4-Substituted 4-Hydroxycyclohexa-2,5-dien-1-ones with Selective Activities against Colon and Renal Cancer Cell Lines

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Received July 1, 2002

The synthesis and antitumor evaluation of a series of new heteroaromatic- and aromatic-substituted hydroxycyclohexadienones ("quinols"), and their imine counterparts, are described. The quinols were synthesized via the addition of a lithiated aromatic moiety to a quinone ketal followed by deprotection. When the aromatic portion of the molecule is a fused heterobicyclic structure (e.g., benzothiazole derivative **7a**), potent in vitro antitumor activity was observed in HCT 116 (GI_{50} = 40 nM) and HT 29 (GI_{50} = 380 nM) human colon as well in as MCF-7 and MDA 468 human breast cancer cell lines. When examined on the NCI Developmental Therapeutics Screening Program in vitro screen (60 human cancer cell lines), active compounds in this series consistently displayed a highly unusual pattern of selectivity; cytotoxicity (LC_{50}) was concentrated in certain colon and renal cell lines only. Analogue **7a** also showed in vivo antitumor activity against human RXF 944XL renal xenografts in nude NMRI mice and is the focus of further study.

Introduction

Phenolic xenobiotics can be modified by cellular systems in a number of ways, e.g., oxidation, glucuronidation, sulfation, methylation, or acetylation,[1] and the bioinstability (under nonphysiological conditions) of certain phenolic protein tyrosine kinase (PTK) inhibitors has been documented. For example, the antitumor PTK inhibitor erbstatin (Figure 1) has a short half-life (<30 min) in fetal calf serum,[2] and the lack of correlation of the activity of the phenolic tyrphostins (Figure 1) with isolated enzymes and their effects in vitro and in vivo is noteworthy.[3] Perhaps most significantly, di- and triphenolic tyrphostins decompose in solution to more active PTK inhibitors,[4] whereas tyrphostins devoid of hydroxy groups have a rapid onset of cellular activity.[5] These observations implicate metabolic oxidation to a quinone (or other) moiety as a potential bioactivating step.

We are interested in using bioactive phenols as starting materials to generate novel and structurally diverse chemical oxidation products that might have enhanced biological properties.[6] Preliminary studies in this area have focused on the oxidation of simple (hydroxyphenyl)benzothiazoles using the hypervalent iodine oxidizing agent [(diacetoxy)iodo]benzene (DAIB). For example, oxidation of 2-(4-hydroxyphenyl)benzothiazole **1** in acetic acid afforded the acetoxycyclohexadienone **2a**; oxidation in alcohols gave the ethers **2b**−**e** (Scheme 1) but the yields deteriorated with the increasing complexity of the alcohol. Oxidation of 2-(3-hydroxyphenyl)benzothiazole **3** in methanol or ethanol similarly gave the acetals **4a** or **4b**, respectively.[7]

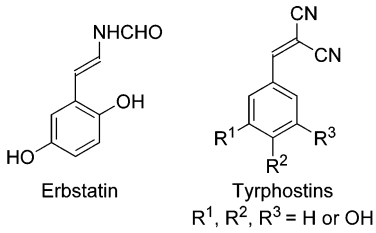
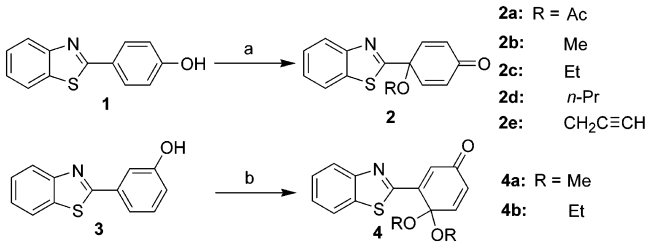


Figure 1. Chemical structures of the protein kinase inhibitors erbstatin and the tyrphostins.

Scheme 1. Oxidation of (Hydroxyphenyl)benzothiazoles Using DAIB[a]



[a] Reagents: (a) DAIB (1 equiv), ROH, 25 °C except R = Ac (50 °C); (b) DAIB (2 equiv), ROH, 25 °C.

Despite its simple structure, the acetoxy derivative **2a** (NSC 696142) showed potent activity in the National Cancer Institute (NCI) in vitro 60-cell panel (mean GI_{50} = 0.36 μM); the starting phenol **1** (NSC 33044) was 100-fold less potent (mean GI_{50} = 33.9 μM). We have now shown that the acetoxy derivative **2a** is unstable in cell culture media, undergoing rapid hydrolysis to the benzothiazole-substituted quinol **7a** (NSC 706704), a compound that retains the characteristic antitumor profile of **2a**. It became a priority to develop a reliable synthetic route to quinols related to **7a** and to glean information on potential biological targets perturbed by

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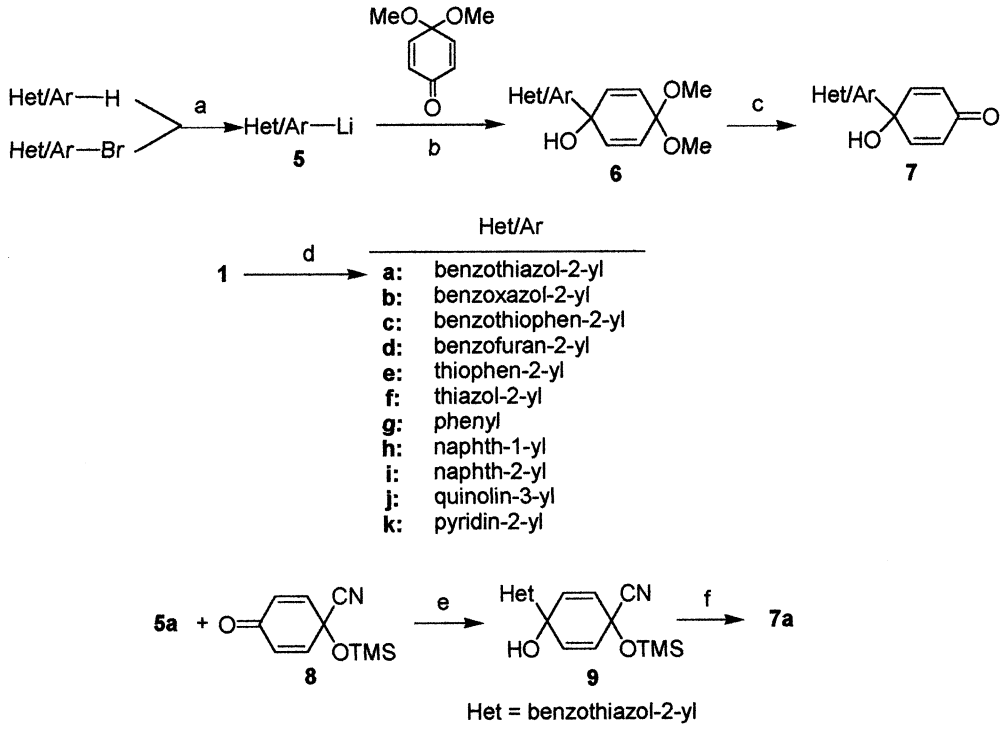
10.1021/jm020984y CCC: $25.00
Published on Web 01/21/2003

Scheme 2. Synthesis of Aromatic and Heteroaromatic Quinols[a]



[a] Reagents: (a) *n*-BuLi, THF, −78 °C; (b) THF, −78 °C; (c) 10% AcOH (aq), acetone, reflux; (d) $PhI(OCOCF_3)_2$, TEMPO, CH_3CN/H_2O (9:1); (e) *n*-BuLi, Et_2O, −100 °C; (f) TBAF·$3H_2O$, THF.

compounds possessing this new pharmacophore that emerged from our "chemistry-driven" approach to drug discovery.[8]

Chemistry

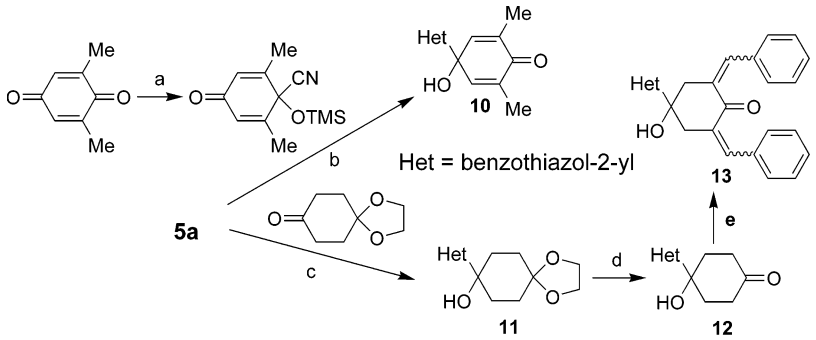
Lithiated heteroaromatic compounds **5a**−**f** were prepared from precursor heterocycles with *n*-butyllithium in THF at −78 °C (not necessary for commercially available phenyllithium **5g**). For the synthesis of the aromatic and heteroaromatic quinols **5h**−**k**, lithium−bromine exchange was used to generate the aryllithium species. Addition of the lithiated substrates to 4,4-dimethoxy-2,5-cyclohexadien-1-one generates the lithiated quinols **6a**−**k** protected as their dimethyl acetals, which were not isolated, but were deprotected in situ using dilute acetic acid[9] to give the required quinols **7a**−**k** (Scheme 2). The optimum yield of **7a** from this route was 67%. A less efficient modification (Experimental Section, method B) involved the addition of 2-lithiobenzothiazole **5a** to 4-cyano-4-trimethylsilyloxy-2,5-cyclohexadienone **8**[10] at −100 °C to give the silyl-protected adduct **9**. Removal of the silyl protecting group with tetrabutylammonium fluoride in THF afforded the same quinol **7a** (24%). Quinol **7a** could also be obtained directly (in low yield) by oxidation of phenol **1** with DAIB in acetonitrile/water. The yield could be improved (to 52%) using [bis(trifluoroacetoxy)iodo]benzene (TAIB) as oxidant together with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) as a radical quenching agent (Experimental Section, method C). Presumably the TEMPO acts to trap any radical species generated from the phenol that might contribute to formation of unwanted byproducts.[11]

The lithiated benzothiazole addition route could also be adapted for the synthesis of quinols substituted in a cyclohexadienone or cyclohexanone moiety. Addition of **5a** to 4-cyano-3,5-dimethyl-4-trimethylsilyloxycyclohexa-2,5-dien-1-one, derived from commercially available 2,6-dimethyl-1,4-benzoquinone via cyanosilylation,[10] followed by quenching afforded quinol **10**. Similarly, reaction of **5a** with 1,4-cyclohexanedione monoethylene ketal gave the protected tetrahydroquinol **11**, which on deprotection using concentrated hydrochloric acid in acetic acid gave **12**, a tetrahydro variant of the lead structure **7a** (method D). Treatment of the hydroxy-substituted cyclohexanone **12** with benzaldehyde under basic conditions gave 4-(benzothiazol-2-yl)-2,6-bis-(phenylmethinyl)-4-hydroxycyclohexanone **13** (Scheme 3, method E).

