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ORIGINAL RESEARCH

Antiproliferative activity of naphthoquinones and indane carboxylic acids from lapachol against a panel of human cancer cell lines

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Abstract

Lapachol (**1**) is a well-studied natural product isolated from plants of the *Bignoniaceae* family and demonstrates diverse biological effects. Historically, chemical transformation of the lapachol scaffold has yielded new derivatives with impressive biological activity and rich chemical diversity. β-lapachone (**2**), α-lapachone (**3**), and 2-acetylfuronaphthoquinone (**4**) are examples of analogs derived from lapachol that show superior antitumor activity compared with the natural product. In the present study, novel indane carboxylic acid: 2,2-dimethyl-2,3-dihydroindeno[1,2-b]pyran-4,5-dione (**9**) and methyl 5-hydroxy-2,2-dimethyl-2,3,4,5-tetrahydroindeno[1,2-b]pyran-5-carboxylate (**10**) and naphthoquinone derivatives were synthesized from lapachol with structural similarities to the antitumor lapachol derivatives. The synthesized compounds were evaluated for antiproliferative activities against a panel of human cancer cell lines including in vitro models for neuroblastoma, melanoma, glioblastoma, and non-small cell lung cancer. As expected, the most potent derivatives were those incorporating β-naphthoquinone and α-naphthoquinono[2,3-b]furan skeletons. Many of these compounds possessed nanomolar to single digit micromolar antiproliferative potency. However, the most interesting analog evaluated was the dione **9** with an indeno[1,2-b]pyran skeleton, which demonstrated potent cytotoxic activity. The current investigation identified several new lead compounds that could be used as starting points for anticancer drug discovery.

Keywords Lapachol · Naphthoquinone · Indane carboxylic acid · Cancer cell lines · Benzilic acid rearrangement

Introduction

Natural products isolated from various traditional herbal plants have had a huge impact on the development of various lead compounds used in the treatment of health issues in societies throughout the world. The tremendous biodiversity of plants in tropical and subtropical regions has led to the identification of equally diverse chemical structures with a wide range of biological activity (Nepomuceno 2011). In 1882, the Italian scientist Emanuele Paterno reported on the isolation of lapachol (**1**) (Fig. 1) from a traditional herbal plant viz., *Tabebuia impetiginosa* (Mart. ex DC) Standl (Castellanos et al. 2009). The first detailed anticancer effects of lapachol (**1**) (Nepomuceno 2011) were evaluated in 1968 and this compound was shown to possess very potent effects toward cancerous tumors in rats (Hussain et al. 2007). Unfortunately, in 1974, the National Cancer Institute demonstrated in a phase I clinical trial that lapachol (**1**) was not an effective treatment of cancer due to lack of a therapeutic window and this essentially terminated further clinical research at the time. However, in 1980, in a

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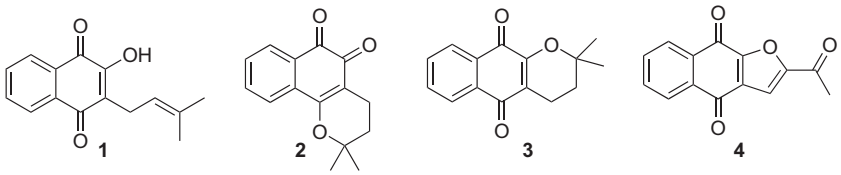


Fig. 1 Structures of lapachol (**1**), β-lapachone (**2**), α-lapachone (**3**), 2-acetylfuronaphthoquinone (**4**)

small study with nine patients from Nice with various cancers (liver, kidney, breast, prostate, and cervix), pure lapachol (**1**) demonstrated the ability to shrink tumors, reduce pain, and achieve complete remissions in three of the patients without significant adverse effects (Hussain et al. 2007).

This discovery and the fact that additional antitumor effects of lapachol (**1**) were found, led to it being considered a good candidate for derivatization to identify new lead compounds through structure–activity relationship (SAR) studies. Moreover, various lapachol analogs have been prepared and studied for the treatment of lung, breast, prostate, melanoma, leukemic, ovarian, glioblastoma, prostate, colon, and renal cancer (Hussain et al. 2007; Fiorito et al. 2014). In addition to its cytotoxic activity, lapachol (**1**) has also been shown to possess very broad biological effects including antibacterial, antiviral, analgesic, anti-inflammatory, and fungicidal activity (Hussain et al. 2007; Oliveira et al. 2002).

