Quinols as Novel Therapeutic Agents. 2.[1] 4-(1-Arylsulfonylindol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-ones and Related Agents as Potent and Selective Antitumor Agents

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A series of substituted 4-(1-arylsulfonylindol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-ones (indolylquinols) has been synthesized on the basis of the discovery of lead compound **1a** and screened for antitumor activity. Synthesis of this novel series was accomplished via the "one-pot" addition of lithiated (arylsulfonyl)indoles to 4,4-dimethoxycyclohexa-2,5-dienone followed by deprotection under acidic conditions. Similar methodology gave rise to the related naphtho-, 1*H*-indole-, and benzimidazole-substituted quinols. A number of compounds in this new series were found to possess in vitro human tumor cell line activity substantially more potent than the recently reported antitumor 4-substituted 4-hydroxycyclohexa-2,5-dien-1-ones[1] with similar patterns of selectivity against colon, renal, and breast cell lines. The most potent compound in the series in vitro, 4-(1-benzenesulfonyl-6-fluoro-1*H*-indol-2-yl)-4-hydroxycyclohexa-2,5-dienone (**1h**), exhibits a mean GI_{50} value of 16 nM and a mean LC_{50} value of 2.24 μM in the NCI 60-cell-line screen, with LC_{50} activity in the HCT 116 human colon cancer cell line below 10 nM. The crystal structure of the unsubstituted indolylquinol **1a** exhibits two independent molecules, both participating in intermolecular hydrogen bonds from quinol OH to carbonyl O, but one OH group also interacts intramolecularly with a sulfonyl O atom. This interaction, which strengthens upon ab initio optimization, may influence the chemical environment of the bioactive quinol moiety. In vivo, significant antitumor activity was recorded (day 28) in mice bearing subcutaneously implanted MDA-MB-435 xenografts, following intraperitoneal treatment of mice with compound **1a** at 50 mg/kg.

Introduction

Metabolic oxidation has been implicated as a potential bioactivating event in the biological mechanism of action of a range of biologically interesting phenols. For example, di- and triphenolic tyrphostins decompose in solution to more active protein tyrosine kinase (PTK) inhibitors,[2] whereas tyrphostins devoid of hydroxy groups have a rapid onset of cellular activity.[3] Our interest in the generation of novel and structurally diverse chemical oxidation products of bioactive phenols[4] as potential therapeutic agents with enhanced biological properties has led to the discovery of a new series of 4-aryl-substituted 4-hydroxycyclohexa-2,5-dien-1-ones (Figure 1) with selective antitumor activities against colon, renal, and breast cancer cell lines.[1,5]

Our previously reported studies on the antitumor properties of 4-substituted 4-hydroxycyclohexa-2,5-dien-1-ones (also known as antitumor quinols)[1] examined the structure−activity relationships (SAR) following variation of the heteroaromatic group. In general, we found that for optimal in vitro antitumor activity as ranked

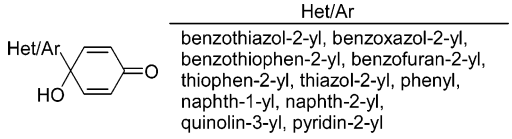

Figure 1. Chemical structures of 4-substituted 4-hydroxycyclohexa-2,5-dien-1-ones.[1]

by mean GI_{50} values in the National Cancer Institute (NCI) panel of 60 human cancer cell lines, a 6/5 heterobicyclic structure was preferred over a 6/6 heterobicyclic structure, which in turn was preferred to a monocycle. As part of this novel series, we wished to synthesize and evaluate the antitumor properties of indol-2-yl-substituted and benzimidazol-2-yl-substituted 4-hydroxycyclohexa-2,5-dien-1-ones. Our synthetic strategy to achieve this objective (Scheme 1) would differ somewhat from the other heteroaromatic-substituted compounds reported, since a protecting group would be required for the acidic NH functionality on both indole and benzimidazole.

Initially the phenylsulfonyl group was tested as an indole protecting group. Deprotonation of the commercially available 1-(phenylsulfonyl)indole, followed by addition to 4,4-dimethoxycylohexadienone and removal of the ketal functionality, to afford 4-(1-benzenesulfonyl-1*H*-indol-2-yl)-4-hydroxycyclohexa-2,5-dienone (X = CH, PG = $PhSO_2$, Scheme 1) was readily accomplished.