Addition of lithiated heterocycles to quinones substituted in the 2 or 3 position, or 2,3-benzo annelation, breaks the plane of symmetry common to structures **7a**−**k**, and racemic mixtures are obtained. Thus, addition of lithiated benzothiazole **5a** to 2-chloro-4,4-dimethoxycyclohexa-2,5-dien-1-one **15**[12] (derived from DAIB oxidation of 2-chloro-4-methoxyphenol **14** in methanol) followed by quenching with aqueous acetic acid furnished the (±)-3-chloroquinol **16** (58%) (Scheme 4). Similarly, addition of lithiated benzothiophene **5c** or benzofuran **5d** to protected 1,4-naphthoquinone **18**, synthesized by DAIB oxidation of 4-methoxy-1-naphthol **17** in methanol, afforded the heterocycle-substituted naphthoquinol **19** or **20**, respectively, as racemic mixtures (method F).

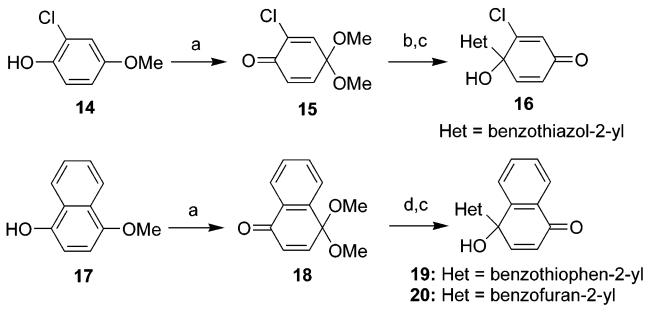
We have shown previously that oxidation of 2-(4-aminophenyl)benzothiazole (**21**, Het = benzothiazol-2-yl) with DAIB in dry toluene yields the corresponding

Scheme 3[a]



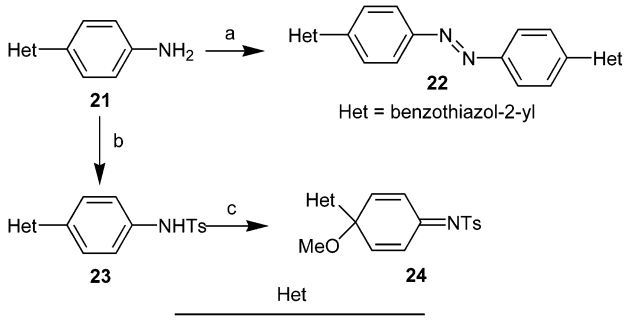
[a] Reagents: (a) TMSCN, PPh_3, CH_2Cl_2; (b) Et_2O, −100 °C, then TBAF·$3H_2O$, THF; (c) THF, −78 °C; (d) concentrated HCl, AcOH, 80 °C; (e) PhCHO, 1 M NaOH (aq), DMSO, reflux.

Scheme 4[a]



[a] Reagents: (a) DAIB, MeOH; (b) **5a**, THF, −78 °C; (c) 10% AcOH (aq), acetone, reflux; (d) **5c** or **5d**, THF, −78 °C.

Scheme 5[a]



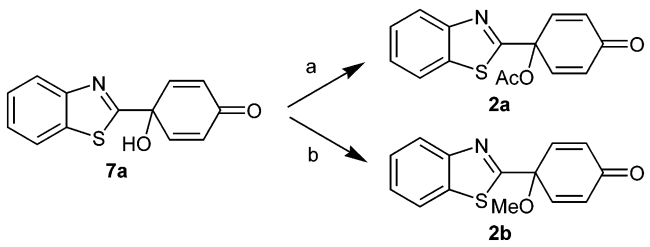
Het	
a:	benzothiazol-2-yl
b:	4-fluorobenzothiazol-2-yl
c:	6-fluorobenzothiazol-2-yl
d:	6-methylbenzothiazol-2-yl
e:	6-methoxybenzothiazol-2-yl

[a] Reagents: (a) DAIB, toluene; (b) *p*-TsCl, pyridine, reflux; (c) $PhI(OCOCF_3)_2$, MeOH.

azobenzene **22**.[13] In contrast, oxidation of the tosyl derivatives **23a**−**e** (synthesized via method G) with TAIB in methanol yielded the 4-methoxyquinoneimines **24a**−**e**, respectively (Scheme 5, method H).

Functionalization of the hydroxy group of the benzothiazole quinol **7a** has also been carried out. Conversion of **7a** to the corresponding ester and methyl ether[7] is readily achieved in yields of 91% and 49%, respectively (Scheme 6). This two-step route to benzothiazole-substituted ester **2a** and methyl ether **2b**, via addition of 2-lithiobenzothiazole to protected quinone followed by deprotection and hydroxyl group functionalization, compares favorably in terms of yield and ease of product isolation to the DAIB oxidation route previously described.[7]

Scheme 6[a]



[a] Reagents: (a) Ac_2O, NEt_3, DMAP, CH_2Cl_2; (b) KO*t*Bu, MeI, THF, −78 to 0 °C.

Biological Results and Discussion

Compound **2a** was highly unstable in cell culture medium (HPLC analysis) with only approximately 9% remaining after 24 h, by which time a major new peak was apparent that increased over time. By 72 h, no original compound could be detected (data not shown). LC−MS was used to determine that the major degradation product of **2a** (mass 285) was **7a** (mass 243). Indeed, when the in vitro activity of **7a** was examined in the NCI panel (see below), its activity fingerprint was very similar to that of **2a**, which, considering the rapid breakdown of **2a** to **7a**, indicates that the hydroxy compound **7a** is the true bioactive species.

In Vitro Activity against Cancer Cell Lines. Initial evaluation of the growth-inhibitory properties of compounds **1** and **7a**−**k** was undertaken in two human colon cell lines (HCT 116 and HT 29) and two mammary carcinoma cell lines (MCF-7 and MDA 468). Selectivity was confirmed following examination of the activity of analogues **7a**−**d** in refractory A549 lung adenocarcinoma cells. The results of MTT assays (see Experimental Section) following 72 h of drug exposure are reported in Table 1 and show that the benzothiazole-substituted quinol **7a** is 150-fold more growth-inhibitory than 2-(4-hydroxyphenyl)benzothiazole **1**, from which it can be derived by oxidation. Other compounds in this initial series, most notably **7b**−**d**, also showed potent growth-inhibitory activity in the colon cell lines examined, possessing IC_{50} values less than 0.5 μM. This observation provided the starting interest to justify the synthesis of further examples of this series of quinols that were also markedly more growth-inhibitory than phenol **1**.

A full range of novel quinols have been evaluated for in vitro activity (48 h drug exposure) across 60 human cancer cell lines through the National Cancer Institute

Table 1. Cell Growth-Inhibitory Activity of Compounds **1** and **7a**−**k**

	IC_{50} (μM)				
compd	HCT 116	HT 29	MCF-7	MDA 468	A549
1	>100	83.7	69.4	0.4	
7a	0.04	0.38	0.35	0.79	2.35
7b	0.18	0.29	0.39	0.35	2.33
7c	0.17	0.55	*a*	*a*	2.43
7d	0.16	0.42	*a*	*a*	2.30
7e	0.66	1.25	0.57	0.72	*a*
7f	0.62	0.72	*a*	*a*	*a*
7g	2.22	3.63	*a*	*a*	*a*
7h	0.42	1.36	0.78	1.77	*a*
7i	0.82	2.5	1.3	1.4	*a*
7j	0.72	0.97	0.77	1.7	*a*
7k	0.55	0.46	0.72	1.5	*a*

[a] Not determined.

(NCI) Developmental Therapeutics Screening Program.[14] The high potency (GI_{50} value) displayed by the acetoxy derivative **2a** is reduced in the related ethers **2b**,**d**. As observed previously, the closely similar activities of **2a** and **7a** suggest that the former is acting as a prodrug modification of the latter. The mean GI_{50} values (Table 2) confirm that with the exception of compounds **10**−**12** all quinols have more potent growth-inhibitory activity than phenol **1**. The most potent compounds at both mean GI_{50} and mean LC_{50} levels are those with a 6/5 heterobicyclic substituent **2a**, **7a**−**d**, **16**, and **20**. Less active are the quinols with monocyclic substituents or 6/6 bicyclic attachments **7e**−**k**. Substituents in the cyclohexadienone moiety have a marked effect on activity. Methyl groups in the 2 and 6 positions (**10**) and cyclohexanone variants **11** and **12** exert a dyschemotherapeutic effect; however, chloro substitution at the 3 position in **16** maintains potency. The series of methyl ethers in which the carbonyl group in the cyclohexandienone unit is replaced by a tosylimino group (**24a**−**e**) were of intermediate activity (cf. methyl ether **2b**). Overall, the most growth-inhibitory compound is the benzothiazole-substituted quinol **7a** (NSC 706704), and on the basis of the limited number of compounds evaluated, a tentative pharmacophore is proposed (Figure 2).

