloro-5-methylbenzonitrile (**13**, R^2 = 3-Cl, 5-Me) with 2-aminothiophenol in PPA, respectively (Scheme 2). However, a variation of this route using 2-aminothiophenol and the ester, methyl 4-amino-3-iodobenzoate (**14**, R^2 = 3-I), in PPA did not afford the expected iodoarylamine **9f**: instead, the deiodinated product **5a** was isolated in 14% yield. Similarly, interaction of 2-aminothiophenol and 4-amino-3,5-diiodobenzoic acid in PPA led only to the detection of **5a** by TLC. Using 4-amino-3-methoxybenzoic acid (**12**, R^2 = 3-OMe) as the acid component gave a mixture of five products. However, this route can be applied for the synthesis of the 2-(4-aminophenyl)-benzoxazole **5b** and -benzimidazole **5c** by employing 2-aminophenol and *o*-phenylenediamine, respectively, to construct the hetero fragment. 2-(4-Nitrophenyl)-benzothiazole (**8a**) is most conveniently formed from

Scheme 2

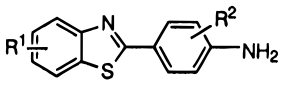


2-aminothiophenol and 4-nitrobenzoyl chloride in pyridine.

2-Phenylbenzothiazoles bearing tertiary amino groups were prepared by two routes. The pyrrolidine **15a** and the pyridine **18** were prepared by oxidative condensation of the thiophenol and 4-(pyrrolidin-1-yl)benzaldehyde or 4-formylpyridine, respectively, in refluxing DMSO,[8] and the amines **15b** and **15c** by displacement of the fluoro group in **15d** by piperidine or morpholine in aqueous sodium hydroxide−DMSO.

Controlled electrophilic substitution of amine **5a** and its 6-methyl analog with bromine in dichloromethane at −5 °C gave monobromo-products **9c** and **9d** (method H). This method was also extended to mono-bromination of **19a** to afford the (aminobromopyridyl)benzothiazole **19b** in 82% yield. More forcing bromination of **5a** in acetic acid gave the (dibromophenyl)benzothiazole **9e** (method I). Monoiodination of **5a**, and its 6-methyl- and 6-methoxy-analog **9m** was achieved with iodine monochloride[9] in acetic acid (method J) to afford **9f**, **9g**, and **9u**, respectively. The facility for iodine loss from **9f**, especially in the presence of copper reagents, was

Table 2. Structure, Yields, and Physical Characteristics of Alkyl-, Halo, Cyano-, Alkoxy-, and Hydroxy-substituted 2-(4-Aminophenyl)benzothiazoles **9**



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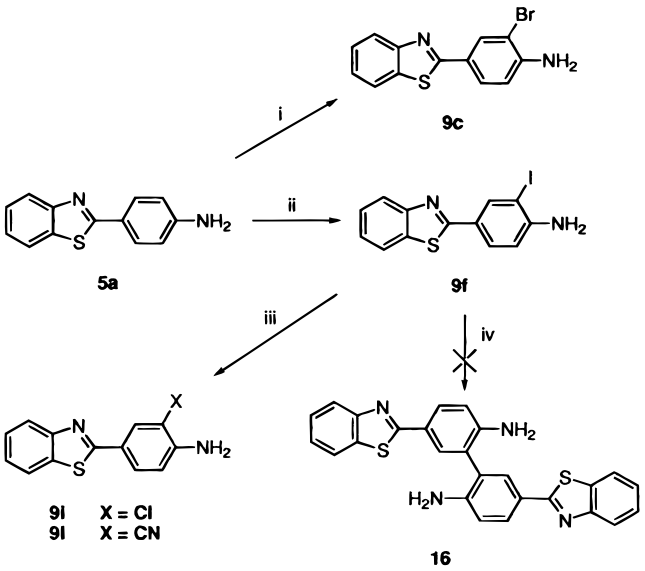
compd	R^1	R^2	synthetic method[f]	yield, %	mp,°C	formula[a]
5a	H	H	E (F)	90 (57)	155−157[b]	−
9a	H	3-Me	F	58	193−195[b]	$C_{14}H_{12}N_2S$
9b	H	3-Et	G	30	118−120[c]	$C_{15}H_{14}N_2S$
9c	H	3-Br	H	79	160−161.5[d]	$C_{13}H_9BrN_2S$
9d	6-Me	3-Br	H	85	188−189.5[d]	$C_{14}H_{11}BrN_2S$
9e	H	3,5-di-Br	I	78	201−203[d]	$C_{13}H_8Br_2N_2S$
9f	H	3-I	J	70	143−144[d]	$C_{13}H_9IN_2S$
9g	6-Me	3-I	J	67	176−178[d]	$C_{14}H_{11}IN_2S$
9h	H	2-Cl	E	93	100−101[c]	−
9i	H	3-Cl	G (K)	54 (63)	159−161[d]	$C_{13}H_9ClN_2S$
9j	H	3-Cl,5-Me	G	62	159−160[d]	$C_{14}H_{11}ClN_2S$
9k	H	3,5-di-Cl	F	64	205.5−207[b]	$C_{13}H_8Cl_2N_2S$
9l	H	3-CN	L	37	209−211[d]	$C_{14}H_9N_3S$
9m	6-OMe	H	E	92	191−193[d]	$C_{14}H_{12}N_2OS$
9n	4,6-di-OMe	H	E	83	181−183[d]	$C_{15}H_{14}N_2O_2S$
9o	5,6-di-OMe	H	E	89	221−222[d]	$C_{15}H_{14}N_2O_2S$
9p	5,7-di-OMe	H	E	90	150−152[d]	$C_{15}H_{14}N_2O_2S$
9q	4-OMe	2-Cl	E	77	148−150[d]	$C_{14}H_{11}ClN_2OS$
9r	5-OMe	2-Cl	E	83	260 dec[d]	$C_{14}H_{11}ClN_2OS$
9s	5-benzyloxy	2-Cl	E	90	119−121[d]	$C_{20}H_{15}ClN_2OS$
9t	6-OMe	2-Cl	E	87	183−185[e]	$C_{14}H_{11}ClN_2OS$
9u	6-OMe	3-I	J	55	179−181[d]	$C_{14}H_{11}IN_2OS$
9v	7-OMe	2-Cl	E	92	246−249[d]	$C_{14}H_{11}ClN_2OS$
9w	H	3-OH	F	60	214−216[d]	$C_{13}H_{10}N_2OS$
9x	6-OH	H	M	89	262−263[d]	$C_{13}H_{10}N_2OS$
9y	5,7-di-OH	H	M	62	294−295[d]	$C_{13}H_{10}N_2O_2S$
9z	5-OH, 7-OMe	H	M	23	282−283[d]	$C_{14}H_{12}N_2O_2S$
9aa	5-OH	2-Cl	N	45	276−279[d]	$C_{13}H_9ClN_2OS$
9bb	6-OH	2-Cl	O	46	184−186[d]	$C_{13}H_9ClN_2OS$

