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Antiproliferative activity of synthetic naphthoquinones related to lapachol. First synthesis of 5-hydroxylapachol

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ABSTRACT

A series of 5-hydroxy-1,4-naphthoquinones analogues was synthesized from juglone (**6**) and their antiproliferative activity against a representative panel of six human solid tumor cell lines has been investigated. The 2,5-dihydroxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione (**4**) and 2,3-dihydro-5-hydroxy-2-(prop-1-en-2-yl)naphtho[2,3-*b*]furan-4,9-dione (**27**) were the most potent antiproliferative agents with GI_{50} values of 0.42–8.1 and 0.80–2.2 μM, respectively. The results provide insight into the correlation between some structural properties of 5-hydroxynaphthoquinones and their antiproliferative activity.

1. Introduction

Quinones are widely distributed in nature and have a variety of roles in organisms. In addition to functional constituents of various biochemical systems such as ubiquinone and vitamin K1, they also may be found in dyes, or acting as defensive compounds. Therefore, quinone derivatives may be toxic to cells by a number of mechanisms including redox cycling,[1] arylation, intercalation, induction of DNA strand breaks, generation of free radicals and alkylation via quinone methide formation.[2] Several clinically important anticancer drugs such as daunorubicin (**1**) and mitomycin C (**2**) contain the quinone moiety as a relevant part of their structures (Fig. 1) and there is an increasing number of reports concerning the biological evaluation of synthetic analogues and new natural products related to this class of compounds.[3] Within the active quinones, lapachol (**3**) a natural naphthoquinone, and many heterocyclic derivatives were investigated during the past years, mainly due to their antibacterial,[4–6] antifungal,[7] trypanocidal[8] and anticancer activities.[9] More recently, 5-hydroxylapachol (**4**) was isolated from the root heart wood of *Tectona grandis* and, like lapachol (**3**), was found to be cytotoxic to *Artemia salina* (brine shrimp) with an LC_{50} of 5 ppm.[10] β-Lapachone (**5**), a cyclization product of lapachol (**3**), has been intensely investigated for clinical use in cancer chemotherapy.[11–13] Although the cytotoxic action of β-lapachone has been known for more than 20 years,[14] the detailed mechanism of action remains largely unknown.

It is well-known that the presence of mono- or dihydroxy groups at C-5 or C-5 and C-8 positions of the naphthoquinone moiety, respectively, induces a higher toxic effect to cells due to an increased efficiency of redox cycling.[15] In this context, we have directed our efforts to obtain novel synthetic derivatives containing a hydroxyl group at the C-5 position. Herein, we report the first synthesis of 5-hydroxylapachol (**4**) and the preparation of new derivatives of (**3**) and (**5**) with diverse substitution patterns at the C-2 and C-3 positions of the naphthoquinone scaffold. These compounds were prepared to establish structure–activity relationships when considering their antiproliferative activities against a representative panel of human solid tumor cell lines comprising A2780 (ovarian), SW1573 (non-small cell lung), WiDr (colon), T-47D (breast), HBL-100 (breast), and HeLa (cervix).

2. Results and discussion

2.1. Chemistry

The target naphthoquinones were synthesized from juglone (**6**) and lapachol (**3**). As shown in Scheme 1, the addition of an aqueous solution of dimethylamine to juglone (**6**) gave a 2.4:1 mixture of

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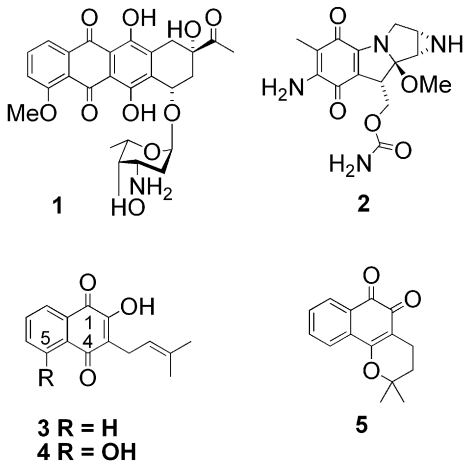
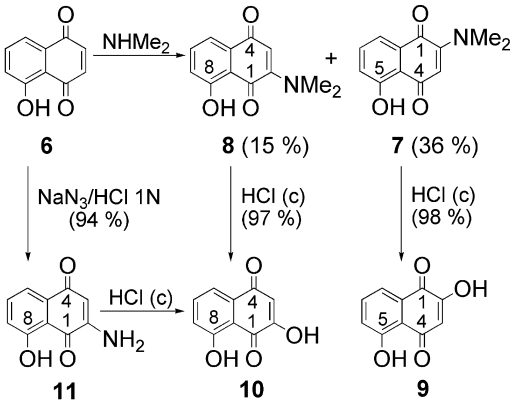


Figure 1. Structure of naturally occurring quinones.



Scheme 1. Preparation of hydroxyjuglones **9** and **10**.

2-dimethylaminojuglone (**7**) and 3-dimethylaminojuglone (**8**) in 51% yield.[16] Subsequent deamination of **7** and **8** with concentrated HCl afforded quantitatively 2-hydroxyjuglone (**9**) and 3-hydroxyjuglone (**10**), respectively. Due to the poor yield of **8** we carried out an alternative approach through the intermediate 3-aminojuglone (**11**) obtained in 94% yield from juglone (**6**) and sodium azide in 1 N HCl solution.[17] The acidic hydrolysis of **11** led to compound **10** in 91% overall yield.

The prenylation of 2-hydroxyjuglone (**9**) with 1-bromo-3-methylbut-2-ene was attempted under different reaction conditions (Scheme 2, Table 1). Treatment of **9** with allyl bromide in the presence of sodium iodide and triethylamine in DMSO (method A) gave the mixture of naphthoquinones **4** and **12–14** in low yield.[18] Interestingly, when DMF was used as the reaction solvent the C-alkylated compound **4** was the major product (57%), and no cyclization products were obtained. Changing the base to potassium or lithium carbonate resulted in low yields of **4** and formation of significant amounts of, *o*-alkylation (**12**), Claisen rearrangement (**15**), and dialkylation (**16**) products. To the best of our knowledge, this is the first reported procedure for the synthesis of the natural product 5-hydroxylapachol (**4**). The formation of the iodinated product **13** may be explained as a result of the electrophilic addition of iodine (arising from the oxidation of iodide by DMSO)[19] to the side chain double bond of **15**, followed by cyclization to give the dihydrofuran ring.[20,21] Similarly, compound **14** could originate from **4**.

Scheme 3 shows the prenylation of 3-hydroxyjuglone (**10**) with 1-bromo-3-methylbut-2-ene. As in the previous case, the reaction of **10** with 1-bromo-3-methylbut-2-ene in the presence of sodium iodide and triethylamine in DMF gave compounds **17**, **18**, and **21** while the reaction in DMSO gave a mixture of compounds **17–20**. When the prenylation was carried out in DMF with potassium carbonate **17**, **18**, **21**, and **22** were obtained.

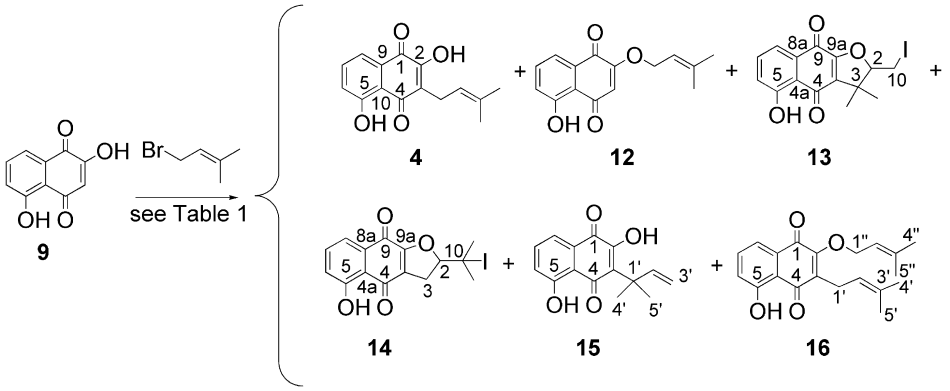
With 5-hydroxylapachol (**4**) in hand, we prepared several derivatives with the purpose of performing SAR studies. The 5-deoxy analogues derived from lapachol (**3**) were also prepared for comparison purposes. Thus, treatment of compound **4** with dimethyl sulfate led to compounds **23** and **24** in 8% and 47% yields, respectively (Scheme 4). In addition, we prepared the set of tricyclic derivatives **25–30** shown in Scheme 5. Treatment of **3** with 3 equiv of CAN in dry acetonitrile gave dihydrofurans **25** (30%) and **26** (51%) via intramolecular cyclization.[22] When the same procedure was applied to **4** the resulting quinones **27** and **28**, were obtained in 7% and 12%, respectively. Treatment of **3** with dilute H_2SO_4 led to α-lapachone (**29**) and β-lapachone (**5**), respectively.[23] The reaction of 5-hydroxylapachol (**4**) with concentrated H_2SO_4 gave only compound **30** in 97% yield.