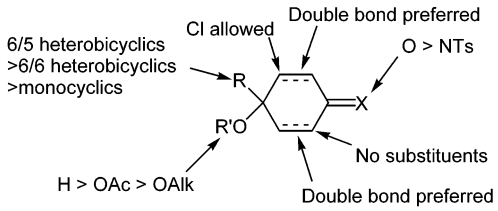


Figure 2. Summary of SAR in antitumor quinols.

The most noteworthy feature of the LC_{50} profiles of the most active compounds (**2a**, **7a**−**d**, **16**, and **20**) is the relative sensitivities of cell lines in the colon and renal cell subpanels. This is illustrated in the mean LC_{50} graph of **7a** (Figure 3), and other active compounds showed this unusual characteristic. In general, the colon cell line HCT 116 was the most sensitive and SW-620 was the least sensitive; ACHN was the most sensitive renal cell line. The selective in vitro activity in colon and renal cell lines in the NCI 60 cell line panel is represented in Table 2, focusing on the mean GI_{50} and mean LC_{50} values across all cell lines and contrasting these values with the mean LC_{50} values in the colon and renal subpanels. The specific LC_{50} values in HCT-116 and SW-620 colon cell lines and in ACHN renal cells are also presented.

In Vivo Activity. Preliminary evaluation of **2a** against a panel of human tumor xenografts in clonogenic assays showed that the highly angiogenic renal

Table 2. Activity of Compounds in the NCI in Vitro 60-Cell Panel

	mean GI_{50} [a] (μM)	mean LC_{50} [a] (μM)	mean LC_{50} in colon subpanel[b] (μM)	mean LC_{50} in renal subpanel[c] (μM)	LC_{50} (μM)		
compd					HCT 116[d]	SW-620[e]	ACHN[f]
1	33.9	>100	>100	>100	>100	>100	>100
2a	0.36	6.76	1.98	1.27	0.52	17.8	0.53
2b	1.91	33.1	8.81	22.4	9.55	>100	24.5
2d	0.71	11.7	7.47	9.55	4.57	>100	3.23
7a	0.23	3.39	1.76	1.57	0.43	>100	0.69
7b	0.46	7.08	1.13	1.64	0.87	>100	4.90
7c	0.25	5.75	1.69	1.40	0.60	>100	0.33
7d	0.52	7.24	3.66	2.40	0.60	>100	1.00
7e	1.78	30.9	32.5	14.4	5.89	>100	35.5
7f	1.35	14.5	6.19	9.17	4.90	26.3	7.94
7g	2.29	21.9	10.6	16.7	6.31	51.3	5.50
7h	1.82	25.7	12.2	17.0	5.37	>100	13.2
7i	0.81	11.7	6.58	4.68	4.47	>100	7.76
7j	1.07	15.8	10.2	9.66	4.26	>100	15.1
7k	1.91	20.4	6.9	11.35	5.24	>100	8.91
10	38.2	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100	>100
13	2.69	47.9	17.4	37.2	5.37	>100	30.9
16	0.46	6.31	2.31	1.80	0.68	>100	0.58
19	2.29	70.8	>100	>100	>100	>100	>100
20	0.74	7.76	7.76	6.48	4.90	3.55	5.75
24a	15.5	83.2	>100	>100	44.7	>100	>100
24b	2.57	27.5	26.9	14.4	57.5	67.6	7.59
24c	1.74	30.2	25.6	19.1	5.25	>100	>100
24d	1.20	19.5	9.92	10.6	1.78	95.5	6.92
24e	1.91	18.6	13.2	11.2	5.01	77.6	8.32

[a] For definitions of mean GI_{50} and mean LC_{50}, see refs 14 and 15. [b] Normally $n = 6$ and excluding the insensitive SW-620 colon cell line. [c] Normally $n = 8$. [d] The most sensitive colon cell line. [e] The least sensitive colon cell line. [f] The most sensitive renal cell line.

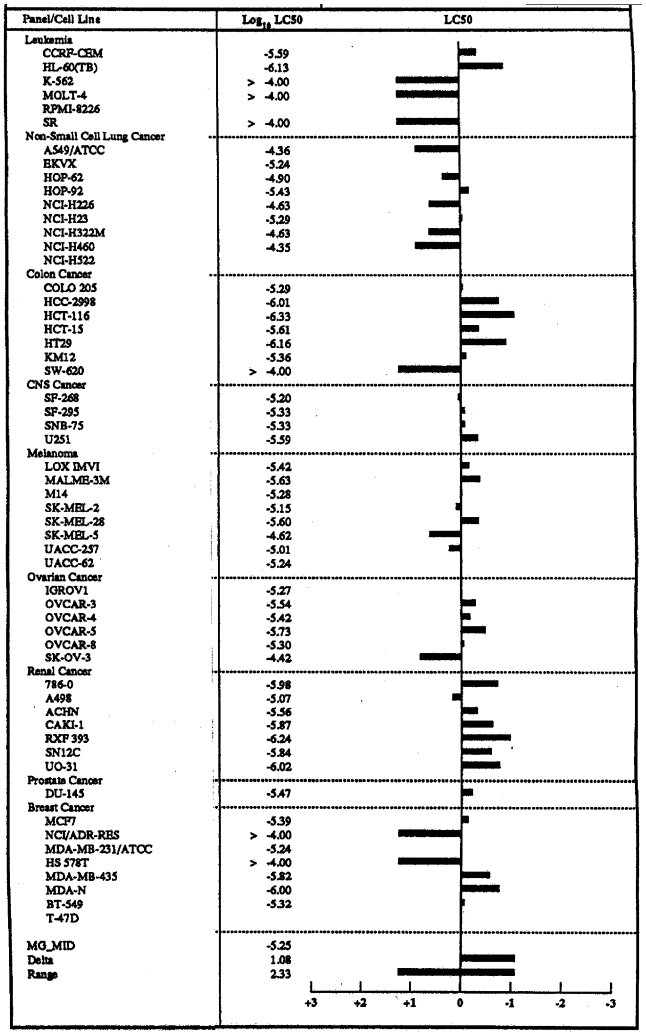


Figure 3. NCI LC_{50} mean graph for compound **7a**.

tumor RXF 944LX was especially sensitive with an IC_{50} less than 0.001 μM (data not shown). The stable quinol **7a** was selected for evaluation in vivo against this tumor.

The drug dose and treatment schedules reported were determined as being tolerated in non-tumor-bearing nude NMRI mice prior to initiation of antitumor tests. Compound **7a** demonstrated antitumor activity against subcutaneously growing human renal cancer xenografts, RXF 944XL, which are highly angiogenic. Compound **7a** (15 mg/kg) administered ip on days 1 and 8 retarded tumor growth by 57% after 10 days (Figure 4A). Treatment on consecutive days (1 and 2, 15 mg/kg ip) elicited superior antitumor activity; 73% growth inhibition was observed (Figure 4B). However, this schedule (15 mg/kg **7a**, days 1 and 2) failed to elicit significant growth inhibition in the human HCT 116 colon xenograft model. In contrast, significant antitumor activity was observed in HT 29 colon xenografts. A tumor growth delay of 7 days accompanied 42.5% growth inhibition, determined 14 days after initial treatment (M. C. Bibby, personal communication). However, in tumor-bearing mice, **7a** was less well tolerated than in tumor-free animals and GI toxicity and body weight loss accompanied antitumor activity.

Table 3. NCI COMPARE Analysis Using **7a** as Seed

compd	PCC value[a]	compound	PCC value[a]
7a	1.00	**7g**	0.48
7b	0.84	**7h**	0.51
7c	0.74	**7i**	0.68
7d	0.67	**7j**	0.76
7e	0.69	**7k**	0.55
7f	0.74		

[a] PCC = Pearson correlation coefficient. For definition, see ref 15.