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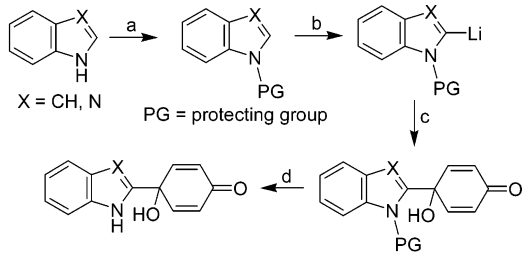
Scheme 1. General Strategy for Preparation of Indol-2-yl- and Benzimidazol-2-yl-Substituted 4-Hydroxycyclohexa-2,5-dien-1-ones[a]



[a] Reagents: (a) "protection"; (b) n-BuLi, THF, −78 °C; (c) 4,4-dimethoxycyclohexadienone, THF, −78 °C; (d) "deprotection".

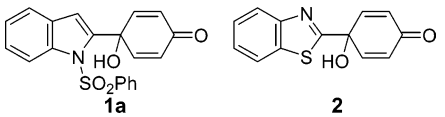
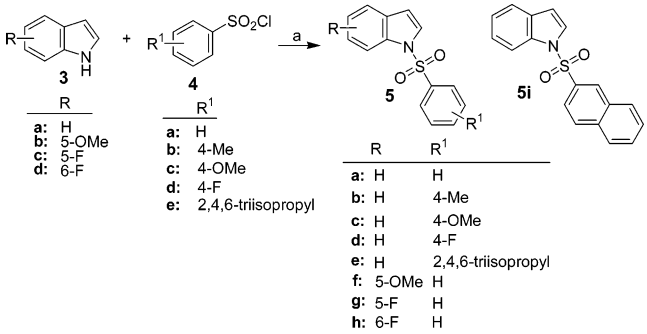


Figure 2. Chemical structures of 4-(benzenesulfonyl)indole- and 4-benzothiazole-substituted quinols.

Scheme 2. Preparation of (Arylsulfonyl)indoles[a]



[a] Reagents: (a) $Bu_4N^+HSO_4^-$, NaOH (aq), toluene, 0 °C.

Removal of the benzenesulfonyl protecting group, however, was not easily achieved under conditions[6] (NaOH in MeOH/H_2O) compatible with maintaining the integrity of the cyclohexadienone structure. Fortuitously, the intermediate 4-(1-benzenesulfonyl-1*H*-indol-2-yl)-4-hydroxycyclohexa-2,5-dienone **1a** (Figure 2), when tested in vitro against the NCI 60-cell-line panel, was found to be almost an order of magnitude more potent (in terms of mean GI_{50}) compared to the most potent compound previously reported, 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-one **2** (Figure 2).[1]

In this paper we report the synthesis and in vitro antitumor evaluation of a new series of potent compounds based on the new lead structure. Preliminary in vivo xenograft activity associated with compound **1a** is also described.

Chemistry

The synthesis of substituted 4-(1-benzenesulfonyl-1*H*-indol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-ones was accomplished as outlined in Scheme 2. Reaction of (substituted)indole **3a**−**d** and tetrabutylammonium hydrogen sulfate (TBAHS) in toluene with sulfonyl chloride **4a**−**e** and aqueous sodium hydroxide[7] gave substituted 1-(benzenesulfonyl)indoles **5a**−**h** in good yield (67−100%). Reaction with naphthalene-2-sulfonyl chloride similarly afforded the indole **5i** (92%).[8]

Lithiated (phenylsulfonyl)indoles were prepared from precursor indoles **5a**−**i** using *n*-butyllithium in THF at −78 °C. Addition of the lithiated substrates to 4,4-dimethoxy-2,5-dien-1-one generates the quinols **6a**−**h** protected as their dimethyl ketals. These intermediate compounds were not isolated but were deprotected in situ using dilute acetic acid to give the required indole-substituted quinols **1a**−**h**[1,9] (Scheme 3). Indole **5i** was converted similarly to the naphthyl analogue **1i**. The preparation of quinols **1a**−**i** from their precursor indoles **5a**−**i** proceeds in low to moderate yield (12−38%), with substantial remaining unreacted starting material in each case. Attempts to vary the conditions of the reaction (e.g. reaction temperature, order of addition of reagents) did not lead to improved yields. However, the concise route employed (two steps from commercially available starting materials) means that sufficient quantities (>100 mg) of material for full chemical characterization and biological screening are readily available.