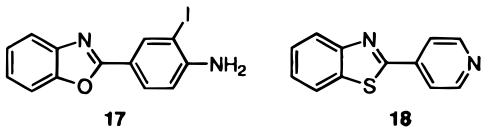
[a] Analyses of all new compounds for C, H, and N agree within ±0.4% of calculated values for indicated atoms. [b] Crystallized from aqueous methanol. [c] Crystallized from aqueous ethanol. [d] Purified by flash chromatography (ethyl acetate−hexane). [e] Crystallized from methanol. [f] Synthetic methods (see Experimental Section for details): E, reduction of the precursor nitro compound with tin(II) chloride dihydrate in ethanol; F, from 2-aminothiophenol and a 4-aminobenzoic acid in polyphosphoric acid; G, from 2-aminothiophenol and a 4-aminobenzonitrile in polyphosphoric acid; H, from the unbrominated precursor with bromine (1 mol equiv) in dichloromethane; I, from the unbrominated precursor with bromine (2.5 mol equiv) in acetic acid; J, from the uniodinated precursor with iodine monochloride in acetic acid; K, from the iodoamine with copper(I) chloride in DMF; L, from the iodoamine with copper(I) cyanide in DMF; M, demethylation of the precursor methoxybenzothiazole with boron tribromide in dichloromethane; N, hydrogenation of **8j** over 10% palladium-charcoal; O, demethylation of **9t** with borane−*NN*-diethylaniline and iodine in methanol.

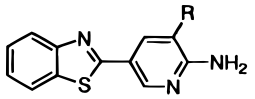
Scheme 3[a]



[a] Reagents: (i) Br_2, CH_2Cl_2; (ii) ICl, AcOH; (iii) CuX (X = Cl, CN), DMF; (iv) Cu, DMF.

exploited to achieve the synthesis of chloroaniline **9i** and cyanoaniline **9l** using copper(I) chloride (method K) or cyanide (method L) in boiling DMF. However, an attempted Ullmann coupling of **9f** to form the 2,2′-biphenyl **16** employing activated copper powder led only to deiodination and the isolation of **5a** in low yield (Scheme 3). Diaminobiphenyls of type **16** are major products from the decomposition of 2-(4-azidophenyl)-benzothiazoles in trifluoromethanesulfonic acid.[1] The iodinated benzoxazole **17** was prepared (66%) from **5b** and iodine monochloride in acetic acid.





Demethylation of the 6-methoxybenzothiazole **9m** with excess boron tribromide in dichloromethane at −70 °C to yield the phenol **9x** proceeded smoothly (89% yield) even in the presence of the potentially complicating 2-(4-aminophenyl) substituent (method M). However, demethylation of the 5,7-dimethoxybenzothiazole **9p** under

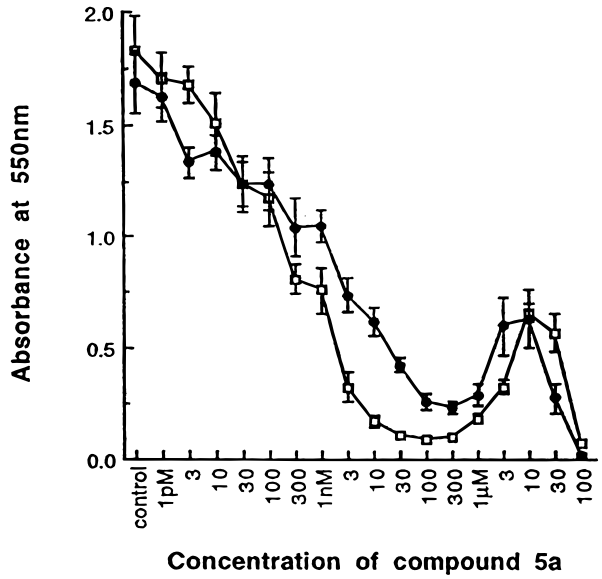


Figure 1. *In vitro* activity of 2-(4-aminophenyl)benzothiazole (**5a**) against MCF-7 (—) and MDA 468 (●—●) cells.

similar conditions was only partial, giving a mixture of the 5,7-dihydroxy- (**9y**) and 5-hydroxy-7-methoxybenzothiazole **9z**. The isomeric 7-hydroxy-5-methoxybenzothiazole structure for the demethylation product was excluded by an NOE difference experiment in DMSO-d_6 degassed with helium. Irradiation of the hydroxyl signal of **9z** resulted in enhancement of H-4 (NOE = 20%) and H-6 (10%); irradiation of the methoxyl singlet resulted in enhancement only at the H-6 signal (7%). Catalytic hydrogenation of 5-(benzyloxy)-2-(2-chloro-4-nitrophenyl)benzothiazole (**8j**) effected both removal of the benzyl group and reduction of nitro to amino affording the aminophenol **9aa** (method N). Removal of the 6-methoxy group of **9t** to yield the 6-hydroxybenzothiazole **9bb** was best accomplished with borane—*N*,*N*-diethylaniline complex and iodine in methanol (method O).

Biological Results and Discussion

***In Vitro* Studies.** The three isomeric 2-(aminophenyl)benzothiazoles were originally prepared as precursors to the corresponding 2-(hydroxyphenyl)benzothiazoles.[2] Evaluation of the prototype benzothiazole **5a** against murine 3T3 cells *in vitro* revealed an IC_{50} value of 55 μM compared with an IC_{50} of 16 μM against the Abelson leukemia virus transformed, tyrosine kinase pp120$^{gag-abl}$ encoded, counterpart (ANN-1). The comparable values for 2-(4-amino-2-chlorophenyl)-6-methoxybenzothiazole (**9t**) were 25 and 2 μM, respectively. However, we have been unable to demonstrate that these compounds elicit pharmacological effects by tyrosine kinase inhibition (see later).

In vitro evaluation of 2-(4-aminophenyl)benzothiazole (**5a**) against MCF-7 mammary carcinoma cells in two laboratories confirmed that the potency of the agent (IC_{50} 0.0003 and 0.0008 μM) was associated with an unusual biphasic dose—response relationship (Figure 1). Maximum growth arrest followed exposure to 100—300 nM; at concentrations between 1 and 30 μM proliferating colonies were observed (the "second proliferative phase") with cytotoxicity at >30 μM. A similar biphasic dose—response profile was seen for **5a** against MDA 468 cells. Continuous exposure of cells to amine **5a** was essential to maintain the second phase (concentrations between 1 and 30 μM). Removal of drug by aspiration of well contents and subsequent washing of cells with sterile PBS before replenishment with medium alone led to rapid cell death at these concentrations but did not affect the growth inhibitory profile at concentrations less than 1 μM. Aspiration of well contents, subsequent washing, and replenishment with medium containing **5a** did not affect the biphasic dose response.

Table 3. *In Vitro* Cytotoxicity of 2-(4-Aminophenyl)benzothiazole **5a** against Human Breast and Other Cancer Cell Lines

cell line	type	receptor status	IC_{50} (μM)[a]
MCF-7[b]	breast	ER^+	0.0003
MCF-7[c]	breast	ER^+	0.0008
MCF-7/Adr	breast	—	>10
MDA 468	breast	ER^- $EGFR^+$	0.0016
MDA 231	breast	$EGFR^+$ $erbB3^+$	0.017
SKBR 3	breast	ER^-	0.0081
ZR 75	breast	ER^+	0.028
T47D	breast	ER^-	>10
DU-145	prostate	—	>30
PC-3	prostate	—	>30
WiDr	colon	—	>30
HT29	colon	—	>30
A204	rhabdomyosarcoma	—	>30
A2780	ovarian	—	>30
IGR-37	melanoma	—	>30
T 24	bladder	—	>30

[a] All IC_{50} values are the mean of at least three determinations. [b] Determined at the University of Nottingham. [c] Determined at TNO, Rijswijk.

