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# Phenylimino-10*H*-anthracen-9-ones as novel antimicrotubule agents—synthesis, antiproliferative activity and inhibition of tubulin polymerization

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ABSTRACT

A novel series of phenylimino-10*H*-anthracen-9-ones and 9-(phenylhydrazone)-9,10-anthracenediones were synthesized and evaluated for interaction with tubulin and for cytotoxicity against a panel of human tumor cell lines. The 10-(3-hydroxy-4-methoxy-phenylimino)-10*H*-anthracen-9-one **15h** and its dichloro analog **16b** were identified as potent inhibitors of tumor cell growth (**16b**, $IC_{50}$ K562 0.11 μM), including multidrug resistant phenotypes. Compound **15h** had excellent activity as an inhibitor of tubulin polymerization. Concentration-dependent cell cycle analyzes by flow cytometry confirmed that KB/HeLa cells treated by **15h** and **16b** were arrested in the G2/M phases of the cell cycle. In competition experiments, **15h** strongly displaced radiolabeled colchicine from its binding site on tubulin, showing $IC_{50}$ values similar to that of colchicine. The results obtained demonstrate that the antiproliferative activity is related to the inhibition of tubulin polymerization.



## 1. Introduction

Strategies to block nuclear and cell division by affecting the mitotic spindle have historically been a successful area of research for the advancement of cancer drugs. Nevertheless, molecules that bind microtubles remain an attractive target for the development of antitumor agents.[1,2] Microtubules are proteinaceous tubes found in nearly all eukaryotic cells. They are obligatory elements of the so-called cytoskeleton, which are formed by polymerization of αβ-tubulin heterodimers. Microtubules play vital roles in eukaryotic cellular processes and are essentially involved in formation and maintenance of the cell shape, in organization of intracellular architecture, chromosome segregation during mitosis, and organelle and macromolecule transport. The great functional variety of microtubule functions seems to be due to different microtubule-associated proteins (MAPs) bound, including motor proteins. Irreversible elimination of microtubules is commonly accompanied by serious cellular dysfunctions and can lead to cell death. As the principle components of the mitotic spindle, microtubules are excellent targets to inhibit cell growth, proliferation and differentiation. Compounds displaying a wide structural heterogeneity have been identified to affect the mitotic spindle. Many of them exert their effects by inhibiting polymerization of tubulin to microtubules, whereby almost all of them interact with the αβ-tubulin dimer and interfere with microtubule dynamics, rather than with MAPs or other proteins.[3–6] The Catharanthus bis-indole alkaloids vinblastine (**1**, Chart 1) and vincristine as well as the taxanes, such as paclitaxel and docetaxel are among the most widely employed antitumor drugs for the treatment of leukemias and lymphomas as well as many types of solid tumors. It was mainly the clinical success of these compounds that has stimulated intensive research aimed at the development of further microtubule-targeting drugs. Colchicine (**2**) effectively functions as an antimitotic agent, but is not used as an anticancer agent, primarily due to its narrow therapeutic window. Many natural products, such as combretastatin A-4[7] (**3**) or the epothilones[8] as well as some synthetic molecules including sulfonamide E-7010[9] (**4**), acridinyl-9-carboxamide D-82318[10] (**5**), benzophenone CKD-516 (**6**)[11] or propenenitrile CC-5079[12] (**7**) (Chart 1) are known to mediate cytotoxic activities through binding to tubulin.

*Abbreviations:* ADR, adriamycine; ITP, inhibition of tubulin polymerization; Kip, Kinase inhibitor protein; MDR, multi-drug resistant; MTP, microtubule protein; n.d., not defined; TBAB, tetra-*n*-butylammonium bromide; XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide; VCR, vincristine.

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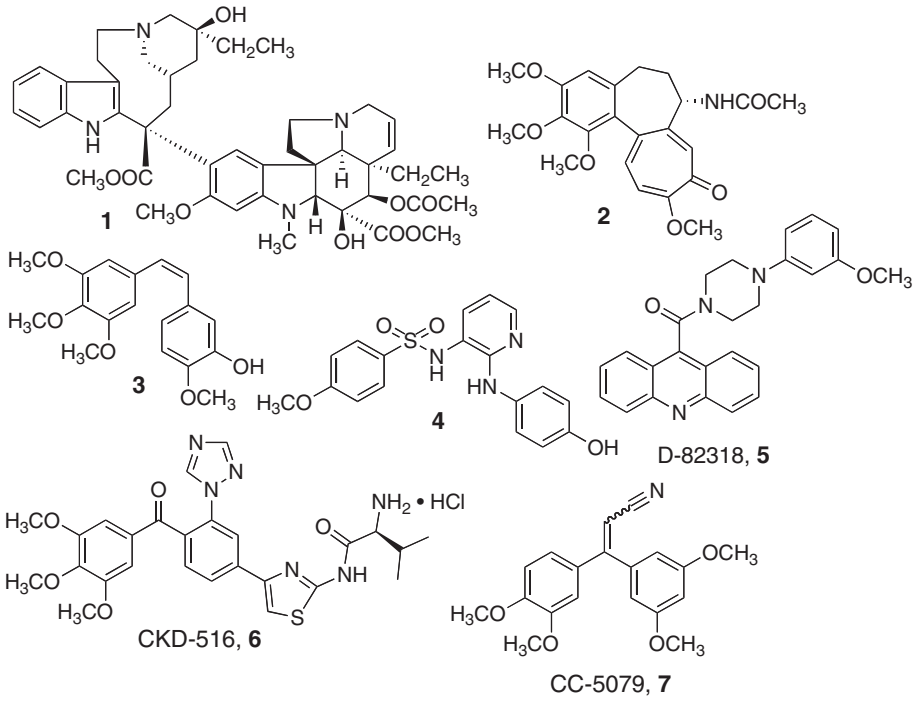



**Chart 1.** Examples of tubulin interacting agents.

In recent years, especially small molecular colchicine site binders derived from natural materials or identified by large-scale screens of compound libraries in combination with traditional medicinal chemistry received a great deal of attention[5,13] However, there are no clinically approved antitumor agents that bind to the colchicine site.

We have been intensively engaged in identifying novel antimitotic anthracenone-based molecules that inhibit tubulin polymerization and have reported the potent in vitro antitumor activity of 10-[(3-hydroxy-4-methoxy-benzylidene)]-10*H*-anthracen-9-one (**8**), 9-[(4-hydroxy-3,5-dimethoxy-benzylidene]-naphtho[2,3-*b*]thiophen-4(9*H*)-one (**9**) and 4-methoxybenzenesulfonic acid ester **10** (Chart 2).[14–16] Very recently, we described a series of 10-(2-*oxo*-2-phenylethylidene)-10*H*-anthracen-9-ones[17] as well as their 1,5- and 1,8-disubstituted[18] derivatives as potent inhibitors of tubulin polymerization, with **11** being a highly active analog.