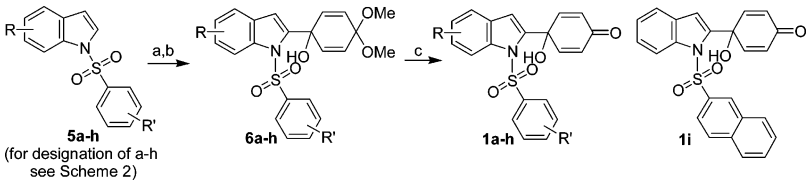
Lithiation of (arylsulfonyl)indoles **5a,b** followed by addition to a protected quinone containing 2,3-benzo annelation (1,4-naphthoquinone dimethyl ketal, prepared via oxidation of 4-methoxy-1-naphthol using iodosobenzene[1]) breaks the plane of symmetry common to structure **1**, to give the benzo-annelated derivatives **7a,b** as racemic mixtures after ketal deprotection (Scheme 4).

For preparation of the benzimidazol-2-yl- and indol-2-yl-substituted quinols **10** and **11** it was necessary to protect the acidic N−H functionality using the pyrrolidinylmethyl[10] and dimethylaminomethyl[11] groups to give intermediates **8** and **9**, respectively, prior to lithiation. Following addition to 4,4-dimethoxycyclohexa-2,5-dien-1-one, deprotection of the ketal functionality using 2 M HCl followed by removal of the azole-protecting group with sodium borohydride yielded quinols **10** and **11** (Scheme 5).

X-ray Crystal Structure and Discussion

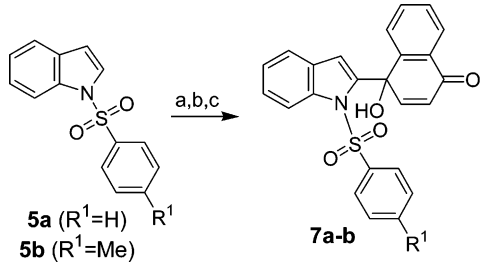
The X-ray crystal structure of the unsubstituted (indolyl)quinol **1a** was determined. The similarity between *a* and *c* axial lengths permitted nonmerohedral twinning according to the twin law $z, -y, x$. Eventually a specimen was found with a minor component fraction of only 0.044. In the final refinement all H atoms were placed in calculated positions except for the important quinol OH group, which underwent stable refinement as a rotating group. The two independent molecules in the structure (Figure 3) differ by rotations of 2−6° about the bonds between rings and have significantly different patterns of hydrogen bonding. The single donor group (quinol O14−H) meets a surfeit of acceptors offering Löwdin charges of −0.506 on O14, −0.289 on carbonyl O15, and −0.675 and −0.652 on sulfonyl O17 and O18, respectively. Both molecules form an intermolecular hydrogen bond to O15. However, in the primed molecule this bond is weaker and an intramolecular interaction with O17′ also appears. Under ab initio molecular orbital optimization with GAMESS[12] in the 6-31G* basis set, the isolated unprimed molecule develops this intramolecular hydrogen bond, the H14··O17 contact closing to 1.875 Å. This restraint on the relative orientation of the rings will influence both the steric accessibility and the electrostatic environment of the bioactive quinol pharmacophore.

Scheme 3. Preparation of (Arylsulfonyl)indole-substituted Quinols[a]



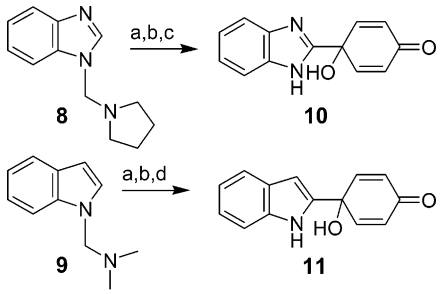
[a] Reagents: (a) n-BuLi, THF, −78 °C; (b) 4,4-dimethoxycyclohexa-2,5-dienone, THF, −78 °C; (c) 10% AcOH (aq), acetone, reflux.