The iodinated quinones **13**, **14** and **20** were obtained in low yield but preliminary testing indicated an interesting biological

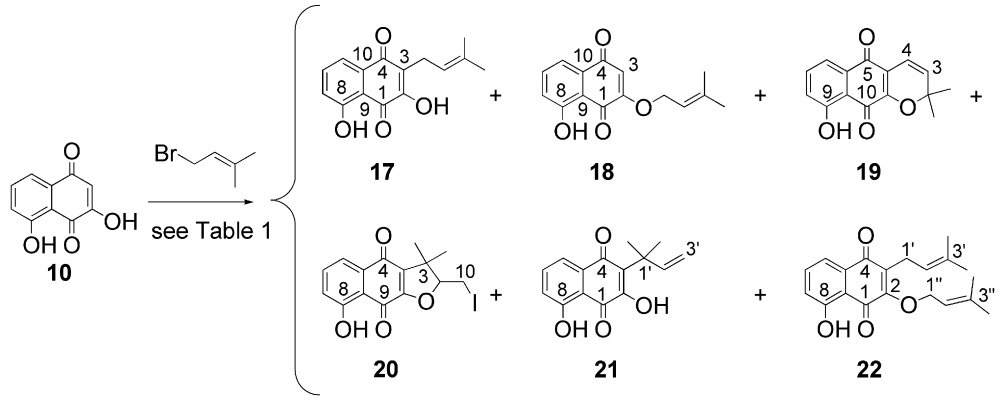
Table 1
Reaction of hydroxyjuglones **9** and **10** with 1-bromo-3-methylbut-2-ene

	Reaction conditions[a]	Products formed (% yield)
9	A	**4** (13), **12** (2), **13** (2), **14** (6)
9	B	**4** (57), **12** (19), **15** (traces)
9	C	**4** (6), **12** (25), **15** (20), **16** (13)
10	A	**17** (10), **18** (19), **19** (24), **20** (0.9)
10	B	**17** (55), **18** (21), **21** (traces)
10	C	**17** (7), **18** (19), **21** (20), **22** (10)

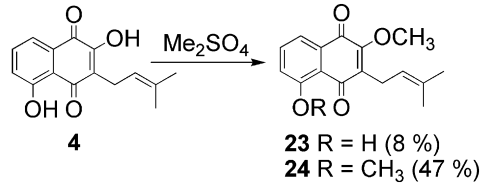
[a] (A) NaI, Et_3N, DMSO; (B) NaI, Et_3N, DMF; (C) K_2CO_3, DMF.



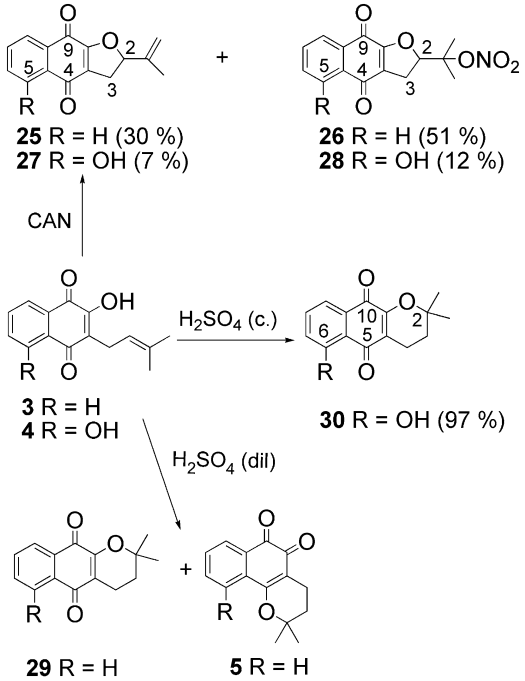
Scheme 2. Reaction of 2-hydroxyjuglone (**9**) with 1-bromo-3-methylbut-2-ene.



Scheme 3. Reaction of 3-hydroxyjuglone (**10**) with 1-bromo-3-methylbut-2-ene.



Scheme 4. Reaction of 5-hydroxylapachol with dimethyl sulfate.



Scheme 5. Reaction of lapachol (**3**) and 5-hydroxylapachol (**4**) with CAN and H_2SO_4.

activity (see below), thus we sought for a more efficient synthetic procedure for their preparation. As shown in Scheme 6, the addition of 3-methylbut-2-en-1-ol to **9** under Mitsunobu's conditions (DIAD, Ph_3P) led to a 5:1 mixture of compounds **12** and **15**. Claisen rearrangement of **12** in ethanol at 60 °C gave **15** in 92% yield. The addition of iodine to compound **15** gave the sought iodinated naphthoquinone **13** (66%) and its isomer **31** (7%). The same reaction sequence applied to 3-hydroxyjuglone (**10**) gave compounds **20** and **32** in 7.1% and 12.5% overall yield, respectively. Compounds **14** (41%) and **33** (6%) were obtained by addition of iodine to 5-hydroxylapachol (**4**).

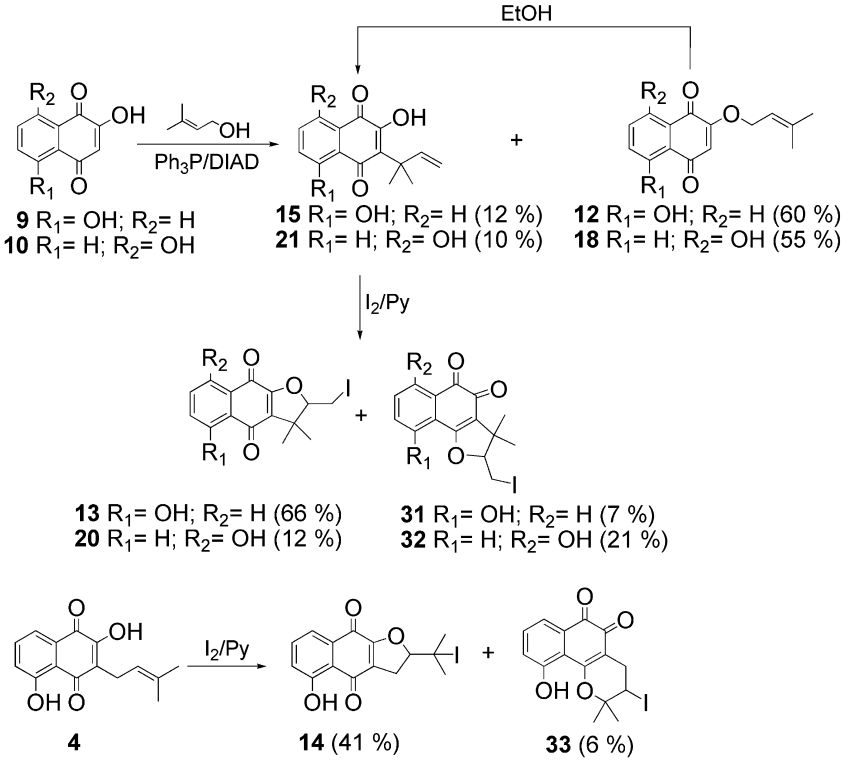
2.2. Biological activity

As a model for the anticancer activity we used the representative panel of human solid tumor cell lines A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon). The in vitro antiproliferative activity of the synthesized naphthoquinones was evaluated using the National Cancer Institute (NCI) protocol[24] after 48 h of drug exposure using the sulforhodamine B (SRB) assay. In this method, for each drug a dose–response curve was generated. The effect was defined as percentage of growth (PG), where 50% growth inhibition (GI_{50}), represented the drug concentration at which PG was +50.[25] The results are summarized in Table 2 together with the lipophilicity (*C* log *P*) of the compounds, evaluated by in silico calculation based on their chemical structures.[26] The data show that lipophilicity differences are not relevant to the observed activities.

The comparison between lapachol (**3**) and the hydroxy derivatives **4** and **17** showed that the presence and position of a hydroxy group attached to the aromatic ring affects the ability to suppress the growth of the tumor cell lines. As shown in Table 2 the results obtained with 5-hydroxylapachol (**4**) were remarkable. In fact, compound **4** was one of the most active products evaluated with GI_{50} values in the range 0.42–8.1 μM. The antiproliferative effect of 8-hydroxylapachol (**17**)[27] carrying the hydroxyl at C-8, was lower than compound **4** although it was comparable to that of lapachol (**3**). Thus, the presence of a phenolic hydroxy group at C-5 seems to play an important role in increasing antiproliferative effect. A similar effect was observed when comparing **25** and **27**, supporting the beneficial role of the C-5 hydroxyl described above.

In this particular context, the influence of the isoprenyl side chain on the antiproliferative activity was evaluated. When the side chain is an 1,1-dimethylallyl group instead of the naturally occurring prenyl group as in compounds **15** and **21**, the activity observed was lower than that of lapachol (**5**) and without differences between the regioisomers. The lack of a side chain as in commercial juglone (**6**) and compounds **7–11** resulted in poor activity. However the presence of a nitrogen atom at C-2 in the quinone ring increased the activity within the group, with compound **11** being the most active. An improved selectivity was also observed when the free hydroxyl groups in the quinone ring of 5- (**4**) and 8-hydroxylapachol (**17**) were converted to the methyl ethers (**16** and **22**). The above results show that the absence of the prenyl side chain produces weakly active derivatives, and suggests that not only the size but the relative position of the side chain is crucial to achieve a better activity profile.

Several compounds assayed were furanonaphthoquinones (**13**, **14**, **20** and **25–28**) and pyranonaphthoquinones (**5**, **19**, **29** and **30**). Some compounds of the series, displayed high antiproliferative



Scheme 6. Preparation of iodinated compounds **13** and **20**.

