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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of damnacanthal, a naturally occurring 9,10-anthraquinone and its analogues, and its biological evaluation against five cancer cell lines

Koushik Saha · Kok Wai Lam · Faridah Abas · A. Sazali Hamzah · Johnson Stanslas · Lim Siang Hui · Nordin H. Lajis

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Abstract Damnacanthal and nordamnacanthal, two naturally occurring 9,10-anthraquinones, and their analogues were synthesized. Cytotoxic activity against five cancer cell lines was evaluated using MTT assay. 2-Bromomethyl-1,3-dimethoxyanthraquinone was found to display the highest activity against all cell lines with IC_{50} range of 2–8 μM. Structure–activity relationship (SAR) assessment was considered to rationalise the cytotoxic effect. Bromomethyl group at position C-2 of the anthraquinone was found to be important in exerting cytotoxic activity of this class of compounds. The presence of the flanking methoxyl or hydroxyl groups at C-1 and C-3 also contributes to this activity. Finally, the antioxidant effect of these compounds was evaluated. MTT assay was used to measure the cytotoxicity against different cancer cell lines. Antioxidant activity was measured by FTC and TBA methods. Only two anthraquinones, damnacanthal and nordamnacanthal, were found to be antioxidative.

Keywords Anthraquinones · Synthesis · Cytotoxicity · Antioxidant activity

Introduction

Anthraquinones continue to attract interest among researchers due to its diverse potential pharmacological uses. 9,10-Anthraquinone derivatives are known to exhibit a quite potent anticancer activity (Jin *et al.*, 2001;

K. Saha · K. W. Lam · F. Abas · N. H. Lajis (✉)
Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia (UPM), 43400 Serdang, Selangor Darul Ehsan, Malaysia
e-mail: nordinlajis@gmail.com

Present Address:
N. H. Lajis
Scientific Chairs Unit, Taibah University, P.O. Box 30001, Madinah al-Munawarah 41311, Saudi Arabia

K. Saha
Department of Chemistry, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
e-mail: ksaha_ju@hotmail.com

K. W. Lam
Drug and Herbal Research Centre Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abd. Aziz, 50300 Kuala Lumpur, Malaysia
e-mail: david_lam_98@yahoo.com

F. Abas
Department of Food Science, Faculty of Food Science and Technology, University Putra Malaysia (UPM), 43400 Serdang, Selangor Darul Ehsan, Malaysia
e-mail: faridah@food.upm.edu.my

A. Sazali Hamzah
Department of Chemistry, Faculty of Applied Science, University Technology MARA, 40450 Shah Alam, Selangor Darul Ehsan, Malaysia
e-mail: asazali@salam.uitm.edu.my

J. Stanslas
Department of Medicine, Faculty of Medicine and Health Science, University Putra Malaysia (UPM), 43400 Serdang, Selangor Darul Ehsan, Malaysia
e-mail: rcxjs@medic.upm.edu.my

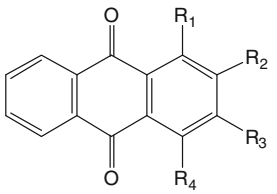
L. S. Hui
Department of Biomedical Science, Faculty of Medicine and Health Science, University Putra Malaysia (UPM), 43400 Serdang, Selangor Darul Ehsan, Malaysia

Zagotto *et al.*, 2000). Anthraquinones are well known for their antioxidant property (Huang *et al.*, 1995; Yen *et al.*, 2000), and have also been reported to exhibit diverse bioactivities including hypotensive, antimicrobial, antiplasmodial, antitumor and cytotoxic effects (Chang *et al.*, 1982; Johnson *et al.*, 1997; Mishra and Gupta, 1982). Unfortunately, anthraquinones are also reported to display undesirable side effects, such as cardiotoxicity (Cardia *et al.*, 2001).

In our previous efforts to discover new bioactive constituents from our plant resources, we isolated a new 2-formyl-1-hydroxyanthraquinone along with ten other known 9,10-anthraquinones, including damnacanthal and nordamnacanthal (Ismail *et al.*, 1997). Both damnacanthal and nordamnacanthal exhibited strong and selective cytotoxic activity against CEM-SS and MCF-7 as compared to HeLa cell lines (Ali *et al.*, 2000). In order to further understand the role of these anthraquinones in their mechanism leading to cell death of anti-proliferative activity towards cancer cell lines, a programme to synthesise these compounds along with their analogues was conducted with the aim of selecting the best candidate for its further larger scale preparation and biological evaluation. We herein report the synthesis of a number of substituted simple 9,10-anthraquinone analogues, followed by the evaluation of their cytotoxic and antioxidant activities, and finally to assess their structure–activity relationship.

Results and discussion

The structure of anthraquinone analogues is represented in Fig. 1. Friedel–Crafts condensation was selected as method of choice for the preparation of the anthraquinone skeleton due to the simplicity of the reaction and less demanding conditions (Wei *et al.*, 2000; Werner *et al.*, 1997; Zhang *et al.*, 1996). Phthalic anhydride and suitable substituted benzenes were used as the starting materials for the desired anthraquinones (**1**–**10**). Methylated hydroxyanthraquinones **11**–**14** as well as **17**–**18**, were prepared from the respective **5** and **8**–**10** using CH_3I in the presence of NaH or $(CH_3)_2SO_4$ in the presence of anhydrous K_2CO_3 (Enger and Iyenrar, 1998; Roberts *et al.*, 1997), in appropriate reaction conditions. Acetylation of hydroxyanthraquinones was accomplished by treating the respective starting materials with Ac_2O with K_2CO_3 used as base. Compound **10** with free unprotected hydroxyl was converted to the unexpected **15** in good yield, upon bromination using *N*-bromosuccinimide in the presence of benzoyl peroxide. However, upon bromination using the same reagent, compounds **16** and **17** gave the expected bromomethyl and dibromomethyl derivatives (**19**–**21**) (Cambie *et al.*, 1992)



Compound	R_1	R_2	R_3	R_4
1	OH	H	Br	H
2	OH	H	H	Br
3	H	OH	Br	H
4	OH	H	H	H
5	H	OH	H	H
6	Br	H	OH	H
7	OH	H	OH	H
8	OH	H	H	OH
9	OH	H	H	CH_3
10	OH	CH_3	OH	H
11	H	OCH_3	H	H
12	OH	H	H	OCH_3
13	OCH_3	H	H	OCH_3
14	OCH_3	H	H	CH_3
15	OH	CH_3	OH	Br
16	OH	CH_3	OAc	H
17	OCH_3	CH_3	OAc	H
18	OCH_3	CH_3	OCH_3	H
19	OCH_3	CH_2Br	OAc	H
20	OCH_3	CH_2Br	OCH_3	H
21	OCH_3	$CHBr_2$	OCH_3	H
22	OH	CH_2Br	OCH_3	H
23	OCH_3	CH_2OEt	OH	H
24	OCH_3	CH_2OEt	OCH_3	H
25	OCH_3	CH_2OEt	OAc	H
26	OCH_3	CHO	OH	H
27	OCH_3	CHO	OH	Br
28	OCH_3	CHO	OCH_3	H
29	OH	CHO	OH	H

Fig. 1 Structure of synthesized anthraquinones

in good yields. Compound **20** was further converted to **22** by demethylation using anhydrous aluminium chloride (Zacharie *et al.*, 1997).