Scheme 4. Preparation of Benzo-Annelated (arylsulfonyl)Indole-Substituted Quinols[a]



[a] Reagents: (a) BuLi, THF, −78 °C; (b) 4,4-dimethoxynaphthalen-1-one, THF, −78 °C; (c) 10% AcOH (aq), acetone, reflux.

Scheme 5. Preparation of Benzimidazol-2-yl- and Indol-2-yl-Substituted Quinols[a]



[a] Reagents: (a) n-BuLi, THF, −78 °C; (b) 4,4-dimethoxycyclohexadienone, THF, −78 °C; (c) 2 M HCl (aq); (d) $NaBH_4$, EtOH/THF, then 10% AcOH (aq), acetone.

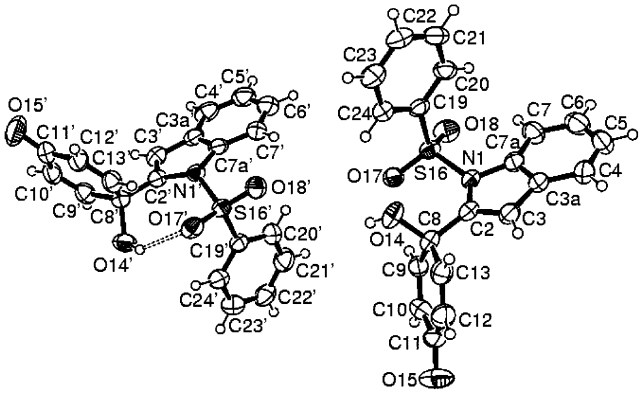


Figure 3. X-ray crystal structure of unsubstituted (indolyl)-quinol **1a**.

Biological Results and Discussion

In Vitro Activity against Cancer Cell Lines. Evaluation of the growth-inhibitory properties of the novel quinols **1a**−**i**, **7a,b**, **10**, and **11** was undertaken in two human colon cancer cell lines (HCT 116 and HT 29) and two mammary carcinoma cell lines (MCF-7 and MDA 468) previously found to be sensitive to "first-generation" 4-substituted-4-hydroxycyclohexa-2,5-dien-1-ones.[1] Following 72 h drug exposure, MTT assays were performed to determine the growth-inhibitory effects of the new compounds.

Table 1. Activity of Compounds in the NCI in Vitro 60-Cell Panel[a]

compd	mean GI_{50} (μM)[b]	mean LC_{50} (μM)[b]	most sensitive cell lines[c] (LC_{50}, μM)
2	0.23	3.39	HCT-116 (0.43), RXF 393 (0.58)
1a	0.039	2.95	HCT-116 (0.033), CAKI-1 (0.053)
1b	0.11	7.24	HCT-116 (0.048), LOX IMVI (0.23)
1c	0.16	6.31	HCT-116 (0.11), LOX IMVI (0.38)
1d	0.23	7.76	HCT-116 (0.36), UO-31 (0.63)
1e	0.43	11.2	HCT-116 (0.55), RXF 393 (0.78)
1f	0.13	6.17	ACHN (0.45), LOX IMVI (0.47)
1g	0.066	3.23	HCT-116 (0.050), LOX IMVI (0.063)
1h	0.016	2.24	HCT-116 (<0.01), ACHN (0.020)
1i	0.19	5.62	HCT-116 (0.37), U251 (0.51)
7a	0.45	7.76	HCT-116 (0.79), LOX IMVI (0.78)
7b	0.39	5.62	HCT-116 (0.45), LOX IMVI (0.72)
10	0.87	13.18	UACC-62 (1.41), HCT-116 (1.86)
11	1.38	23.99	RXF 393 (3.39), HCT-116 (5.13)

[a] All compounds were screened twice, with concurrent results. The data presented above represents values for one particular test date across most of the group of compounds. [b] For definitions of mean GI_{50} and mean LC_{50} see ref 13. [c] Cancer cell line origin: HCT-116 (colon); CAKI-1, ACHN, RXF 393, UO-31 (renal), LOX IMVI, UACC-62 (melanoma); U251 (CNS).