Table 2
Lipophilicity and GI_{50} values for the in vitro screening of naphthoquinones against human solid tumor cells[a]

Compds	Cell line						
	C log *P*	A2780 (ovarian)	HBL-100 (breast)	HeLa (cervix)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
3	3.7	1.9 (±0.5)	7.8 (±4.6)	2.3 (±0.8)	34 (±6.5)	76 (±29)	36 (±9.8)
4	4.0	0.57 (±0.16)	0.60 (±0.12)	0.42 (±0.11)	0.70 (±0.23)	8.1 (±3.7)	6.3 (±1.9)
5	1.7	1.1 (±0.8)	0.69 (±0.39)	0.81 (±0.45)	0.76 (±0.39)	2.3 (±0.4)	2.0 (±0.2)
6	3.7	64 (±34)	66 (±28)	89 (±17)	50 (±13)	77 (±30)	81 (±26)
7	2.4	16 (±4.0)	30 (±2.8)	19 (±1.8)	35 (±5.5)	38 (±8.0)	32 (±4.5)
8	2.4	2.4 (±1.2)	5.7 (±1.3)	7.1 (±3.6)	5.3 (±2.2)	34 (±4.8)	17 (±5.5)
9	2.0	>100	>100	>100	>100	>100	>100
10	2.0	28 (±8.1)	>100	90 (±14)	86 (±13.2)	>100	>100
11	2.3	3.1 (±0.9)	2.9 (±0.3)	5.7 (±2.4)	2.0 (±0.8)	4.0 (±0.8)	3.5 (±0.3)
12	3.7	12 (±4.1)	27 (±2.7)	18 (±3.0)	17 (±10.6)	28 (±5.3)	18 (±7.4)
13	4.7	1.7 (±0.5)	1.9 (±0.3)	1.7 (±0.4)	1.3 (±0.3)	1.8 (±0.4)	2.0 (±0.03)
14	4.6	2.1 (±1.2)	2.0 (±0.5)	2.4 (±0.7)	2.3 (±0.5)	2.3 (±0.4)	1.9 (±0.2)
15	3.9	26 (±5.1)	31 (±3.0)	28 (±1.6)	36 (±6.3)	27 (±2.3)	31 (±1.6)
16	5.7	1.0 (±0.3)	1.6 (±0.2)	1.9 (±0.2)	4.3 (±0.3)	21 (±4.5)	11 (±3.8)
17	4.0	3.9 (±0.6)	21 (±4.1)	2.6 (±0.4)	18 (±2.7)	30 (±4.7)	19 (±2.4)
18	3.7	2.0 (±0.5)	19 (±6.1)	4.5 (±1.6)	7.7 (±1.6)	23 (±6.1)	7.3 (±3.2)
19	3.3	2.4 (±0.1)	3.9 (±1.5)	2.3 (±0.3)	4.8 (±1.4)	19 (±2.6)	19 (±6.0)
20	4.7	1.6 (±0.3)	0.41 (±0.05)	1.3 (±0.3)	0.40 (±0.13)	2.5 (±0.4)	2.0 (±0.2)
21	3.9	21 (±1.9)	16 (±1.8)	24 (±5.3)	3.8 (±1.3)	30 (±4.6)	23 (±1.8)
22	5.7	5.9 (±0.7)	20 (±1.4)	20 (±0.6)	5.1 (±2.4)	28 (±6.9)	22 (±6.1)
23	4.1	1.8 (±0.4)	20 (±6.9)	3.5 (±0.6)	4.8 (±0.2)	22 (±4.3)	20 (±2.7)
24	3.9	21 (±5.6)	17 (±2.3)	18 (±1.0)	5.8 (±1.7)	45 (±8.1)	28 (±5.5)
25	3.4	2.6 (±0.2)	2.3 (±0.2)	2.1 (±0.9)	1.9 (±0.9)	21 (±4.6)	2.7 (±0.3)
26	0.7	2.1 (±0.3)	2.1 (±0.2)	1.8 (±0.2)	2.5 (±0.5)	20 (±8.0)	4.8 (±1.6)
27	3.7	0.82 (±0.36)	0.94 (±0.36)	0.80 (±0.41)	0.83 (±0.35)	2.2 (±0.7)	2.0 (±0.1)
28	1.0	2.2 (±0.2)	2.8 (±0.5)	2.2 (±0.34)	2.8 (±1.3)	23 (±2.8)	18 (±2.1)
29	3.2	4.0 (±0.4)	14 (±4.5)	15 (±2.6)	3.1 (±0.8)	25 (±3.5)	26 (±0.6)
30	3.5	3.2 (±0.9)	2.6 (±0.8)	12 (±1.2)	2.7 (±0.7)	21 (±4.7)	18 (±1.6)

[a] GI_{50} values are given in μM and are mean of three to ten experiments, standard deviation is given in parentheses.

activity, with the furanonaphthoquinone **27** exhibiting the highest activity (GI_{50} values of 0.80–2.2 μM) comparable to β-lapachone (**5**). When comparing the activity of **27** with those of the analogue furanonaphthoquinones **14** and **28**, the observed decrease in activity may be ascribed to the replacement of the exocyclic double bond by an oxygenated function like a nitrate group or by iodine.

3. Conclusion

In summary, we described the first synthesis of the antiproliferative agent, 5-hydroxylapachol (**4**), through prenylation of 2-hydroxyjuglone (**9**). The conventional structure–activity relationships indicate that antiproliferative activity is favored by naphthoquinones possessing a 5-hydroxy group in the aromatic ring. Additionally, this activity is favored in furanonaphthoquinones with compound **27** being the most active member of this and all series. This result is in agreement with recent findings by Iida et al.[28] and point toward 5-hydroxynaphthoquinones as a promising group of lapachone analogues.

4. Experimental

4.1. General procedure

Melting points were taken on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna 550 FT-IR spectrophotometer. NMR spectra were acquired at 500.13 (^{1}H NMR) and 125.77 MHz (^{13}C NMR) on a Bruker Avance II 500 or at 200.13 (^{1}H NMR) and 50.32 MHz (^{13}C NMR) on a Bruker AC 200 spectrometer. Chemical shifts are given in ppm downfield from TMS as internal standard, *J* values are given in Hz. Multiplicity determinations and 2D spectra were obtained using standard Bruker software. Low-resolution EI mass spectra (LR EIMS) were collected on a Shimadzu QP-5000 mass spectrometer at 70 eV by direct inlet. High-resolution EI mass spectra (HR EIMS) were determined on an Agilent LCTOF mass spectrometer. Elemental analyses were performed on an Exeter CE 440 analyzer. All solvents were of AR grade except dichloromethane (DCM) which was of LR grade and distilled before use. When necessary, the purification of solvents and starting materials was carried out using standard procedures. Lapachol (2-hydroxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione, **3**) and juglone (5-hydroxynaphthalene-1,4-dione, **6**) were used as starting materials to synthesize the training set of naphthoquinones. Lapachol (**3**) was isolated by extraction of the powdered wood of *Tabebuia impetiginosa* (*Bignoniaceae*) with a cold solution of sodium carbonate.[18,29] Juglone (**6**) was purchased from Sigma–Aldrich Co. The α-lapachone (3,4-dihydro-2,2-dimethyl-2*H*-benzo[*g*]chromene-5,10-dione, **29**) and β-lapachone (3,4-dihydro-2,2-dimethyl-2*H*-benzo[*h*]chromene-5,6-dione, **5**) were obtained by intramolecular cyclization of lapachol (**3**) using sulfuric acid,[23] whereas compounds **25** and **26** were obtained by intramolecular cyclization of **3** using CAN following known procedures.[22] Reactions were monitored using thin-layer chromatography (TLC) on aluminum-backed precoated Silica Gel 60 F254 plates (E Merck). In general naphthoquinones are highly colored and were visible on a TLC plate; colorless compounds were detected using UV light. Flash chromatography was carried out using Silica Gel 60 (230–400 mesh) with the solvent system indicated in the individual procedures. All solvent ratios are quoted as vol/vol. Full characterization data are included for known compounds where these data are incompletely reported in the literature. All new compounds were characterized by HRMS, IR and NMR. Assignment of ^{1}H and ^{13}C spectra was made using COSY, HSQC and HMBC spectra for all new compounds.

4.2. Chemistry

4.2.1. 2-(Dimethylamino)-5-hydroxy-1,4-naphthalenedione (2-*N*,*N*-dimethylaminojuglone, 7) and 2-(dimethylamino)-8-hydroxy-1,4-naphthalenedione (3-*N*,*N*-dimethylaminojuglone, 8)

The reaction of juglone (**6**) (1 g, 5.75 mmol) with an aqueous solution of dimethylamine (1.7 mL, 37% w/v) was carried out following the procedure described by Thomson.[16] The reaction product was purified by column chromatography on silica gel (hexanes/EtOAc, gradient) to give **7** (450 mg, 36% yield) as a bright red solid; mp 148–149 °C (from EtOH) (lit.[16] 147 °C). The ^{1}H and ^{13}C NMR data were consistent with that reported in the literature.[30] MS *m/z* (%) 217 (M^+, 100), 202 (27), 188 (12), 174 (8), 160 (8), 121 (6), 89 (8), 68 (9), 63 (11), 44 (24). Anal. Calcd for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.12; H 5.09; N, 6.44. Further elution gave compound **8** (187 mg, 15%) as violet crystals; mp 157–158 °C (from EtOH) (lit.[16] 156 °C). The ^{1}H NMR data were consistent with that reported in the literature.[30] ^{13}C NMR (125.77 MHz, $CDCl_3$): 188.3 (C-4), 182.0 (C-1), 161.7 (C-8), 151.6 (C-2), 136.8 (C-6), 132.9 (C-10), 122.5 (C-7), 117.9 (C-5), 115.4 (C-9), 108.2 (C-3), 43.0 (CH_3). MS *m/z* (%) 217 (M^+, 100), 202 (36), 188 (8), 174 (10), 160 (7), 121 (5), 89 (8), 68 (7), 63 (11), 44 (32).