Derivatives of lapachol are also of interest due to their diverse biological properties recently identified. β-lapachone **(2)** and α-lapachone **(3)** isolated from *T. impetiginosa* (Castellanos et al. 2009) and 2-acetylfuronaphthoquinone **(4)** isolated from the Bignoniaceae *Newbouldia laevis* (Eyong et al. 2005) also possess unique properties. For example, β-lapachone **(2)** displays a broad spectrum of biological and pharmacological effects including antifungal, anti-inflammatory, anticancer, antibacterial, antiangiogenic, DNA damaging, and antitrypanocidal activity (Hussain et al. 2007; Queiroz et al. 2008; Rios-Luci et al. 2012; Li et al. 2000; Lim et al. 2015; Ramos-Perez et al. 2014; Perez-Sacau et al. 2007; Sunassee et al. 2013; da Silva et al. 2007). Interestingly, it has been reported that β-lapachone **(2)** is able to selectively induce cell death in various human cancer cells without killing non-transformed cells and in combination therapy with taxol it displays potent anticancer effects against many cultured cancer cells including ovarian, prostate, lung, breast, colon, melanoma, and pancreatic cell lines (Hussain et al. 2007). In addition, α-lapachone **(3)** has been shown to possess trypanocidal effects toward *Trypanosoma cruzi* and its analogs display antileishmanial activity toward *Leishmania amazonensis* and *L. braziliensis* (Souza-Silva et al. 2014), while 2-acetylfuronaphthoquinone **(4)** is a potential chemopreventive agent (Ueda 2005).

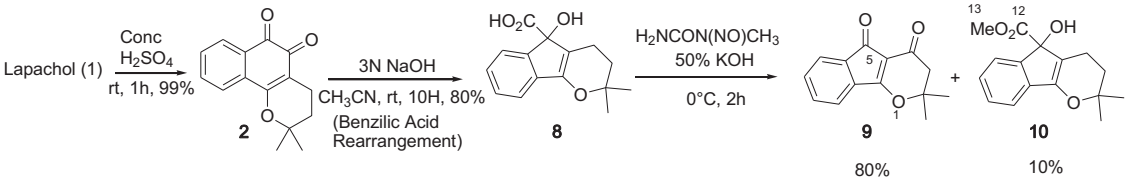
Results and discussion

Chemical transformation

The aim of this study was to prepare new derivatives of lapachol that are structurally related to β-lapachone, α-lapachone, and 2-acetylfuronaphtoquinone with potentially enhanced biological activity. In addition, we sought to probe how changes to the stereochemistry and electronics of the core structures influence bioactivity. Finally, from previous studies, quinones represent a class of generally cytotoxic moieties, which lead to a variety of generally undesirable effects in vivo, including acute cytotoxicity, immunotoxicity, and carcinogenesis. Quinones, often considered nuisance pharmacophores (Dahlin et al. 2015), are Michael acceptors, and cellular damage can occur through alkylation of crucial cellular proteins and/or DNA. Alternatively, quinones are highly redox active molecules, which can engage in redox cycles with their semi-quinone radicals, leading to formation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical. Production of ROS can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including lipids, proteins, and DNA (Bolton et al. 2000; Monks et al. 1992). In a pharmacomodulation attempt to remove these quinone groups, orthoquinones were converted to indane carboxylic acids via benzilic acid rearrangement and to phenazines using a Schiff base reaction to avoid these potential pitfalls associated with drugs that possess a quinone moiety.

To obtain new derivatives for anticancer screening, lapachol (**1**) was treated with conc. H_2SO_4 at room temperature (rt) to afforded β-lapachone (**2**), which undergoes a benzilic acid rearrangement to yield compound **8** when treated with 3 N sodium hydroxide as previously described (Eyong et al. 2013). Treatment of **8** with diazomethane generated in situ from nitrosomethylurea (NMU) afforded an unexpected dione derivative **9** as the major compound alongside the expected methyl ester **10** (Scheme 1).