Consistently the most sensitive cell line among those examined was the colon-derived HCT 116, with GI_{50} values spanning a 10-fold range (GI_{50} 0.044−0.774 μM), with compounds **1h** (44 nM) and **1g** (68 nM) being the most potent. GI_{50} values in the other cell lines tested were generally found to be in the sub-micromolar range. In the case of HT 29, GI_{50} values ranged over a 5-fold range from 94 nM (compound **11**) to 444 nM (**7a**); for breast cell line MCF-7, values ranged from 210 nM (**10**) to 435 nM (**7a**); and for MDA, 468 values ranged from 262 nM (**1h**) to 545 nM (**11**).

The full range of novel new quinols was also evaluated for in vitro activity across 60 human cancer cell lines in the NCI Developmental Therapeutics Screening Program.[13] Table 1 shows the results of this comprehensive screening protocol in a condensed form, listing for each compound the mean GI_{50} value and mean LC_{50} value across the 60 human cancer cell lines (48 h drug exposure). Since the most interesting selectivity profiles are observed in the LC_{50} panel, the two most sensitive cell lines in the panel are indicated for each compound along with LC_{50} values for the cell lines concerned. Typically, the most sensitive cell lines at the LC_{50} level are found in the colon and renal cell panels. For comparison, the NCI screen values for 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-one (**2**), the most potent compound from the previously reported quinol series, are included in Table 1.

Inspection of the above NCI screening data indicates that all members of the (arylsulfonylindolyl)quinol series (**1a**−**i**) possess potent in vitro antiproliferative

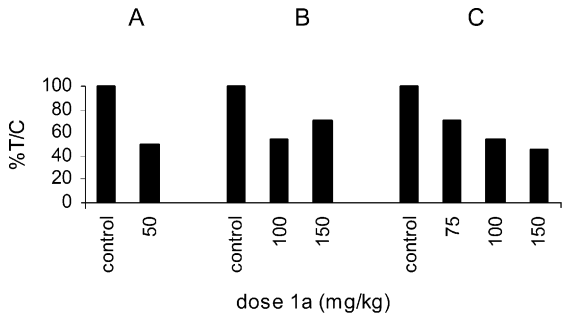


Figure 4. In vivo activity of compound **1a** against MDA-MB-435 breast (A), HCT 116 colon (B), and CAKI-1 renal (C) tumor xenografts. Data recorded 28 days after xenograft implantation.

activity, with mean GI_{50} values across the 60 cell lines ranging from 16 nM (**1h**) to 430 nM. The unsubstituted (1*H*-indolyl)quinol **12** and its benzimidazole counterpart **11** were however markedly less potent across the range of cell lines examined, emphasizing the importance of the arylsulfonyl substituent for endowing compounds with potent antitumor properties.

The most potent compound in the series is the 4-(1-benzenesulfonyl-1*H*-6-fluoroindol-2-yl)-4-hydroxycyclohexa-2,5-dienone **1h**, both in terms of mean GI_{50} (16 nM) and mean LC_{50} (2.24 μM). Notably, this compound ranks as more than 10-fold more potent (mean GI_{50}) compared to the most potent member of the quinol series, 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-one, **2**, reported previously.[1] Increasing the steric bulk around the arylsulfonyl group (e.g. the 2,4,6-triisopropylphenyl derivative **1e**) leads to a decrease in activity across the panel. Fusion of a second aromatic ring onto the core cyclohexadienone motif to give the corresponding benzo-annelated quinols **7a** and **7b** also has a dyschemotherapeutic effect, confirming our working hypothesis that two "Michael acceptor" electrophilic β-carbons are necessary for optimal antitumor activity.

The most sensitive cell line to (arylsulfonyl)indolyl quinols (**1a**−**i**) in all but one case (**1f**) was found to be HCT-116 colon; again this observation correlates with the sensitivity of HCT-116 to previously reported 4-substituted-4-hydroxycyclohexa-2,5-dien-1-ones.[1] The LC_{50} values indicate that the most potent compound (**1h**) in the two most sensitive cell lines (HCT-116, <10 nM; CAKI-1, 20 nM) is substantially more than an order of magnitude more potent than the corresponding LC_{50} values for the two cell lines most sensitive to **2** (HCT-116, 0.43 μM; RXF 393, 0.58 μM) and represents a significant step forward in enhancing the in vitro antitumor potency of the quinol series.