4.2.2. 2,5-Dihydroxy-1,4-naphthalenedione (2-hydroxyjuglone, 9)

A suspension of **7** (1.52 g, 7.0 mmol) in conc. HCl (75 mL) was heated under reflux for 5 h. After cooling, the reaction mixture was diluted with water, and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc, 2:8) to give **9** (1.30 g, 98%) as red crystals; mp 219–220 °C (dec.) (lit.[31] 218–220 °C (dec.)). The ^{1}H and ^{13}C NMR data were consistent with that reported in the literature.[32] MS *m/z* (%) 190 (M^+, 100), 162 (25), 134 (35), 121 (91), 105 (14), 92 (26), 69 (23), 63 (39), 51 (30).

4.2.3. 2,8-Dihydroxy-1,4-naphthalenedione (3-hydroxyjuglone, 10)

A suspension of compound **8** (or **11**) (2.4 mmol) in conc. HCl (165 mL) was heated under reflux for 24 h. The mixture was poured into cold water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated. The residue was purified by column chromatography on silica gel (hexane/EtOAc, 2:8) and crystallized from dilute acetic acid to afford **10** (442 mg, 97%) as orange crystals; mp 219–221 °C (from acetic acid) (lit.[31] 218–222 °C). The ^{1}H and ^{13}C NMR data were consistent with that reported in the literature.[32] MS *m/z* (%) 190 (M^+, 100), 162 (24), 134 (17), 121 (62), 105 (11), 92 (26), 69 (22), 63 (30), 51 (19).

4.2.4. 2-Amino-8-hydroxynaphthalene-1,4-dione (3-aminojuglone, 11)

To a stirred solution of juglone (**6**) (100 mg, 0.57 mmol) in 4.8 mL of methanol under an argon atmosphere was added a solution of sodium azide (220 mg, 3.38 mmol) in 1.6 mL of water and acidified to pH 4 (with 1 N HCl). The reaction was heated at 30–35 °C for 22 h, and then the mixture was cooled, and extracted with EtOAc. The organic layer was washed successively with water and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc, gradient) to give **11** (101 mg, 94%) as red crystals; mp 254–255 °C (lit.[17] 253–254 °C). The ^{1}H NMR data were consistent with that reported in the literature.[17] ^{13}C NMR (125.77 MHz, CD_3OD): 188.4 (C-4), 185.6 (C-1), 163.7 (C-8), 153.4 (C-2), 139.4 (C-6), 135.9 (C-10), 124.2 (C-7), 116.2 (C-9), 112.1 (C-5), 104.5 (C-3). MS *m/z* (%) 189 (M^+, 100), 173 (2.3), 162 (61), 145 (6), 133 (38), 121 (56), 104 (15), 92 (51), 68 (24), 63 (36), 41 (27).

4.2.5. Reaction of 2-hydroxyjuglone (9) with 1-bromo-3-methylbut-2-ene

4.2.5.1. Method a. The reaction of **9** (108 mg, 0.57 mmol) with 1-bromo-3-methylbut-2-ene (76.5 μL, 0.66 mmol) in DMSO was carried following the procedure described by Jiang et al.[18] The reaction mixture was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient) to give quinones **4** (19 mg, 13%), **12** (2.9 mg, 2%), **13** (4.4 mg, 2%), and **14** (13.1 mg, 6%).

*4.2.5.1.1. 2,5-Dihydroxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione (5-hydroxylapachol, **4**).* Orange crystals; mp 143–144 °C (lit.[10] 144–145 °C). The spectroscopic data (^{1}H NMR, ^{13}C NMR, and MS) were consistent with that reported in the literature.[10]

*4.2.5.1.2. 2-(3-Methylbut-2-enyloxy)-5-hydroxynaphthalene-1,4-dione (**12**).* Dark yellow crystals; mp 135–136 °C (from isopropanol). IR (KBr) 3404, 2936, 2910, 1689, 1633, 1590, 1452, 1376, 1241, 852, 740 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 12.26 (1H, s, OH-5), 7.66 (1H, dd, *J* = 7.5, 1.1 Hz, H-8), 7.56 (1H, t, *J* = 8.0 Hz, H-7), 7.26 (1H, dd, *J* = 8.4, 1.1 Hz, H-6), 6.08 (1H, s, H-3), 5.48 (1H, tsept, *J* = 6.7, 1.3 Hz, H-2′), 4.60 (2H, d, *J* = 6.9 Hz, H-1′), 1.81 (3H, s, C*H*$_3$-4′), 1.76 (3H, s, C*H*$_3$-5′). ^{13}C NMR (50.32 MHz, $CDCl_3$): 190.9 (C-4), 179.53 (C-1), 161.0 (C-5), 160.2 (C-2), 140.9 (C-3′), 135.4 (C-7), 131.1 (C-9), 125.0 (C-6), 119.5 (C-8), 116.9 (C-2′), 114.1 (C-10), 110.0 (C-3), 66.7 (C-1′), 25.8 (C-4′), 18.4 (C-5′). MS *m/z* (%) 258 (M^+, 10), 243 (10), 225 (2), 215 (3), 197 (1), 190 (99), 173 (3), 162 (33), 145 (6), 134 (11), 121 (33), 105 (56), 89 (23), 69 (91), 63 (36), 41 (100). HRMS found: 258.0894 $(M)^+$ calcd for $C_{15}H_{14}O_4$: 258.0892.

*4.2.5.1.3. 2,3-Dihydro-5-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[2,3-b]furan-4,9-dione (**13**).* Yellow crystals; mp 114–115 °C. IR (KBr) 2967, 2927, 2872, 1730, 1682, 1633, 1454, 765, 705 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 12.29 (1H, s, OH-5), 7.61 (1H, dd, *J* = 7.4, 1.1 Hz, H-8), 7.53 (1H, t, *J* = 7.9 Hz, H-7), 7.24 (1H, dd, *J* = 8.4, 1.0 Hz, H-6), 4.76 (1H, t, *J* = 7.0 Hz, H-2), 3.47 (1H, dd, *J* = 11.0, 7.1 Hz, H-10a), 3.39 (1H, dd, *J* = 11.0, 7.0 Hz, H-10b), 1.64 (3H, s, C*H*$_3$-11), 1.44 (3H, s, C*H*$_3$-12). ^{13}C NMR (125.77 MHz, $CDCl_3$): 188.4 (C-4), 177.1 (C-9), 161.4 (C-5), 158.0 (C-9a), 135.1 (C-7), 131.5 (C-8a), 130.5 (C-3a), 125.9 (C-6), 119.4 (C-8), 115.0 (C-4a), 94.0 (C-2), 46.0 (C-3), 27.6 (C-11), 19.7 (C-12), -2.1 (C-10). MS *m/z* (%) 384 (M^+, 100), 369 (M–CH_3, 15), 257 (M–I, 22), 242 (M–I–CH_3, 39), 215 (16), 199 (8), 187 (15), 128 (11), 115 (17), 92 (15), 69 (12), 63 (18), 41 (34). HRMS found: 384.9932 $(M+H)^+$ calcd for $C_{15}H_{14}O_4I$: 384.9937.

*4.2.5.1.4. 2,3-Dihydro-5-hydroxy-2-(2-iodopropano-2-yl)naphtho[2,3-b]furan-4,9-dione (**14**).* Orange crystals; mp 113–114 °C. IR (KBr) 2975, 2920, 2846, 1677, 1627, 1612, 1457, 1260, 770, 686 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 12.19 (1H, s, OH-5), 7.64 (1H, dd, *J* = 7.5, 1.1 Hz, H-8), 7.54 (1H, t, *J* = 8.0 Hz, H-7), 7.24 (1H, dd, *J* = 8.4, 1.1 Hz, H-6), 4.30 (1H, dd, *J* = 8.2, 5.5 Hz, H-2), 3.36 (1H, dd, *J* = 19.0, 5.5 Hz, H-3a), 3.18 (1H, dd, *J* = 19.1, 8.3 Hz, H-3b), 1.66 (3H, s, C*H*$_3$-11), 1.60 (3H, s, C*H*$_3$-12). ^{13}C NMR (125.77 MHz, $CDCl_3$): 188.9 (C-4), 178.7 (C-9), 161.0 (C-5), 154.3 (C-9a), 135.4 (C-7), 131.0 (C-8a), 125.0 (C-6), 119.4 (C-8), 118.7 (C-3a), 113.9 (C-4a), 80.8 (C-2), 30.3 (C-3), 27.2 (C-10), 26.5 (C-11), 24.9 (C-12). MS *m/z* (%) 384 (M^+, 61), 257 (M-I, 76), 215 (100), 187 (16), 121 (23), 92 (19), 69 (98), 63 (30), 41 (48). HRMS found: 384.9937 $(M+H)^+$ calcd for $C_{15}H_{14}O_4I$: 384.9937.