These compounds are characterized by possessing an enone moiety between the anthracenone and the terminal aromatic ring.[17] Our present strategy for the synthesis of novel antitubulin compounds is based on the modification of the linker between the anthracenone and the terminal phenyl ring and incorporation of a C=N-bond. In this connection, anthracenone–based oxime derivatives as represented by **12** have very recently been described by our group.[19] We now report on phenylimino-10*H*-anthracen-9-ones, in which the benzylidene C=C-double bond linker is replaced by a C=N-bond. Several compounds of these novel 10-phenylimino-10*H*-anthracen-9-ones inhibited the growth of various tumor cell lines, acted in a cell cycle dependent manner and were found to be potent inhibitors of tubulin polymerization. Inhibition of tubulin polymerization of the most active compound is comparable or superior to those of the reference compounds, such as nocodazole, podophyllotoxin and colchicine.

## 2. Results and discussion

### 2.1. Chemistry

Various methods for the preparation of a quinone imine functionality are available.[20] Phenylimino-10*H*-anthracen-9-ones can

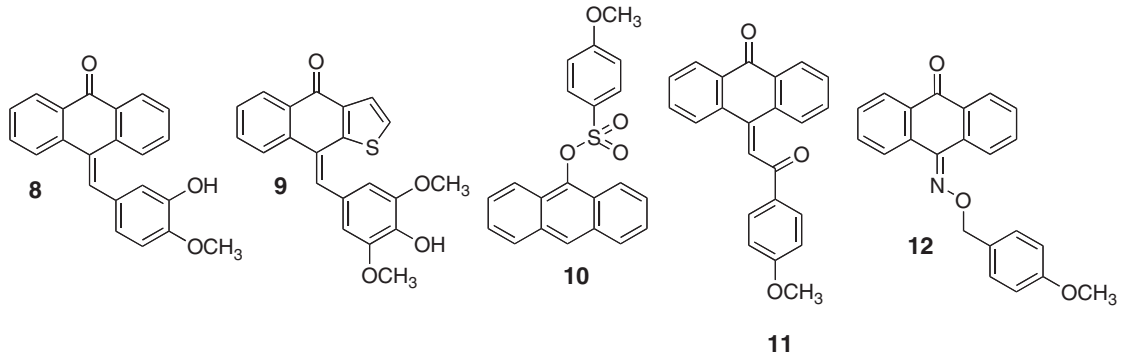



**Chart 2.** Benzylidenes **8** and **9**, sulfonate **10**, phenacylidene **11** and oxime ether **12**.

be obtained from 10-bromoanthrone **14a** and nitrosodimethylaniline.[21] However, bromination is not required in the case of hydroxy-substituted 10*H*-anthracen-9-ones or 1-chloro-10*H*-anthracen-9-one.[22,23] Reaction of 10,10-dibromo-9-anthrones with nitrogen-containing nucleophiles provides another synthetic strategy.[24] A different approach was taken by Hall and co-workers,[25] who performed the synthesis of polyaromatic quinone imines using $TiCl_4$/Dabco.

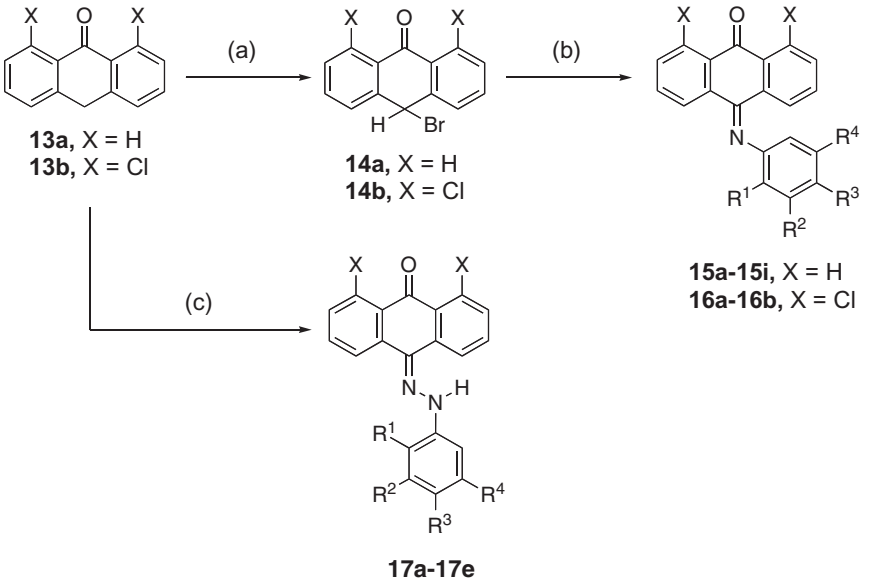
In our case, we obtained the desired phenylimino-10*H*-anthracen-9-ones **15a**–**15i** and the dichloro analogs **16a**–**16c** in a one-pot reaction by boiling the mono-brominated anthrones **14a** or **14b** with 2 equiv of commercially available aromatic amines in dry benzene under reflux in the presence of air (Scheme 1). Mono-brominated **14a** and **14b** were obtained from **13a** and **13b** as described.[26,27] We suggest that the formation of phenyliminoanthracenones is due to the air oxidation of intermediate 10-phenylamino-10*H*-anthracen-9-ones, which could not be isolated.[28] For the preparation of the structurally related azo compounds **17a**–**17e**, CH-acidic 10*H*-anthracen-9-one **13a** was reacted with diazonium salts according to a literature procedure.[29] In good agreement with literature findings,[30] we confirmed by $^1$H NMR that these compounds exist in the hydrazone tautomeric form rather than their azo forms under the measurement conditions used.

### 2.2. In vitro cell growth inhibition assay

The compounds were preliminary screened for antiproliferative activity against the human erythroid leukemia cell line K562,[31] which has widely been used for the screening of potential antitumor compounds. Cell proliferation was determined by directly counting the cells with a hemocytometer after 48 h treatment. Table 1 summarizes the effects of phenylimino compounds **15a**–**15i** and **16a**–**16c** on the growth of K562 cells.