In Vivo Activity. The in vivo antitumor activity of compound **1a** has been examined in three xenograft models taken from the NCI human cell line panel: MDA-MB-435 breast, HCT 116 colon and CAKI-1 renal tumors implanted sc into the flanks of Ncr:nu/nu female mice. Preliminary data, recorded 28 days after implantation, revealed significant (50%) growth inhibition of MDA-MB-435 tumors following intraperitoneal (ip) treatment of animals with 50 mg/kg **1a** in PBS/Tween (volume of 0.2 mL/20 g of body weight) administered on days 8−12 (Figure 4). Compound **1a** was well-tolerated by mice; a moderate body weight loss was recorded, but blood parameters remained unaltered. HCT 116 tumor growth inhibition, determined on days 4, 8, and 12, was transient and not significant, after treatment of animals ip with 100 and 150 mg/kg **1a**. Body weight loss was moderate, however, and again hematological parameters were not adversely influenced. In mice bearing CAKI-1 tumors, dose-dependent antitumor activity was detected following ip treatment (days 12, 16, and 20) with 75, 100, or 150 mg/kg **1a** (29%, 45%, 55% tumor growth inhibition respectively, measured on day 28; Figure 4). Body weight loss was dose-dependent, and 2/6 toxic deaths were observed in the group receiving 150 mg/kg **1a**. Hematological parameters remained unaffected. The conclusions following preliminary study of the in vivo antitumor activity of this compound using an unoptimized schedule indicate that activity may be restricted by poor aqueous solubility, which in turn results in limited bioavailability. Optimization of the drug formulation in conjunction with the synthesis of more water-soluble analogues of **1a** is underway and will be reported in due course.

Mechanism of Action. Initial studies toward identification of a mechanistic target(s) underpinning this class of substituted hydroxycyclohexadienone ("quinol") structure were carried out previously by NCI COMPARE[14] analysis using the lead compound AW 464 (NSC 706704) as seed.[1] Among compounds (natural products and small synthetic molecules) exhibiting significant Pearson correlation coefficients (PCCs > 0.7), structurally unrelated inhibitors of thioredoxin/thioredoxin reductase[15] with similar profiles of in vitro antitumor activity (toxicity in colon, renal, and breast cancer cell lines) were of particular interest. We have screened both the first-generation[1] and the present series of antitumor quinols as inhibitors of human thioredoxin signaling and conducted further investigations into features of their antitumor mechanism. For example, compound **1a** was found to be an irreversible inhibitor of human thioredoxin signaling with an IC_{50} value of 5.7 μM. Details of our mechanistic studies will be published separately. Drug development work continues in our laboratory with the aim of identifying a clinical candidate molecule from this series of novel thioredoxin-inhibitory agents.

Conclusions

A novel series of substituted 4-(1-arylsulfonylindol-2-yl)-4-hydroxycyclohexa-2,5-dien-1-ones (indolylquinols) **1a**−**i** have been synthesized via a two-step procedure involving initial N-protection of an indole using an arylsulfonyl chloride, followed by C-2 lithiation and addition to benzoquinone ketal, and subsequent ketal deprotection. C-2 lithiation of (arylsulfonyl)indoles **5a,b** followed by addition to a protected quinone containing 2,3-benzo annelation (1,4-naphthoquinone dimethyl -ketal) leads to the benzo-annelated derivatives **7a,b**. The corresponding 1*H*-benzimidazole and 1*H*-indole-substituted quinols **10** and **11** were similarly prepared via protection of the acidic N−H functionality using the pyrrolidinylmethyl and dimethylaminomethyl groups, respectively, prior to lithiation. The crystal structure of the unsubstituted indolylquinol **1a** exhibits two independent molecules, both participating in intermolecular hydrogen bonds from quinol OH to carbonyl O. A number of these novel compounds were found to possess in vitro human tumor cell line activity substan-

tially more potent than the recently reported antitumor 4-substituted 4-hydroxycyclohexa-2,5-dien-1-ones[1] with similar patterns of selectivity against colon, renal, and breast cell lines. For example, the most potent compound in the series, the 6-fluoro analogue **1h**, exhibits a mean GI_{50} value of 16 nM and mean LC_{50} value of 2.24 μM in the NCI 60-cell-line screen, with LC_{50} activity in the HCT 116 human colon cancer cell line below 10 nM. In vivo, significant antitumor activity was recorded (day 28) in mice bearing subcutaneously implanted MDA-MB-435 xenografts, following intraperitoneal treatment of mice with compound **1a** at 50 mg/kg.