NCI COMPARE Analysis. COMPARE is a computer algorithm developed to match patterns of in vitro activities.[15] Compounds with significant Pearson correlation coefficients (PCCs) greater than 0.7 often share mechanisms of action, and the method can be used to interrogate the database for molecular targets expressed in different cell types. Table 3 lists PCC values for compounds **7a**−**k** using the lead compound **7a** as the seed in the GI_{50} panel. This result establishes (as expected) that these compounds all probably share a common mechanism of action. Moreover, COMPARE analysis also uncovered chemically unrelated structures that had PCCs greater than 0.7. These included the natural products 22-hydroxytingenone (NSC 684506), heliangolide (NSC 335753), and arnebin (NSC 140377), together with many small synthetics characterized chemically as being "double Michael acceptors". Of particular interest are structurally unrelated inhibitors of thioredoxin/thioredoxin reductase that possess similar profiles of in vitro antitumor activity, eliciting toxicity in colon, renal, and certain breast cancer cell lines.[16]

The hydroxycyclohexadienone pharmacophore of compounds **7a**−**k** is not represented in antitumor agents in any known clinical class, although related antitumor epoxyquinols are better known (e.g., manumycin A[17] and LL-C 10037α[18]). Two reports, however, of compounds

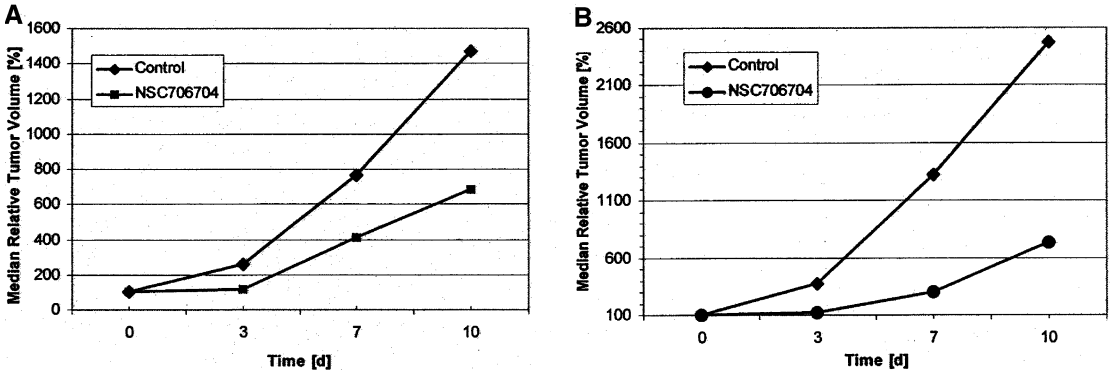


Figure 4. In vivo activity against renal RXF 944XL xenografts. Animals were treated with compound **7a** (NSC 706704, 15 mg/kg, ip) on days 1 and 8 (A) or on days 1 and 2 (B).

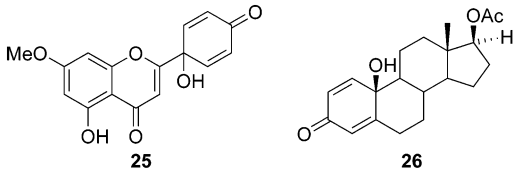


Figure 5. Structures of related hydroxycyclohexadienone antitumor agents.

showing antitumor activity and containing the hydroxycyclohexadienone structures have appeared, namely, a hydroxylated flavone-substituted quinol **25**[19] and an oxidized estrone **26**[20] (Figure 5).

Conclusions

A novel series of (hetero)aromatic quinols **7a**−**k** have been synthesized via a one-pot procedure involving addition of a lithiated aromatic group to a protected quinone followed by deprotection. In addition, quinols bearing substituents on the cyclohexadienone ring (**10**, **13**, and **16**), the tetrahydro derivative **12**, and benzo-fused analogues **19** and **20** have been prepared. The synthesis of the corresponding benzothiazole quinolimines **24a**−**e** has been accomplished from *p*-toluenesulfonyl-protected 2-(4-aminophenyl)benzothiazoles **23a**−**e.** A number of these quinols, most notably those containing a 6/5 bicyclic heterocycle (e.g., **7a**−**d**), exhibit potent and selective antitumor activity in vitro, with (unusually) activity at the LC_{50} level being concentrated in certain colon and renal cell lines. In vivo xenograft activity was also observed for compound **7a** in the renal tumor cell line RXF 944XL. Additionally, we have shown that compound **7a** forms mono- and diadducts with aliphatic and aromatic thiols, and biochemical and gene array investigations have identified thioredoxin as the purported molecular target for the new series of quinols. These results will be published separately.

Experimental Section

All new compounds were characterized by elemental microanalysis (C, H, and N values within 0.4% of theoretical values). Melting points were determined using 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 recorded on a Mattson 2020 GALAXY series FT-IR spectrophotometer. Mass spectra were recorded on an AEI MS-902 and VG Micromass 7070E spectrometers. TLC systems for routine monitoring of reaction mixtures and for confirming the homogeneity of analytical samples used Kieselgel 60F_{254} (0.25 mm) silica gel TLC aluminum sheets. Sorbsil silica gel C 60-H (40−60 μm) was used for flash chromatographic separations. All reactions were carried out under an inert atmosphere using anhydrous reagents and solvents. THF was dried and purified before use by distillation from sodium benzophenone. All other commercial materials were used as received.

General Method for the Synthesis of Aromatic and Heteroaromatic Quinols 7a−k. Method A. A solution of the appropriate aromatic or heteroaromatic compound **5a**−**k** (26.0 mmol) in THF (30 mL) was added to a solution of *n*-butyllithium (17.8 mL of a 1.6 M solution in hexanes, 28.5 mmol) in THF (30 mL) at −78 °C with stirring. After being stirred at −78 °C for 1 h, the aryllithium compound solution was transferred via cannula to a solution of 4,4-dimethoxy-2,5-cyclohexadien-1-one (3.6 mL, 26.0 mmol) in THF (60 mL). After being stirred for 2 h at −78 °C, the reaction mixture was poured into brine (100 mL) and the layers were separated. The aqueous layer was extracted using dichloromethane (3 × 100 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield the protected aromatic quinol **6a**−**k**. The protected product was dissolved in acetone (40 mL), and 10% aqueous acetic acid was added. The mixture was heated at reflux for 2 h and then allowed to cool, and the acetone was removed in vacuo. The aqueous phase was extracted using dichloromethane (3 × 50 mL), dried ($MgSO_4$), filtered, and concentrated to give the aromatic or heteroaromatic quinol, which was purified by flash column chromatography (eluant = dichloromethane) when necessary.

4-(Benzothiazol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (7a): yield 67%; mp 63−66 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (1H, dd, J = 1.0, 8.0 Hz, H-4′), 7.89 (1H, dd, J = 1.0, 7.8 Hz, H-7′), 7.52 (1H, td, J = 1.3, 8.0 Hz, H-5′), 7.43 (1H, td, J = 1.3, 7.8 Hz, H-6′), 7.02 (2H, dd, J = 1.9, 10.0 Hz, H-3, H-5), 6.35 (2H, d, J = 10.0 Hz, H-2, H-6); IR 3482, 3165, 1667, 1622, 1150, 1081, 764 cm^{-1}; MS (CI) m/z 244 ($M^+ + 1$). Anal. ($C_{13}H_9NO_2S$) C, H, N.

4-(Benzoxazol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (7b): yield 30%; mp 116−117 °C; ^{1}H NMR ($CDCl_3$) δ 7.78 (1H, dd, J = 1.8, 7.9 Hz, H-4′), 7.58 (1H, dd, J = 1.8, 8.0 Hz, H-7′), 7.44 (2H, m, H-5′, H-6′), 7.12 (2H, d, J = 9.3 Hz, H-3, H-5), 6.45 (2H, d, J = 9.3 Hz, H-2, H-6); IR 3421, 3213, 1666, 1622, 1167, 1064, 925, 856, 744 cm^{-1}; MS (EI) m/z 227 (M^+), 211, 119. Anal. ($C_{13}H_9NO_3$) C, H, N.

4-(Benzothiophen-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (7c): yield 60%; mp 185−188 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (1H, dd, J = 4.3, 5.0 Hz, ArH), 7.73 (1H, dd, J = 4.3, 5.0 Hz, ArH), 7.37 (2H, m, ArH), 7.26 (1H, s, H-3′), 7.10 (2H, d, J = 8.3 Hz, H-3, H-5), 6.31 (2H, d, J = 8.3 Hz, H-2, H-6), 2.71 (1H, s, OH); IR 3408, 1661, 1615, 1117, 1062, 905, 748 cm^{-1}; MS (CI) m/z 243 ($M^+ + 1$), 225 ($-H_2O$). Anal. ($C_{14}H_{10}O_2S$) C, H, N.

4-(Benzofuran-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (7d): yield 82%; mp 106.5−108.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.57 (1H, d, J = 7.5 Hz, ArH), 7.49 (1H, d, J = 7.5 Hz, ArH), 7.29 (2H, m, ArH), 7.16 (2H, d, J = 10.3 Hz, H-3, H-5), 6.77 (1H, s, H-3′), 6.34 (2H, d, J = 10.3 Hz, H-2, H-6), 2.83 (1H, s, OH); IR 3368, 3270, 1669, 1622, 1175, 1036, 905, 745 cm^{-1}; MS (CI) m/z 227 ($M^+ + 1$), 209 ($-H_2O$). Anal. ($C_{14}H_{10}O_3 \cdot {}^1/_4H_2O$) C, H.

4-Hydroxy-4-(thiophen-2-yl)-2,5-cyclohexadien-1-one (7e): yield 70%; mp 103 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (1H, m, ArH), 7.07 (2H, d, J = 10.1 Hz, H-3, H-5), 7.02 (2H, m, ArH), 6.22 (2H, d, J = 10.1 Hz, H-2, H-6), 3.44 (1H, s, OH); IR 3327, 3105, 1664, 1612, 1173, 1034, 906, 697 cm^{-1}; MS (CI) m/z 193 ($M^+ + 1$), 175 ($-H_2O$). Anal. ($C_{10}H_8O_2S$) C, H.

4-Hydroxy-4-(thiazol-2-yl)-2,5-cyclohexadien-1-one (7f): yield 57%; mp 138−139 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, d, J = 3.2 Hz, ArH), 7.46 (1H, d, J = 3.2 Hz, ArH), 7.01 (2H, d, J = 10.1 Hz, H-3, H-5), 6.33 (2H, d, J = 10.1 Hz, H-2, H-6), 4.41 (1H, s, OH); IR 3109, 2805, 1674, 1628, 1497, 1148, 1069, 937, 910, 750 cm^{-1}; MS (EI) m/z 193 (M^+), 177, 85. Anal. ($C_9H_7NO_2S$) C, H, N.