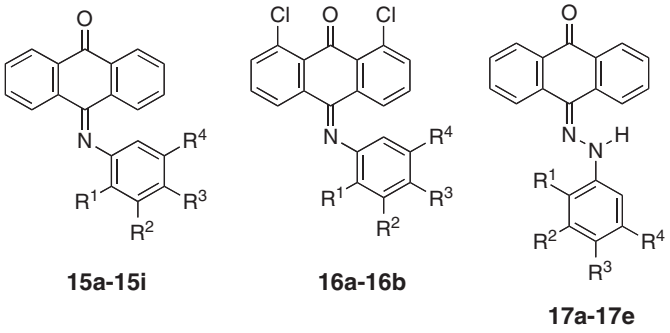
The observed antiproliferative activities depended on the substitution pattern of the terminal phenyl ring. Six compounds (**15b**, **15d**, **15f**, **15h**, **16a** and **16b**) showed activities in the submicromolar range, with $IC_{50}$ values ranging from 0.1 to 0.7 μM, but were in general slightly less active than the most active analogs of our recently described series.[14–16] Compound **15h**, bearing an isovanilline partial structure, displayed strong antiproliferative activity ($IC_{50}$ K562: 0.13 μM). It was the most potent inhibitor of tumor cell growth among the compounds tested, but was less potent than adriamycin ($IC_{50}$ K562: 0.01 μM) and colchicine ($IC_{50}$ K562 0.02 μM). Moreover, this compound was 5–6 fold less potent than the recently described close analog **8** (**15h**, 0.13 μM vs 0.02 μM for **8**). Similar activity was found for the 1,8-dichloro compound **16b** ($IC_{50}$ K562 0.11 μM), indicating that a carbonyl group being flanked by two chloro atoms is obviously tolerated. Again, the 3-hydroxy-4-methoxyphenyl appeared to be beneficial for inhibitory potency. Nevertheless, as an important finding, we documented a loss of potency when replacing the benzylidene C=C-bond by a C=N-bond. The unsubstituted compound **15a** revealed weak antiproliferative activity ($IC_{50}$ K562: 6.8 μM). Interestingly, dimethoxy-substituted derivatives **15c**–**15f** showed variable potencies, suggesting that steric factors account for the activity of these compounds. Compound **15c** was found to be a weak inhibitor (K562, $IC_{50}$ 9.3 μM) of tumor cell growth, whereas the constitutional isomer **15d** displayed a nearly 13-fold increase in antiproliferative activity (K562, $IC_{50}$ 0.73 μM) as compared to that of **15c**. Activity of **15f** was slightly less than that of **15d**. Analog **15e** (K562, $IC_{50}$ 3.40 μM) showed substantially reduced activity. Introduction of a 3,4,5-trimethoxyphenyl group in **15g**, a well defined pharmacophore for the inhibition of tubulin polymerization found in colchicine, combretastatin A-4 and podophyllotoxin, lead to a dramatic drop in cytotoxicity in comparison with **15b**. This is in agreement with our previous reports.[14–16] In addition, 10*H*-anthracen-9-one **13a** displayed only low activity (data not shown), indicating that the phenylimino structure is closely related to the antiproliferative activity.

To determine whether a structurally related azo group changes the SAR, a group of anthraquinone-derived phenylhydrazones with differently substituted phenyl rings were synthesized. The anticancer properties of some of them have recently been described.[32] The hydrazones depicted in Table 1 revealed a dramatic loss of cytotoxicity, documenting that the antiproliferative activity is obviously closely related to a C=C- or C=N-double bond and that a hydrazone group in this position is detrimental to potency (**15b** vs **17b**, **15f** vs **17d**). Interestingly, if the terminal aromatic ring was substituted with trimethoxy (**17e**), growth inhibition increased markedly as compared to that of **15g**.




**Scheme 1.** $R^1$–$R^4$ are defined in Table 1. Reagents and conditions: (a) $Br_2$, $CH_2Cl_2$, rt; (b) aromatic amine, reflux, 2–3 h; (c) aromatic amine, HCl 37%/$H_2O$/THF, $NaNO_2$, 0 °C.

**Table 1**
Antiproliferative activity of compounds **15a–15i**, **16a–16b** and **17a–17e** against K562 cells and anti–tubulin activities




| Compd | $R^1$ | $R^2$ | $R^3$ | $R^4$ | K562[a] $IC_{50}$ (μM) | ITP[b] $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|
| **15a** | H | H | H | H | 6.80 | 1.45 |
| **15b** | H | H | $OCH_3$ | H | 0.68 | 1.9 |
| **15c** | H | $OCH_3$ | $OCH_3$ | H | 9.3 | 3.6 |
| **15d** | H | $OCH_3$ | H | $OCH_3$ | 0.73 | 1.6 |
| **15e** | $OCH_3$ | H | $OCH_3$ | H | 3.4 | 1.6 |
| **15f** | $OCH_3$ | H | H | $OCH_3$ | 0.87 | ND |
| **15g** | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | >30 μM | >10 |
| **15h** | H | OH | $OCH_3$ | H | 0.13 | 0.44 |
| **15i** | H | $OCH_2O$ | | H | 2.67 | ND |
| **16a** | H | H | $OCH_3$ | H | 0.52 | ND |
| **16b** | H | OH | $OCH_3$ | H | 0.11 | 1.11 |
| **17a** | H | H | H | H | ND | >10 |
| **17b** | H | H | $OCH_3$ | H | 8.41 | >10 |
| **17c** | H | $OCH_3$ | H | H | 9 | >10 |
| **17d** | $OCH_3$ | H | H | $OCH_3$ | > 80 | >10 |
| **17e** | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | 2.74 | >10 |
| Colchicine | | | | | 0.02 | 1.4 |
| Nocodazole | | | | | ND | 0.76 |
| Podophyllotoxine | | | | | ND | 0.35 |
| Vinblastine sulphate | | | | | 0.001 | 0.13 |
| Adriamycine | | | | | 0.01 | ND |

[a] $IC_{50}$, concentration of drug required for 50% inhibition of cell growth (K562). Cells were treated with drugs for 48 h. $IC_{50}$ values are the means of at least three independent determinations (SD <10%).
[b] ITP = inhibition of tubulin polymerization; $IC_{50}$ values were determined after 30 min at 37 °C and represent the concentration for 50% inhibition of the maximum tubulin assembly rate.

## 2.3. Effect on growth of different solid tumor cell lines

To further evaluate the antiproliferative properties, the effect of the active compounds **15h** and **16b** against a panel of five tumor cell lines derived from solid tumors was measured by cellular metabolic activity using the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) assay.[33]

Both compounds were nearly as potent as nocodazole and showed strong activities with $IC_{50}$ values in the range of 0.1–0.2 μM toward several proliferating cell lines (Table 2). Moreover, the compounds were not active against RKO cells (human colon adenocarcinoma) with ectopic inducible expression of cyclin-dependent kinase inhibitor $p27^{kip1}$.[34] By contrast, growth of proliferating RKO cells was strongly inhibited by **15h** and **16b** with $IC_{50}$ values of 0.17 and 0.16 μM, respectively, indicating activity toward cycling cells. A major limitation of the treatment of many human cancers is the development of multiple drug resistance (MDR) in patients and a loss of efficacy over time, meaning that cancer cells do not respond to chemotherapy by developing a broad spectrum resistance to several anticancer drugs, among them antitubulin

**Table 2**
Cytotoxic activity of **15h** and **16b** against different tumor cell lines

| | $IC_{50}$[a] (μM) | | | | | |
|---|---|---|---|---|---|---|
| Compd | KB/HeLa (cervix) | SKOV3 (ovary) | SF268 (glioma) | NCI-H460 (lung) | $RKOp27^{kip1}$ (human colon adenocarcinoma) not induced *versus* induced | |
| **15h** | 0.22 | 0.16 | 0.34 | 0.21 | 0.17 | >9 |
| **16b** | 0.15 | 0.10 | 0.15 | 0.18 | 0.16 | >9 |
| Paclitaxel | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | >3 |
| Nocodazole | 0.14 | 0.17 | 0.30 | 0.15 | 0.11 | >10 |
| Colchicine | 0.03 | 0.05 | 0.05 | 0.07 | 0.02 | >10 |

[a] $IC_{50}$ values were determined from XTT proliferation assays after incubation with test compound for 48 h. All experiments were performed at least in two replicates ($n = 2$), and $IC_{50}$ data were calculated from dose–response curves by nonlinear regression analysis.