4.2.5.2. Method b. To 2-hydroxyjuglone (**9**) (50 mg, 0.26 mmol), in a dried round bottom flask, was added 1-bromo-3-methylbut-2-ene (37 μL, 0.316 mmol), sodium iodide (47.4 mg, 0.316 mmol), triethylamine (0.045 mL, 0.316 mmol) and DMF (0.350 mL). The mixture was stirred at room temperature under a nitrogen atmosphere for 1 h, and then heated to 40 °C. After 4 h, the reaction mixture was cooled diluted with water and extracted with CH_2Cl_2. The organic layer was washed successively with $NaHCO_3$ (5% w/v) and water, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **4** (39 mg, 57%), **12** (12.6 mg, 19%) and traces of **15**.

*4.2.5.2.1. 2,5-Dihydroxy-3-(2-methylbut-3-en-2-yl)naphthalene-1,4-dione (**15**).* Orange crystals; mp 123–124 °C. IR (KBr) 3246, 2989, 2951, 2926, 1725, 1661, 1631, 1485, 1463, 1267, 765, 702 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 12.55 (1H, s, OH-5), 7.97 (1H, s, OH-2), 7.62 (1H, dd, *J* = 7.4, 1.2 Hz, H-8), 7.52 (1H, dd, *J* = 8.2, 7.6 Hz, H-7), 7.28 (1H, dd, *J* = 8.4, 1.2 Hz, H-6), 6.28 (1H, dd, *J* = 17.5, 10.6 Hz, H-2′), 5.00 (1H, dd, *J* = 17.5, 0.8 Hz, H-3′), 4.97 (1H, dd, *J* = 10.6, 0.9 Hz, H-3′), 1.57 (6H, s, CH_3-4′ and 5′). ^{13}C NMR (125.77 MHz, $CDCl_3$): 191.3 (C-4), 181.5 (C-1), 161.5 (C-5), 153.5 (C-2), 148.1 (C-2′), 134.7 (C-7), 128.5 (C-9), 127.5 (C-3), 126.7 (C-6), 118.9 (C-8), 115.2 (C-10), 109.8 (C-3′), 41.0 (C-1′), 28.5 (C-4′ and C-5′). MS *m/z* (%) 258 (M^+, 63), 243 (100), 229 (12), 215 (22), 197 (13), 187 (11), 169 (10), 159 (8), 141 (11), 131 (9), 121 (18), 103 (7), 92 (17), 77 (16), 63 (13). HRMS found: 259.0970 $(M+H)^+$ calcd for $C_{15}H_{15}O_4$: 259.0970.

4.2.5.3. Method c. A solution of **9** (50.0 mg, 0.26 mmol) in DMF (1.15 mL) was added to K_2CO_3 (36.5 mg, 0.26 mmol) or Li_2CO_3 (19.2 mg, 0.26 mmol) in DMF (0.350 mL), and stirred for 15 min at room temperature under an argon atmosphere. 1-Bromo-3-methylbut-2-ene (0.0761 mL, 0.66 mmol) in DMF (0.115 mL) was added dropwise over 15 min, stirring was continued for 15 min at the same temperature, and then the reaction mixture was heated to 40 °C. After 2 h the mixture was cooled and water was added to stop the reaction. The reaction mixture was extracted with ether and the organic layer was washed successively with NaCl (ss), water, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was fractionated by column chromatography on silica gel (cyclohexane/toluene, gradient), to give quinones **4** (3.7 mg, 6%), **12** (16.6 mg, 25%), **15** (13.7 mg, 20%) and **16** (11 mg, 13%).

*4.2.5.3.1. 2-(3-Methylbut-2-enyloxy)-5-hydroxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione (**16**).* Yellow oil. IR (KBr) 3386, 2962, 2927, 2857, 1730, 1627, 1462, 1265 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 12.31 (1H, s, OH-5), 7.56 (1H, dd, *J* = 7.5, 1.7 Hz, H-8), 7.53 (1H, t, *J* = 7.7 Hz, H-7), 7.21 (1H, dd, *J* = 7.8, 1.7 Hz, H-6), 5.45 (1H, tsept, *J* = 7.7, 1.9 Hz, H-2″), 5.10 (1H, tsept, *J* = 7.0, 1.7 Hz H-2′), 4.93 (2H, d, *J* = 7.3 Hz, H-1″), 3.27 (2H, d, *J* = 7.1 Hz, H-1′), 1.77 (3H, s, C*H*$_3$-5′), 1.76 (3H, s, C*H*$_3$-4″), 1.72 (3H, s, C*H*$_3$-5″), 1.68 (3H, d, *J* = 0.7 Hz, C*H*$_3$-4′). ^{13}C NMR (125.77 MHz, $CDCl_3$): 190.9 (C-4), 181.3 (C-1), 160.9 (C-5), 157.6 (C-2), 139.7 (C-3″), 135.2 (C-7), 134.7 (C-3), 133.7 (C-3′), 131.7 (C-9), 124.4 (C-6), 119.9 (C-2′), 119.8 (C-2″), 118.8 (C-8), 114.5 (C-10), 70.1 (C-1″), 25.8 (C-4″), 25.8 (C-4′), 22.5 (C-1′), 18.1 (C-5″), 17.9 (C-5′). MS *m/z* (%) 326 (M^+,1), 279 (12), 258 (61), 243 (100), 225 (10), 215 (21), 197 (8), 187 (7), 131 (5), 121 (17), 104 (7), 83(11), 71(21), 58 (47), 41(32). HRMS found: 327.1595 $(M+H)^+$ calcd for $C_{20}H_{23}O_4$: 327.1596.

4.2.6. Reaction of 3-hydroxyjuglone (10) with 1-bromo-3-methylbut-2-ene

4.2.6.1. Method a. The reaction of 3-hydroxyjuglone (**10**) (162 mg, 0.855 mmol) with 1-bromo-3-methylbut-2-ene (0.115 mL, 0.99 mmol) was carried out following the procedure described for 2-hydroxyjuglone (method a). The resulting mixture was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **17** (22.1 mg, 10%), **18** (41.9 mg, 19%), **19** (5.4 mg, 2.4%) and **20** (3.0 mg, 0.9%).

*4.2.6.1.1. 3,5-Dihydroxy-2-(3-methylbut-2-enyl)naphthalene-1,4-dione (**17**)[27].* Orange needles; mp 122–123 °C (from EtOH) (lit.[33] 121.5–123.0 °C). ^{1}H NMR (500.13 MHz, $CDCl_3$): 11.11 (1H, s, OH-8), 7.65 (1H, dd, *J* = 7.5, 1.5 Hz, H-5), 7.62 (1H, t, *J* = 7.7 Hz, H-6), 7.19 (1H, dd, *J* = 8.1, 1.5 Hz, H-7), 5.18 (1H, m, H-2′), 3.29 (2H, d, *J* = 7.4 Hz, H-1′), 1.78 (3H, s, C*H*$_3$-5′), 1.68 (3H, s, C*H*$_3$-4′). ^{13}C NMR (125.77 MHz, $CDCl_3$): 185.0 (C-1), 183.7 (C-4), 161.1 (C-8), 152.3 (C-2), 137.5 (C-6), 134.1 (C-3′), 132.7 (C-10), 124.7 (C-3), 123.1 (C-7), 119.7 (C-5), 119.3 (C-2′), 113.0 (C-9), 25.8 (C-5′), 22.7 (C-1′), 17.9 (C-4′). MS *m/z* (%) 258 (M^+, 100), 244 (69), 225 (31), 215 (54), 195 (25), 187 (21), 175 (24), 165 (15), 149 (30), 131 (23), 121 (2), 103 (23), 92 (46), 77 (50), 65 (59), 41 (72).

*4.2.6.1.2. 2-(3-Methylbut-2-enyloxy)-8-hydroxynaphthalene-1,4-dione (**18**).* Orange needles; mp 133–135 °C (from EtOH). IR (KBr) 3564, 3068, 2969, 2915, 1648, 1597, 1580, 1457, 1300, 1205, 826, 730 cm^{-1}.^{1}H NMR (500.13 MHz, $CDCl_3$): 11.79 (1H, s, OH-8), 7.62 (2H, m, H-5 and H-6), 7.22 (1H, dd, *J* = 6.9, 2.8, H-7), 6.13 (1H, s, H-3), 5.49 (1H, tsept, *J* = 6.6, 1.8 Hz H-2′), 4.58 (2H, d, *J* = 6.9 Hz, H-1′), 1.81 (3H, s, CH_3-4′), 1.76 (3H, s, CH_3-5′). ^{13}C NMR (50.32 MHz, $CDCl_3$): 185.2 (C-1), 184.1 (C-4), 161.9 (C-8), 159.1 (C-2), 141.0 (C-3′), 137.0 (C-6), 132.0 (C-10), 123.7 (C-7), 118.8 (C-5), 116.9 (C-2′), 114.3 (C-9), 111.0 (C-3), 66.5 (C-1′), 25.8 (C-4′), 18.4 (C-5′). MS *m/z* (%) 258 (M^+, 90), 240 (21), 231 (47), 213 (13), 203 (12), 192 (100), 173 (12), 161 (16), 145 (12), 133 (11), 121 (23), 105 (74), 89 (30), 68 (82), 63 (53), 53 (62); HRMS found: 258.0899, calcd for $C_{15}H_{14}O_4$: 258.0892.