Table 4. *In Vitro* Activity of 2-(4-Aminophenyl)benzothiazoles and Related Compounds against MCF-7 and MDA 468 Mammary Carcinoma Cell Lines

IC_{50} range (μM)[a]	MCF-7	MDA 468
<0.0001—0.001	**5a* 9a 9a[b,c] 9c 9d 9f 9g 9i 9u**	**9a 9a[b,c] 9c 9d 9f 9g 9i 9u**
>0.001—0.01	**9b 9h* 9k 9m***	**5a* 5a[b] * 9b 9k**
>0.01—0.1	**5b* 9e 9j 9l 19b**	**9e 9l 18 19b**
>0.1—1	**15a***	**5b* 5c 9h* 9j 9x**
>1—10	**5c 9x 15b 15c 18**	**9n 9o 9w 15b 15c 18 19a**
>10	**9n 9o 9p 9q 9s 9w 9y 19a**	**9m* 9p 9q 9s**

[a] Initial cytotoxic event. [b] Methanesulfonic acid salt. [c] Ethanesulfonic acid salt. * Biphasic dose—response relationship.

Inhibition of growth appears to be selective for certain mammary carcinoma lines (Table 3) as **5a** was inactive (IC_{50} > 30 μM) against a range of colon, rhabdomyosarcoma, ovarian, melanoma, prostatic, and bladder cell lines (Table 3). MCF-7/Adr (an MCF-7 variant cell line with acquired resistance to adriamycin by expression of the MDR phenotype) appeared resistant to the growth inhibitory properties of **5a** (IC_{50} > 10 μM).

In order to determine whether compound **5a** was cytotoxic or merely cytostatic, assays were performed to measure lactate dehydrogenase leakage from treated cells. Following a drug exposure period of 96 h a biphasic cytotoxic response was observed. Maximum toxicity occurred between 10 and 300 nM in both MCF-7 and MDA 468 cell lines. In MCF-7 cells LC_{50} values of 0.001 μM and 24 μM were estimated for the initial and second phase cytotoxic events. Estimated LC_{50} values of 0.001 and 38 μM were obtained in MDA 468 cells.

Structure—Activity Relationships. In MTT assays the cytotoxicities (IC_{50} values) of 2-(4-aminophenyl)benzothiazoles and related compounds against MCF-7 and MDA 468 cells extend over a 6 log span (Table 4). There is good overall consistency in response between

Table 5. *In Vivo* Evaluation of 2-(4-Aminophenyl)benzothiazole (**5a**) and 2-(3-Aminophenyl)benzothiazole against Human Breast Carcinoma BO

compound	schedule	dose[a] (mg/kg/inj)	BWC[b] (%)	RTV[c] (T/C %)	evaluation[d]
5a	qd 27, 34, 41	100	−7	52*	(+)
	qd 27, 34, 41	10	−4	52*	(+)
	qd 27, 34, 41	1	−5	41*	+
2-(3-aminophenyl)benzothiazole	qd 27, 34, 41	200	−6	32*	++
	qd 27, 34, 41	20	−3	48*	+
	qd 27, 34, 41	2	2	78	−

[a] By ip route. [b] Body weight change. [c] Relative tumor volume. [d] (+), T/C % ≥51%; +, T/C % = 36−50%; ++, T/C = 21−35%. * Significant versus controls ($p < 0.05$).

Table 6. *In Vivo* Evaluation of 2-(4-Amino-3-methylphenyl)benzothiazole (**9a**) and 2-(4-Amino-3-iodophenyl)benzothiazole **(9f)** against Human Breast Carcinoma Xenografts

compound	tumor[a]	optimum dose[b] (mg/kg/inj)	schedule	WBC[c] (% of controls)	platelets (% of controls)	BWC[d] (%)	RTV[e] (T/C%)	evaluation[f]
9a	BO	25	qd 27, 34, 41	n.t.	n.t.	−9	35*	++
	MCF-7	12.5	qd 12, 19, 26	100	108	−12	31*	++
	MT-1	6.25	qd 7, 14, 21	n.t.	n.t.	−3	34*	++
	MT-3	12.5	qd 7, 14, 21	n.t.	n.t.	−3	22*	++
9f	BO	200	qd 27, 34, 41	n.t.	n.t.	−14	97	−
	MCF-7	200	qd 12, 19, 26	112	106	−14	68*	(+)

[a] Implanted sc. [b] By ip route. [c] White blood cells. [d] Body weight change. [e] Relative tumor volume. [f] See footnote *d*, Table 7. n.t. Not tested. * Significant versus controls ($p < 0.05$).

the two cell lines. Discrepancies arise when compounds eliciting a biphasic dose response (indicated with an asterix) induce an initial phase IC_{50} value in one cell line only. To aid evaluation of structure−activity relationships the data presented in Table 4 refers to IC_{50} ranges calculated for the initial cytotoxic event (see Figure 1). Further analysis of the biphasic nature of the response to selected compounds, and the influence of exposure time and drug biotransformation *in vitro*, will be published separately.

The lead benzothiazole **5a** is 10-fold more cytotoxic against the MCF-7 and MDA 468 lines than the benzoxazole **5b** but approximately 10000-fold more active than the benzimidazole **5c** congener. *In vitro* potency is retained in the methane- and ethanesulfonic acid salts of **5a.** The tertiary amine analogs **15a**−**c** have IC_{50} values in the 0.1−10 μM range. Introduction of alkoxy or hydroxy groups into the benzothiazole nucleus, in the absence of additional substituents in the arylamine residue, has a dyschemotherapeutic effect. Particularly striking is the inactivity of 2-(4-aminophenyl)-5,7-dihydroxybenzothiazole (**9y**) (IC_{50} 80 μM)—actually, the least active of all the compounds evaluated—against the MCF-7 cell line, despite this molecule having the same overall disposition of hydroxy groups in the heterocyclic nucleus as quercetin (**1**), genistein (**2**), and the trihydroxybenzothiazole (**3**).[2]

Introduction of a Cl substituent into the 2′-position of the aminophenyl group of **5a** gave compound **9h** with reduced activity compared to the parent amine especially against the MDA 468 cell line (IC_{50} 0.6 μM). However, a rich seam of activity extends through compounds with a 3′-methyl- (**9a**), 3′-ethyl- (**9b**), 3′-bromo- (**9c**), 3′-iodo- (**9f**)-, and 3′-chloro- (**9i**) substituents. Particularly effective against both cell lines (IC_{50} < 0.0001 μM) are 2-(4-amino-3-bromophenyl)-6-methylbenzothiazole (**9d**) and the iodo analog **9g**. In contrast, 3′-cyano- (**9l**) and 3′-hydroxy- (**9w**) substituents reduced activity markedly compared to the parent amine **5a**. A similar dyschemotherapeutic effect was observed in 3′,5′-disubstituted compounds **9e**, **9j**, and **9k**. The 3′-iodo derivative in the benzoxazole series **17** is approximately 10-fold less active than **9f**, confirming the enhanced potency of the benzothiazole series of compounds over the benzoxazoles.