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 determined on a Perkin-Elmer Spectrum One FT-IR spectrometer. Mass spectra were recorded on an AEI MS-902 or a VG Micromass 7070E spectrometer. TLC systems for routine monitoring of reaction 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 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 1-(Arylsulfonyl)-indoles (5a−i).[6] To a vigorously stirring solution of indole (8.5 mmol, **3a**−**d**) and tetrabutylammonium hydrogen sulfate (TBAHS) (1.28 mmol) in toluene (25 mL) at 0 °C was added 50% aqueous sodium hydroxide (25 mL) and sulfonyl chloride (12.8 mmol, **4a**−**e**). The resultant solution was stirred at room temperature for 16 h. After this time, the organic layer was separated; washed with 1 N HCl (2 × 25 mL), saturated aqueous $NaHCO_3$ (2 × 25 mL), water (25 mL), brine (25 mL); dried ($MgSO_4$); and evaporated to dryness to yield 1-(arylsulfonyl)indoles (**5a**−**h**), which were used in subsequent steps without further purification. 1-(2-Naphthylsulfonyl)indole (**5i**) was prepared in a similar manner from indole and 2-naphthylsulfonyl chloride.

General Method for the Synthesis of 4-(1-Arylsulfonyl-1*H*-indol-2-yl)-4-hydroxycyclohexa-2,5-dienones (1a−i). To a stirring solution of *n*-butyllithium (3.3 mL, 1.6 M in hexanes, 5.2 mmol) in THF (7 mL) at −78 °C was added a solution of 1-(arylsulfonyl)indole (3.5 mmol, **5a**−**i**) in THF (7 mL) dropwise, under a nitrogen atmosphere. Following addition, the solution was stirred at −78 °C for 1.5 h. After this time, the resultant solution was added via cannula to a stirring solution of 4,4-dimethoxycyclohexa-2,5-dienone (0.54 g, 3.5 mmol) in THF (14 mL) at −78 °C. Following addition, the solution was stirred at −78 °C for 2 h. After this time, the resultant solution was poured into brine (25 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was washed with water (3 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting oil (**6a**−**i**) was redissolved in acetone (20 mL), and 10% aqueous acetic acid (20 mL) added followed by heating at reflux for 1 h. After this time, the solution was allowed to cool to room temperature and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was washed with water (3 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. Purification by flash column chromatography (4:1 hexane: EtOAc) yielded products **1a**−**i** in low to moderate yields.

General Method for the Synthesis of 4-(1-Arylsulfonyl-1*H*-indol-2-yl)-4-hydroxy-4*H*-naphthalen-1-ones (7a,b). To a stirring solution of *n*-butyllithium (3.3 mL, 1.6 M in hexanes, 5.2 mmol) in THF (7 mL) at −78 °C was added a solution of sulfonylindole (**5a**, **5b**, 3.5 mmol) in THF (7 mL) dropwise, under a nitrogen atmosphere. Following addition, the solution was stirred at −78 °C for 1.5 h. The resultant solution was added via cannula to a stirring solution of freshly prepared 4,4-dimethoxy-4*H*-naphthalen-1-one (0.54 g, 3.5 mmol) in THF (14 mL) at −78 °C. Following addition, the solution was stirred at −78 °C for 2 h. After this time, the resultant solution was poured into brine (25 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was washed with water (3 × 20 mL), brine (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The dark oil was redissolved in acetone (20 mL) and 10% aqueous acetic acid (20 mL) and heated at reflux for 1 h. After this time, the solution was allowed to cool to room temperature and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was washed with water (3 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. Purification by flash column chromatography (4:1 hexane: EtOAc) gave the required benzo-annelated (indolyl)quinol (**7a**, **7b**) as white solids.