*4.2.6.1.3. 9-Hydroxy-2,2-dimethyl-2H-benzo[g]chromene-5,10-dione (α-caryopterone, **19**).* Orange needles; mp 144–145 °C (dec.) (from EtOH) (lit.[33] 144–145.5 °C (dec)). ^{1}H NMR (500.13 MHz, $CDCl_3$): 11.88 (1H, s, OH-9), 7.64 (1H, dd, *J* = 7.5, 1.1 Hz, H-6), 7.59 (1H, t, *J* = 7.8 Hz, H-7), 7.21 (1H, dd, *J* = 8.3, 1.0 Hz, H-8), 6.64 (1H, d, *J* = 10.0 Hz, H-4), 5.74 (1H, d, *J* = 10.0 Hz, H-3), 1.57 (6H, s, CH_3-11 and 12); ^{13}C NMR (125.77 MHz, $CDCl_3$): 184.7 (C-10), 181.0 (C-5), 161.5 (C-9), 152.1 (C-10a), 136.7 (C-7), 131.6 (C-5a), 131.3 (C-3), 124.0 (C-8), 119.0 (C-6), 118.6 (C-4a), 115.4 (C-4), 114.5 (C-9a), 80.7 (C-2), 28.4 (CH_3-11 and 12). MS *m/z* (%) 256 (M^+, 21), 241 (100), 228 (3), 213 (25), 182 (5), 173 (5), 149 (7), 121 (16), 92 (13), 77 (8), 63 (21), 43 (18).

*4.2.6.1.4. 2,3-Dihydro-8-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[2,3-b]furan-4,9-dione (**20**).* Orange oil. IR (KBr) 2962, 2922, 1640, 1609, 1457, 1370, 1276, 752, 707 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 11.62 (1H, s, OH-8), 7.60 (2H, m, H-5 and H-6), 7.20 (1H, dd, *J* = 7.4, 2.2 Hz, H-7), 4.74 (1H, t, *J* = 6.7 Hz, H-2), 3.68 (1H, dd, *J* = 11.0, 6.8 Hz, H-10a), 3.60 (1H, dd, *J* = 11.1, 6.7 Hz, H-10b), 1.62 (3H, s, CH_3-11), 1.43 (3H, s, CH_3-12). ^{13}C NMR (125.77 MHz, $CDCl_3$): 182.6 (C-9), 181.1 (C-4), 161.9 (C-8), 157.3 (C-9a), 137.0 (C-6), 133.45 (C-4a), 130.6 (C-3a), 124.1 (C-7), 119.1 (C-5), 114.5 (C-8a), 93.1 (C-2), 46.1 (C-3), 27.5 (C-10), 27.2 (C-11), 19.8 (C-12). MS *m/z* (%) 384 (M^+, 0.6), 323 (36), 257 (M^+−I, 100), 242 (66), 213 (40), 199 (25), 185 (25), 128 (30), 115 (52), 103 (27), 92 (71), 77 (37), 63 (43), 41 (48); HRMS found: 383.9849, calcd for $C_{15}H_{13}O_4I$: 383.9859.

4.2.6.2. Method b. The reaction of 3-hydroxyjuglone (**10**) (81 mg, 0.428 mmol) with 1-bromo-3-methylbut-2-ene (57.4 μL, 0.50 mmol) was carried out as described for compound **9** (method b). The resulting residue was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **17** (60.7 mg, 55%), **18** (23.2 mg, 21%) and traces of **21**.

*4.2.6.2.1. 3,5-Dihydroxy-2-(2-methylbut-3-en-2-yl)naphthalene-1,4-dione (**21**)[27].* Orange needles; mp 118–119 °C (dec.) (from EtOH). IR (KBr) 3313, 2956, 2927, 2867, 1734, 1635, 1459, 1286, 752, 705 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 10.92 (1H, s, OH-8), 7.72 (1H, s, OH-2), 7.63 (1H, t, *J* = 7.9 Hz, H-6), 7.58 (1H, dd, *J* = 7.5, 1.2 Hz, H-5), 7.17 (1H, dd, *J* = 8.4, 1.2 Hz, H-7), 6.27 (1H, dd, *J* = 17.5, 10.6 Hz, H-2′), 5.01 (1H, dd, *J* = 17.5, 0.9 Hz, H-3′), 4.97 (1H, dd, *J* = 10.6, 0.9 Hz, H-3′), 1.55 (6H, s, CH_3-4′ and 5′). ^{13}C NMR (125.77 MHz, $CDCl_3$): 185.2 (C-1), 184.0 (C-4), 160.7 (C-8), 152.5 (C-2), 147.9 (C-2′), 137.9 (C-6), 133.9 (C-10), 129.6 (C-3), 122.3 (C-7), 119.8 (C-5), 112.5 (C-9), 109.8 (C-3′), 41.1 (C-1′), 28.1 (C-4′ and 5′). MS *m/z* (%) 258 (M^+, 60), 243 (100), 229 (16), 215 (40), 197 (14), 187 (10), 175 (9), 159 (6), 141 (12), 128 (14), 115 (36), 103 (13), 92 (30), 77 (30), 63 (44), 41 (54); HRMS found: 259.0968 $(M+H)^+$, calcd for $C_{15}H_{15}O_4$: 259.0970.

4.2.6.3. Method c. The reaction of 3-hydroxyjuglone (**10**) (108 mg, 0.57 mmol) with 1-bromo-3-methylbut-2-ene (76.5 μL, 0.66 mmol) was carried out as described for compound **9** (method c). The resulting mixture was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **17** (10.3 mg, 7%), **18** (27.9 mg, 19%), **21** (29.5 mg, 20%) and **22** (18.6 mg, 10%).

*4.2.6.3.1. 3-(3-Methylbut-2-enyloxy)-5-hydroxy-2-(3-methylbut-2-enyl)naphthalene-1,4-dione (**22**)[27].* Yellow crystals; mp 72–73 °C (from EtOH). IR (KBr) 3418, 2967, 2920, 2872, 1635, 1609, 1457, 1273, 752, 715 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 11.88 (1H, s, OH-8), 7.60 (1H, dd, *J* = 7.5, 1.4 Hz, H-5), 7.57 (1H, t, *J* = 7.9 Hz, H-6), 7.20 (1H, dd, *J* = 8.1, 1.4 Hz, H-7), 5.48 (1H, tsept, *J* = 7.2, 1.4 Hz H-2″), 5.08 (1H, tsept, *J* = 7.2, 1.4 Hz, H-2′), 4.86 (2H, d, *J* = 7.3 Hz, H-1″), 3.29 (2H, d, *J* = 7.3 Hz, H-1′), 1.77 (6H, s, CH_3-5′ and 4″), 1.73 (3H, s, CH_3-5″), 1.67 (3H, d, *J* = 1.1 Hz CH_3-4′); ^{13}C NMR (125.77 MHz, $CDCl_3$): 187.0 (C-1), 184.6 (C-4), 161.4 (C-8), 156.3 (C-2), 139.8 (C-3″), 137.2 (C-3′), 136.3 (C-6), 133.7 (C-3), 132.2 (C-10), 123.7 (C-7), 119.9 (C-2′), 119.7 (C-2″), 118.9 (C-5), 114.4 (C-9), 70.4 (C-1″), 25.8 (C-4″), 25.8 (C-4′), 23.4 (C-1′), 18.1 (C-5″), 17.9 (C-5′). MS *m/z* (%) 326 (M^+, 0.6), 258 (48), 243 (67), 225 (5), 215 (12), 204 (7), 187 (5), 175 (14), 165 (13), 149 (19), 128 (5), 121 (14), 103 (5), 92 (13), 69 (100), 55 (19), 41 (35); HRMS $(M+H)^+$ 326.1532, calcd for $C_{20}H_{22}O_4$: 326.1518.

4.2.7. 5-Hydroxy-2-methoxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione (23) and 2,5-dimethoxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione (24)

5-Hydroxylapachol (**4**) (17.8 mg, 0.069 mmol) was added to a stirred mixture of potassium carbonate (52 mg, 0.38 mmol) and acetone (1.3 mL) at room temperature followed by dimethyl sulfate (0.0103 mL, 0.11 mmol). After 20 h the starting material had disappeared (tlc), the solvent was removed under vacuum and the solid was extracted with EtOAc, washed with brine and water, dried over anhydrous sodium sulfate, and the solvent evaporated under reduced pressure. The residue was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **23** (1.5 mg, 8%) and **24** (4.0 mg, 47%).