Replacement of the 2-(4-aminophenyl) group of **5a** with a 2-(pyridin-4-yl) (**18**) or 2-(2-aminopyridin-5-yl) residue (**19a**) gave compounds cytotoxic only at concentrations >1 μM. Introduction of a bromo group to give 2-(2-amino-3-bromopyridin-5-yl)benzothiazole (**19b)** enhanced activity >100-fold over the unbrominated aminopyridine **19a**, but potency was still considerably less than that of the bromoarylamine **9c**.

***In Vivo* Studies.** As a preliminary to the *in vivo* tests the maximum tolerated doses (MTDs) of four test compounds administered as single doses (ip) in female BDF1 mice were assessed. Whereas the MTDs of the parent amine **5a**, a reference isomeric compound 2-(3-aminophenyl)benzothiazole, and 2-(4-amino-3-iodophenyl)benzothiazole (**9f**) were about 200 mg/kg, the corresponding value for the 2-(4-amino-3-methylphenyl) analog **9a** was only 25 mg/kg. Possibly these discrepancies reflect differences in solubility and/or routes of metabolism of the agents. Both compound **5a** and the 2-(3-aminophenyl) isomer elicited inhibitory effects against the breast carcinoma BO (Table 5), but in the former case both tumor growth and body weight were influenced relatively independent of dose. To some extent this mimics the unusual biphasic dose−response seen in *in vitro* tests on this compound (eg Figure 1). The isomer 2-(3-aminophenyl)benzothiazole gave a more classical *in vivo* dose−response relationship (Table 5).

The *in vivo* activity of the amines **9a** and **9f** in a panel of four or two, respectively, xenotransplanted breast carcinomas is recorded in Table 6. Whereas compound **9f** displayed only borderline activity in one of the models, compound **9a** induced a consistent tumor growth inhibition in all four tumors. One representative test against the MCF-7 carcinoma is documented in Figure 2. Although compound **9a** is toxic at the top dose of 25 mg/kg with only one survivor, the surviving animal was tumor free and no overall change in white blood cell or platelet counts were measured, indicating that bone marrow toxicity is not dose-limiting. The activity of **9a** against the ER^- tumors MT-1 and MT-3 is notable because these tumors are predictive for the clinical

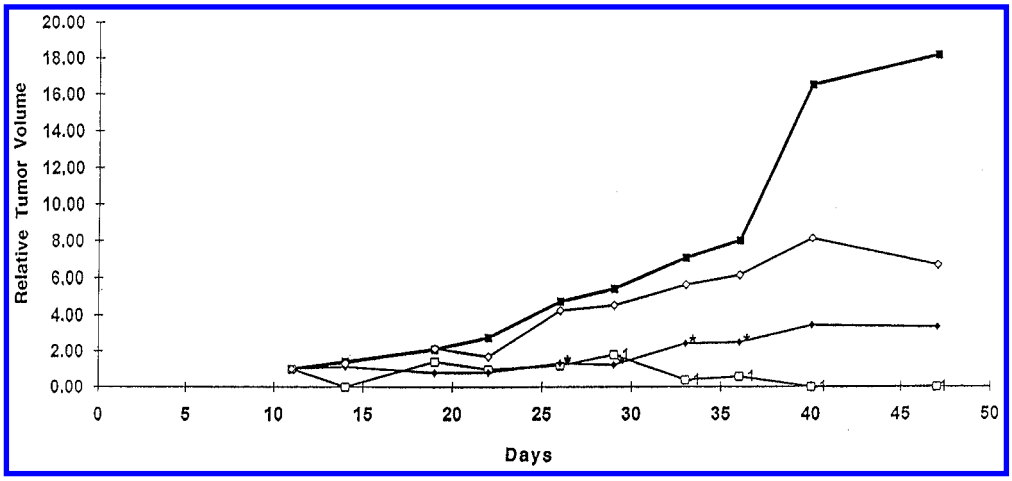


Figure 2. In vivo activity of 2-(4-amino-3-methylphenyl)benzothiazole (**9a**) against MCF-7 in nude mice administered on days 12, 19, and 26. Doses: ■—■, saline control; ◊—◊, 6.25 mg/kg/inj; ♦—♦, 12.5 mg/kg/ inj; □—□, 25 mg/kg/ inj. *Significant versus controls ($p < 0.05$).

activity of cyclophosphamide, adriamycin, and mitoxantrone and are exquisitively sensitive to hexadecylphosphocholine and other ether lipids.[10] In contrast these tumors are completely unresponsive to methotrexate and vincristine, and only modestly sensitive to cisplatin.

Mechanism Studies. Our search for a pharmacological mechanism to explain the unusual activity of this new series of compounds has not been illuminating. Thus (data not shown) compound **5a** has no effect on the assembly of microtubules in P388 cells; it does not inhibit human topoisomerase II (IC50 > 100 μM). At concentrations of 2, 5, and 10 μM, **5a** had no effect on aromatase and lyase obtained from human placental microsomes; also **5a** did not modulate protein kinase C (PKC) activity. The cell cycle regulators of G2/M transition, cdc 2 kinase, and cdc 25 phosphatase obtained from starfish oocytes were not affected by **5a**, **9a**, **9c**, or **9f** at concentrations of <50 μM.

Western blot assays performed using antiphosphotyrosine primary antibody demonstrated no inhibition of tyrosine phosphorylation following treatment with compounds **5a** and **9f** in unstimulated and EGF-stimulated MCF-7 or MDA 468 cells. **5a** (1, 10 and 100 μM) did not compete with EGF for EGF receptor in MCF-7 and MDA 468 cell lines. In the HBL-316 cell line, which is genetically engineered to overexpress a point mutation-activated erbB2 kinase, no inhibition of total phosphotyrosine levels measured by immunoassay, was detected. EGF (5, 50, and 100 ng/mL), diethylstilboestrol, α- and β-oestradiol (0.1 nM−1 μM) did not interfere with the biphasic nature of the dose response in MCF-7 cells to compound **5a** in phenol red free medium supplemented with charcoal stripped FCS, or medium supplemented with 1% FCS. Moreover, **5a** did not compete with oestradiol for estrogen receptors (ER). Thus it appears unlikely that the benzothiazoles subvert ER or EGFR dependent pathways to elicit their response. Concentrations of insulin (10 ng/mL), IGF I (10 nM), and IGF II (5 nM) which have been reported to stimulate the growth of breast cancer cell lines[11,12] did not affect the dose−response profiles nor the estimated IC_{50} values for growth arrest in MCF-7 cells induced by **5a**.