The bromomethyl derivatives, **19** and **20** were successfully converted to the respective ethoxymethyl derivatives **23** and **24** by treating them with ethanol in the presence of aqueous sodium hydroxide. 2-Formylanthraquinones, **26**–**28** were obtained when the respective ethoxymethyl derivatives **23**–**25** were brominated with *N*-bromosuccinimide in the presence of benzoyl peroxide followed by hydrolysis with aqueous acetic acid (Roberts *et al.*, 1997).

Table 1 Cytotoxic activity of anthraquinones (IC_{50} in μM)

Compound	MCF7	MES-SA	MES-SA/DX5	DU145	H460
1	–	–	–	–	–
2	36	14	20	27	20
3	–	–	–	–	–
4	–	–	–	–	–
5	69	–	–	72	66
6	27	56	29	44	43
7	55	30	21	29	23
8	–	–	–	–	–
9	–	–	–	–	–
10	56	38	35	32	42
11	68	–	–	70	68
12	79	–	–	74	82
13	70	–	–	69	71
14	55	19	–	48	54
15	28	20	30	31	19
16	56	–	–	37	68
17	26	–	–	27	–
18	–	–	–	–	–
19	6	–	–	5	26
20	8	2	2	4	5
21	27	10	7	28	23
22	34	4	–	26	30
23	35	–	–	50	–
24	–	–	–	–	–
25	30	–	–	23	–
26	11	–	–	26	25
27	19	–	–	34	32
28	55	5	–	32	65
29	36	18	–	42	40

–, not active with IC50 of >100 μM

Compound **28** was converted to **29** by demethylation using anhydrous aluminium chloride.

The anthraquinone analogues were evaluated for their cytotoxic activity against five different cell lines: breast cancer (MCF7), human uterine sarcoma (MES-SA), multidrug-resistant variant human uterine sarcoma (MES-SA/DX5), prostate cancer (DU145) and lung cancer (H460). The highest concentration of the compound used for the MTT assay was 100 μM with the lowest 0.1 μM using 10-fold serial dilution. Table 1 shows the overall results of cytotoxic activity of the analogues.

2-Bromomethyl-1,3-dimethoxyanthraquinone **20** was found to be the most cytotoxic compound against all cell lines tested. The IC_{50} values for this compound range between 2 and 8 μM. However, this compound did not show any selectivity against any particular cell line. Additional bromine in dibromo derivative **21** seemed to decrease the cytotoxicity of **20** in all the cancer cell lines. This may be related to the increase in the calculated lipophilicity (ALOGPs) with the predicted value of 4.44. The addition of the second bromo atom in the bromoalkyl group could result in the increase in the hydrophobic character, which is crucial in determining the capability of the compound to cross the cell membranes. Another derivative of bromomethylanthraquinone **19** exhibited strong cytotoxicity against MCF7 (IC_{50}: 6 μM) and DU145 (IC_{50}: 5 μM) cell lines. On the other hand, compound **22**, which also bears bromomethyl group at position-2 but with hydroxyl group at position-1, showed a degree of selectivity against human uterine sarcoma (MES-SA) cell line with IC_{50} value of 4 μM. However, both the compounds **19** and **22** failed to match the activity of compound **20**. It is noteworthy that the 2-formyl analogues of these compounds also showed some degree of selectivity towards cancer cell lines tested

Further inspection on the solubility (ALOGpS) of compounds **20**, **21**, **19** and **22** demonstrated that they are less sufficient for fast absorption due to the lower (calculated) solubility. According to the study on the rate-limiting steps of human oral absorption of 238 drugs, the absorption is usually very low if the calculated solubility is less than 0.00001 mg/L (Zhao *et al.*, 2002). Therefore, it is critical to improve the solubility of these compounds to permit dissolution and absorption in the future studies. Compound **22** showed promising results by displaying only moderate activity against other cell lines and could be a potential candidate for further drug development. Other compounds including **14**, **21**, **28** and **29** also showed selectivity against MES-SA cell line. Damnacanthal (**26**), although exhibited only moderate activity against DU145 and H460, displayed some selectivity against MCF7 with IC_{50} value 11 μM.

The overall results indicated that anthraquinones containing bromomethyl group at C-2 with dimethoxy or methoxy and acetoxy at C-1 and C-3, respectively, (**19** and **20**) markedly increased cytotoxic activity while the presence of hydroxyl at C-1 instead of methoxy reduced the cytotoxicity but increased the selectivity (**22**). The presence of a formyl group at C-2 with methoxy at C-1 and hydroxy at C-3 also caused significant cytotoxicity (**26** and **27**). Comprehensively, the theoretical study of these compounds showed that they fulfilled Lipinski rule-of-5 and drug-like properties. Another important physicochemical criterion is the polar surface area (PSA), which is based on the summation of tabulated surface contributions of polar fragments. In this experiment, we calculated the polar surface area of the compounds using the topological PSA (TPSA) procedure (see Table 2). All the compounds were generally within the desired limits of TPSA in the range of 50–100 $Å^2$, suggesting a potentially good intestinal absorption, blood–brain barrier penetration and permeability across cell membranes.