The dione **9** was obtained as a red solid (mp 178–180 °C) from silica gel purification by gradient elution (20 → 30%, hexane/ethyl acetate). The molecular formula was determined to be $C_{14}H_{12}O_3$ on the basis of LCMS ($APCI^+$) *m/z* Calcd for $C_{14}H_{13}O_3$ $[M+H]^+$ 229.08647, found 229.08606. The ^1H NMR spectrum (600 MHz, $CDCl_3$) of compound **9**



Scheme 1 New indane derivatives from lapachol (**1**)

Table 1 ^{1}H and ^{13}C NMR data (600 and 150 MHz) of compounds **9** and **10** (400 and 100 MHz) (δ_H, $CDCl_3$, J in Hz)

Position	Compound 9		Compound 10
	^{1}H	^{13}C	^{1}H
2	–	93.8	–
3	2.95 (s, 2H)	39.5	1.63 (t, $J = 8.4$, 2H)
4	–	175.9	2.68 (t, $J = 8.4$, 2H)
4a	–	115.1	–
5	–	181.5	–
5a	–	128.1	–
6	8.15 (m, 1H)	129.5	8.05 (m, 1H)
7	7.60 (m, 1H)	132.0	7.69 (m, 1H)
8	7.65 (m, 1H)	134.6	7.69 (m, 1H)
9	7.65 (m, 1H)	124.7	8.05 (m, 1H)
9a	–	131.1	–
9b	–	168.9	–
10	1.61 (s, 3H)	28.6	1.29 (s, 3H)
11	1.61 (s, 3H)	28.6	1.29 (s, 3H)
12	–	–	–
13	–	–	4.14 (s, 3H)

exhibited signals indicative of four aromatic protons at δ_H 8.15 (1H, m), 7.65 (2H, m), and 7.60 (1H, m), a methylene group at δ_H 2.95 (2H, s) and two methyl groups at δ_H 1.61 (6H, s). The ^{13}C NMR spectrum (150 MHz, $CDCl_3$) exhibited 13 carbon signals that were assigned by a DEPT from multiplicity edited HSQC experiment as four methine sp^2 at 134.6, 132.0, 129.5, and 124.7, one methylene at 39.5, two methyl at 28.6 appearing as a single peak, and seven carbon signals including six sp^2 carbon atoms at 181.5 (C=O), 175.9 (C=O), 168.9 (=C–O), 131.1 (C=C), 128.1 (C=C), 115.1 (C=C) and an sp^3 carbon atom at 93.8 (C–O). The ^{1}H and ^{13}C NMR data (Table 1) were assigned using ^{1}H–^{1}H COSY, HSQC, and HMBC spectra (see Supporting Information). In the ^{1}H–^{1}H COSY, correlations were observed between H-6 (δ_H 8.15, 1H, m) and H-7 (7.60, 1H, m), between H-7 (7.60, 1H, m) and H-8 (7.65, 1H, m), and between H-8 (7.65, 1H, m) and H-9 (7.65, 1H, m) indicating AA′BB′ spin system of four aromatic protons. HMBC spectrum showed correlations of H-3 (δ_H 2.95, 2H, s) with C-4 (175.9), C-9b (168.9), C-4a (115.1), C-2 (93.8), C-10 (28.6), and C-11 (28.6) supporting H-3 connectivity in ^{2}J or ^{3}J and ^{4}J. Decarboxylation followed by benzylic oxidation occurs under the reaction conditions or during workup to form dione **9**. Further investigations into the mechanism for the reaction resulting in compound **9** will be pursued.

Compound **10** was obtained as a white solid from silica gel purification by gradient elution (2.5 → 5%, hexane/ethyl acetate). The molecular formula was determined to be $C_{16}H_{18}O_4$ on the basis of HRMS (ESI-TOF) *m/z* Calcd for $C_{16}H_{19}O_4$ $[M+H]^+$ 275.1283, found 275.1295. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **10** exhibited signals indicative of four aromatic protons at δ_H 8.05 (2H, m) and 7.70 (2H, m), attributed to an AA′BB′ spin system. A singlet at 4.14 (3H, s) attributed to a methoxy group. Two set of triplets at 2.69 (2H, t, $J =$ 8.4 Hz) and at 1.63(2H, t, $J = 8.4$ Hz) corresponding to two adjacent methylene groups. A singlet at δ_H 1.29 (6H, s) for two methyl groups. Compound **10** is the expected ester from previously reported carboxylic acid **8**. The ^{1}H NMR spectra of compound **10** is very similar to compound **8** with just the appearance of the new methyl ester peak in compound **10**. See SI including a stacked spectra with compounds **8** and **10**.