To aid future studies to elucidate the mode(s) of action of this series of compounds, MCF-7 cell lines have been established with acquired resistance to **5a** by long term exposure to 10 nM or 10 μM drug. These cells yielded estimated IC_{50} values of 48 μM and 57 μM, respectively, when rechallenged with drug. In addition, certain compounds have been evaluated in the NCI tumor cell panel. Compounds **9a**, **9c**, **9f**, and **9i**, four of the most active compounds described in the present work (Table 4), also show potent activities and disease selectivity, particularly in the NCI breast and ovarian subpanels but also against certain non-small cell lung (NCI-H226 and NCI-H460), colon (HCC-2998), and renal cell lines (A498 and TK-10), with biphasic dose−response relationships in the sensitive lines. In these evaluations, cells were exposed to drug for only 48 h compared with the longer drug exposures detailed in this paper. The cytotoxicity fingerprints of these four compounds are remarkably consistent. When 2-(4-amino-3-iodophenyl)-benzothiazole (**9f**) was used as a seed in a COMPARE analysis[13] the three closest compounds in a database of 48 000 profiles were the structurally related benzothiazoles **9a**, **9c**, and **9f** (Pearson correlation coefficient > 0.75). Moreover, as the compounds do not COMPARE with any clinical agents of known pharmacological type they may be operating by a new mechanism. Further details of these NCI results and the activities of 2-(4-aminophenyl)benzothiazoles in lung and ovarian cell panels will be published separately.

Conclusion

The new 2-(4-aminophenyl)benzothiazoles reported in this paper are simple to prepare, the molecules are chemically robust and display potent inhibitory properties in a range of cell types *in vitro*. No pharmacological mechanism has yet been adduced to explain these subtle and selective effects, particularly against breast cancer cell lines. For one example, 2-(4-amino-3-methylphenyl)benzothiazole (**9a**), this effectiveness extends to useful inhibition of a panel of human breast cancer models in nude mice. The possibility that the compounds might act by a novel mechanism makes the search for a pharmaceutically suitable candidate for clinical trial an urgent one.

Experimental Section

All new compounds listed in the tables were characterized by elemental microanalysis (C, H, and N values ±0.4% of theoretical values). Mass spectra were recorded on an AEI MS-902 or a VG Micromass 7070E spectrometer. IR spectra were determined in KBr on a Mattson 2020 GALAXY series

FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC250 or ARX250 spectrometers. TLC systems for routine monitoring of reaction mixtures and confirming the homogeneity of analytical samples employed Kieselgel 60F_{254} (0.25 mm) with either $CHCl_3$ or $CHCl_3$−2% ethanol as developing solvents. Sorbsil silica gel C 60-H (40−60 μm) and an ethyl acetate−hexane solvent mixture was used for flash chromatographic separations.

General Method for the Synthesis of Substituted Nitrobenzanilides 6. Method A. A mixture of the substituted aniline (0.02 M) and the appropriate nitrobenzoyl chloride (0.02 M) in pyridine (50 mL) was stirred under reflux (2 h) and poured into water. The precipitate was collected, washed with water, and purified as indicated in Table 1.

General Methods for the Synthesis of Substituted Nitrothiobenzanilides 7. Method B. A mixture of the substituted nitrobenzanilide (0.01 M) and Lawesson's reagent (0.6 mol equiv) in HMPA (30 mL) was stirred at 100 °C for 6 h and poured into water. The precipitate was collected and washed with water. Yields, physical characteristics, and purification methods are listed in Table 1.

Method C. As Method B, but using refluxing chlorobenzene (5−10 mL) for 3 h as solvent. Products precipitated slowly from the cooled reaction mixtures.

General Method for the Jacobson Synthesis of Substituted 2-(4-Nitrophenyl)benzothiazoles 8. Method D. Substituted nitrothiobenzanilides **7** (0.05 M) were wetted with a little ethanol, and 30% aqueous sodium hydroxide solution (8 mol equiv) was added. The mixture was diluted with water to provide a final solution/suspension of 10% aqueous sodium hydroxide. Aliquots of this mixture (5 mL) were added at 1 min intervals to a stirred solution of potassium ferricyanide (4 mol equiv) in water at 80−90 °C. The reaction mixture was heated for a further 0.5 h and then allowed to cool. Products **8** were collected and washed with water. Details of yields, physical characteristics, and purification methods are given in Table 1.

Jacobson Cyclization of Thiobenzanilide 7g. Cyclization of this thiobenzanilide by method D gave a mixture which was separated by flash chromatography using ethyl acetate−hexane (1: 4) as eluant. The minor product was 2-(2-chloro-4-nitrophenyl)-6-methoxybenzothiazole **8h** (24%). (For details of physical characteristics see Table 1). The major product (72%) was the imidoyl sulfide **10**; mp 136−137 °C; ^{1}H NMR ($CHCl_3$) δ 3.15 (s, 3 H, OMe), 3.29 (s, 1 H, SH, exchangeable with CD_3OD), 3.31 (s, 3 H, OMe). Anal. ($C_{28}H_{20}Cl_2N_4O_6S_2$) C, H, N.

General Methods for the Synthesis of Substituted 2-(4-Aminophenyl)benzothiazoles 9. Method E. A mixture of the appropriate 2-(4-nitrophenyl)benzothiazoles **8** (0.015 M) and tin(II) chloride dihydrate (0.075 M) in boiling ethanol (50 mL) was stirred under nitrogen for 4 h. Ethanol was removed by vacuum evaporation, the residue was extracted into ethyl acetate (3 × 100 mL), and the combined organic fraction shaken with 2M aqueous sodium hydroxide solution (3 × 100 mL) followed by water (2 × 100 mL). The organic layer was evaporated to leave a residue of the amine. Yields, physical characteristics, and purification methods are listed in Table 2.

Method F. 2-Aminothiophenol **11** (R^1 = H) (0.053 M) and the appropriate substituted benzoic acids **12** (0.05 M) were heated in polyphosphoric acid (PPA; 85 g) at 220 °C for 4 h, cooled, and poured into ice-cold 10% aqueous sodium carbonate. The solid product was collected, washed (water), and purified as indicated in Table 2.

Method G. As Method F but reacting together 2-aminothiophenol and a substituted benzonitrile **13** in PPA.

Method H. A solution of bromine (0.32 g) in dichloromethane (10 mL) was added at −5 °C to a solution of a 2-(4-aminophenyl)benzothiazole (1 mol equiv) in dichloromethane (50 mL). After being stirred at −5 °C for 2 min the mixture was shaken with ice−water (400 mL) for 1 h. The separated organic layer was washed with 10% aqueous sodium thiosulfate (2 × 50 mL) and water (2 × 60 mL), dried ($MgSO_4$), and vacuum evaporated to furnish a residue which was purified by flash chromatography.

Method I. A solution of 2-(4-aminophenyl)benzothiazole (**5a**) (0.6 g) in acetic acid (15 mL) was mixed with a solution of bromine (0.98 g, 2.5 mol equiv) in acetic acid at 25 °C and then warmed to 80 °C for 2 h. The acetic acid was removed by vacuum evaporation and the residue partitioned between 10% aqueous sodium bicarbonate (150 mL) and dichloromethane (150 mL). The separated organic layer was purified as in method H to yield 2-(4-amino-3,5-dibromophenyl)benzothiazole **9e** as a yellow solid (78%). Physical characteristic are recorded in Table 2.