Table 2 Calculated physicochemical properties for compounds **1–29**

Compound	MW	Volume	TPSA	HBA	HBD	ALOGPs	ALOGpS	ALOGpS (g/L)
1	303.111	208.481	54.370	3	1	3.92 (±0.41)	−4.01	0.029
2	303.111	208.481	54.370	3	1	3.93 (±0.43)	−4.05	0.027
3	303.111	208.481	54.370	3	1	3.61 (±0.39)	−3.97	0.032
4	224.215	190.595	54.370	3	1	3.13 (±0.38)	−3.12	0.170
5	224.215	190.595	54.370	3	1	2.89 (±0.32)	−3.37	0.095
6	287.112	200.463	34.142	2	0	3.90 (±0.35)	−4.52	0.009
7	240.214	198.613	74.598	4	2	3.13 (±0.37)	−4.55	0.007
8	240.214	198.613	74.598	4	2	2.93 (±0.69)	−2.92	0.290
9	238.242	207.156	54.370	3	1	3.52 (±0.46)	−3.23	0.052
10	254.241	215.174	74.598	4	2	3.13 (±0.46)	−3.23	0.150
11	238.242	208.123	43.376	3	0	3.13 (±0.37)	−4.55	0.007
12	254.241	216.141	63.604	4	1	3.08 (±0.43)	−3.34	0.120
13	268.268	233.669	52.610	4	0	3.01 (±0.44)	−3.85	0.038
14	252.269	224.684	43.376	3	0	3.45 (±0.42)	−4.69	0.005
15	333.130	233.059	74.598	4	2	3.82 (±0.60)	−3.61	0.081
16	296.278	251.685	80.675	5	1	3.23 (±0.39)	−4.05	0.026
17	310.305	269.213	69.681	5	0	3.20 (±0.54)	−4.46	0.012
18	282.295	250.230	52.610	4	0	3.34 (±0.54)	−4.30	0.014
19	389.201	287.339	69.681	5	0	3.55 (±0.34)	−5.15	0.003
20	361.191	268.356	52.610	4	0	3.71 (±0.48)	−5.05	0.003
21	440.087	286.267	52.610	4	0	4.44 (±0.64)	−5.51	0.001
22	347.164	250.828	63.604	4	1	3.75 (±0.46)	−4.57	0.009
23	312.321	275.290	72.838	5	1	2.99 (±0.57)	−3.97	0.033
24	326.348	292.818	61.844	5	0	3.16 (±0.55)	−4.33	0.015
25	354.358	311.802	78.915	6	0	3.00 (±0.41)	−4.40	0.014
26	282.251	235.124	80.675	5	1	2.83 (±0.54)	−3.64	0.065
27	361.147	253.010	80.675	5	1	3.52 (±0.69)	−4.32	0.017
28	296.278	252.652	69.681	5	0	2.72 (±0.53)	−4.28	0.002
29	268.224	217.596	91.669	5	2	2.93 (±0.89)	−3.20	0.170

Antioxidant activity of the anthraquinones was measured using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The FTC method measures the amount of peroxide in the initial stage of lipid peroxidation. Low absorbance value in the FTC method indicates high level of antioxidant activity. Only three anthraquinones, damnacanthal (**26**), nordamnacanthal (**29**) and commercial alizarin displayed stronger antioxidant activity than vitamin E, although these compounds showed lower activity than butylated hydroxy toluene (BHT). Alizarin exhibited stronger activity than compound **26** and **29** (Fig. 2). All other anthraquinones were found to be inactive.

During the oxidation process, peroxide is gradually decomposed to lower molecular compounds and the relative concentrations are measured using TBA method. Figure 3 shows the absorbance value of active

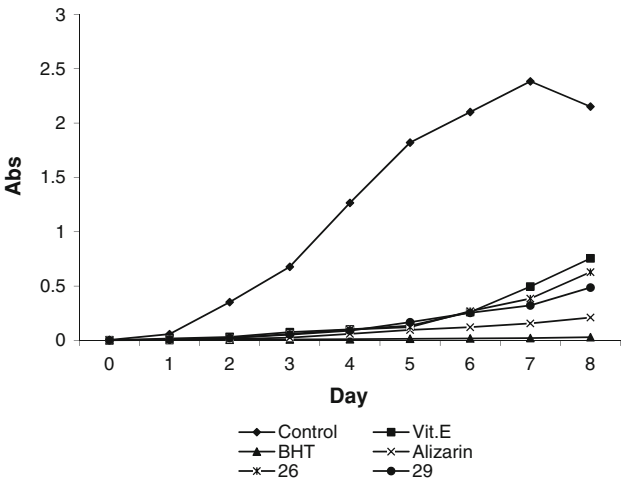


Fig. 2 Antioxidant activity using FTC method

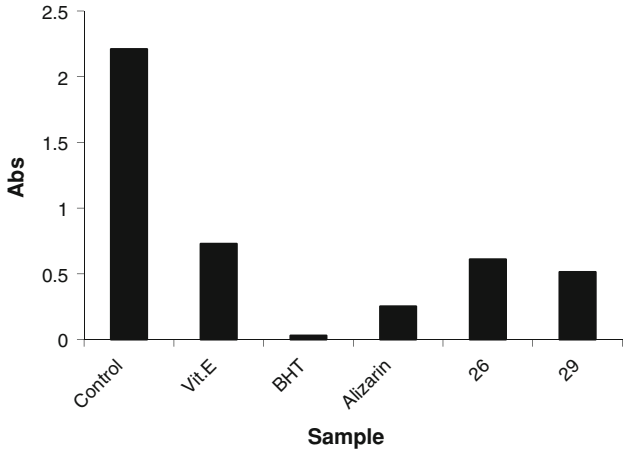


Fig. 3 Antioxidant activity using TBA method

anthraquinones and the standards. The absorbance value was measured on the final day (8th day) of FTC assay. The results were found to be consistent with the results of the assay using FTC method. Anthraquinones containing *ortho*-dihydroxy moiety exhibited antioxidant activity, e.g. alizarin. The presence of formyl group next to a hydroxyl in anthraquinone derivative also plays an important role in their antioxidant activity (**26** and **29**).

Experimental

Melting points were determined on a hot stage melting point apparatus XSP-12 model 500X and are uncorrected. UV spectra were recorded on a CARY 100 Conc UV–Visible spectrophotometer in $CHCl_3$ or MeOH. IR spectra were recorded on a Perkin Elmer RXI FT-IR spectrometer as KBr disc. Mass spectra were measured on Finnigan Mat SSQ 710 spectrometer with ionization induced by electron impact at 70 eV. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using Varian 500 MHz NMR spectrometer. Column chromatography was performed on Silica gel 60 Merck 9385 (230–400 mesh ASTM).