Previously synthesized compounds **5**, **6, 7, 8, 11–23** (Eyong et al. 2013, 2015a; Tanis et al. 1988), which are structurally related to α-lapachone, β-lapachone, and 2-acetylfuronapthoquinone were re-synthesized to evaluate their anticancer activities in a comprehensive panel of human cancer cell line assays. Lapachol (**1**) under the Hooker oxidation condition afforded nor-lapachol (**5**). Treatment of compound **5** with conc. H_2SO_4 afforded nor-β lapachone (**6**), which underwent benzilic acid rearrangement using 3 N NaOH to give hydroxy acid **7** (Eyong et al. 2013).

Lapachol also afforded 3-bromo-β-lapachone (**11**) upon bromination and the benzilic acid rearrangement of **11** provides compound **12** (Eyong et al. 2013). Finally, hydrogen peroxide oxidation of lapachol afforded the Hooker intermediate **13** which on esterification provided methyl ester **14** (Eyong et al. 2013).

In an attempt to obtain napthoquinones stereoselectively for SAR studies, lapachol **(1)** was converted to lomatiol (**15)** under allylic oxidation conditions (Tanis et al. 1988). Subsequent treatment of lomatiol with m-CPBA afforded

compounds **16** and **17** as a 1:1 mixture of diastereomers, while under the Sharpless asymmetric epoxidation conditions lomatiol afforded compounds **18** and **19** in 2:3 diastereomeric ratio. On bromination, lomatiol afforded monobrominated compounds **20**, **21**, **22**, and **23** (Eyong et al. 2015a).

These naphthoquinones together with some previously isolated and synthesized quinone derivatives were screened for their cytotoxicity, including 7-methoxy-2-acetylfuronapthoquinone (**24**), 2,3-dihydro-6-hydroxy-2-(prop-1-en-2-yl)naphtha[2,3-b]furan-4,9-dione (**25**), 2-acetylfuronapthoquinone (**4**), knipholone (**26**), knipholone anthrone (**27**), 3-hydroxydehydroiso-α-lapachone (**28**), 2-(prop-1-en-2-yl)naphtha[2,3-b]furan-4,9-dione (**29**), 3,4 dehydro-α-lapachone (**30**), tithoniamarin (**31**), new-bouldiaquinone (**32**), acetyllapachol (**33**), 4-(1,4-dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2-methylbut-2-enal (**34**), 2-hydroxy-3-(4,4-dimethoxy-3-methylbutyl)naphthalene-1,4-dione (**35**), 1,4-dihydro-2-((3-(hydroxymethyl)-3-methyloxiran-2-yl)methyl) 1,4-dioxonaphthalen-3-yl acetate (**36**), 4-(1,4-dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2-methylbutanal (**37**), acetyllomatiol (**38**), methoxy lomatiol (**39**), 1,4-dihydro-2-((3-(hydroxymethyl)-3-methyloxiran-2-yl)methyl)-1,4-dioxonaphthalen-3-yl acetate (**40**), lapachol-OMe (**41**), 1a-methoxy-7a-(3-methylbut-2-en-1-yl)-1a,7a-dihydronaphtho[2,3-b]oxirene-2,7-dione (**42**), 6-hydroxy-6-(3-methylbut-2-enyl)benzo[a]phenazin-5(6H)-one (**43**), β-lapachone-o-phenylene diamine (**44**), and 3-bromo-5-hydroxy-2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromene-6 carbaldehyde (**45**) (Eyong et al. 2005, 2006, 2008 2013, 2015a, 2015b; Kuete et al. 2007; Induli et al. 2013) (Fig. 2).