Method J. To a solution of a 2-(4-aminophenyl)benzothiazole (0.013 M) in acetic acid (35 mL) was added dropwise over 10 min at 25 °C a solution of iodine monochloride (0.017 M) in acetic acid (35 mL). The mixture was stirred at 25 °C for a further 1.5 h and excess acetic acid removed by vacuum evaporation. The residue was partitioned between aqueous sodium bicarbonate−dichloromethane and the separated organic layer purified as in method H.

Method K. A mixture of 2-(4-amino-3-iodophenyl)benzothiazole (**9f**) (0.3 g) and copper(I) chloride (0.16 g) was boiled in DMF (20 mL) for 4 h. The reaction mixture was concentrated by vacuum evaporation and the residue stirred with water. Organic products were extracted into ethyl acetate, dried ($MgSO_4$), and chromatographed on silica gel using ethyl acetate−hexane (2:5) as eluting solvent. 2-(4-Amino-3-chlorophenyl)benzothiazole (**9i**) was isolated (63%) as a cream solid (see Table 2).

Method L. Interaction of the benzothiazole **9f** with copper(I) cyanide in boiling DMF as in method K gave 2-(4-amino-3-cyanophenyl)benzothiazole (**9l**) (37%) (see Table 2).

General Methods for the Synthesis of Hydroxy-Substituted 2-(4-Aminophenyl)benzothiazoles. Method M. To a stirred suspension of the methoxy-substituted 2-(4-aminophenyl)benzothiazole (0.01 M) in dry dichloromethane (25 mL) under nitrogen at −70 °C was added a 1 M solution of boron tribromide (2 mol equiv for each methoxy group plus a further 3 mol equiv)[2] dropwise over 30−45 min. The mixture was maintained at −70 °C for 1 h and then allowed to warm slowly to 20 °C and stirred overnight. The reaction mixture was recooled to −70 °C and quenched by the dropwise addition of methanol until no further reaction occurred. The mixture was poured into 8% aqueous sodium hydroxide solution (50 mL). The aqueous phase was separated, neutralized to pH 7 with 5 M hydrochloric acid, and extracted three times with a mixture of dichloromethane−methanol (4:1). The combined organic layers were dried ($MgSO_4$) and evaporated to yield hydroxy-substituted 2-phenylbenzothiazoles**.** The crude products were purified by flash column chromatography employing ethyl acetate−hexane (3:2) as solvent. Yields and physical characteristics of the products are listed in Table 2.

Method N. A solution of 5-(benzyloxy)-2-(2-chloro-4-nitrophenyl)benzothiazole **8j** (0.064 g) in acetone was hydrogenated at 30 psi over a 10% palladium-charcoal catalyst (0.025 g). After removal of catalyst through Celite and evaporation of solvent, the residue was purified by flash chromatography, eluting with ethyl acetate−hexane (1:2), to yield 2-(4-amino-2-chlorophenyl)-5-hydroxybenzothiazole (**9aa**) (45%): see Table 2 for physical characteristics.

Method O. Compound **9t** (0.068 g) was reacted with borane−*N*,*N*-diethylaniline complex (0.057 g) and iodine (0.8 g) in methanol at room temperature overnight. After removal of solvent the residue was dissolved in ethyl acetate (50 mL) and shaken successively with 5% aqueous sodium thiosulfate (2 × 50 mL) and water (2 × 100 mL). The organic layer was concentrated and the residue chromatographed using ethyl acetate−hexane (1:1) as eluting solvent. The 6-hydroxybenzothiazole **9bb** was isolated in 46% yield (see Table 2 for physical characteristics).

2-(4-Aminophenyl)benzothiazole (5a). This compound was prepared from reduction of 2-(4-nitrophenyl)benzothiazole[1] with tin(II) chloride dihydrate in ethanol: mp 155−157 °C (lit.,[14] 157 °C); ^{1}H NMR (DMSO-d_6) δ 8.00 (d, 1H, *J* 7.1 Hz, H-7), 7.88 (d, 1H, *J* 7.7 Hz, H-4), 7.75 (d, 2H, *J* 8.6 Hz, H-2′, H-6′), 7.45 (dt, 1H, *J* 1.3, 7.6 Hz, H-5), 7.33 (dt, 1H, *J* 1.2, 7.5 Hz, H-6), 6.66 (d, 2H, *J* 8.7 Hz, H-3′, H-5′), 5.90 (brs, 2H, NH_2); IR 3458, 3296, 3179, 1637, 1604, 1479, 1432, 1312, 1239, 1186, 963, 826, 760, 730, 715 cm^{-1}.

2-(4-Aminophenyl)benzoxazole (5b). 2-Aminophenol (1.5 g) and 4-aminobenzoic acid (1.89 g) were reacted in PPA at 190 °C according to General Method F. The benzoxazole **5b** (62%) had mp 176−178 °C (MeOH−H_2O) (lit.,[15] mp 185 °C).

2-(4-Aminophenyl)benzimidazole (5c). Similarly prepared, from 2-aminoaniline and 4-aminobenzoic acid, the benzimidazole **5c** (70%) had mp 244.5−246 °C (EtOH−H_2O) (lit.,[16] mp 246−247 °C).

2-(4-Amino-3-methylphenyl)benzothiazole (9a). Prepared from 4-amino-3-methylbenzoic acid and 2-aminothiophenol at 220 °C in PPA according to General Method F. ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1H, *J* 8.0 Hz, H-7), 7.90−7.86 (m, 2H, H-4, H-2′), 7.79 (dd, 1H, *J* 2.1, 8.2 Hz, 6′-H), 7.48 (dt, 1H, *J* 1.3, 7.7 Hz, 5-H), 7.37 (dt, 1H, *J* 1.2, 7.6 Hz, 6-H), 6.76 (d, 1H, *J* 8.2 Hz, H-5'), 3.98 (br s, 2H, NH_2), 2.28 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.2 (C), 154.7 (C), 148.0 (C), 135.0 (C), 130.2 (CH), 127.4 (CH), 126.4 (CH), 124.8 (CH), 124.3 (C), 122.9 (CH), 122.6 (C), 121.8 (CH), 115.0 (CH), 17.6 (CH_3); IR 3469, 3323, 3198, 1625, 1481, 1438, 1403, 1310, 1237, 753 cm^{-1}; MS (EI) *m*/*z* 240 (M^+), 223.

2-(4-Amino-3-bromophenyl)benzothiazole (9c). Compound **5a** was brominated with bromine in dichloromethane at −5 °C according to General Method H to give **9c**. ^{1}H NMR (DMSO-d_6) δ 8.08−8.05 (m, 2H, H-7, H-2′), 7.95 (d, 1H, *J* 8.0 Hz, H-4), 7.78 (dd, 1H, *J* 1.6, 8.5 Hz, H-6′), 7.50 (t, 1H, *J* 7.6 Hz, H-5), 7.39 (t, 1H, *J* 7.5 Hz, H-6), 6.91 (d, 1H, *J* 8.5 Hz, H-5′), 6.13 (brs, 2H, NH_2); ^{13}C NMR (DMSO-d_6) δ 167.3 (C), 154.5 (C), 149.7 (C), 134.8 (C), 131.8 (CH), 128.8 (CH), 127.3 (CH), 125.6 (CH), 122.9 (2CH), 122.6 (C), 115.9 (CH), 107.9 (C); IR 3440, 3291, 3174, 1625, 1600, 1476, 1404, 1320, 1225, 754, 724 cm^{-1}; MS (EI) *m*/*z* 306 ($M^+ + 1$), 225 ($M^+ + 1$, − Br), 198.