4-Hydroxy-4-phenyl-2,5-cyclohexadien-1-one (7g):[8] yield 79%; mp 102−103 °C (lit.[8] 99−100 °C); ^{1}H NMR ($CDCl_3$) δ 7.49−7.31 (5H, m, ArH), 6.89 (2H, d, J = 10.1 Hz, H-3, H-5), 6.19 (2H, d, J = 10.1 Hz, H-2, H-6).

4-Hydroxy-4-(naphth-1-yl)-2,5-cyclohexadien-1-one (7h): synthesized according to method A from 1-bromonaphthalene; yield 89%; mp 117−118 °C; ^{1}H NMR ($CDCl_3$) δ 8.49 (1H, m, ArH), 7.89 (3H, m, ArH), 7.52 (3H, m, ArH), 7.26 (2H, d, J = 9.0 Hz, ArH), 6.37 (2H, d, J = 10.1 Hz, H-2, H-6), 2.67 (1H, s, OH); IR 3439, 3300, 1663, 1620, 1381, 1344, 1036, 1017, 862, 781 cm^{-1}; MS (EI) m/z 236 (M^+), 220, 128. Anal. ($C_{16}H_{12}O_2$) C, H.

4-Hydroxy-4-(naphth-2-yl)-2,5-cyclohexadien-1-one (7i): synthesized according to method A from 2-bromonaphthalene; yield 50%; mp 137 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 (1H, d, J = 1.8 Hz, H-1), 7.84 (3H, m, ArH), 7.49 (3H, m, ArH), 6.97 (2H, d, J = 10.0 Hz, H-3, H-5), 6.28 (2H, d, J = 10.0 Hz, H-2, H-6); IR 3422, 3052, 1661, 1620, 1393, 1117, 1065, 976,

868, 818, 750 cm^{-1}; MS (EI) *m/z* 236 (M^+), 220, 128. Anal. ($C_{16}H_{12}O_2$) C, H.

4-Hydroxy-4-(quinolin-3-yl)-2,5-cyclohexadien-1-one (7j): synthesized according to method A from 3-bromoquinoline; yield 42%; mp 183−184 °C; ^1H NMR ($CDCl_3$) δ 8.93 (1H, s, H-2), 8.34 (1H, d, J = 2.3 Hz, H-4), 8.13 (1H, d, J = 8.0 Hz, ArH), 7.85 (1H, d, J = 9.0 Hz, ArH), 7.76 (1H, dt, J = 1.5, 7.8 Hz, ArH), 7.60 (1H, dt, J = 1.0, 7.6 Hz, ArH), 6.97 (2H, d, J = 10.0 Hz, H-3, H-5), 6.31 (2H, d, J = 10.0 Hz, H-2, H-6); IR 3048, 2766, 1663, 1624, 1497, 1279, 1163, 1069, 868, 748 cm^{-1}; MS (EI) *m/z* 237 (M^+), 221, 129. Anal. ($C_{15}H_{11}NO_2 \cdot {}^1/_4H_2O$) C, H, N.

4-Hydroxy-4-(pyridin-2-yl)-2,5-cyclohexadien-1-one (7k): synthesized according to method A from 2-bromopyridine; yield 55%; mp 116−117 °C; ^1H NMR ($CDCl_3$) δ 8.58 (1H, m, ArH), 7.72 (1H, dt, J = 1.8, 7.6 Hz, ArH), 7.31 (2H, m, ArH), 6.76 (2H, d, J = 10.3 Hz, H-3, H-5), 6.28 (2H, d, J = 10.3 Hz, H-2, H-6); IR 3131, 1670, 1626, 1439, 1397, 1167, 1105, 1063, 963, 862, 795 cm^{-1}; MS (EI) *m/z* 187 (M^+), 171, 79. Anal. ($C_{11}H_9NO_2 \cdot {}^1/_4H_2O$) C, H, N.

Alternative Synthesis of 4-(Benzothiazol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (7a) via 4-Cyano-4-trimethylsilyloxycyclohexa-2,5-dien-1-one (8).[10] Method B. To benzothiazole (0.750 g, 5.55 mmol) dissolved in dry diethyl ether (100 mL) at −78 °C under a nitrogen atmosphere was added *n*-butyllithium (2.52 mL of 2.5 M solution in hexanes, 1 equiv) dropwise. The reaction mixture was stirred for 1 h and then cooled to −100 °C. Then 4-cyano-4-trimethylsilyloxycyclohexa-2,5-dien-1-one **8**[9] (1.07 g, 5.55 mmol, 1 equiv) dissolved in diethyl ether (10 mL) was then added dropwise with stirring. The reaction mixture became dark-green. After 2 h, water (20 mL) was added and the reaction mixture was warmed to room temperature. The organic layer was separated, washed with water (40 mL), dried over magnesium sulfate, and concentrated under vacuum. The residue **9** was dissolved in THF (25 mL), and tetrabutylammonium fluoride hydrate (4.35 g, 16.7 mmol, 3 equiv) was added. The reaction mixture was stirred at room temperature for 4 h and then diluted with water (100 mL) and extracted with diethyl ether (3 × 50 mL). The organic layers were combined, dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by flash column chromatography, eluting with ethyl acetate/hexane 2:8. The yield of 4-(benzothiazol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (**7a**) was 0.32 g (24%).

Alternative Synthesis of 4-(Benzothiazol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (7a) via DAIB Oxidation in CH_3CN/H_2O in the Presence of TEMPO.[10] Method C. TEMPO (34 mg, 0.22 mmol) was added to a solution of 2-(4-hydroxyphenyl)benzothiazole (0.25 g, 1.1 mmol) in 9:1 v/v acetonitrile/water (10 mL). DAIB (0.71 g, 2.2 mmol) was then added, and the reaction mixture was stirred at room temperature for 1 h. The acetonitrile was removed in vacuo. Then more water (10 mL) was added and the crude product was extracted with diethyl ether (3 × 10 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography, eluting with ethyl acetate/hexane 2:8. The yield of 4-(benzothiazol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (**7a**) was 0.14 g (52%).

Synthesis of 4-(Benzothiazol-2-yl)-4-hydroxy-2,6-dimethylcyclohexa-2,5-dien-1-one (10). To 2,6-dimethyl-1,4-benzoquinone (2 g, 14.7 mmol) and trimethylsilyl cyanide (1.45 g, 14.7 mmol) dissolved in dry dichloromethane under a nitrogen atmosphere was added triphenylphosphine (5 mg). The reaction mixture was heated at reflux for 1 h and then cooled to room temperature. The solvent was removed under vacuum, and the residue was treated with hexane. The crystalline solid (4-cyano-3,5-dimethyl-4-trimethylsilyloxycyclohexa-2,5-dien-2-one) was collected on a filter washed with a little cold hexane and dried under vacuum. The yield was 3.35 g (97%). The product (0.862 g, 6.4 mmol) was used directly in the next step (method B) without further purification to yield the title compound (**10**) as an off-white solid (0.919 g, 3.4 mmol) in 53% yield: mp 132 °C; ^1H NMR ($CDCl_3$) δ 8.07 (1H, m, benzothiazole H-4/7), 7.92 (1H, m, benzothiazole H-4/7), 7.57 (1H, m, benzothiazole H-5/6), 7.50 (1H, m, benzothiazole H-5/6), 6.81 (2H, s, H-3, H-5), 4.28 (1H, s, OH), 2.02 (6H, s, CH_3 × 2); IR (KBr disk) 2359, 1669, 1632, 1507, 1433, 1373, 1057, 768 cm^{-1}; MS (AP^+) 272 ($M^+ + 1$), 254 ($M^+ + 1 - H_2O$). Anal. ($C_{15}H_{13}NO_2S$) C, H, N.

Synthesis of 4-(Benzothiazol-2-yl)-1,1-ethylenedioxy-4-hydroxycyclohexane (11). To benzothiazole (3.46 g, 25.6 mmol) dissolved in dry THF under a nitrogen atmosphere at −78 °C was added *n*-butyllithium (17.1 mL of 1.5 M solution, 1 equiv) dropwise. The reaction mixture was stirred for 1 h, and then 1,4-cyclohexanedione monoethylene ketal dissolved in dry THF (15 mL) was added in one portion. The reaction mixture was stirred at −78 °C for a further 3 h, and then water (10 mL) was added. The mixture was warmed to room temperature, and the white precipitate was collected on a filter, washed with water (2 × 20 mL), and then dried under vacuum. The yield was 4.403 g (59%): mp 165 °C; ^1H NMR ($CDCl_3$) δ 8.05 (1H, m, benzothiazole H-4/7), 7.89 (1H, m, benzothiazole H-4/7), 7.47 (1H, m, benzothiazole H-5/6), 7.39 (1H, m, benzothiazole H-5/6), 4.02 (4H, s, ethylene 2 × CH_2), 3.16 (1H, s, OH), 2.41−2.31 (4H, m, cyclohexanone 2 × CH_2), 1.82−1.72 (4H, m, cyclohexanone 2 × CH_2); IR (KBr disk) 2897, 1501, 1437, 1368, 1038, 997, 764 cm^{-1}; MS (AP^+) 292 ($M^+ + 1$), 274 ($M^+ + 1 - H_2O$). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

Synthesis of 4-(Benzothiazol-2-yl)-4-hydroxycyclohexan-1-one (12). Method D. A solution of the ethylene ketal **11** (1.0 g, 3.4 mmol) in acetic acid (40 mL) and concentrated HCl (10 mL) was heated at 80 °C for 2 h with stirring. The volume was reduced on a rotary evaporator, and the remaining solution was carefully neutralized with saturated sodium hydrogen carbonate solution and then extracted with diethyl ether (3 × 50 mL). The organic layers were combined, washed with water (2 × 50 mL), and dried over magnesium sulfate. Removal of the solvent under vacuum gave the product **12** (0.618 g) as an off-white solid (73%): mp 134 °C; ^1H NMR ($CDCl_3$) δ 8.00 (1H, m, benzothiazole H-4/7), 7.91 (1H, m, benzothiazole H-4/7), 7.49 (1H, m, benzothiazole H-5/6), 7.41 (1H, m, benzothiazole H-5/6), 3.66 (1H, s, OH), 2.97−2.85 (4H, m, cyclohexanone CH_2), 2.53−2.34 (4H, m, cyclohexanone CH_2); IR (KBr disk) 2913, 1690, 1505, 1427, 1312, 1198, 889, 768 cm^{-1}; MS (AP^+) 248 ($M^+ + 1$), 230 ($M^+ + 1 - H_2O$). Anal. ($C_{13}H_{13}NO_2S$) C, H, N.