Cytotoxic activity

The compounds were evaluated in a panel (Dasari et al. 2015; Zhao et al. 2015) of human cell lines containing in vitro models for cancers typically associated with dismal prognosis, such as BE(2)-C, Kelly, SKNSH, CHLA-90 (all neuroblastoma) (Esposito et al. 2017; Zhao et al. 2016), A549 (non-small cell lung cancer) (Rossi et al. 2017), SKMEL-28 (melanoma) (Amann et al. 2017), and U373 (glioblastoma) (Osuka and Van Meir 2017).

First, the compounds were tested at a single concentration of 10 μM and the percent residual growth of cancer cells was recorded after a 72-h treatment (Table 2).

In most cases, the active compounds did not discriminate between the cell lines used with the exception of a few, such as β-lapachone derivative **21**, which was essentially inactive in three neuroblastoma cell lines, yet showed potent activity against the other cell lines in the panel.

Fourteen of the most potent compounds were then selected and evaluated against A549, SKMEL-28, and U373 cell lines to determine their IC_{50} values (Table 3). Most of these compounds possess the β-naphthoquinone and the α-naphthoquinono[2,3-b]furan structural skeletons. Indeed, a literature search reveals a significant number of reports describing antiproliferative properties of compounds in these classes (Rios-Luci et al. 2012; Yamashita et al. 2007). Our results show that submicromolar to single digit micromolar IC_{50} values are associated with compounds in which the β-naphthoquinone ring is fused with a saturated pyran (**2**, **11**, **20**, **21**) or a furan (**6**, **22**, **23**) moiety.

In the α-naphthoquinono[2,3-b]furan derivatives, the furan ring can be aromatic (**4**, **24**, **29**) or saturated (**16 and 28**) and retain activity, exhibiting submicromolar to low micromolar IC_{50} values. Within the α-naphthoquinono[2,3-b]furan class, the acetyl furan **4** led to the highest cytotoxic activity indicating that the carbonyl of the acetoxyl group on the furan ring may play an important role in bioactivity (cf. isopropylidene furan **29**).

Knipholone anthrone (**27**) exhibited a mean IC_{50} of ca. 1 μM, a value that is consistent with the literature data (Habtemariam 2010). It should be noted that this compound was recently found to induce necrotic cell death in cancer cells, identifying it as a promising anticancer agent to fight cancers with intrinsic resistance to apoptosis (Kornienko et al. 2013; Aksenov et al. 2015). Activity was found in the indeno[1,2-b]pyran skeleton, as represented with the product of the benzilic acid rearrangement, dione **9**, showing submicromolar to single digit micromolar potency depending on the cell line tested.

Conclusion

In this study, the cytotoxic activity of some previously isolated quinones and derivatives of lapachol, which are structurally related to known anticancer agents (i.e. β-lapachone, α-lapachone, and 2-acetylfuronaphthoquinone), and two new indeno[1,2-b]pyran derivatives were evaluated in a panel of human cancer cell lines. Fourteen of the tested compounds were most active with submicromolar to low micromolar IC_{50} values. Among these compounds, seven are based on the β-naphthoquinone and five on the α-naphthoquinono[2,3-b]furan structural skeletons. These results are comparable with values described in the literature for these classes of compounds (Rios-Luci et al. 2012; Yamashita et al. 2007). Among the two new indeno[1,2-b]pyran derivatives, the further oxidized, benzilic acid rearrangement product, dione **9** was the most interesting finding given that anticancer activity with this structural skeleton appears to be unprecedented. Given the ease of synthesis and potent cytotoxic activity of dione **9** from commercially available lapachol (**1**), this tricyclic pyranone-fused indenone represents a compelling starting