2-(4-Amino-3-iodophenyl)benzothiazole (9f). Prepared from iodination of the amine **5a** with iodine monochloride according to General Method J; ^{1}H NMR (DMSO-d_6) δ 8.28 (d, 1H, *J* 2.1 Hz, 2′-H), 8.05 (d, 1H, *J* = 7.6 Hz, H-7), 7.94 (d, 1H, *J* 7.7 Hz, H-4), 7.79 (dd, 1H, *J* 2.1, 8.5 Hz, H-6′), 7.49 (dt, 1H, *J* 1.2, 7.7 Hz, H-5), 7.38 (dt, 1H, *J* 1.2, 7.6 Hz, H-6), 6.86 (d, 1H, *J* 8.5 Hz, H-5′), 5.98 (brs, 2H, NH_2); ^{13}C NMR (DMSO-d_6) 166.4 (C), 153.6 (C), 151.8 (C), 137.5 (CH), 134.0 (C), 128.8 (CH), 126.6 (CH), 124.9 (CH), 123.0 (C), 122.20 (CH), 122.18 (CH), 114.0 (CH), 82.6 (C, C-3′); IR 3426, 3286, 3173, 1623, 1587, 1473, 1435, 1398, 1310, 1225, 973, 874, 753, 723 cm^{-1}; MS (EI) *m*/*z* 352 (M^+), 260, 225 (M^+ − I).

2-(4-Amino-3-chlorophenyl)benzothiazole (9i). Compound **9i** was prepared from condensation of 4-amino-3-chlorobenzonitrile with 2-aminothiophenol in PPA at 230 °C according to General Method G: ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 1H, *J* 2.0 Hz, H-2′), 8.05 (d, 1H, *J* 8.0 Hz, H-7), 7.89 (d, 1H, *J* 7.9 Hz, H-4), 7.81 (dd, 1H, *J* 2.0, 8.4 Hz, H-6′), 7.50 (dt, 1H, *J* 1.3, 7.7 Hz, H-5), 7.37 (dt, 1H, *J* 1.2, 7.6 Hz, H-6), 6.85 (d, 1H, *J* 8.4 Hz, H-5′), 4.43 (brs, 2H, NH_2); ^{13}C NMR δ ($CDCl_3$) 167.4 (C), 154.5 (C), 145.8 (C), 135.1 (C), 129.0 (CH), 127.7 (CH), 126.7 (CH), 125.2 (CH), 125.1 (C), 123.1 (CH), 121.9 (CH), 119.7 (C), 115.8 (CH); IR 3451, 3300, 1626, 1475, 1406, 1384, 1224, 754, 723 cm^{-1}; MS (EI) *m*/*z* 260 (M^+), 225 (M^+ − Cl), 108.

2-[(4-Pyrrolidin-1-yl)phenyl]benzothiazole (15a). Interaction of 2-aminothiophenol (1.5 g, 12.5 mmol) and 4-(pyrrolidin-1-yl)benzaldehyde (2.24 g, 12.5 mmol) in DMSO (50 mL) at 180 °C for 15 min gave the pyrrolidinobenzothiazole **15a** (2.8 g, 80%) when the mixture was diluted with water: mp 240−241 °C (EtOH−DMSO) (lit.,[8] mp 241−243 °C).

2-[4-(Piperidin-1-yl)phenyl]benzothiazole (15b). A mixture of 2-(4-fluorophenyl)benzothiazole[17] (0.8 g, 3.49 mmol), piperidine (3.0 mL), 50% aqueous KOH (1.5 mL), and DMSO (5 mL) was heated at 100 °C for 24 h and cooled, and the melt was poured into water. An ethyl acetate extract furnished **15b** (0.43 g, 42%): mp 171−173 °C (MeOH) (lit.,[8] mp 175−176 °C).

2-[4-(Morpholin-4-yl)phenyl]benzothiazole (15c). Similarly prepared, from 2-(4-fluorophenyl)benzothiazole and morpholine, this compound (50% yield) had mp 272−273 °C (MeOH) (lit.,[8] mp 277−278 °C).

2-(Pyridin-4-yl)benzothiazole (18). From 2-aminothiophenol and 4-formylpyridine, according to the method used to prepare compound **15a**, the pyridylbenzothiazole **18** (85%) had mp 131−133.5 °C (lit.,[8] mp 135−136 °C).

2-(2-Aminopyridin-5-yl)benzothiazole (19a). Prepared from 2-aminothiophenol and 6-aminonicotinic acid according to General Method F, the benzothiazole **19a** (59%) had mp 188−189 °C; ^{1}H NMR (DMSO-d_6) δ 8.65 (d, 1 H, *J* 2.3 Hz, H-2′), 8.08−8.01 (m, 2 H, H-6′,4), 7.95 (d, 1 H, *J* 8.1 Hz, H-7), 7.49 (t, 1 H, *J* 7.3 Hz, H-5), 7.38 (t, 1 H, *J* 7.4 Hz, H-6), 6.77 (brs, 2 H, NH_2), 6.59 (d, 1 H, *J* 8.8 Hz, H-5′); IR (KBr) 3370, 3150, 1610, 1470, 1400, 955, 840, 750 cm^{-1}; MS (EI) *m*/*z* 228 ($M^+ + 1$). Anal. ($C_{12}H_9N_3S$) C, H, N.

2-(2-Amino-3-bromopyridin-5-yl)benzothiazole (19b). The amine **19a** was brominated in dichloromethane according to General Method H. The crude product was chromatographed, eluting with hexane−ethyl acetate (2:1), to give title compound (82%): mp 232−233.5 °C, ^{1}H NMR (DMSO-d_6) δ 8.66 (d, 1 H, *J* 2.0 Hz, H-2′), 8.33 (d, 1 H, *J* 2.0 Hz, H-6), 8.11 (d, 1 H, *J* 7.9 Hz, H-4), 7.98 (d, 1 H, *J* 8.0 Hz, H-7), 7.53 (t, 1 H, *J* 7.5 Hz, H-5), 7.42 (t, 1 H, *J* 7.5 Hz, H-6), 7.06 (brs, 2 H, NH_2); IR (KBr) 3420, 3275, 3150, 1650, 1465, 1400, 1220, 755 cm^{-1}; MS (EI) *m*/*z* 308 ($M^+ + 2$), 305 ($M^+ - 1$). Anal. ($C_{12}H_8BrN_3S$) C, H, N.