Synthesis of 4-(Benzothiazol-2-yl)-2,6-bis(phenylmethinyl)-4-hydroxycyclohexanone (13). Method E. A solution of the hydroxycyclohexanone **12** (0.124 g, 0.5 mmol) and benzaldehyde (0.212 g, 2.0 mmol) dissolved in DMSO (1 mL) and 1 M sodium hydroxide solution (5 mL) was heated at reflux for 3 h. The reaction mixture was cooled and diluted with ethanol (25 mL), and 1 M HCl solution (5 mL) was added. Concentration under vacuum to a volume of ∼10 mL gave a white precipitate that was collected on a filter and washed with water. The solid was purified by flash column chromatography, eluting with ethyl acetate/hexane 2:8. The product was a yellow solid 0.120 g (58%): mp 184 °C; ^1H NMR ($CDCl_3$) δ 8.07 (2H, s, methylene C−H × 2), 8.04 (1H, m, benzothiazole H-4/7), 7.91 (1H, m, benzothiazole H-4/7), 7.54−7.36 (12H, m, benzothiazole H-5,6, Ph-H × 10), 3.72 (2H, dd, J = 2.6, 15.9 Hz, H-3,5), 3.51 (2H, dd, J = 7.3, 14.5 Hz), 3.05 (1H, s, OH); IR (KBr disk) 1672, 1599, 1447, 1242, 1193, 1179, 986, 768 cm^{-1}; MS (AP^+) 424 ($M^+ + 1$), 406 ($M^+ + 1 - H_2O$). Anal. ($C_{27}H_{21}NO_2S \cdot {}^1/_2H_2O$) C, H, N.

4-(Benzothiazol-2-yl)-3-chloro-4-hydroxy-2,5-cyclohexadien-1-one (16): synthesized according to method A from benzothiazole and 2-chloro-4,4-dimethoxycyclohexa-2,5-dien-1-one **15**;[11] yield 58%; mp 140−143 °C; ^1H NMR ($CDCl_3$) δ 8.06 (1H, dd, J = 0.8, 7.5 Hz, H-4′), 7.90 (1H, dd, J = 0.8, 7.5 Hz, H-7′), 7.55 (1H, dt, J = 1.5, 8.0 Hz, H-5′), 7.46 (1H, dt, J = 1.5, 8.0 Hz, H-6′), 6.99 (1H, d, J = 9.8 Hz, H-5), 6.60 (1H, d, J = 1.8 Hz, H-2), 6.36 (1H, dd, J = 1.8, 9.8 Hz, H-6); IR 3376, 3061, 1657, 1616, 1314, 1144, 1074, 980, 910, 750, 592 cm^{-1}; MS (CI) *m/z* 278/280 ($M^+ + 1$), 260/262. Anal. ($C_{13}H_8ClNO_2S$) C, H, N.

General Method for the Synthesis of Heteroaromatic Naphthoquinols 19 and 20. Method F. A solution of 4-methoxy-1-naphthol **17** (2.76 g, 15.8 mmol) and DAIB (6.10 g, 18.9 mmol) in methanol (75 mL) was stirred at 25 °C under a nitrogen atmosphere for 1 h. The resultant dark-blue solution was poured into a saturated $NaHCO_3$ (aq) solution (75 mL), and then the mixture was concentrated to approximately 50 mL in vacuo. The blue oil was extracted with dichloromethane (3 × 75 mL), and the organic layer was washed with water (2 × 75 mL) and brine (2 × 75 mL) and then dried ($MgSO_4$), filtered, and concentrated in vacuo (water bath temperature less than 40 °C) to yield 4,4-dimethoxy-4*H*-naphthalen-1-one **18** as a dark-blue semisolid that was used without further purification: ^{1}H NMR ($CDCl_3$) δ 8.15 (1H, d, J = 8.0 Hz, ArH), 7.40−7.85 (3H, m, ArH), 6.90 (1H, d, J = 12.2 Hz, H-3), 6.55 (1H, d, J = 12.2 Hz, H-2), 3.15 (6H, s, OCH_3).

To a solution of *n*-butyllithium (3.4 mL of a 1.6 M solution in hexanes, 5.4 mmol) in THF (6 mL) at −78 °C was slowly added a solution of benzothiophene or benzofuran (4.9 mmol) in THF (6 mL) with stirring under a nitrogen atmosphere. Following addition, the solution was stirred at −78 °C for 1 h. The lithiated heteroaromatic was then added via cannula to a stirring solution of freshly prepared 4,4-dimethoxy-4*H*-naphthalen-1-one **18** (4.9 mmol) in THF (12 mL) at −78 °C, followed by stirring for a further 2 h. The resulting solution was then poured into brine (20 mL) and extracted using dichloromethane (3 × 20 mL). The combined organic layers were washed with water (2 × 15 mL) and brine (2 × 15 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The dark oil was redissolved in acetone (10 mL), 10% aqueous acetic acid solution (10 mL) was added, and the mixture was heated under reflux for 1 h. The solution was then allowed to cool and was extracted with dichloromethane (3 × 20 mL). The organic layers were washed with water (2 × 15 mL) and brine (2 × 15 mL) and then dried ($MgSO_4$), filtered, and concentrated in vacuo to yield the crude product. Purification by column chromatography (4:1 hexane/EtOAc) then yielded the desired product.

4-Benzothiophen-2-yl-4-hydroxy-4*H*-naphth-1-one (19): yield 52%; mp 150−152 °C; ^{1}H NMR ($CDCl_3$) δ 8.20 (1H, d, J = 7.5 Hz, ArH), 7.92−7.81 (2H, m, ArH), 7.72−7.65 (2H, m, ArH), 7.49 (1H, d, J = 4.0 Hz, ArH), 7.40 (1H, td, J = 1.5, 7.5 Hz, ArH), 7.38−7.33 (2H, m, ArH), 7.30 (1H, d, J = 10.1 Hz, H-3), 7.09 (1H, s), 6.65 (1H, s, H-3′), 6.45 (1H, d, J = 10.1 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 185.1, 151.9, 149.9. 146.9, 140.1, 139.9, 133.6, 129.7, 128.9, 128.6, 126.4, 126.3, 124.7, 124.6, 123.9, 122.6, 121.2, 70.7; IR 3430, 1648, 1597, 1434, 1276, 1155, 1036, 760 cm^{-1}; MS (CI) m/z 293 (M^+ + 1). Anal. ($C_{18}H_{12}O_2S$) C, H.

4-(Benzofuran-2-yl)-4-hydroxy-4*H*-naphth-1-one (20): yield 65%; mp 105−107 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (1H, d, J = 7.2 Hz, ArH), 7.80 (1H, d, J = 5.3 Hz, ArH), 7.65 (1H, t, J = 7.5 Hz, ArH), 7.55−7.49 (2H, m, ArH), 7.40 (1H, d, J = 7.5 Hz, ArH), 7.28−7.12 (3H, m, ArH), 6.58 (1H, s, H-3′), 6.40 (1H, d, J = 10.2 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 157.0, 155.0, 148.5. 143.3, 133.8, 131.0, 130.4, 128.6, 128.5, 128.0, 127.0, 125.2, 123.6, 121.8, 111.9, 104.7, 69.4; IR 3311, 1657, 1624, 1451, 1240, 1170, 1038, 765 cm^{-1}; MS (CI) m/z 277.1 (M^+ + 1), 259. Anal. ($C_{18}H_{12}O_3$) C, H.

General Method for the Synthesis of 2-[4-(*p*-Tosylamino)]phenylbenzothiazoles. Method G. The precursors 4- and 6-fluoro-[21] and 6-methoxy[22] 2-(4-aminophenyl)benzothiazoles have been described previously. 2-(4-Aminophenyl)-6-methylbenzothiazole is commercially available (Aldrich Chemical Co.). To a solution of the 2-(4-aminophenyl)benzothiazole (0.2 g) in pyridine (2 mL) was added *p*-toluenesulfonyl chloride (1.5 equiv). The reaction mixture was heated under reflux for 10 min and then was cooled, and water (5 mL) was added. The precipitate formed was collected by filtration, washed with water, and dried in vacuo.

2-[4-(*p*-tosylamino)]phenylbenzothiazole (23a): yield 96%; mp 176 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (1H, m, H-4′/7′), 7.95 (2H, d, J = 8.6 Hz, toluenesulfonyl H-2, H-6), 7.85 (1H, m, H-4′/7′), 7.73 (2H, d, J = 8.6 Hz, H-2, H-6), 7.47 (1H, m, H-5′/6′), 7.36 (1H, m, H-5′/6′), 7.26−7.18 (5H, m, toluenesulfonyl H-3, H-5, H-3, H-5, NH), 2.37 (3H, s, CH_3); IR 1608, 1486, 1395, 1158, 1091, 763, 665, 590 cm^{-1}; MS (CI) m/z 381 (M^+ + 1). Anal. ($C_{20}H_{16}N_2O_2S_2$) C, H, N.