4.2.7.1. 5-Hydroxy-2-methoxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione (5-hydroxy-2-methoxylapachol, 23). Yellow crystals; mp 60–61 °C. IR (KBr) 2954, 2930, 2854, 1674, 1633, 1601, 1457, 1310, 1266, 762, 697 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 12.29 (1H, s, OH-5), 7.57 (1H, dd, *J* = 7.5, 1.7 Hz, H-8), 7.54 (1H, t, *J* = 7.7 Hz, H-7), 7.22 (1H, dd, *J* = 7.8, 1.7 Hz, H-6), 5.11 (1H, tsept, *J* = 7.2, 1.4 Hz, H-2′), 4.14 (3H, s, OCH_3), 3.27 (2H, d, *J* = 7.3 Hz, H-1′), 1.78 (3H, s, CH_3-5′), 1.69 (3H, d, *J* = 1.1 Hz, CH_3-4′). ^{13}C NMR (125.77 MHz, $CDCl_3$): 190.9 (C-4), 181.2 (C-1), 160.9 (C-5), 158.0 (C-2), 135.3 (C-7), 133.9 (C-3′), 131.6 (C-9), 124.6 (C-3), 124.5 (C-6), 119.8 (C-2′), 118.9 (C-8), 114.4 (C-10), 61.3 (OCH_3), 25.8 (C-4′), 22.4 (C-1′), 17.9 (C-5′). MS *m/z* (%) 272 (M^+, 81), 257 (51), 239 (42), 229 (71), 211 (46), 197 (20), 187 (16), 173 (26), 165 (24), 149 (19), 128 (13), 115 (39), 103 (15), 92 (29), 77 (25), 63 (39); HRMS found: 272.1055, calcd for $C_{16}H_{16}O_4$: 272.1049.

4.2.7.2. 2,5-Dimethoxy-3-(3-methylbut-2-enyl)naphthalene-1,4-dione (2,5-dimethoxylapachol, 24). Orange oil. IR (KBr) 2930, 2841, 1667, 1614, 1580, 1470, 1444, 1239, 762 cm^{-1}. ^{1}H NMR (500.13 MHz, $CDCl_3$): 7.70 (1H, dd, *J* = 7.6, 1.1 Hz, H-8), 7.60 (1H, dd, *J* = 8.4, 7.7 Hz, H-7), 7.26 (1H, dd, *J* = 8.4, 0.9 Hz, H-6), 5.13 (1H, tsept, *J* = 7.1, 1.3, Hz, H-2′), 4.05 (3H, s, OCH_3 at C-2), 3.99 (3H, s, OCH_3 at C-5), 3.27 (2H, d, *J* = 7.3 Hz, H-1′), 1.77 (3H, s, CH_3-5′), 1.66 (3H, d, *J* = 1.0 Hz, CH_3-4′). ^{13}C NMR (125.77 MHz, $CDCl_3$): 184.7 (C-4), 181.8 (C-1), 159.3 (C-5), 155.7 (C-2), 136.4 (C-3), 134.1 (C-7), 133.9 (C-9), 133.4 (C-3′), 120.3 (C-2′), 119.6 (C-10), 118.9 (C-8), 117.9 (C-6), 60.8 (OCH_3 at C-2), 56.5 (OCH_3 at C-5), 25.8 (C-4′), 23.2 (C-1′), 17.9 (C-5′). MS *m/z*

(%) 286 (M^+, 20), 271 (16), 255 (13), 243 (14), 228 (11), 179 (13), 149 (9), 135 (11), 115 (12), 105 (11), 91 (11), 76 (33), 63 (18), 43 (100); HRMS found: 287.1281 $(M+H)^+$, calcd for $C_{17}H_{19}O_4$: 287.1283.

4.2.8. Reaction of 5-hydroxylapachol (4) with CAN

The reaction of 5-hydroxylapachol (**4**) (30.0 mg, 0.12 mmol) with CAN (191 mg, 0.35 mmol) was carried out following the procedure described by Eyong et al.[22] The reaction residue was purified by flash chromatography (silica gel, eluting with mixtures of hexane/EtOAc, gradient), to give **27** (2.1 mg, 7%) and **28** (4.3 mg, 12%).

4.2.8.1. 2,3-Dihydro-5-hydroxy-2-(prop-1-en-2-yl)naphtho[2,3-*b*]-furan-4,9-dione (5-hydroxydehydroiso-α-lapachone, 27). Orange crystals; mp 118–119 °C (from EtOH) (lit.[34] 118–121 °C). IR (KBr) 3595, 2961, 2926, 1676, 1636, 1455, 1282, 1226, 832, 765 cm^{-1}. The spectroscopic data (NMR, MS) were consistent with that reported in the literature.[34]

4.2.8.2. 2,3-Dihydro-5-hydroxy-2-(2-nitratepropan-2-yl)naphtho[2,3-*b*]furan-4,9-dione (28). Orange crystal; mp 157–158 °C (from EtOH). IR (KBr) 3412, 2946, 2920, 2846, 1677, 1638, 1627, 1606, 1456, 1292 cm^{-1}.^{1}H NMR (500.13 MHz, $CDCl_3$): 12.13 (1H, s, OH-5), 7.65 (1H, dd, J = 7.4, 1.1 Hz, H-8), 7.56 (1H, t, J = 7.9 Hz, H-7), 7.27 (1H, dd, J = 7.9, 0.8 Hz, H-6), 5.19 (1H, dd, J = 11.0, 8.8 Hz, H-2), 3.27 (1H, dd, J = 17.4, 11.0 Hz, H-3a), 3.19 (1H, dd, J = 17.4, 8.9 Hz, H-3b), 1.71 (3H, s, C*H*$_3$-11), 1.68 (3H, s, C*H*$_3$-12); ^{13}C NMR (125.77 MHz, $CDCl_3$): 187.8 (C-4), 176.4 (C-9), 161.3 (C-5), 160.4 (C-9a), 135.3 (C-7), 131.7 (C-8a), 125.9 (C-6), 123.7 (C-3a), 119.7 (C-8), 114.7 (C-4a), 89.7 (C-10), 88.4 (C-2), 28.1 (C-3), 21.4 (C-11), 20.3 (C-12). MS *m/z* (%) 319 (M^+, 16), 257 ($M-NO_3$, 3.5), 215 (71), 159 (16), 131 (15), 103 (12), 92 (8), 77 (22), 59 (20), 43 (100); HRMS found: 320.0774 $(M+H)^+$ calcd for $C_{15}H_{14}NO_7$: 320.0770.

4.2.9. 3,4-Dihydro-6-hydroxy-2,2-dimethyl-2*H*-benzo[*g*]chromene-5,10-dione (6-hydroxy-α-lapachone, 30)

The reaction of 5-hydroxylapachol (**4**) with H_2SO_4 was carried out following the procedure described in Ref. 23. The reaction residue was purified by column chromatography on silica gel (hexane/EtOAc, gradient) to give **30** (97%) as yellow crystals; mp 179–180 °C (lit.[30] 178–180 °C). The ^{1}H NMR spectrum was consistent with that reported in the literature.[33] ^{13}C NMR (125.77 MHz, $CDCl_3$): 190.1 (C-5), 179.3 (C-10), 160.9 (C-6), 155.3 (C-10a), 135.0 (C-8), 131.2 (C-9a), 124.7 (C-7), 119.5 (C-4a), 119.1 (C-9), 114.1 (C-5a), 78.6 (C-2), 31.3 (C-3), 26.5 (CH_3-11), 16.1 (C-4). MS *m/z* (%) 258 (M^+, 74), 243 (M-CH_3, 100), 215 (15), 173 (29), 149 (14), 121 (15), 89 (18), 74 (14), 63 (39), 41 (85).

4.2.10. Synthesis of 2-(3-methylbut-2-enyloxy)-5-hydroxynaphthalene-1,4-dione (12) and 2,5-dihydroxy-3-(2-methylbut-3-en-2-yl)naphthalene-1,4-dione (15) using Mitsunobu's conditions

2-Hydroxyjuglone (**9**) (92.8 mg, 0.5 mmol), 3-methylbut-2-en-1-ol (0.0609 mL, 0.6 mmol) and triphenylphosphine (157 mg, 0.6 mmol) were dissolved in dry THF (5 mL), and then DIAD (0.12 mL, 0.6 mmol) in THF (2.5 mL) was added. The reaction was stirred overnight at room temperature and then concentrated in vacuo. The residue was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **12** (77.4 mg, 60%) and **15** (15.5 mg, 12%), respectively.

4.2.11. Synthesis of 2-(3-methylbut-2-enyloxy)-8-hydroxynaphthalene-1,4-dione (18) and 3,5-dihydroxy-2-(2-methylbut-3-en-2-yl)naphthalene-1,4-dione (21) using Mitsunobu's conditions

3-Hydroxyjuglone (**10**) (100.0 mg, 0.54 mmol), 3-methylbut-2-en-1-ol (0.162 mL, 1.6 mmol) and triphenylphosphine (419 mg, 1.6 mmol) were dissolved in dry THF (5 mL), and then DIAD (0.12 mL, 0.6 mmol) in THF (2.5 mL) was added. The reaction was stirred 1 h at room temperature and then concentrated in vacuo. The residue was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **18** (55%) and **21** (10%).

4.2.12. Synthesis of 2,5-dihydroxy-3-(2-methylbut-3-en-2-yl)naphthalene-1,4-dione (15) by Claisen rearrangement

Compound **12** (77.0 mg, 0.3 mmol) in EtOH (4 mL) was heated at 60 °C overnight. The reaction mixture was cooled to room temperature, and the solvent evaporated under reduced pressure. The residue was fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinone **15** (70.8 mg, 92%).