**Table 3**
Antiproliferative activity of **15h** and **16b**, paclitaxel, nocodazole and vindesine against tumor cell lines with different resistance phenotypes (XTT assay)

| | $IC_{50}$[a] (μM) | | | | | |
|---|---|---|---|---|---|---|
| | LT12 | LT12 MDR | L1210 | L1210 VCR-resistant | P388 | P388 ADR-resistant |
| **15h** | 0.18 | 0.14 | 0.29 | 0.22 | 0.23 | 0.23 |
| **16b** | 0.31 | 0.16 | 0.19 | 0.19 | 0.23 | 0.30 |
| Paclitaxel | 0.006 | 0.40 | 0.06 | >5 | 0.04 | >5 |
| Nocodazole | 0.04 | 0.07 | 0.06 | 0.07 | 0.07 | 0.05 |
| Vindesine | 0.001 | 0.26 | 0.02 | >5 | 0.01 | 1.10 |

[a] $IC_{50}$ values were determined from XTT proliferation assays after incubation with test compound for 48 h. All experiments were performed at least in two replicates ($n = 2$), and $IC_{50}$ data were calculated from dose–response curves by nonlinear regression analysis.

agents.[35,36] Multiple drug resistance is mediated, among other factors, by overexpression of transmembrane cellular pumps, such as the 170 kDa P-glycoprotein (Pgp),[37] encoded by the *mdr*1 gene and the 180 kDa MDR protein (MRP).[38] Important aspects concerning the key mechanisms of antimicrotubule drug resistance have recently been reviewed.[39] The antiproliferative activity of **15h** and **16b** against tumor cell lines with different resistance phenotypes was evaluated in an XTT based assay.

On the whole, as documented by the $IC_{50}$ data (Table 3), **15h** and **16b** were effective against the cell lines tested and retained activity in cell lines with various multiple drug resistance phenotypes. This feature was distinct from paclitaxel and vindesine because LT12MDR, L1210VCR and P388ADR cell lines were more resistant to these chemotherapeutics than the non-resistant cell lines. Therefore, the phenylimino-10*H*-anthracen-9-ones **15h** and **16b** are inhibitors of tumor cell proliferation and are poor substrates for transport by overexpression of Pgp170. However, the reference compound nocodazole was more potent than any other compound examined in this assay.

## 2.4. Effect on cell cycle progression

By targeting the mitotic spindle, microtubule inhibitors arrest the cell cycle during metaphase phase. As a consequence, mitosis is blocked at the transition from metaphase to anaphase. To gain further insight into the mode of action, the most active compounds **15h** and **16b** were assayed for their effects on cell cycle using an established KB/HeLa (human cervical epitheloid carcinoma) cell-based assay system. Sub-confluent KB/HeLa cells were exposed to test compounds and cell cycle dependent DNA content was determined by flow cytometry using propidium iodide in permeabilized cells. The percentage of cells in G2/M phase after 24 h was plotted against different concentrations of the compounds. The concentration for 50% cells arrested in G2/M phase was found to be 0.36 μM for **15h** (Table 4), thus being three-fold less active than **16b**.

As the antiproliferative activities against K562 cells were comparable, different susceptibilities are possibly due to the fact that both cell lines probably differ in a number of important issues, such as enzymatic equipment or the effects of regulatory proteins expressed within the cells. Compound **16b** ($EC_{50}$ 0.12 μM) showed activities similar to nocodazole ($EC_{50}$ 0.09 μM) and was slightly more active than the recently described **8** ($EC_{50}$ 0.2 μM).[14] In summary, the effect of compound **16b** and—to a lesser extent of **15h**—on cell cycle progression correlated with their strong antiproliferative and antitubulin activities (see below) and was similar to that observed for the majority of antimitotic agents.

**Table 4**
Flow cytometrical cell cycle analysis of KB/HeLa cells treated with **15h**, **16b** and reference compounds vincristine, colchicine, paclitaxel and nocodazole

| | **15h** | **16b** | Colchicine | Nocodazole | Paclitaxel | Vincristine |
|---|---|---|---|---|---|---|
| $EC_{50}$[a] (nM) | 359 | 121 | 14 | 91 | 49 | 2.4 |

[a] $EC_{50}$ values were determined from dose–response cell cycle analysis experiments and represent the concentration for 50% cells arrested in G2/M phase after 24 h. All experiments were performed at least in two replicates ($n = 2$), and $IC_{50}$ data were calculated from dose–response curves by nonlinear regression analysis (GraphPad Prism™).

## 2.5. In vitro tubulin polymerization assays

To investigate whether the antiproliferative activities of these compounds were related to the interaction with the microtubule system, we selected fourteen compounds to measure their antitubulin activities and compared their activity with that of the reference antitubulin drugs colchicine, podophyllotoxin, nocodazole, and vinblastine (Table 1).

In the presence of GTP and $Mg^{2+}$, αβ-tubulin is known to self assemble (polymerize) in vitro into microtubules at physiological temperature (37 °C). This reaction is accompanied by an increase of turbidity within the protein solution, enabling to record microtubule polymerization by time-dependent measurements in a spectrophotometer. The turbidity curves, usually obtained at 340–360 nm, reveal a sigmoid behavior with a plateau level reached after 30 min at the conditions realized in this study (Fig. 1). Inhibition of tubulin polymerization is reflected by a decreased level of steady state turbidity as exemplified for **15h** (Fig. 1).

With this assay, it is possible to detect and to characterize drugs acting on tubulin polymerization and causing microtubule depolymerization. Therefore, this assay provides a convenient in vitro method to pre-investigate the effect of a drug on tubulin polymerization/depolymerization. The results obtained with the test agents are summarized in Table 1. For comparison, the data of the potent antimitotic compounds colchicine, podophyllotoxin, nocodazole and vinblastine are also presented. In general, inhibition of tubulin polymerization correlated well with drug-caused growth inhibition. For instance, compounds **15a**, **15b**, **15d** and **15e**, were found to be strong inhibitors of tubulin polymerization, with activities being in the range of colchicine (1.4 μM). The hydroxy–methoxy substituted compound **15h** ($IC_{50}$ 0.44 μM) has been proved an excellent inhibitor of tubulin polymerization. This is in agreement with **15h** ranking among the most antiproliferative active compounds together with **16b**. As a tubulin polymerization inhibitor, the activity of **15h** was comparable or superior to the reference compounds, with the exception of vinblastine sulfate. Also, the 1,8-dichloro analog **16b** proved to be strongly active ($IC_{50}$ 1.11 μM) and exhibited virtually the same activity as colchicine. This again indicates that the 3-hydroxy-4-methoxy substitution pattern plays an essential role to exhibit strong antitubulin and antiproliferative activities within the phenyliminoanthracenone series of compounds. The 3-hydroxy-4-methoxyphenyl fragment is a much more active moiety than 4-methoxy (**15b**) or 3,4,5-trimethoxy (**15g**) in this series. This is consistent with the results from the previously described 10-benzylidene-10*H*-anthracen-9-