4-Fluoro2-[4-(*p*-tosylamino)]phenylbenzothiazole (23b): yield 96%; mp 233 °C; ^{1}H NMR (DMSO-d_6) δ 8.59 (1H, broad d, NH), 7.99 (2H, d, J = 8.7 Hz, toluenesulfonyl H-2, H-6), 7.95 (1H, m, benzothiazole-H), 7.74 (2H, d, J = 8.7 Hz, H-2, H-6), 7.45 (2H, m, 2 × benzothiazole-H), 7.41 (2H, d, J = 8.7 Hz, H-3, H-5), 7.31 (2H, d, J = 8.7 Hz, toluenesulfonyl H-3, H-5), 2.35 (3H, s, CH_3); IR 1609, 1466, 1331, 1244, 1157, 922, 837, 671 cm^{-1}; MS (CI) m/z 399 (M^+ + 1). Anal. ($C_{20}H_{15}FN_2O_2S_2$) C, H, N.

6-Fluoro-2-[4-(*p*-tosylamino)]phenylbenzothiazole (23c): yield 95%; mp 249 °C; ^{1}H NMR (DMSO-d_6) δ 8.59 (1H, broad d, NH), 8.05 (2H, m, 2 × benzothiazole-H), 7.99 (2H, d, J = 7.5 Hz, toluenesulfonyl H-2. H-6), 7.74 (2H, d, J = 8.3 Hz, H-2, H-6), 7.40 (1H, m, benzothiazole-H), 7.39 (2H, d, J = 8.3 Hz, H-3, H-5), 7.30 (2H, d, J = 7.5 Hz, toluenesulfonyl H-3, H-5), 2.34 (3H, s, CH_3); IR 1607, 1566, 1470, 1452, 1333, 1157, 812, 665, 577 cm^{-1}; MS (CI) m/z 399 (M^+ + 1). Anal. ($C_{20}H_{15}FN_2O_2S_2$) C, H, N.

6-Methyl-2-[4-(*p*-tosylamino)]phenylbenzothiazole (23d): yield 99%; mp 207 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (2H, d, J = 8.7 Hz, toluenesulfonyl H-2, H-6), 7.93 (1H, m, benzothiazole-H), 7.74 (2H, d, J = 8.4 Hz, H-2, H-6), 7.72 (1H, m, benzothiazole-H), 7.40−7.19 (5H, m, benzothiazole-H, H-3, H-5, toluenesulfonyl H-3, H-5), 2.52 (3H, s, CH_3), 2.40 (3H, s, CH_3); IR 3293, 1488, 1333, 1159, 819, 676, 571 cm^{-1}; MS (CI) m/z 395 (M^+ + 1). Anal. ($C_{21}H_{18}N_2O_2S_2$) C, H, N.

6-Methoxy-2-[4-(*p*-tosylamino)]phenylbenzothiazole (23e): yield 91%; mp 216−218 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (3H, d, J = 8.4 Hz, toluenesulfonyl H-2, H-6, H-4′), 7.72 (2H, d, J = 8.3 Hz, H-2, H-6), 7.36 (1H, d, J = 2.5 Hz, H-7′), 7.26 (2H, d, J = 8.4 Hz, toluenesulfonyl H-3, H-5), 7.10 (1H, dd, J = 2.5, 9.0 Hz, H-5′), 6.95 (1H, s, NH), 3.92 (3H, s, OCH_3), 2.40 (3H, s, CH_3); IR 1609, 1520, 1462, 1333, 1263, 1157, 924, 664 cm^{-1}; MS (CI) m/z 411 (M^+ + 1). Anal. ($C_{21}H_{18}N_2O_3S_2$) C, H, N.

General Method for the Synthesis of *N*-[4-(Benzothiazol-2-yl)-4-methoxy-2,5-cyclohexadienylidene]-(4-methyl)phenylsulfonamides. Method H. To a solution of the *N*-(*p*-toluenesulfonyl)-2-(4-aminophenyl)benzothiazole (0.1 g) was added TAIB (1.1 equiv) in one portion. The resulting suspension was stirred at room temperature for 5 h, and the precipitate formed was collected by filtration, washed with ice cold methanol (2 mL), and dried in vacuo.

***N*-[4-(Benzothiazol-2-yl)-4-methoxy-2,5-cyclohexadienylidene]-(4-methyl)phenylsulfonamide (24a):** yield 73%; mp 216 °C; ^{1}H NMR ($CDCl_3$) δ 8.18 (1H, m), 8.03 (1H, m), 7.88 (1H, d, J = 8.3 Hz), 7.65 (1H, dd, J = 2.1, 10.3 Hz), 7.55−7.48 (5H, m), 7.24 (1H, dd, J = 2.8, 10.2 Hz), 7.12 (1H, dd, J = 2.8, 9.9 Hz), 6.67 (1H, dd, J = 2.0, 9.8 Hz), 3.41 (3H, s, OCH_3), 2.45 (3H, s, CH_3); IR 1609, 1597, 1543, 1316, 1300, 1150, 1076, 862 cm^{-1}; MS (CI) m/z 411 (M^+ + 1), 381 ($M^+ - OCH_3$). Anal. ($C_{21}H_{18}N_2O_3S_2 \cdot {}^1/_2H_2O$) C, H, N.

***N*-[4-(4-Fluorobenzothiazol-2-yl)-4-methoxy-2,5-cyclohexadienylidene]-(4-methyl)phenylsulfonamide (24b):** yield 78%; mp 176 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 (3H, d, J = 7.7 Hz, benzothiazole-H, toluenesulfonyl H-2, H-6), 7.82 (1H, d, J = 7.6 Hz, benzothiazole-H), 7.50 (2H, d, J = 7.7 Hz, toluenesulfonyl H-3, H-5), 7.35 (2H, m), 7.00 (2H, t, J = 8.8 Hz), 6.78 (1H, d, J = 9.9 Hz), 3.58 (3H, s, OCH_3), 2.60 (3H, s, CH_3); IR 1655, 1609, 1543, 1468, 1316, 1154, 862, 675 cm^{-1}; MS (CI) m/z 429 (M^+ + 1), 397 ($M^+ - OCH_3$). Anal. ($C_{21}H_{17}FN_2O_3S_2 \cdot H_2O$) C, H, N.

***N*-[4-(6-Fluorobenzothiazol-2-yl)-4-methoxy-2,5-cyclohexadienylidene]-(4-methyl)phenylsulfonamide (24c):** yield 78%; mp 135 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (4H, m), 7.61 (1H, dd, J = 2.1, 8.0 Hz), 7.39 (2H, d, J = 7.9 Hz), 7.25 (1H, m), 6.87 (2H, m), 6.64 (1H, m), 3.46 (3H, s, OCH_3), 2.48 (3H, s, CH_3); IR 1651, 1609, 1544, 1454, 1317, 1152, 858, 675 cm^{-1}; MS (CI) m/z 429 (M^+ + 1), 397 ($M^+ - OCH_3$). Anal. ($C_{21}H_{17}FN_2O_3S_2$) C, H, N.

***N*-[4-(6-Methylbenzothiazol-2-yl)-4-methoxy-2,5-cyclohexadienylidene]-(4-methyl)phenylsulfonamide (24d):** yield 67%; mp 182 °C; ^{1}H NMR ($CDCl_3$) δ 7.93−7.83 (4H, m), 7.70 (1H, m), 7.37 (2H, d, J = 8.2 Hz), 7.29 (1H, m), 6.93−6.83 (2H, m), 6.62 (1H, dd, J = 2.0, 10.0 Hz), 3.43 (3H, s, OCH_3), 2.49 (3H, s, CH_3); IR 1653, 1599, 1545, 1316, 1155, 1078, 862, 677 cm^{-1}; MS (CI) m/z 425 ($M^+ + 1$), 395 ($M^+ - OCH_3$). Anal. ($C_{22}H_{20}N_2O_3S_2$) C, H, N.

***N*-[4-(6-Methoxybenzothiazol-2-yl)-4-methoxy-2,5-cyclohexadienylidene]-(4-methyl)phenylsulfonamide (24e):** yield 67%; mp 166−168 °C; ^{1}H NMR ($CDCl_3$) δ 7.90 (4H, m, H-2, H-6, toluenesulfonyl H-2, H-6), 7.37 (3H, m, H-7′, toluenesulfonyl H-3, H-5), 7.10 (1H, dd, J = 2.5, 9.0 Hz, H-5′), 6.90 (2H, m, H-3, H-5), 6.63 (1H, m, H-4′), 3.90 (3H, s, OCH_3), 2.48 (3H, s, CH_3); IR 1651, 1605, 1545, 1464, 1316, 1158, 860, 677 cm^{-1}; MS (CI) m/z 441 ($M^+ + 1$), 409 ($M^+ - OCH_3$). Anal. ($C_{21}H_{18}N_2O_3S_2 \cdot {}^1/_2H_2O$) ($C_{22}H_{20}N_2O_4S_2$) C, H, N.

Quinol Functionalization: Conversion of 7a to the Corresponding Acetate Ester 2a. Triethylamine (0.38 mL, 2.7 mmol) and DMAP (44 mg, 0.36 mmol) were added to a solution of 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (0.438 g, 1.8 mmol) in dichloromethane (20 mL). Acetic anhydride (0.25 mL, 2.7 mmol) was then slowly added. The reaction mixture was stirred at room temperature for 30 min and then washed with 1 M HCl (20 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield the acetate ester **2a** (0.469 g) in 91% yield.