Synthesis of Salts of 2-(4-Aminophenyl)benzothiazoles. 2-(4-Aminophenyl)benzothiazole Methanesulfonic Acid Salt. To a solution of the aminophenylbenzothiazole **5a** (0.5 g) in ethyl acetate (65 mL) was added, dropwise, methanesulfonic acid (0.215 g) at 25 °C. The salt was collected and washed with ethyl acetate to give a yellow powder (0.67 g, 94%): mp 261−262 °C; ^{1}H NMR (DMSO-d_6) 2.41 (s, 3 H, CH_3). Anal. ($C_{14}H_{14}N_2S_2O_3$) C, H, N. Similarly prepared were the following:

2-(4-Aminophenyl)benzothiazole ethanesulfonic acid salt: a yellow solid (90%), mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 2.52 (q, 2 H, CH_2), 1.10 (t, 3 H, CH_3). Anal. ($C_{15}H_{16}N_2S_2O_3$) C, H, N.

2-(4-Amino-3-methylphenyl)benzothiazole methanesulfonic acid salt: a yellow solid (94%), mp 199−201 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3 H, CH_3SO_3), 3.30 (s, 3 H, CH_3). Anal. ($C_{15}H_{14}N_2S_2O_3$) C, H, N.

2-(4-Amino-3-methylphenyl)benzothiazole ethanesulfonic acid salt: as a yellow solid (93%), mp 206−209 °C; ^{1}H NMR (DMSO-d_6) 2.54 (q, 2 H, CH_2), 2.33 (s, 3 H, CH_3), 1.11 (t, 3 H, CH_3) Anal. ($C_{16}H_{16}N_2S_2O_3$) C, H, N.

***In Vitro* Cytotoxicity Assays.** Stock solutions of drugs (10 mM) were prepared in DMSO and stored protected from light at 4 °C for 4 weeks. Compounds tested were stable up to 50 °C in a pH range of 1−10 (>28 days; data not shown). Serial dilutions were prepared in medium prior to assay so that the final concentration of DMSO did not exceed 0.25%.

3T3 and ANN-1 Cells. Cells (2×10^4) were grown in Dulbecco's modified Eagles medium, supplemented with 10% fetal calf serum. Assays were conducted according to a published method.[18]

MCF-7, MDA 468, MCF-7/Adr, SKBR 3, ZR 75, and MDA 231, T47D Human Breast Carcinoma Cell Lines, DU145 and PC3 Human Prostate Carcinoma Cell Lines. Cells were cultured in an atmosphere of 5% CO_2 in RPMI 1640 medium containing 2 μM L-glutamine supplemented with 10% foetal bovine serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin. Cells were routinely subcultured twice weekly to maintain logarithmic growth. Cells were seeded into 96-well plates at a density of $2.5-3 \times 10^2$ per well and allowed to adhere for 4 h before drug was introduced. A final concentration range between 1 pM and 100 μM was achieved ($n = 8$). Cultures were incubated for 10 days (MDA 468) or 7 days (all other cell lines). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, final concentration 400 mg/mL) was added to each well. The following 4 h incubation period allows metabolism of MTT by mitochondrial dehydrogenases of viable cells to form an insoluble formazan product. Medium was aspirated and formazan solubilized by addition of DMSO (100 μL) and glycine buffer (25 μL). Absorbance, as a measure of viable cell number, was read at 550 nm on an Anthos Labtec systems plate reader.

MCF-7 Breast, WiDr and HT29 Colon, A204 Rhabdomyosarcoma, A2780 Ovarian, IGR-37 Melanoma, and T 24 Bladder Tumor Cell Lines. Cells were seeded at a density of 10^4 in Dulbecco's medium and exposed to drug for 120 h. The end-point was determined by the addition of 100

μL of saline containing 0.002% w/v propidium bromide (Sigma), 0.3% drawing ink (Staedtler marsmatic 745), and Triton X-100.[2] The plates were kept at 4 °C overnight before reading with an automated scanning stage. Fluorescence intensity was measured in arbitrary units by a photomultiplier. An HP-87 computer controlled the movement of the stage and collected and processed the data from the multiplier. For each compound, a dose−response curve was obtained and the IC_{50} value (the drug concentration producing 50% inhibition of cell growth) was calculated.

Agent Cytotoxicity. This was estimated by measuring the leakage of lactate dehydrogenase (LDH) from cells damaged by toxic insult. Cells were seeded into 24-well plates in medium supplemented with 1% FCS at a density of 5×10^4/well and allowed 4 h to attach before drug was administered (final concentration 1 nM−100 μM, $n = 3$/control $n = 6$). Following 96 h exposure, medium was collected, centrifuged to pellet any debris and assayed for LDH activity. Concurrently, cells were counted using a haemocytometer. The oxidation of NADH to NAD^+ by LDH was measured spectrophotometrically by following the decrease in absorption at 340 nM (Leathwood and Plummer, 1969). An amount of 2.4 mL of PBS, pH 7.4, 0.1 mL of NADH (3.5 mM), and 0.4 mL of medium sample were added to a cuvette. The assay mixture was allowed to equilibrate at 37 °C before initiating the reaction by addition of 0.1 mL of sodium pyruvate solution (32 mM). The rate of change of absorbance over 5 min was monitored on a Pye Unicam SP8-400 UV/vis spectrophotometer. Maximal release of LDH, representing 100% cell death, was determined following lysis of untreated cells in 1% Triton-X 100. LDH release was measured in untreated cells to obtain a value representing natural cell death. Agent cytotoxicity was expressed as % Triton- releasable LDH activity/10^5 cells, and the drug concentration which elicited 50% toxicity (LC_{50} value) calculated.

***In Vivo* Antitumor Tests.** Four human breast carcinomas xenotransplanted into female Ncr: nu/nu (Taconic, Germantown, USA) or male Bom: NMRI-nu/nu mice (Bomholtgaard, Ry, Denmark) were used for the evaluation of antitumor activity. Mice weighing 20−25 g at the start of experiments were held under sterile conditions at 24−26 °C, 50% relative humidity, and 12 h light-dark rhythm in laminar flow shelves. The animals received autoclaved food and bedding (Sniff, Soest, Germany); the drinking water was filtered and acidified (pH 4.0).

The following breast carcinoma cell lines were used: BO (T61, gift of Prof. Fortmeyer, Frankfurt, Germany); MCF-7 (NCI, USA); MT-1 and MT-3.[10] The tumors BO and MCF-7 are ER^+ models, the carcinomas MT-1 and MT-3 ER^- ones. Tumors were transplanted subcutaneously (sc) as pieces (2×2 mm) into the left flank of 5−8 nude mice/experimental group. Drug treatment was initiated when the tumors reached a diameter of 4−5 mm. Compounds were solubilized with Tween 80 (maximum 10% of final volume) and suspended in saline. Suspensions were prepared freshly for each drug administration and injected in a volume of 0.2 mL/20 g body weight employing a once-weekly schedule ($\times$ 3). Tumor size was measured twice weekly with a caliper. Median tumor volume/group was related to the first treatment day and expressed as relative tumor volume (RTV). For the estimation of toxicity, body weight was determined twice weekly and the mean percentage body weight change (BWC) was calculated. In one experiment blood was obtained from the retroorbital venous plexus of mice and blood cells were determined with a Coulter counter (Model T41).

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Supporting Information Available: ^1H NMR chemical shifts, coupling contants, and ^{13}C NMR chemical shifts for **6a**−**i**, **7a**−**i**, **8b**−**8k**, **9b**, **9d-e**, **9g, 9j-z**, **9aa,** and **9bb** (4 pages). Ordering information is given on any current masthead page.

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