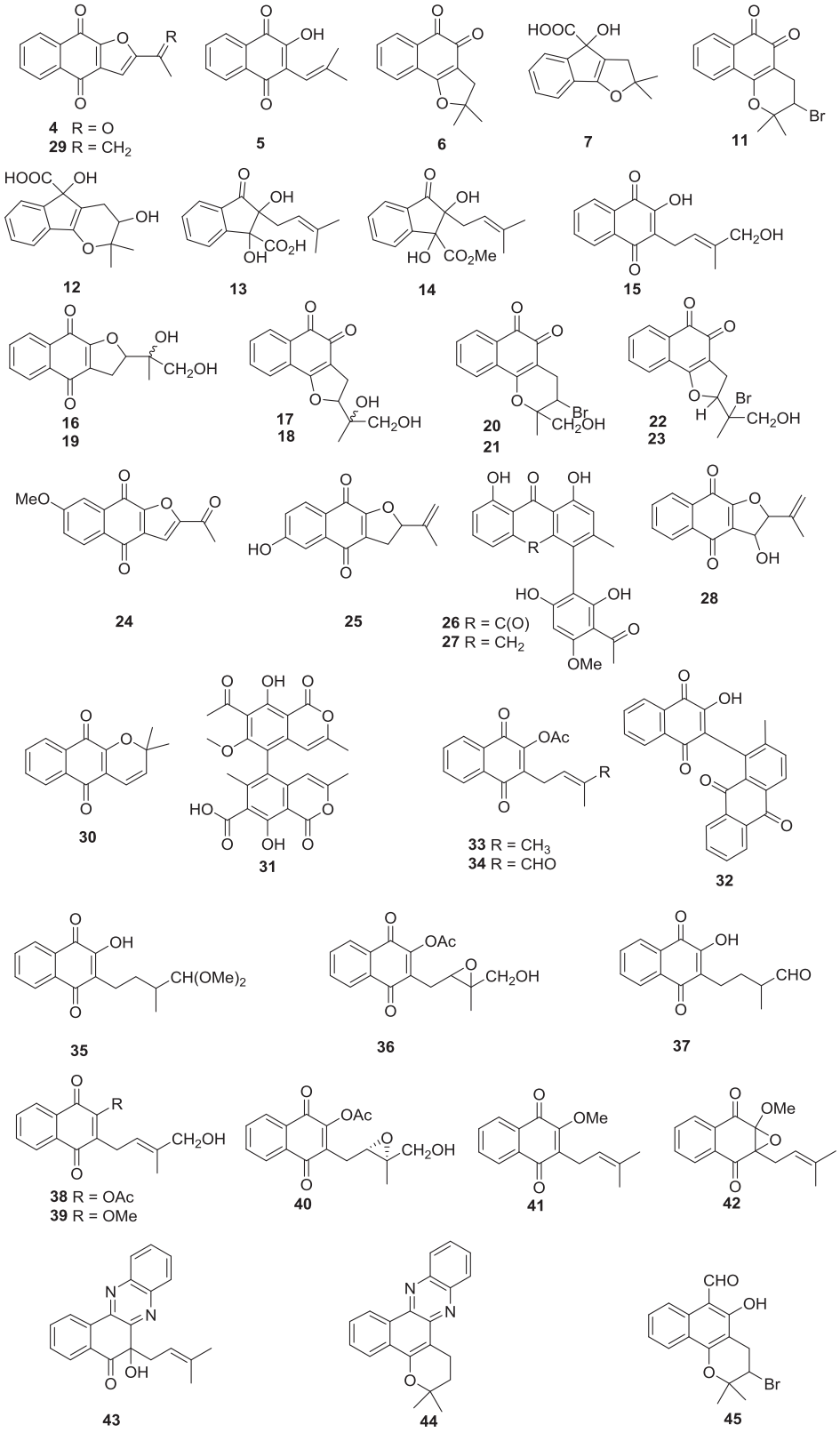


Fig. 2 Previously isolated and synthesized quinone derivatives

Table 2 Percent residual growth of cancer cell lines after a 72 h of treatment with 10 μM of each compound as determined by the colorimetric MTT assay