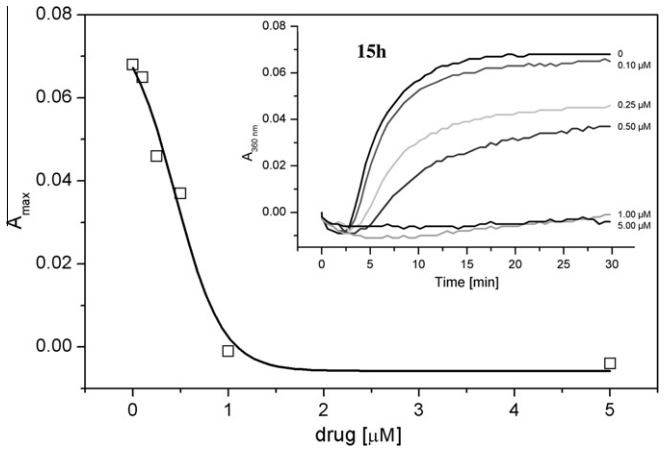



**Fig. 1.** Inhibition of in vitro polymerization of tubulin (in total 1.2 mg/ml protein; ~85% tubulin plus ~15% microtubule-associated proteins) at 37 °C by various concentrations of **15h**; turbidity was recorded at 360 nm. The steady state tubulin assembly level (see insert) in the absence of inhibitor was set 100%. $IC_{50}$ values were determined by sigmoidal fitting the plot of the steady state levels of tubulin assembly (taken after 30 min) against drug concentration and represent the concentration for 50% inhibition of the maximum tubulin polymerization level.

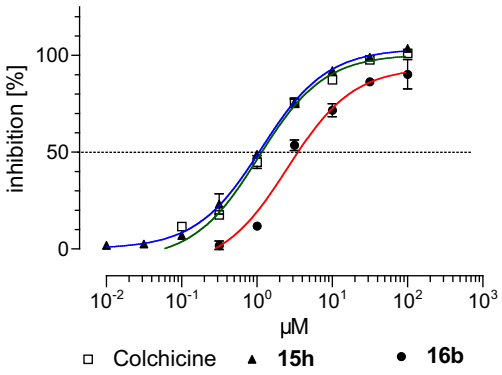



**Fig. 2.** [$^3$H] colchicine competition binding assay of **15h**, **16b** and colchicine. Radiolabeled colchicine, unlabeled compound and biotin-labeled tubulin were incubated together for 2 h at 37 °C.

ones,[14] where we identified the isovanilline-derived **9** (Chart 2) as the most active representative. Also, anthracenedione phenylhydrazones **17a**–**17e** did not show any appreciable antitubulin activity. Based on the diffraction map[40] of a close structural analog of colchicine bound to αβ-tubulin, computer simulation models have recently been employed to explain empirical structure–activity relationships of colchicine site inhibitors (CSIs) of tubulin polymerization.[41,42] Although **9** has no structural resemblance to colchicine, it docked preferentially at the same site.[43,44] The most active analog **15h** contains some potential pharmacophore fragments such as the oxygen atom of the anthracenone carbonyl group as well as the oxygen atom and the methyl group of the methoxy substituent. According to the described pharmacophore model[41] and consistent with our experimental data, a phenolic hydroxy group being flanked by one or two methoxy groups (**8**, **9**, Chart 2) is an important determinant for activity within the anthracenone derived CSIs.[14,15] Both the methoxy group and the terminal aryl ring are essential features for activity.[41,42] It has recently been hypothesized that the terminal isovanillinyl ring in **8** possibly binds to the same region of the protein as the pseudoaromatic tropone ring C in colchicine.[42] Interestingly, as documented by our earlier work, the terminal isovanillinyl partial structure could not be successfully replaced by trimethoxy—a prominent pharmacophore present in many CSIs—with retention of high activity. A similar observation has been made with trimethoxy analog **15g**, displaying a dramatic drop in potency in comparison with **15h** for both inhibition of tubulin polymerization and antiproliferative activity. This finding indicates that the significantly larger hydrophobic trimethoxy moiety in **15g** relative to the methoxy in **15h** is not well tolerated in the terminal aryl ring in the anthracenone based CSIs. The anthracenone carbonyl oxygen probably has importance as a hydrogen bond acceptor. The novel compounds **15a**–**15i** and **16a**–**16c** embody a phenylimino group as linker between two aryl groups, which might provide additional hydrogen bonding features. However, while **15h** effectively binds to tubulin in vitro, it is slightly less efficient in inhibiting K562 cell growth as compared with the recently described inhibitors **8** and **11** (Chart 2). Drugs affecting tubulin are classified by the region of the protein to which they bind. The taxol site, the colchicine site, and the Vinca alkaloid domain are established binding regions. Therefore, we examined **15h** and **16b** for an inhibitory effect on the binding of [$^3$H]colchicine to tubulin using a competitive scintillation proximity assay.[45] It was observed that **15h** and **16b** competitively inhibited [$^3$H]colchicine binding to biotinylated tubulin (Fig. 2) with $IC_{50}$ values of 1.1 (**15h**) and 2.6 μM (**16b**) versus colchicine (1.06 μM). Consistent with these findings, **16b** was nearly two times less potent than **15h** as an inhibitor of tubulin polymerization (Table 1). Notably, while potencies for inhibition of tubulin polymerization differ only slightly, we found **15h** to be approximately three-fold less active than **8** in the [$^3$H]colchicine binding assay.

Moreover, the compounds did not compete with [$^3$H]paclitaxel (data not shown) and no stabilization of the colchicine binding was observed, as it is documented for Vinca site binders.[46,47] Thus, we conclude that antimitotic activity of these compounds is preferentially due to interaction with the colchicine binding site.

## 3. Conclusion

The present study describes new synthetic inhibitors of tubulin polymerization, based on a phenylimino-10*H*-anthracen-9-one molecular skeleton. Similar to many other inhibitors of tubulin polymerization, selected compounds were efficacious in inhibiting tumor cell proliferation with $IC_{50}$ values at the submicromolar level. The best results for inhibition of tumor cell growth were obtained with the 3-hydroxy-4-methoxyphenyl analogs **15h** and **16b**. In accordance with our previous findings, the substitution pattern in the terminal phenyl ring and the nature of the linker group are critical for strong inhibition of tumor cell proliferation and inhibition of tubulin polymerization. As those analogs with the greatest inhibitory effects on cell growth strongly inhibited tubulin assembly, we conclude that tubulin is the molecular target of the compounds. Compounds **15h** and **16b** are good to excellent inhibitors of tubulin polymerization and interact most likely with tubulin at the colchicine site. Compound **15h** strongly displaced radiolabeled colchicine from its binding site in the tubulin, showing $IC_{50}$ values similar to that of colchicine.