Synthesis of 4-(Benzimidazol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (10). To *N*-(*N*-pyrrolidinomethyl)benzimidazole[10] (0.571 g, 2.84 mmol) in dry THF (20 mL) under nitrogen was added *n*-butyllithium (1.25 mL of 2.5 M solution, 3.12 mmol) dropwise with stirring at −78 °C. After 1 h, 4,4-dimethoxycyclohexadienone (0.437 g, 2.84 mmol) was added dropwise with stirring. After a further 2 h, the reaction was allowed to rise to room temperature, 2 M HCl (20 mL) was added, and the volume was reduced under vacuum. The pH was adjusted to 6−7 with 1 M sodium hydrogen carbonate solution to form a white precipitate which was collected by filtration, washed with water, and dried under vacuum to give a white solid (0.30 g, 47%).

Synthesis of 4-(Indol-2-yl)-4-hydroxy-2,5-cyclohexadien-1-one (11). To *N*-(dimethylaminomethyl)indole[11] (0.57 g, 3.2 mmol) dissolved in THF (10 mL) under nitrogen was added *n*-butyllithium (2.02 mL of 1.6 M solution, 3.2 mmol) at −78 °C. After 10 min the reaction was warmed to 0 °C over 35−40 min and then cooled again to −78 °C, and 4,4-dimethoxycyclohexadienone (0.5 g, 3.2 mmol) was added. After stirring at −78 °C for 1 h, the reaction was warmed to room temperature over several hours, quenched by the addition of water (20 mL), and then extracted with diethyl ether (2 × 20 mL). The organic fractions were combined, dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in ethanol:THF (1:1, 10 mL), sodium borohydride (0.135 g, 3.5 mmol, 1.1 equiv) added, and the reaction stirred at reflux for 5 h. After cooling to room temperature, the solvent was removed in vacuo. The residue was dissolved in acetone (20 mL), 10% aqueous acetic acid (20 mL) was added, and the reaction was stirred overnight. After cooling to room temperature, the acetone was removed under reduced pressure and the aqueous phase extracted with ether (2 × 20 mL). The combined organic layers were washed with saturated sodium hydrogen carbonate solution (20 mL) and water (20 mL), dried, and concentrated under vacuum. The crude product was purified by flash column chromatography (ethyl acetate/hexane 1:4) to give a white solid (0.19 g, 26%).

In Vitro Assays. Compounds were prepared as 10 mM top stocks, dissolved in DMSO, and stored at 4 °C, protected from light for a maximum period of 4 weeks. Human-derived cell lines (HCT 116, HT29 colon carcinoma; MCF-7 (ER+), MDA 468 (ER−) breast carcinoma) were routinely cultivated at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal calf serum and subcultured twice weekly to maintain continuous logarithmic growth. Cells were seeded into 96-well microtiter plates at a density of 5 × 10^3 per well and allowed 24 h to adhere before drugs were introduced (final concentration 0.1−100 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 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-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.[13] 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 drug exposure by sulforhodamine B colorimetry.

In Vivo Evaluation. All animal experiments were performed according to the German Animal Protection Law, permit TVV G0247/98. Female Ncr:nu/nu mice were housed in filter cages under pathogen-free, standardized environmental conditions (22 °C temperature, 50 ± 10% relative humidity, 12 h light−dark rhythm). Mice received autoclaved food and bedding (Ssniff, Soest, Germany) and acidified drinking water (pH 4.0) ad libitum.

Xenografts were generated by sc inoculation of 10^7 cells from routine cell culture into the flanks of the mice on day zero. When tumors were palpable (approximately 5−6 mm in diameter) treatment was initiated. Compound **1a** and control vehicle (PBS/Tween) were administered ip in a volume of 0.2 mL/20 g of body weight. Tumor size was measured twice weekly by calliper, and volumes computed by Excel software according to the calculation $V = (\text{width}^2 \times \text{length})/2$. Relative tumor volume (RTV) was expressed as tumor volume in animals receiving drug treatment/tumor volume in untreated control animals (T/C) and represented as percentages. Body weight was determined twice weekly, and blood was taken from the retrobulbar venous plexus (3−6 days after initiation of treatment). Blood parameters, such as white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and platelets, were determined by Coulter counter (model T840). Mice were sacrificed when tumor size reached 15% body weight, or when body weight loss exceeded 20%. Mice that died before sacrifice of controls were termed "toxic deaths".

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Supporting Information Available: Physical and spectroscopic data for compounds **1a**−**i**, **7a,b**, **10**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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