General procedure for anthraquinone synthesis

A mixture of anhydrous aluminium chloride (60 g, 450 mmol) and sodium chloride (12 g, 205 mmol) was heated until it completely melted (external temperature, 125–130 °C). Phthalic anhydride (6.7 g, 45 mmol) and benzene derivative (41 mmol) were mixed well and slowly introduced into the melt of aluminium chloride and sodium chloride. The mixture was heated with stirring at 165–170 °C for 45 min. After cooling, the reaction mixture was decomposed by adding a mixture of ice and hydrochloric acid. The acidic mixture was then briefly heated under reflux and filtered after cooling. The crude residue was extracted with ethyl acetate and the compounds were purified from ethyl acetate extract by using column chromatography.

*3-Bromo-1-hydroxyanthraquinone (**1**), 4-bromo-1-hydroxyanthraquinone (**2**), 3-bromo-2-hydroxyanthraquinone (**3**), 1-hydroxyanthraquinone (**4**) and 2-hydroxyanthraquinone (**5**)*

Phthalic anhydride (6.7 g, 45 mmol) and 4-bromophenol (7.05 g, 41 mmol) were reacted according to the general procedure to produce **1** (2.97 g, 24 %), **2** (1.36 g, 11 %), **3** (0.99 g, 8 %), **4** (0.37 g, 4 %) and **5** (0.28 g, 3 %) with the total yield of 50 %.

*3-Bromo-1-hydroxyanthraquinone (**1**)* Yellow crystals; mp 188–189 °C; UV ($CHCl_3$) λ_{max} 403, 334, 284, 257 nm; IR (KBr disc) v 3437 (OH), 2931, 1674 (C=O, unchelated), 1638 (C=O, chelated), 1591 (C=C, aromatic), 1466, 1355, 1291, 1253, 1033 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.63 (1H, s, 1-OH), 8.34–8.30 (2H, m, H-5 & H-8), 7.96 (1H, d, $J = 2.0$ Hz, H-4), 7.87–7.85 (2H, m, H-6 & H-7), 7.52 (1H, d, $J = 2.0$ Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 188.4 (C-9), 181.6 (C-10), 163.2 (C-1), 135.2 (C-7), 134.8 (C-6), 134.3 (C-14), 133.3 (C-12), 133.2 (C-11), 131.8 (C-13), 127.9 (C-8), 127.3 (C-5), 127.1 (C-2), 123.1 (C-4), 115.3 (C-3); MS *m/z* (rel. int.) 304 ($[M + 2]^+$, 66), 302 (M^+, 63), 276 (7), 274 (6), 248 (11), 246 (14), 223 (18), 195 (15), 167 (21), 139 (100), 113 (14), 97 (33), 83 (29), 69 (96).

*4-Bromo-1-hydroxyanthraquinone (**2**)* Orange needles; mp 195–196 °C [lit. 197–198 °C, Dictionary of Organic Compounds (1965)]; UV ($CHCl_3$) λ_{max} 414, 330, 272, 255 nm; IR (KBr disc) v 3438 (OH), 1670 (C=O, unchelated), 1636 (C=O, chelated), 1590 (C=C, aromatic), 1447, 1410, 1348, 1244, 1115, 788 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.29 (1H, s, 1-OH), 8.31–8.28 (2H, m, H-5 & H-8), 7.90 (1H, d, $J = 9.0$ Hz, H-3), 7.85–7.81 (2H, m, H-6 & H-7), 7.17 (1H, d, $J = 9.0$ Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 188.3 (C-9), 181.4 (C-10), 163.2 (C-1), 144.1 (C-3), 135.4 (C-7), 134.3 (C-12), 134.2 (C-6), 132.3 (C-11), 130.2 (C-14), 128.0 (C-8), 126.8 (C-5), 125.4 (C-2), 118.0 (C-13), 113.4 (C-4); MS *m/z* (rel. int.) 304 ($[M + 2]^+$, 93), 302 (M^+, 100), 276 (14), 274 (15), 248 (20), 246 (21), 223 (5), 195 (5), 167 (6), 139 (69), 113 (12), 97 (6), 69 (28).

*3-Bromo-2-hydroxyanthraquinone (**3**)* Yellow amorphous compound; mp 262–263 °C [lit. 267–268 °C, Dictionary of Organic Compounds (1965)]; UV (MeOH) λ_{max} 465, 311, 284, 247 nm; IR (KBr disc) v 3413 (OH), 1668 (C=O, unchelated), 1572 (C=C, aromatic), 1336, 1304,

1278, 718 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.00 (1H, br.s, 2-OH), 8.24 (1H, s, H-1), 8.17–8.15 (2H, m, H-5 &H-8), 7.92–7.90 (2H, m, H-6 & H-7), 7.65 (1H, s, H-4); ^{13}C NMR (DMSO-d_6) δ 182.8 (C-10), 181.2 (C-9), 160.4 (C-2), 135.4 (C-6), 135.0 (C-7), 134.8 (C-13), 133.7 (C-11), 133.6 (C-12), 132.9 (C-1), 127.4 (C-5 & C-8), 126.6 (C-14), 117.5 (C-3), 113.5 (C-4); MS *m/z* (rel. int.) 304 ($[M + 2]^+$, 98), 302 (M^+, 100), 276 (19), 274 (18), 248 (17), 246 (18), 223 (15), 195 (18), 167 (21), 139 (89), 123 (14), 83 (36), 69 (94), 50 (25).

*1-Hydroxyanthraquinone (**4**)* Yellow-orange needles; mp 190–191 °C [lit. 195–196 °C, Dictionary of Natural Products (2003)]; UV ($CHCl_3$) λ_{max} 405, 330, 270, 253 nm; IR (KBr disc) *v* 3436 (OH), 1674 (C=O, unchelated), 1638 (C=O, chelated), 1592 (C=C, aromatic), 1452, 1259, 1227, 762 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.64 (1H, s, 1-OH), 8.35–8.32 (2H, m, H-5 & H-8), 7.86 (1H, dd, $J = 7.5$ & 1.0 Hz, H-4), 7.85–7.83 (2H, m, H-6 & H-7), 7.71 (1H, t, $J = 8.0$ & 7.5 Hz, H-3), 7.34 (1H, dd, $J = 8.0$ & 1.0 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 188.9 (C-9), 182.7 (C-10), 162.8 (C-1), 137.0 (C-3), 134.9 (C-7), 134.4 (C-6), 133.9 (C-12), 133.7 (C-14), 133.5 (C-11), 127.7 (C-8), 127.2 (C-5), 124.6 (C-2), 119.8 (C-4), 116.4 (C-13); MS *m/z* (rel. int.) 224 (M^+, 100), 196 (19), 168 (36), 139 (51), 113 (7), 98 (10), 84 (13), 70 (44), 50 (19).