Quinol Functionalization: Conversion of 7a to the Corresponding Methyl Ether 2b. To 4-benzothiazol-2-yl-4-hydroxycyclohexadienone (0.1 g, 0.41 mmol) in dry THF (5 mL) at −78 °C under N_2 was added potassium *tert*-butoxide (0.069 g, 0.615 mmol, 1.5 equiv) suspended in THF (2 mL) with stirring. After 20 min, methyl iodide (0.088 g, 0.615 mmol, 1.5 equiv) was added and the reaction mixture was warmed to 0 °C for 4 h. Water (20 mL) was added, and the reaction was warmed to room temperature and extracted with diethyl ether (2 × 20 mL). The combined organic layers were combined, washed with water (2 × 10 mL), and dried over magnesium sulfate. Removal of the solvent in vacuo gave a solid, which was stirred with hexane then collected on a filter, washed with hexane, and dried under vacuum. The yield of quinol methyl ether was 49%.

Biological Experiments. In Vitro Assays. Nottingham Culture Procedure. Compounds were prepared as 10 mM top stocks, dissolved in DMSO, stored at 4 °C, and protected from light for a maximum period of 4 weeks. Human-derived cell lines (HCT 116, HT29 colon carcinoma; MCF-7 (ER+), MDA 468 (ER-) breast carcinoma; A549 lung adenocarcinoma) were routinely cultivated at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 2 mM l-glutamine and 10% fetal calf serum and subcultured twice weekly to maintain continuous logarithmic growth. Cells were seeded into 96-well microtiter plates at a density of 5×10^3 per well and were 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 of exposure, MTT was added to each well (final concentration of 400 g/mL). Incubation at 37 °C for 4 h allowed reduction of MTT by viable cells to an insoluble formazan product. Well contents were aspirated, and formazan was solubilized by addition of DMSO/glycine buffer (pH 10.5) (4:1). Absorbance was read on an Anthos Labtec systems plate reader at 550 nm as a measure of cell viability; thus, cell growth or drug toxicity was determined.

NCI Growth-Inhibitory Determination. Cell culture and drug application procedures have been described previously.[14] Briefly, cell lines were inoculated into a series of 96-well microtiter plates, with varied seeding densities depending on the growth characteristics of each cell line. Following a 24 h drug-free incubation, test agents were added at five 10-fold dilutions with a maximum concentration of 100 μM. Cellular protein levels were determined after 48 h of drug exposure by sulforhodamine B colorimetry.

In Vivo Evaluation. Six to eight week old outbred nude mice (NMRI genetic background) were housed in Macrolon cages under laminar air flow on natural daylight cycles. Experiments were conducted according to UKCCCR guidelines. Human-derived renal cell carcinoma RXF 944XL hypernephroma fragments (25 mg) were implanted sc in both flanks of the animals. When xenografts were clearly palpable (volume 100−200 mm^3), animals were randomly allocated into treatment groups. Drug (15 mg/kg **7a**) was administered ip on days 1 and 8 or on days 1 and 2. Control animals received vehicle alone (10% DMSO + 0.05% Tween 80/saline, or 10% DMSO in aracus oil). Tumor growth was monitored twice weekly by serial caliper measurement. To evaluate tolerance of animals to compound **7a**, non-tumor-bearing mice received 15 mg/kg according to the selected schedules.

Acknowledgment. This work was supported by Cancer Research U.K. We thank Dr. Ven Narayanan and his colleagues (NCI) for access to the NCI in vitro screening facility, Mr. Charlie Matthews (Nottingham) for cell culture assistance, and Prof. Mike Bibby and colleagues (Cancer Research Unit, University of Bradford) for in vivo studies in colon xenografts.

References

(1) Gibson, G. G.; Skett, P. *Introduction to Drug Metabolism, 3rd Edition*; Nelson Thornes: Cheltenham, U.K., 2001; pp 1−36.
(2) Umezawa, K.; Imoto, M. Use of erbstatin as protein tyrosine kinase inhibitor. *Methods Enzymol.* **1991**, *201*, 379−385.
(3) Rambas, L.; McMurray, J. S.; Budde, R. J. A. The degree of inhibition of protein tyrosine kinase activity by Tyrphostin 23 and 25 is related to their instability. *Cancer Res.* **1994**, *54*, 867−869.
(4) Faaland, C. A.; Mermelstein, F. H.; Hayastin, J.; Laskin, J. D. Rapid uptake of tyrphostin into A431 human epidermoid cells is followed by delayed inhibition of epidermal growth factor (EGF) stimulated EGF receptor tyrosine kinase activity. *Mol. Cell Biol.* **1991**, *11*, 2697−2703.
(5) Reddy, K. B.; Mangold, G. L.; Tandon, A. K.; Yoneda, T.; Mundy, G. R.; Zilberstein, A. Inhibition of breast cancer cell growth in vitro by a tyrosine kinase inhibitor. *Cancer Res.* **1992**, *52*, 3631−3641.
(6) Wells, G.; Seaton, A.; Stevens, M. F. G. Structural studies on bioactive compounds. 32. Oxidation of tyrphostin protein tyrosine kinase inhibitors with hypervalent iodine reagents. *J. Med. Chem.* **2000**, *43*, 1550−1562.
(7) Wells, G.; Bradshaw, T. D.; Diana, P.; Seaton, A.; Westwell, A. D.; Stevens, M. F. G. Antitumor benzothiazoles. 10. The synthesis and antitumor activity of benzothiazole substituted quinol derivatives. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 513−515.
(8) Patent application filed July 6, 2001 (U.K.).
(9) Capparelli, M. P.; DeSchepper, R. E.; Swenton, J. S. Structural and solvent/electrolyte effects on the selectivity and efficiency of the anodic oxidation of *para*-substituted aromatic ethers. *J. Org. Chem.* **1987**, *52*, 4953−4961.
(10) Livinghouse, T. Trimethylsilyl cyanide: cyanosilylation of *p*-benzoquinone. *Org. Synth.* **1981**, *60*, 126−132.
(11) McKillop, A.; McLaren, L.; Watson, R. J.; Taylor, R. J. K.; Lewis, N. A concise synthesis of the novel antibiotic aranorosin. *Tetrahedron Lett.* **1993**, *34*, 5519−5522.
(12) McKillop, A.; Perry, D. H.; Edwards, M.; Antus, S.; Farkas, L.; Nogradi, M.; Taylor, E. C. Thallium in organic synthesis. XLII. Direct oxidation of 4-substituted cyclohexa-2,5-dienones using thallium(III) nitrate. *J. Org. Chem.* **1976**, *41*, 282−287.
(13) Stevens, M. F. G.; Shi, D.-F.; Castro, A. Antitumour benzothiazoles. Part 2. Formation of 2,2′-diaminobiphenyls from the decomposition of 2-(4-azidophenyl)benzazoles in trifluoromethanesulfonic acid. *J. Chem. Soc., Perkin Trans. 1* **1996**, 83−93.
(14) Boyd, M. R.; Paull, K. D. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res.* **1995**, *34*, 91−104.
(15) Weinstein, J. N.; Myers, T. G.; O'Connor, P. M.; Friend, S. H.; Fornace, A. J.; Kohn, K. W.; Fojo, T.; Bates, S. E.; Rubinstein, L. V.; Anderson, N. L.; Buolamwini, J. K.; van Osdol, W. W.; Monks, A. P.; Scudiero, D. A.; Sausville, E. A.; Zaharevitz, D. W.; Bunow, B.; Viswanadhan, V. N.; Johnson, G. S.; Wittes, R. E.; Paull, K. D. An information-intensive approach to the molecular pharmacology of cancer. *Science* **1997**, *275*, 343−349.

(16) Kunkel, M. W.; Kirkpatrick, D. L.; Johnson, J. I.; Powis, G. Cell line-directed screening assay for inhibitors of thioredoxin reductase signalling as potential anti-cancer drugs. *Anti-Cancer Drug Des.* **1997**, *12*, 659−670.
(17) Alcaraz, L.; Macdonald, G.; Ragot, J. P.; Lewis, N.; Taylor, R. J. K. Manumycin A: synthesis of the (+)-enantiomer and revision of stereochemical assignment. *J. Org. Chem.* **1998**, *63*, 3526−3527.
(18) Wipf, P.; Kim, Y. Synthesis of the antitumor antibiotic LL-C10037α. *J. Org. Chem.* **1994**, *59*, 3518−3519.
(19) Wada, H.; Fujita, H.; Murakami, T.; Saiki, Y.; Chen, C. M. Chemical and chemotaxonomical studies of ferns. LXXIII. New flavonoids with modified B-ring from the genus Pseudophegopteris (Thelypteridacae). *Chem. Pharm. Bull.* **1987**, *35*, 4757−4762.
(20) Milić, D. R.; Kapor, A.; Markov, B.; Ribar, B.; Strümpel, M.; Juranić, Z.; Gašić, M. J.; Šolaja, B. A. X-ray crystal structure of 10*β*-hydroxy-4*β*,5*β*-epoxyestr-1-en-3,17-dione and antitumor activity of its congeners. *Molecules* **1999**, *4*, 338−352.
(21) Hutchinson, I.; Chua, M.-S.; Browne, H. L.; Trapani, V.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. Antitumor benzothiazoles. 14. Synthesis and in vitro biological properties of fluorinated 2-(4-aminophenyl)benzothiazoles. *J. Med. Chem.* **2001**, *44*, 1446−1455.
(22) Kashiyama, E.; Hutchinson, I.; Chua, M.-S.; Stinson, S. F.; Phillips, L. R.; Kaur, G.; Sausville, E. A.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. Antitumor benzothiazoles. 8. Synthesis, metabolic formation, and biological properties of the C- and N-oxidation products of antitumor 2-(4-aminophenyl)-benzothiazoles. *J. Med. Chem.* **1999**, *42*, 4172−4184.

JM020984Y