As typically observed for agents that inhibit tubulin polymerization, **15h** and **16b** also induced G2/-M arrest. Notably, no growth inhibitory effect was found in cell cycle-arrested cells. Whereas the effectiveness of numerous clinically useful drugs is limited by the fact that they are substrates for the efflux pumps Pgp170 and MRP, both compounds were active toward parental tumor cell lines and multidrug resistant cell lines. This feature was distinct from those of paclitaxel and vindesine. Due to their attractive in vitro antitumor activities, we believe that compounds of this structural class are attractive for further structural modifications and that our findings may contribute to the design of novel antitumor agents. Investigations on the role of the phenylimino-10*H*-anthracen-9-ones for antimitotic activity are in progress and results from related modifications will be reported in due course.

## 4. Experimental protocols

### 4.1. Chemistry and chemical methods

Melting points were determined with a Kofler melting point apparatus and are uncorrected. Spectra were obtained as follows: $^1$H NMR spectra were recorded with a Varian Mercury 400 plus (400 MHz) or a Varian Gemini 200 (200 MHz) spectrometer, using tetramethylsilane as an internal standard. Fourier-transform IR spectra were recorded on a Bio-Rad laboratories Typ FTS 135 spectrometer and analysis was performed with WIN-IR Foundation software. Compound purity was determined by combustion analysis performed at the Münster microanalysis laboratory, using a Vario EL III CHNOS elemental analyzer (Elementar Analysensysteme GmbH), and all tested compounds showed values within ±0.4% of the calculated composition. Compound purity is ⩾95%. Mass spectra recorded in the EI mode were performed by a MAT GCQ Finnigan instrument. Atmospheric pressure chemical ionization (APCI) method was performed with a microTOF-QII apparatus (Bruker). All organic solvents were appropriately dried or purified prior to use. Aromatic amines as well as 10*H*-anthracen-9-one (anthrone) were obtained from commercial sources. Analytical TLC was done on Merck silica 60 $F_{254}$ alumina coated plates (E. Merck, Darmstadt). Chromatography refers to column chromatography using Acros 60–200 mesh silica gel. In most cases, the concentrated pure fractions obtained by chromatography using the indicated eluents were treated with a small amount of hexane to induce precipitation. All new compounds displayed $^1$H NMR, FTIR, and MS spectra consistent with the assigned structure. Yields have not been optimized. Chromatography solvent (vol %): EE = ethyl acetate; H = hexane; M = methanol; MC = methylene chloride. Elemental analyzes were within ±0.4% of calculated values, except where stated otherwise.

### 4.2. 10-Phenylimino-10*H*-anthracen-9-ones

#### 4.2.1. 10-Bromo-10*H*-anthracen-9-one (14a)

Based on literature procedures,[26,27] to a mechanically stirred suspension of 10*H*-anthracen-9-one **13a** (10 g, 51.5 mmol) in chloroform (80 mL) $Br_2$ (51 mmol, 8.15 g, 2.6 mL) was added dropwise. The suspension was stirred at room temperature for 30 min and great amounts of HBr evolved. Then, the solution was concentrated. The light yellow precipitate was collected, washed with hexane (100 mL) and dried in vacuum at room temperature (6.10 g, 44%): mp 148–149 °C, Lit.[26] 148 °C.

#### 4.2.2. 10-Bromo-1,8-dichloro-10*H*-anthracen-9-one (14b)

The title compound was prepared from **13b** according to a literature protocol.[48]

#### 4.2.3. 10-Phenylimino-10*H*-anthracen-9-one (15a)[28]

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and aniline (0.68 g, 7.32 mmol) in a similar manner as described for the preparation of **15e**. Purification by chromatography (MC) afforded **15a** as a red powder (0.24 g, 19%): mp 117–119 °C; FTIR 1654 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 8.33–7.38 (m, 8H), 6.26–6.01 (m, 3H), 3.76 (s, 6H); MS *m*/*z* 284 (17), 283 (82), 282 (100), 328 (28), 312 (23); Anal. ($C_{20}H_{13}NO$) Calcd: C, 84.78; H, 4.62; N, 4.94. Found: C, 84.71, H, 4.59; N, 4.85.

#### 4.2.4. 10-(4-Methoxyphenylimino)-10*H*-anthracen-9-one (15b)[49]

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and 4-methoxyaniline (0.90 g, 7.32 mmol) in a similar manner as described for the preparation of **15e**. Purification by chromatography (MC/MeOH 9.8:0.2) afforded **15b** as an orange-red powder (0.14 g, 12% yield): mp 140–141 °C; FTIR 1665 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 8.47 (d, 1H, *J* = 7.6 Hz), 8.33–8.25 (m, 2H), 7.79–7.60 (m, 2H), 7.52 (t, 1H, *J* = 6.7 Hz), 7.32–7.27 (m, 2H), 6.95 (d, 2H, *J* = 6.7 Hz), 6.85 (d, 2H, *J* = 6.7 Hz), 3.84 (s, 3H); MS *m*/*z* 315 (6), 314 (20), 313 (92), 298 (100); Anal. ($C_{21}H_{15}NO_2$), Calcd: C, 80.49; H, 4.82; N, 4.47. Found: C, 80.29; H, 4.62; N, 4.27.

#### 4.2.5. 10-(3,4-Dimethoxyphenylimino)-10*H*-anthracen-9-one (15c)

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and 1,2-dimethoxybenzene (1.12 g, 7.32 mmol) in a similar manner as described for the preparation of **15e**.

Purification by chromatography (MC) afforded **15c** as an orange-red powder (0.25 g, 20% yield): mp 158–159 °C; FTIR 1666 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 8.47 (d, 1H, *J* = 7.3 Hz), 8.33–8.26 (m, 2H), 7.77–7.66 (m, 2H), 7.62–7.53 (m, 1H), 7.40–7.30 (m, 2H), 6.86 (d, 1H, *J* = 8.4 Hz), 6.48 (d, 1H, *J* = 2.4 Hz), 6.37 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz), 3.91 (s, 3H), 3.81 (s, 3H); MS *m*/*z* 345 (3), 344 (23), 343 (95), 328 (100); Anal. ($C_{22}H_{17}NO_3$) Calcd: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.61; H, 4.62; N, 4.08.

#### 4.2.6. 10-(3,5-Dimethoxyphenylimino)-10*H*-anthracen-9-one (15d)

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and 2,4-dimethoxyaniline (1.12 g, 7.32 mmol) in a similar manner as described for the preparation of **15e**. Purification by chromatography (MC) afforded **15d** as fine, red needles (90 mg, 7% yield): mp 182–184 °C; FTIR 1656 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 8.33–7.38 (m, 8H), 6.26–6.01 (m, 3H), 3.76 (s, 6H); MS *m*/*z* 343 (100, $M^+$), 328 (28), 312 (23); Anal. ($C_{22}H_{17}NO_3$) Calcd: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.72; H, 4.69; N, 3.97.