*2-Hydroxyanthraquinone (**5**)* Yellow amorphous solid; mp 298–299 °C [lit. 302–303 °C, dictionary of natural products (2003)]; UV (MeOH) λ_{max} 373, 329, 270, 242 nm; IR (KBr disc) *v* 3370 (OH), 1672 (C=O, unchelated), 1578 (C=C, aromatic), 1341, 1305, 1281, 720 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.08 (1H, br.s, 2-OH), 8.18-8.16 (2H, m, H-5 & H-8), 8.10 (1H, d, $J = 8.5$ Hz, H-4), 7.91–7.88 (2H, m, H-6 & H-7), 7.51 (1H, d, $J = 2.5$ Hz, H-1), 7.25 (1H, dd, $J = 8.5$ & 2.5 Hz, H-3); ^{13}C NMR (DMSO-d_6) δ 183.4 (C-9), 181.9 (C-10), 163.8 (C-2), 135.9 (C-13), 135.3 (C-7), 134.7 (C-6), 133.9 (C-12), 133.8 (C-11), 130.6 (C-4), 127.3 (C-8), 127.2 (C-5), 125.9 (C-14), 122.3 (C-3), 112.9 (C-1); MS *m/z* (rel. int.) 224 (M^+, 100), 196 (35), 168 (35), 139 (78), 113 (9), 98 (7), 84 (12), 63 (21).

*1-Bromo-3-hydroxyanthraquinone (**6**)*

Reaction between phthalic anhydride (6.7 g, 45 mmol) and 3-bromophenol (7.05 g, 41 mmol) gave four products including **1** (3.96 g, 32 %), **2** (0.12 g, 1 %), **4** (0.46 g, 5 %) and **6** (1.36 g, 11 %). The total yield was 49 %.

*1-Bromo-3-hydroxyanthraquinone (**6**)* Yellow amorphous compound; mp 180–181 °C [lit. 187 °C, dictionary of organic compounds (1965)]; UV (MeOH) λ_{max} 366, 277, 243 nm; IR (KBr disc) *v* 3393 (OH), 1655 (C=O, unchelated), 1576 (C=C, aromatic), 1329, 1290, 1245, 715 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.50 (1H, br.s, 3-OH), 8.16–8.11 (2H, m, H-5 & H-8), 7.93–7.86 (2H, m, H-6 & H-7), 7.59 (1H, d, $J = 2.5$ Hz, H-4), 7.46 (1H, d, $J = 2.5$ Hz, H-2); ^{13}C NMR (DMSO-d_6) δ 182.3 (C-10), 180.6 (C-9), 162.6 (C-3), 138.2 (C-14), 135.5 (C-6), 134.8 (C-11), 134.5 (C-7), 132.8 (C-12), 128.1 (C-2), 127.6 (C-5), 126.9 (C-8), 124.2 (C-1), 122.9 (C-13), 114.3 (C-4); MS *m/z* (rel. int.) 304 ($[M + 2]^+$, 22), 302 (M^+, 24), 276 (11), 274 (11), 248 (8), 246 (9), 223 (6), 195 (14), 167 (17), 139 (100), 113 (13), 87 (12), 69 (23), 50 (38).

*1,3-Dihydroxyanthraquinone or xanthopurpurin (**7**)*

When phthalic anhydride (6.7 g, 45 mmol) and resorcinol (4.51 g, 41 mmol) were used as starting material, two products **7** (0.98 g, 11 %) and 3,3-di(2′,4′-dihydroxyphenyl)phthalide (2.29 g, 31 %) were found with total yield of 42 %.

*1,3-Dihydroxyanthraquinone or xanthopurpurin (**7**)* Yellow amorphous compound; mp 268 °C [lit. 268–270 °C, Dictionary of Natural Products (2003)]; UV (MeOH) λ_{max} 413, 280, 241 nm; IR (KBr disc) *v* 3394 (OH), 1630 (C=O), 1340, 1320, 1161, 779, 712 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.75 (1H, s, 1-OH), 11.35 (1H, br.s, 3-OH), 8.22–8.15 (2H, m, H-5 & H-8), 7.95–7.89 (2H, m, H-6 & H-7), 7.16 (1H, d, $J = 2.0$ Hz, H-4), 6.63 (1H, d, $J = 2.0$ Hz, H-2); MS *m/z* (rel. int.) 240 (M^+, 100), 212 (17), 184 (27), 155 (10), 128 (21), 106 (11), 92 (11), 77 (17), 69 (21), 51 (23).

*1,4-Dihydroxyanthraquinone or quinizarin (**8**)*

Reaction between phthalic anhydride (6.7 g, 45 mmol) and hydroquinone (4.51 g, 41 mmol) produced **8** (4.33 g) with 44 % yield.

*1,4-Dihydroxyanthraquinone or quinizarin (**8**)* Orange-red crystals; mp 197–198 °C [lit. 194 °C, Dictionary of Natural Products (2003)]; UV ($CHCl_3$) λ_{max} 476, 328, 280, 250 nm; IR (KBr disc) *v* 3437 (OH), 2926, 1630 (C=O, chelated), 1591 (C=C, aromatic), 1453, 1358, 1257, 1226, 790 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.92 (2H, s, 1-OH & 4-OH), 8.37–8.35 (2H, m, H-5 & H-8), 7.86–7.85 (2H, m, H-6 & H-7), 7.33 (2H, s, H-2 & H-3); ^{13}C NMR ($CDCl_3$) δ 187.2 (C-9 & C-10), 158.1 (C-1 & C-4), 134.8 (C-6 & C-7), 133.7 (C-11 & C-12), 129.6 (C-2 & C-3), 127.3 (C-5 & C-8), 113.0 (C-13 & C-14); MS *m/z* (rel. int.) 240 (M^+, 100), 212 (10), 183 (17), 155 (11), 128 (14), 102 (11), 77 (6).

*1-Hydroxy-4-methylanthraquinone (**9**)*

Reaction between phthalic anhydride (6.7 g, 45 mmol) and *p*-cresol (4.43 g, 41 mmol) gave compound **9** (4.39 g, 45 %).