4.2.13. Synthesis of 3,5-dihydroxy-2-(2-methylbut-3-en-2-yl)naphthalene-1,4-dione (21) by Claisen rearrangement

Compound **21** (90% yield) was obtained from compound **18** following the procedure described previously for compound **15**.

4.2.14. Synthesis of 2,3-dihydro-5-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[2,3-*b*]furan-4,9-dione (13) and 2,3-dihydro-9-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[1,2-*b*]furan-4,5-dione (31)

Compound **15** (18.2 mg, 0.070 mmol) in dichloromethane (2 mL) was treated at room temperature with a solution of iodine (57.6 mg, 0.46 mmol) in a mixture of dichloromethane (1.9 mL) and pyridine (0.25 mL). The reaction mixture was stirred for 40 min at room temperature, followed by addition of cold water. The organic phase was washed with 10% sodium carbonate followed by cold water and dried with anhydrous Na_2SO_4. The solvent was evaporated under vacuum and the residue fractionated by column chromatography on silica gel (hexane/EtOAc, gradient), to give quinones **13** (17.7 mg, 66%) and **31** (1.9. mg, 7%).

4.2.14.1. 12,3-Dihydro-9-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[1,2-b]furan-4,5-dione (31). Orange crystals; mp 154–155 °C. IR (KBr) 3450, 2925, 2854, 1727, 1608, 1461, 1259, 1039, 945 cm^{-1}.^{1}H NMR (500.13 MHz, $CDCl_3$): 7.89 (1H, s, OH-9), 7.71 (1H, dd, J = 7.5, 1.0 Hz, H-6), 7.48 (1H, dd, J = 8.4, 7.5 Hz, H-7), 7.22 (1H, dd, J = 8.5, 1.0 Hz, H-8), 4.88 (1H, dd, J = 9.4, 4.8, H-2), 3.49 (1H, dd, J = 11.1, 4.8 Hz, H-10a), 3.46 (1H, dd, J = 11.1, 9.4 Hz, H-10b), 1.55 (3H, s, C*H*$_3$-11), 1.37 (3H, s, C*H*$_3$-12). ^{13}C NMR (125.77 MHz, $CDCl_3$): 180.5 (C-5), 175.3 (C-4), 166.4 (C-9b), 155.1 (C-9), 133.7 (C-7), 131.3 (C-5a), 125.0 (C-8), 123.6 (C-6), 122.2 (C-3a), 110.7 (C-9a), 96.8 (C-2), 44.2 (C-3), 26.6 (C-11), 19.8 (C-12), 0.0 (C-10). MS *m/z* (%) 384 (M^+), 369 (M-CH_3, 7), 257 (M–I, 29), 242 ($M-I-CH_3$, 17), 229 (57), 214 (31), 199 (8), 187 (23), 127 (19), 115 (18), 92 (22), 69 (100), 63 (61), 41 (82). HRMS found: 384.9933 $(M+H)^+$ calcd for $C_{15}H_{14}O_4I$: 384.9937.

4.2.15. Synthesis of 2,3-dihydro-8-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[2,3-*b*]furan-4,9-dione (20) and 2,3-dihydro-6-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[1,2-*b*]furan-4,5-dione (32)

Compounds **20** (12%) and **32** (21%) were obtained from compound **21** following the procedure described previously for compound **13**.

4.2.15.1. 2,3-Dihydro-6-hydroxy-2-(iodomethyl)-3,3-dimethylnaphtho[1,2-b]furan-4,5-dione (32). Orange crystal; mp 215–217 °C. IR (KBr) 3384, 2981, 2914, 1643, 1440, 1210, 1041, 952 cm^{-1}. ^1H NMR (500.13 MHz, $CDCl_3$): 11.89 (1H, s, OH-6), 7.57 (1H, dd, *J* = 8.7, 7.3 Hz, H-8), 7.26 (1H, dd, *J* = 7.3, 1.0 Hz, H-9), 7.13 (1H, dd, *J* = 8.7, 1.0 Hz, H-7), 4.77 (1H, dd, *J* = 7.2, 6.7 Hz, H-2), 3.43 (2H, m, H-10a and H-10b), 1.56 (3H, s, C*H*$_3$-11), 1.36 (3H, s, C*H*$_3$-12). ^{13}C NMR (125.77 MHz, $CDCl_3$): 185.0 (C-5), 174.9 (C-4), 166.4 (C-9b), 164.5 (C-6), 137.7 (C-8), 127.1 (C-9a), 123.2 (C-7), 123.1 (C-3a), 117.7 (C-9), 113.4 (C-5a), 95.1 (C-2), 45.3 (C-3), 27.0 (C-11), 19.6 (C-12), −0.6 (C-10). MS *m*/*z* (%) 384 (M^+, 33), 369 (18), 356 (5), 257 (M^+−I, 24), 242 (27), 229 (60), 214 (47), 213 (38), 199 (19), 187 (33), 127 (15), 115 (19), 92 (24), 69 (70), 63 (43), 41 (100); HRMS found: 383.9863, calcd for $C_{15}H_{13}O_4I$: 383.9859.

4.2.16. Synthesis of 2,3-dihydro-5-hydroxy-2-(2-iodopropano-2-yl)naphtho[2,3-*b*]furan-4,9-dione (14) and 3,4-dihydro-10-hydroxy-3-iodo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5,6-dione (33)

5-Hydroxylapachol (**4**) (40 mg, 0.16 mmol) in dichloromethane (4.5 mL) was treated at room temperature with a solution of iodine (130 mg, 0.51 mmol) dissolved in a mixture of dichloromethane (4.2 mL) and pyridine (0.5 mL). The reaction mixture was stirred for 30 min at room temperature, followed by addition of cold water. The organic phase was washed with 10% sodium carbonate (3 × 12 mL), followed by cold water (3 × 12 mL). After drying over sodium sulfate, the solvent was evaporated under vacuum. The residue was submitted to column chromatography on silica gel (hexane/EtOAc, gradient) and the quinones **14** (41%) and **33** (6%) were isolated

4.2.16.1. 3,4-Dihydro-10-hydroxy-3-iodo-2,2-dimethyl-2*H*-benzo[*h*]chromene-5,6-dione (33). Red crystals; mp 125–126 °C. IR (KBr) 3419, 2984, 1695, 1645, 1606, 1589, 1456, 1385, 1292, 1267, 833, 781, 662 cm^{-1}. ^1H NMR (500.13 MHz, $CDCl_3$): 8.79 (1H, s, OH-10), 7.74 (1H, dd, *J* = 7.5, 1.3 Hz, H-7), 7.42 (1H, dd, *J* = 8.3, 7.8 Hz, H-8), 7.20 (1H, dd, *J* = 8.4, 1.3 Hz, H-9), 4.39 (1H, dd, *J* = 8.7, 5.5 Hz, H-3), 3.34 (1H, dd, *J* = 18.2, 5.5 Hz, H-4a), 3.14 (1H, dd, *J* = 18.2, 8.7 Hz, H-4b), 1.78 (3H, s, C*H*$_3$-11), 1.74 (3H, s, C*H*$_3$-12). ^{13}C NMR (125.77 MHz, $CDCl_3$): 178.6 (C-6), 177.5 (C-5), 163.3 (C-10b), 155.9 (C-10), 132.6 (C-8), 130.9 (C-6a), 126.3 (C-9), 123.4 (C-7), 113.9 (C-10a), 111.7 (C-4a), 83.8 (C-2), 30.3 (C-4), 27.1 (C-11), 26.1 (C-3), 25.1 (C-12). MS *m*/*z* (%) 384 (M^+, 20), 257 (M^+−I, 21), 215 (38), 175 (9), 121 (12), 69 (54); HRMS found: 383.9862, calcd for $C_{15}H_{13}O_4I$: 383.9859.

4.3. Cells, culture and plating

The human solid tumor cell lines A2780 (ovarian), SW1573 (non-small cell lung), WiDr (colon), T-47D (breast), HBL-100 (breast), and HeLa (cervix) were used in this study. The cell lines were a kind gift of Professor G. J. Peters (Cancer Center Amsterdam, The Netherlands). Cells were maintained in 25 cm^2 culture flasks in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (100 units penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions displaying >97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 μL per well at densities of 7500 (HBL-100 and SW1573), 15,000 (A2780, HeLa and T-47D) and 20,000 (WiDr) cells per well, based on their doubling times.

4.3.1. Chemosensitivity testing

Chemosensitivity tests were performed using the SRB assay of the NCI with slight modifications. Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicates at different dilutions in the range 1–100 μM. The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which time cells were precipitated with 25 μL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 492 nm, using BioTek's PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium. The percentage growth (PG) was calculated with respect to untreated control cells (*C*) at each of the drug concentration levels based on the difference in OD at the start (*T*0) and end of drug exposure (*T*), according to NCI formulas. Therefore, if T is greater than or equal to *T*0 the calculation is $100 \times [(T - T0)/(C - T0)]$. If *T* is less than *T*0 denoting cell killing the calculation is $100 \times [(T - T0)/(T0)]$. The effect is defined as percentage of growth, where 50% growth inhibition (GI_{50}) represents the concentration at which PG is +50. With these calculations a PG value of 0 corresponds to the amount of cells present at the start of drug exposure, while negative PG values denote net cell kill.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.02.032.

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