Compound	Residual growth (%)							
	BE(2)-C	Kelly	SKNSH	CHLA-90	A549	SKMEL-28	U373	Mean
1	77	89	81	95	83	75	73	82
2	1	6	1	2	2	3	4	3
4	2	1	28	5	2	2	4	6
5	81	68	82	88	72	83	61	76
6	2	1	1	2	2	1	5	2
7	95	82	93	94	102	94	82	92
8	93	81	96	98	67	88	70	85
9	3	3	2	3	2	3	6	3
10	13	88	88	89	NT[a]	NT	NT	69
11	2	2	1	1	1	1	4	2
12	95	82	93	94	85	78	71	85
13	101	48	80	86	101	110	99	89
14	90	59	92	86	74	75	63	77
15	91	76	99	98	69	94	63	84
16	1	1	1	3	2	3	6	2
17	83	66	89	96	NT	NT	NT	83
18	38	76	74	85	78	87	74	73
19	1	2	2	3	NT	NT	NT	2
20	4	4	2	2	1	2	6	3
21	2	97	74	87	1	2	4	38
22	28	95	79	86	13	7	65	53
23	1	2	2	32	1	3	5	7
24	3	4	4	5	2	2	5	4
25	37	69	76	66	29	18	39	47
26	93	65	101	96	64	68	74	80
27	2	5	95	7	3	4	14	19
28	6	69	57	65	2	3	37	34
29	1	44	58	34	1	2	31	25
30	68	79	88	96	88	63	86	81
31	82	65	93	85	78	76	94	82
32	76	79	88	95	40	48	77	72
33	24	84	81	97	66	76	48	68
34	87	61	89	93	56	67	56	73
35	85	73	92	94	67	66	65	78
36	78	89	87	98	51	64	78	78
37	36	99	80	91	17	77	72	67
38	9	84	91	97	58	80	58	68
39	16	77	62	74	59	20	64	53
40	2	35	54	29	3	3	41	24
41	58	91	85	94	93	77	84	83
42	4	88	87	101	78	98	78	76
43	92	64	90	89	67	84	73	80
44	94	75	89	88	77	68	78	81
45	72	90	66	85	NT	NT	NT	78

[a]Not tested

Table 3 In vitro growth inhibition (IC_{50}) after a 72 h of treatment with each compound as determined by the colorimetric MTT assay

Compound	Structural skeleton	IC_{50} (μM)				
		A549	SKMEL-28	U373	IC_{50} ± SEM	
2	β-naphthoquinone	0.03	0.05	0.24	0.1	0.07
11	β-naphthoquinone	3	2	2	2.2	0.2
20	β-naphthoquinone	3	3	2	2	0.4
21	β-naphthoquinone	2	1	1	1.4	0.5
6	β-naphthoquinone	3	2	3	2.5	0.1
22	β-naphthoquinone	5	2	NT	3.5	1.3
23	β-naphthoquinone	0.3	0.4	2.2	1.0	0.6
4	α-naphthoquinono[2,3-b]furan	0.01	0.02	0.22	0.09	0.07
24	α-naphthoquinono[2,3-b]furan	0.2	0.3	2	0.9	0.7
29	α-naphthoquinono[2,3-b]furan	3	2	NT	2.4	0.4
16	α-naphthoquinono[2,3-b]furan	2	3	3	2.6	0.2
28	α-naphthoquinono[2,3-b]furan	3	3	3	3.0	0.0
27	anthrone	0.1	2	2	1.1	0.5
9	indeno[1,2-b]pyran	0.8	0.6	2.0	1.1	0.5

point for further derivative synthesis and mode of action studies.

Experimental

Synthetic procedures

Synthesis of 2,2-dimethyl-2,3-dihydroindeno[1,2-b]pyran-4,5-dione (9) and methyl 5-hydroxy-2,2-dimethyl-2,3,4,5-tetrahydroindeno[1,2-b]pyran-5-carboxylate (10)

To a stirred solution of lapachol (**1**), (242 mg, 1.0 mmol, 1 equiv) in CH_2Cl_2 (7 mL) was added conc. H_2SO_4 (0.8 ml, 1.5 mmol, 1.5 equiv), the resulting reaction mixture was stirred for 3 h, at room temperature. The orange suspension was filtered and washed with ice cold water (3 × 5 ml), the crude product was dried under vacuum and column chromatographic purification of the crude compound over silica gel using 20–30% EtOAc in hexane as solvent gradient afforded pure β-lapachone (**2**) (218 mg, 90% yield), as an orange solid (Eyong et al. 2013).