*1-Hydroxy-4-methylanthraquinone (**9**)* Yellow crystals; mp 174–175 °C [lit. 175–176 °C, Dictionary of Organic compounds (1965)]; UV ($CHCl_3$) λ_{max} 416, 325, 271, 252 nm; IR (KBr disc) *v* 3435 (OH), 2929, 1638 (C=O), 1365, 1282, 1248, 789, 724 cm^{-1}; ^1H NMR ($CDCl_3$) δ13.20 (1H, s, 1-OH), 8.32–8.28 (2H, m, H-5 & H-8), 7.85–7.79 (2H, m, H-6 & H-7), 7.52 (1H, d, $J = 9.0$ Hz, H-2), 7.25 (1H, d, $J = 9.0$ Hz, H-3), 2.78 (3H, s, -CH_3); MS *m/z* (rel. int.) 238 (M^+, 100), 210 (12), 181 (34), 152 (25), 119 (6), 96 (12), 76 (24), 51 (12).

*1,3-Dihydroxy-2-methylanthraquinone or rubiadin (**10**)*

Reaction between phthalic anhydride (6.7 g, 45 mmol) and 2-methylresorcinol (5.08 g, 41 mmol) produced **10** (4.16 g) with 40 % yield.

*1,3-Dihydroxy-2-methylanthraquinone or rubiadin (**10**)* Yellow needles; mp 281–282 °C [lit. 280–283 °C, Leistner (1975)]; UV (MeOH) λ_{max} 411, 279, 245 nm; IR (KBr disc) *v* 3402 (OH), 1661 (C=O, unchelated), 1624 (C=O, chelated), 1591 (C=C, aromatic), 1338, 1310, 1122, 712 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.05 (1H, s, 1-OH), 11.18 (1H, s, 3-OH), 8.15–8.08 (2H, m, H-5 & H-8), 7.89–7.83 (2H, m, H-6 & H-7), 7.20 (1H, s, H-4), 2.03 (3H, s, -CH_3); MS *m/z* (rel. int.) 254 (M^+, 100), 226 (10), 197 (9), 152 (9), 115 (9), 76 (12).

*2-Methoxyanthraquinone (**11**)*

Sodium hydride (39 mg, 1.6 mmol) and 2 molar methyl iodide solution in *tert*-butyl methyl ether (4 mL, 8 mmol) were added successively into the solution of compound **5** (179 mg, 0.8 mmol) in dimethylformamide (10 mL) and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was poured into crushed ice and the product **11** (171 mg, 90 %) was purified by crystallization.

*2-Methoxyanthraquinone (**11**)* Yellow crystals; mp 194–196 °C [lit. 195–196 °C, Dictionary of Natural Products (2003)]; UV ($CHCl_3$) λ_{max} 371, 330, 269, 248 nm; IR (KBr disc) *v* 3075, 2987, 1675 (C=O, unchelated), 1591 (C=C, aromatic), 1330, 1304, 1081, 851, 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.33–8.30 (2H, m, H-5 & H-8), 8.28 (1H, d, $J = 8.5$ Hz, H-4), 7.81–7.78 (2H, m, H-6 & H-7), 7.76 (1H, d, $J = 2.5$ Hz, H-1), 7.30 (1H, dd, $J = 8.5$ & 2.5 Hz, H-3), 4.01 (3H, s, –OCH_3); MS *m/z* (rel. int.) 238 (M^+, 100), 209 (32), 195 (11), 180 (16), 167 (15), 152 (24), 139 (41), 113 (5), 89 (4), 63 (6).

*1-Hydroxy-4-methoxyanthraquinone (12) and 1,4-dimethoxyanthraquinone (**13**)*

Dimethyl sulphate (4 mL, 42.3 mmol) was added slowly into the mixture of **8** (96 mg, 0.4 mmol) and anhydrous potassium carbonate (1.6 g, 11.8 mmol) in dry acetone (40 mL) and the reaction mixture was refluxed for 6 h. The reaction mixture was then poured into crushed ice and filtered. The product **12** (62 mg, 60 %) and **13** (21 mg, 20 %) were purified by column chromatography.

*1-Hydroxy-4-methoxyanthraquinone or quinizarin 4-methyl ether (**12**)* Orange-red crystals; mp 188–189 °C [lit. 189 °C, Dictionary of Natural Products (2003)]; UV ($CHCl_3$) λ_{max} 458, 324, 274, 253 nm; IR (KBr disc) *v* 3436 (OH), 3068, 2928, 1630 (C=O), 1595 (C=C, aromatic), 1474, 1352, 1243, 1182, 1015, 785, 724 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.02 (1H, s, 1-OH), 8.32–8.29 (2H, m, H-5 & H-8), 7.84–7.76 (2H, m, H-6 & H-7), 7.43 (1H, d, $J = 9.5$ Hz, H-2), 7.35 (1H, d, $J = 9.5$ Hz, H-3), 4.05 (3H, s, –OCH_3); MS *m/z* (rel. int.) 254 (M^+, 74), 225 (100), 211 (17), 197 (28), 183 (27), 152 (30), 139 (16), 127 (21), 105 (7), 77 (8).

*1,4-Dimethoxyanthraquinone or quinizarin 1,4-dimethyl ether (**13**)* Yellow amorphous solid; mp 144–145 °C [lit. 143 °C, Dictionary of natural Products (2003)]; UV ($CHCl_3$) λ_{max} 424, 322, 250 nm; IR (KBr disc) *v* 2956, 1665 (C=O, unchelated), 1270, 1254, 1055, 976, 803 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.19–8.17 (2H, m, H-5 & H-8), 7.73–7.72 (2H, m, H-6 & H-7), 7.37 (2H, s, H-2 & H-3), 4.02 (6H, s, –OCH_3); MS *m/z* (rel. int.) 268 (M^+, 58), 239 (100), 221 (32), 193 (38), 181 (20), 165 (35), 152 (26), 126 (20), 105 (8), 77 (6).

*1-Methoxy-4-methylanthraquinone (**14**)*

Compound **14** (101 mg, 100 %) was formed when **9** (95 mg, 0.4 mmol) was methylated with dimethyl sulphate (2 mL, 21.2 mmol) and anhydrous potassium carbonate (0.8 g, 5.9 mmol). The reaction time was 22 h at reflux condition.

*1-Methoxy-4-methylanthraquinone (**14**)* Yellow crystals; mp 127–128 °C [lit. 128 °C, Dictionary of Organic Compounds (1965)]; UV ($CHCl_3$) λ_{max} 389, 321, 254 nm; IR

(KBr disc) ν 2970, 2928, 1670 (C=O, unchelated), 1593 (C=C, aromatic), 1325, 1255, 1037, 980, 724 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21–8.16 (2H, m, H-5 & H-8), 7.74–7.72 (2H, m, H-6 & H-7), 7.52 (1H, d, $J = 9.0$ Hz, H-2), 7.26 (1H, d, $J = 9.0$ Hz, H-3), 4.03 (3H, s, $-OCH_3$), 2.76 (3H, s, $-CH_3$); MS *m/z* (rel. int.) 252 (M^+, 100), 235 (27), 223 (80), 209 (35), 195 (14), 178 (29), 165 (64), 152 (60), 126 (5), 77 (6), 63 (5).