#### 4.2.7. General procedure for the preparation of compounds 15a–15i and 16a–16c. 10-(2,4-Dimethoxyphenylimino)-10*H*-anthracen-9-one (15e)

Bromoanthrone **14a** (3.66 mmol) and 2,4-dimethoxyaniline (1.12 g, 7.32 mmol) were suspended in absolute benzene (50 mL) and heated under reflux in the presence of air (2–3 h, TLC control). The solvent was thereafter evaporated under reduced pressure, and then the residue was purified by chromatography (MC) to afford **15e** as a dark-red powder (0.40 g, 32% yield): mp 142–144 °C; FTIR; 1665 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.51–8.49 (m, 1H), 8.30–8.24 (m, 2H), 7.73–7.70 (m, 1H), 7.65–7.61 (m, 1H), 7.53–7.49 (m, 1H), 7.46–7.44 (m, 1H), 7.32–7.28 (m, 1H), 6.77–6.74 (m, 1H), 6.53–6.50 (m, 2H), 3.84 (s, 3H), 3.58 (s, 3H); MS *m*/*z* 345 (4), 344 (27), 343 (100), 342 (23), 328 (44); Anal. ($C_{22}H_{17}NO_3$), Calcd: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.82; H, 4.89; N, 3.80.

#### 4.2.8. 10-(2,5-Dimethoxyphenylimino)-10*H*-anthracen-9-one (15f)

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and 2,5-dimethoxyaniline (1.12 g, 7.32 mmol) in a similar manner as described for the preparation of **15e**. Purification by chromatography (MC) afforded **15f** as a red powder (0.15 g, 12% yield): mp 134 °C; FTIR 1675 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.51–7.31 (m, 8H), 6.77–6.50 (m, 3H), 3.84 (s, 3H), 3.58 (s, 3H); MS *m*/*z* 343 (100%, $M^+$); $C_{22}H_{17}NO_3$, Calcd: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.83; H, 4.59; N, 3.78.

#### 4.2.9. 10-(3,4,5-Trimethoxyphenylimino)-10*H*-anthracen-9-one (15g)

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and 3,4,5-trimethoxyaniline (1.34 g, 7.32 mmol) in a similar

manner as described for the preparation of **15e**. Purification by chromatography (EE/PE 8:2) afforded **15g** as a red powder (0.08 g, 6% yield): mp 210–212 °C; FTIR 1667 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.31–7.26 (m, 8H), 6.08 (s, 2H), 3.88 (s, 3H), 3.77 (s, 6H); MS *m*/*z* 373 (49), 360 (4), 359 (25), 358 (100), 316 (2), 315 (20), 314 (81), 286 (47) ; Anal. ($C_{23}H_{19}NO_4$) C, H, N.

#### 4.2.10. 10-(3-Hydroxy-4-methoxy-phenylimino)-10*H*-anthracen-9-one (15h)

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and 5-amino-2-methoxyphenol (1.02 g, 7.32 mmol) as described for the preparation of **15e**. Purification by chromatography (MC/MeOH 9.8:0.2) afforded **15h** as orange-red crystals (0.21 g, 18% yield): mp 171–172 °C; FTIR 3381, 1673 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.45 (d, 1H, *J* = 7.82 Hz), 8.31–8.25 (m, 2H), 7.74–7.70 (m, 1H), 7.66–7.63 (m, 1H), 7.54–7.50 (m, 1H), 7.41–7.39 (m, 1H), 7.32–7.29 (m, 1H), 6.83 (d, 1H, *J* = 8.4 Hz), 6.55 (d, 1H, *J* = 2.4 Hz), 6.27 (dd, 1H, *J* = 8.5 Hz, *J* = 2.5 Hz), 5.69 (s, 1H), 3.92 (s, 3H); MS *m*/*z* 331 (3), 330 (22), 329 (100), 316 (2), 315 (20), 314 (81), 286 (47); Anal. ($C_{21}H_{15}NO_3$), Calcd: C, 76.58; H, 4.59; N, 4.25. Found: C, 76.19; H, 4.19; N, 4.08.

#### 4.2.11. 10-(3,4-Methylenedioxyphenylimino)-10*H*-anthracen-9-one (15i)

The title compound was prepared from **14a** (2.14 g, 3.66 mmol) and 3,4-(methylenedioxy)aniline (1.00 g, 7.32 mmol) in a similar manner as described for the preparation of **15e**. Purification by chromatography (MC) afforded **15i** as a orange-red crystals (0.32 g, 27 % yield): mp 148–150 °C; FTIR 1668 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.44–8.42 (m, 1H), 8.32–8.25 (m, 2H), 7.72–7.70 (m, 1H), 7.66–7.63 (m, 1H), 7.56–7.52 (m, 1H), 7.43–7.41 (m, 1H), 7.36–7.34 (m, 1H), 6.80 (d, 1H, *J* = 8.2 Hz), 6.43 (d, 1H, *J* = 1.96 Hz), 6.27 (dd, 1H, *J* = 8.02 Hz, *J* = 1.96 Hz), 6.07 (s, 2H); MS (*m*/*z*), 342 (23), 328 (44); Anal. ($C_{21}H_{13}NO_3$) Calcd: C, 77.05; H, 4.00; N, 4.28. Found: C, 76.80; H, 3.71; N, 4.06.

#### 4.2.12. 1,8-Dichloro-[10-(4-methoxy-phenylimino)]-10*H*-anthracen-9-one (16a)

The title compound was prepared from **14b** (1.02 g, 3.00 mmol) and 4-methoxyaniline (0.74 g, 6 mmol) in a similar manner as described for the preparation of **15e**. Purification by chromatography (EE/PE 1:1) afforded **16a** as orange-red crystals (0.35 g, 30% yield): mp 229 °C; FTIR 1685 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.22 dd, 1H, *J* = 8.2 Hz, *J* = 1.2 Hz), 7.61–7.59 (m, 1H), 7.56–7.52 (m, 1H), 7.49–7.46 (m, 1H), 7.16–7.12 (m, 1H), 7.09–7.06 (m, 1H), 6.86 (d, 2H, *J* = 9.0 Hz), 6.81 (d, 2H, *J* = 9.0 Hz), 3.82 (s, 3H); MS *m*/*z* 385 (11), 381 (100), 366 (82); Anal. ($C_{21}H_{13}Cl_2NO_2$) Calcd: C, 65.99; H, 3.43; N, 3.66. Found: C, 65.83; H, 3.10; N, 3.52.

#### 4.2.13. 1,8-Dichloro-[10-(3-hydroxy-4-methoxy-phenylimino)]-10*H*-anthracen-9-one (16b)

The title compound was prepared from **14b** (1.03 g, 3 mmol) and 5-amino-2-methoxyphenol (1.60 g, 6 mmol) in a similar manner as described for the preparation of **15e**. Purification by chromatography (MC) afforded **16b** as fine-red crystals (0.22 g, 18% yield): mp 247 °C; FTIR 3446, 1678 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.20 (dd, 1H, $J_o$ = 7.62 Hz, $J_m$ = 1.17 Hz), 7.61–7.59 (m, 1H), 7.56–7.54 (m, 1H), 7.49–7.46 (m, 1H), 7.15–7.14 (m, 2H), 6.76 (d, 1H, *J* = 8.40 Hz), 6.57 (d, 1H, *J* = 2.5 Hz), 6.25 (dd, 1H, *J* = 8.5 Hz, *J* = 2.5 Hz), 5.66 (s, 1H), 3.89 (s, 3H); MS *m*/*z* 399 (65), 398 (22), 397 (100); Anal. ($C_{21}H_{13}Cl_2NO_3$), Calcd: C, 63.34; H, 3.29; N, 3.52. Found: C, 63.23; H, 3.33; N, 3.32.