A solution of β-lapachone (**2**) (50 mg, 0.2 mmol) in acetonitrile (2 ml) at 25 °C was treated with 3 N NaOH solution (1 ml). The resultant mixture was stirred for 10 h at 25 °C, then the mixture was acidified to pH 5 using 5% HCl (2 ml) and the solvent was removed under reduced pressure. The residue was diluted with water (5 ml) and CH_2Cl_2 (5 ml). The layers were separated, the aqueous layer was extracted with CH_2Cl_2 (2 × 5 ml) and the combined organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give crude compound, which was purified by column chromatography over silica gel using 10–20% EtOAc in hexane as solvent gradient afforded pure compound **8** (43 mg, 80% yield) as a red solid (s).

Methylation of compound **8** with diazomethane. To a stirred slurry of 50% aq. KOH (10 ml) in Et_2O (5.0 ml) at 0 °C, N-nitroso-N-methylurea (22.5 mg, 0.16 mmol, 2 equiv) was added slowly. The resulting yellow colored organic layer containing diazomethane was separated, dried over KOH pellets, and added to compound **8** (20.8 mg, 0.08 mmol, 1 equiv) in Et_2O (2 ml), the reaction mixture was stirred for 2 h and the solvent was removed under reduced pressure. Column chromatographic purification of the crude products over silica gel using hexane-EtOAc gradient systems afforded the unexpected dione **9** (18.4 mg, 80% yield) and ester **10** (2.2 mg, 10% yield). dione **9**: ^{1}H NMR (600 MHz, $CDCl_3$) 8.15 (m, 1H), 7.60 (m, 1H), 7.65 (m, 1H), 7.65 (m, 1H), 2.95 (s, 2H), 1.61 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 181.5, 175.9, 168.9, 134.6, 132.0, 131.1, 129.5, 128.1, 124.7, 115.1, 93.8, 39.5, 28.6, 28.6. LCMS (APCI+) *m/z* calcd for $C_{14}H_{13}O_3$ [M+H]+: 229.08592; found: 229.08606. Ester **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–8.04 (m, 2H), 7.70–7.68 (m, 2H), 4.14 (s, 3H), 2.70–2.66 (m, 2H), 1.75–1.65 (m, 2H) 1.29 (s, 6H). HRMS (ES+) *m/z* calcd for $C_{16}H_{19}O_4$ [M+H]+: 275.1278; found: 275.1297.

Biological assays

Cell lines

BE(2)-C and SK-N-SH cells were purchased from the American Type Culture Collection (ATCC). Kelly cells

were obtained from the cell line repository at the Greehey Children's Cancer Research Institute, University of Texas Health Science Center at San Antonio. CHLA-90 cells were obtained from Children's Oncology Group. The neuroblastoma cell lines were maintained in DMEM/F12 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (P/S) under standard cell culture conditions. The A549 cells (DSMZ) were cultured in RPMI culture medium supplemented with 10% heat-inactivated FBS. SKMEL-28 cells (ATCC) and U373 GBM cells (ECACC) were cultured in RPMI culture medium supplemented with 10% heat-inactivated FBS (Life Technologies code 10270106). Cell culture media were supplemented with 4 mM glutamine (Lonza code BE17-605E), 100 µg/ml gentamicin (Lonza code 17-5182), and P/S (200 units/ml and 200 µg/ml) (Lonza code 17-602E) at 37 °C with 5% CO_2.

Cell proliferation assay

The effect of the investigated compounds on cell proliferation was determined by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay. The compounds were dissolved in DMSO or MeOH/CH_2Cl_2 at a concentration of either 10 or 50 mM prior to cell treatment. The cells were trypsinized and seeded at various cell concentrations depending on the cell type. The cells were grown for 24–72 h, treated with test compounds at required concentrations and incubated for 72 h in 100 or 200 µl media depending on the cell line used. The number of experiments and replicates varied depending on the cell line. Cells treated with 0.1% DMSO, 1% CH_2Cl_2, or 1% MeOH were used as a negative control. The effect of compound treatment on cell survival was evaluated by comparing compound-treated cells to cells treated with control using two-tailed student's t test, with $p < 0.05$ considered as statistically significant. The IC_{50} corresponds to the concentration of the compound of interest that reduces by 50% the growth of the cancer cell line of interest after having cultured it for 72 h in the presence of the compound in comparison to the untreated control condition.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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