*4-Bromo-1,3-dihydroxy-2-methylanthraquinone (**15**)*

A mixture of compound **10** (203 mg, 0.8 mmol), *N*-bromosuccinimide (370 mg, 2.08 mmol) and benzoyl peroxide (20 mg) in carbon tetrachloride (50 mL) was refluxed for 24 h. The solvent was evaporated and the product was washed with warm water and then dissolved in a small amount of acetone. The solution was poured into crushed ice and the precipitates were collected by filtration. Column chromatography was used to purify the product **15** (159 mg, 60 %).

*4-Bromo-1,3-dihydroxy-2-methylanthraquinone (**15**)* Yellow-orange amorphous solid; mp 290–292 °C; UV ($CHCl_3$) λ_{max} 422, 335, 273, 250 nm; IR (KBr disc) ν 3409 (OH), 2931, 1672 (C=O, unchelated), 1618 (C=O, chelated), 1577 (C=C, aromatic), 1420, 1362, 1295, 1197, 829, 722 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.87 (1H, s, 1-OH), 8.32–8.28 (2H, m, H-5 & H-8), 7.82–7.80 (2H, m, H-6 & H-7), 7.15 (1H, s, 3-OH), 2.35 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$) δ 187.2 (C-9), 181.8 (C-10), 163.3 (C-1), 157.5 (C-3), 134.8 (C-7), 134.3 (C-6), 134.0 (C-12), 132.7 (C-11), 128.2 (C-14), 127.8 (C-8), 126.7 (C-5), 119.7 (C-2), 112.0 (C-13), 104.2 (C-4), 9.7 (CH_3); MS *m/z* (rel. int.) 334 ($[M + 2]^+$, 100), 332 (M^+, 98), 306 (16), 304 (16), 292 (17), 290 (17), 253 (67), 225 (86), 197 (28), 169 (16), 139 (26), 115 (15), 77 (6), 55 (5).

*3-Acetoxy-1-hydroxy-2-methylanthraquinone (**16**)*

Acetic anhydride (1.4 ml, 14.6 mmol) was added dropwise into the mixture of anhydrous potassium carbonate (0.8 g, 5.84 mmol) and compound **10** (370 mg, 1.46 mmol) in dry acetone (25 mL) and the reaction mixture was stirred for 20 h at room temperature. The mixture was then poured into crushed ice and filtered. The product **16** (431 mg, 100 %) was purified by crystallization.

*3-Acetoxy-1-hydroxy-2-methylanthraquinone (**16**)* Yellowish orange crystals; mp 190–191 °C [lit. 191 °C, Dictionary of Natural Products (2003)]; UV ($CHCl_3$) λ_{max} 409, 331, 285, 262, 248 nm; IR (KBr disc) ν 3435 (OH), 2964, 2930, 1764 (C=O, ester), 1667 (C=O, unchelated), 1618 (C=O, chelated), 1594 (C=C, aromatic), 1420, 1330, 1282, 1217, 1102, 1014, 781 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.13 (1H, s, 1-OH), 8.33–8.28 (2H, m, H-5 & H-8), 7.83-7.81 (2H, m, H-6 & H-7), 7.55 (1H, s, H-4), 2.42 (3H, s, $-COCH_3$), 2.22 (3H, s, $-CH_3$); MS *m/z* (rel. int.) 296 (M^+, 2), 254 (100), 236 (11), 226 (20), 208 (7), 197 (11), 180 (9), 152 (11), 115 (8).

*3-Acetoxy-1-methoxy-2-methylanthraquinone (**17**)*

Compound **17** (451 mg, 100 %) was formed when **16** (431 mg, 1.46 mmol) was methylated with dimethyl sulphate (4.9 ml, 51.1 mmol) and anhydrous potassium carbonate (2.01 g, 14.6 mmol) in dry acetone at reflux condition for 22 h.

*3-Acetoxy-1-methoxy-2-methylanthraquinone (**17**)* Pale yellow crystals; mp 173–174 °C [lit. 174 °C, Roberts *et al.*, (1997)]; UV ($CHCl_3$) λ_{max} 406, 336, 280, 260 nm; IR (KBr disc) ν 2940, 1764 (C=O, ester), 1673 (C=O, unchelated), 1582 (C=C, aromatic), 1330, 1279, 1195, 1099, 716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.30–8.24 (2H, m, H-5 & H-8), 7.86 (1H, s, H-4), 7.83–7.75 (2H, m, H-6 & H-7), 3.97 (3H, s, $-OCH_3$), 2.42 (3H, s, $-COCH_3$), 2.27 (3H, s, $-CH_3$); MS *m/z* (rel. int.) 310 (M^+, 8), 268 (100), 250 (84), 239 (56), 222 (48), 194 (39), 165 (44), 152 (38), 139 (22), 115 (12).

*1,3-Dimethoxy-2-methylanthraquinone (**18**)*

Compound **10** (400 mg, 1.57 mmol) was methylated with dimethyl sulphate (10.4 mL, 110 mmol) in the presence of anhydrous potassium carbonate (4.26 g, 31.4 mmol) at reflux condition for 22 h to form **18** (444 mg) as 100 % product.

*1,3-Dimethoxy-2-methylanthraquinone or rubiadin 1,3-dimethyl ether (**18**)* Yellow crystals; mp 160 °C [lit. 159–160 °C, Roberts *et al.* (1997)]; UV ($CHCl_3$) λ_{max} 352, 279, 240 nm; IR (KBr disc) ν 2941, 1668 (C=O, unchelated), 1577 (C=C, aromatic), 1325, 1287, 1135, 979, 718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.30–8.23 (2H, m, H-5 & H-8), 7.80–7.72 (2H, m, H-6 & H-7), 7.65 (1H, s, H-4), 4.04 (3H, s, $-OCH_3$), 3.93 (3H, s, $-OCH_3$), 2.29 (3H, s, $-CH_3$); MS *m/z* (rel. int.) 282 (M^+, 100), 264 (43), 253 (36), 236 (42), 221 (30), 193 (31), 181 (39), 165 (44), 152 (44), 139 (24), 111 (10), 83 (14), 57 (10).