#### 4.2.14. 9-[(2-Phenyl)hydrazone]-9,10-anthracenedione (17a)

The title compound was prepared from **13a** (3.88 g, 20 mmol) and aniline (1.86 g, 20 mmol) in a similar manner as described for the preparation of **17d**. Purification by silica gel chromatography (EE/PE 1:1) afforded **17a** as a red powder (1.20 g, 20% yield): mp 173 °C; FTIR 1629 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 9.17 (s, 1H), 8.47–8.45 (m, 1H), 8.39 (d, 1H, *J* = 8.22 Hz), 8.30 (d, 1H, *J* = 7.83 Hz), 8.23 (dd, 1H, *J* = 7.8 Hz, *J* = 0.8 Hz), 7.80–7.76 (m, 1H), 7.70–7.61 (m, 2H), 7.50–7.46 (m, 1H), 7.38–7.32 (m, 4H), 7.04–6.80 (m, 1H); MS *m*/*z* 298 (100), 297 (88), 270 (13), 269 (14); Anal. ($C_{20}H_{14}N_2O$) C, H, N, Calcd: C, 80.52; H, 4.73; N, 9.39. Found: C, 80.14; H, 4.60; N, 9.15.

#### 4.2.15. 9-[2-(4-Methoxyphenyl)hydrazone]-9,10-anthracenedione (17b)[50]

The title compound was prepared from **13a** (3.88 g, 20 mmol) and 4-methoxyaniline (2.46 g, 20.0 mmol) in a similar manner as described for the preparation of **17d**. Purification by silica gel chromatography (EE/PE 1:1) afforded **17b** as a dark-red powder (4.46 g, 68% yield): mp 184–185 °C; FTIR 1651 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 9.12 (s, 1H), 8.47 (dd, 1H, $J_o$ = 7.82 Hz, *J* = 0.8 Hz), 8.38 (d, 1H, *J* = 7.8 Hz), 8.32–8.9 (m, 1H), 8.24–8.22 (m, 1H), 7.81–7.77 (m, 1H), 7.68–7.65 (m, 1H), 7.61–7.57 (m, 1H), 7.49–7.45 (m, 1H), 7.28 (d, 2H, *J* = 9.0 Hz), 6.93 (d, 2H, *J* = 9.0 Hz), 3.81 (s, 3H); MS (APCI) calcd for $C_{21}H_{16}N_2O_2$ $[M+H]^+$ 329.13; found 329.1310; Anal. ($C_{21}H_{16}N_2O_2$) Calcd: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.53; H, 4.79; N, 8.38.

#### 4.2.16. 9-[2-(3-Methoxyphenyl)hydrazone]-9,10-anthracenedione (17c)

The title compound was prepared from **13a** (3.88 g, 20.00 mmol) and 3-methoxyaniline (2.46 g, 20 mmol) in a similar manner as described for the preparation of **17d**. Purification by chromatography (MC) afforded **17c** as a fine orange powder (1.83 g, 28% yield): mp 128–131 °C; FTIR 1654 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 9.18 (s, 1H), 8.42–8.39 (m, 2H), 8.37–8.35 (m, 1H), 8.28–8.25 (m, 1H), 8.22–8.19 (m, 1H), 7.74–7.71 (m, 1H), 7.69–7.63 (m, 1), 7.58–7.47 (m, 1H), 7.28–7.23 (m, 1H), 7.00–6.99 (m, 1H), 6.87–6.84 (m, 1H), 6.59–6.55 (m, 1H), 3.87 (s, 3H); MS (APCI) calcd for $C_{21}H_{16}N_2O_2$ $[M+H]^+$ 329.13; found 329.1313; Anal. ($C_{21}H_{16}N_2O_2$) Calcd: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.78; H, 5.00; N, 8.25.

#### 4.2.17. Preparation of 9-[(2,5-Dimethoxyphenyl)hydrazone)]-9,10-anthracenedione (17d)

A solution of sodium nitrite (1.4 g, 20 mmol, in 6 mL $H_2O$) was added to a solution of 2,5-dimethoxyaniline (3.06 g, 20 mmol) in HCl 37% (6.7 mL)/$H_2O$ (21 mL)/THF (5 mL) at 0 °C. Then, this solution was added dropwise to an ethanolic (60 mL) suspension of **13a** (3.88 g, 20 mmol) and NaOH (150 mmol, 6.0 g, in 16 mL $H_2O$). The mixture was stirred until the reaction was complete and then poured into ice water (500 mL). The product precipitated and was collected by filtration and subsequently dried (Dean stark). Removal of toluene and purification of the residue by column chromatography gave the product. Purification by silica gel chromatography (EE/PE 1:1) afforded **17d** as dark-red needles (4.37 g, 61% yield): mp 183–184 °C; FTIR 1644, 1521 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 9.66 (s, 1H), 8.50–8.48 (m, 1H), 8.41–8.33 (m, 2H), 8.25–8.22 (m, 1H), 7.82–7.77 (m, 1H), 7.67–7.60 (m, 2H), 7.51–7.45 (m, 1H), 7.36 (d, 1H, *J* = 3.0 Hz), 6.79 (d, 1H, *J* = 8.8 Hz), 6.46 (dd, 1H, *J* = 8.8 Hz, *J* = 3 Hz), 3.85 (s, 6H); MS *m*/*z* 357.98 (100); Anal. ($C_{22}H_{18}N_2O_3$) Calcd: C, 73.73; H, 5.06; N, 7.82. Found: C, 73.92; H, 5.00; N, 7.77.

#### 4.2.18. 9-[2-(3,4,5-Trimethoxyphenyl)hydrazone]-9,10-anthracenedione (17e)

The title compound was prepared from **13a** (3.88 g, 20 mmol) and 3,4,5-trimethoxyaniline (3.66 g, 20 mmol) in a similar manner as described for the preparation of **17d**. Purification by silica gel

chromatography (EE/PE 1:1) afforded **17e** as fine orange crystals (5.36 g, 69 % yield): mp 200–201 °C; FTIR 1649, 1594, 1500 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 9.17 (s, 1H), 8.38–8.35 (m, 1H), 8.32–8.26 (m, 2H), 8.21–8.18 (m, 1H), 7.75–7.64 (m, 2H), 7.55–7.44 (m, 2H), 6.61 (s, 2H), 3.93 (s, 6H), 3.83 (s, 3H); MS *m*/*z* 385 (11), 381 (100), 366 (82); Anal. ($C_{23}H_{20}N_2O_4$) Calcd: C, 71.12; H, 5.19; N, 7.21. Found: C, 71.05; H, 4.87; N, 7.06.

### 4.3. Biological assay methods

These were described previously in full detail.[15]

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## References and notes

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