*3-Acetoxy-2-bromomethyl-1-methoxyanthraquinone (**19**)*

Compound **19** (376 mg, 100 %) was formed when compound **17** (300 mg, 0.97 mmol) was brominated with *N*-bromosuccinimide (329 mg, 1.84 mmol) and benzoyl peroxide (30 mg) in carbon tetrachloride at reflux condition for 24 h.

3-Acetoxy-2-bromomethyl-1-methoxyanthraquinone ***(19)*** Yellow solid; mp 165–166 °C [lit. 169–170 °C, Hirose (1960)]; UV ($CHCl_3$) λ_{max} 335, 283, 262 nm; IR (KBr disc) *v* 3069, 2929, 1775 (C=O, ester), 1677 (C=O, unchelated), 1578 (C=C, aromatic), 1327, 1285, 1136, 1076, 987, 916 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.29–8.23 (2H, m, H-5 & H-8), 7.95 (1H, s, H-4), 7.81–7.77 (2H, m, H-6 & H-7), 4.62 (2H, s, $–CH_2Br$), 4.11 (3H, s, $–OCH_3$), 2.47 (3H, s, $–COCH_3$); MS *m/z* (rel. int.) 390 ($[M + 2]^+$, 1), 388 (M^+, 1), 309 (17), 267 (100), 238 (17), 209 (11), 181 (16), 152 (21), 139 (15), 83 (10).

2-Bromomethyl-1,3-dimethoxyanthraquinone (20) and 2-dibromomethyl-1,3-dimethoxyanthraquinone ***(21)***

Compound **18** (400 mg, 1.41 mmol) was brominated with *N*-bromosuccinimide (479 mg, 2.68 mmol) in the presence of benzoyl peroxide (40 mg) to form compounds **20** (398 mg, 78 %) and 21 (56 mg, 9 %).

2-Bromomethyl-1,3-dimethoxyanthraquinone ***(20)*** Yellow crystals; mp 154–155 °C; UV ($CHCl_3$) λ_{max} 366, 340, 280, 244 nm; IR (KBr disc) *v* 2939, 1670 (C=O, unchelated), 1578 (C=C, aromatic), 1330, 1288, 1231, 1161, 1112, 987, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.31–8.25 (2H, m, H-5 & H-8), 7.83–7.75 (2H, m, H-6 & H-7), 7.70 (1H, s, H-4), 4.72 (2H, s, $–CH_2–$), 4.13 (3H, s, $–OCH_3$), 4.11 (3H, s, $–OCH_3$); MS *m/z* (rel. int.) 362 ($[M + 2]^+$, 2), 360 (M^+, 3), 281 (100), 267 (6), 236 (7), 223 (4), 181 (5), 165 (7), 152 (9), 139 (6), 76 (2).

2-Dibromomethyl-1,3-dimethoxyanthraquinone ***(21)*** Yellow crystals; mp 145–146 °C; UV ($CHCl_3$) λ_{max} 369, 339, 280, 245 nm; IR (KBr disc) *v* 3042, 2933, 1674 (C=O, unchelated), 1577 (C = C, aromatic), 1331, 1287, 1130, 984, 712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.31–8.26 (2H, m, H-5 & H-8), 7.85–7.76 (3H, m, H-4, H-6 & H-7), 7.42 (1H, s, $–C\underline{H}Br_2$), 4.26 (3H, s, $–OCH_3$), 4.05 (3H, s, $–OCH_3$); MS *m/z* (rel. int.) 442 ($[M + 4]^+$, <1), 440 ($[M + 2]^+$, 1), 438 (M^+, <1), 361 (100), 359 (99), 280 (65), 251 (50), 222 (12), 194 (13), 165 (16), 151 (15), 138 (9), 76 (4).

2-Bromomethyl-1-hydroxy-3-methoxyanthraquinone ***(22)***

Anhydrous aluminium chloride (167 mg, 1.12 mmol) was introduced into the solution of **20** (50 mg, 0.14 mmol) in dichloromethane (30 mL). Pyridine (0.04 mL, 0.56 mmol) was then added dropwise and the reaction mixture was refluxed for 24 h, acidified with dilute hydrochloric acid and extracted with ethyl acetate. The product **22** (33 mg, 68 %) was purified by column chromatography.

2-Bromomethyl-1-hydroxy-3-methoxyanthraquinone ***(22)*** Yellow crystals; mp 193–194 °C; UV ($CHCl_3$) λ_{max} 410, 338, 277, 247 nm; IR (KBr disc) *v* 3483 (OH), 2930, 1631 (C=O), 1599 (C=C, aromatic), 1376, 1335, 1303, 1134, 718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.15 (1H, s, 1-OH), 8.32–8.29 (2H, m, H-5 & H-8), 7.85–7.80 (2H, m, H-6 & H-7), 7.45 (1H, s, H-4), 4.89 (2H, s, $–CH_2Br$), 4.00 (3H, s, $–OCH_3$); MS *m/z* (rel. int.) 348 ($[M + 2]^+$, 1), 346 (M^+, 1), 284 (22), 269 (60), 267 (35), 255 (100), 253 (11), 238 (13), 223 (20), 208 (16), 181 (20), 152 (20), 139 (34), 77 (8).

2-Ethoxymethyl-3-hydroxy-1-methoxyanthraquinone ***(23)***

Compound **19** (376 mg, 0.97 mmol), ethanol (37 mL) and 10 % aq. NaOH (5 mL) were taken together and stirred the solution for 24 h at room temperature. Addition of water (125 mL) and 10 % aq. HCl (6 mL) into the reaction mixture gave precipitates which were filtered and dried. The compound **23** (227 mg, 75 %) was finally purified by column chromatography.

2-Ethoxymethyl-3-hydroxy-1-methoxyanthraquinone ***(23)*** Yellow solid; mp 201–202 °C [lit. 202–204 °C, Roberts *et al.* (1997)]; UV ($CHCl_3$) λ_{max} 371, 334, 277, 247 nm; IR (KBr disc) *v*3309 (OH), 2976, 2932, 1672 (C=O, unchelated), 1570 (C=C, aromatic), 1283, 1094, 977, 716 cm^{-1}; ^1H NMR (acetone-d_6) δ 9.90 (1H, s, 3-OH), 8.27–8.19 (2H, m, H-5 & H-8), 7.92–7.86 (2H, m, H-6 & H-7), 7.60 (1H, s, H-4), 4.79 (2H, s, $–CH_2O–$), 3.97 (3H, s, $–OCH_3$), 3.68 (2H, q, $J = 7.0$ Hz, $–O\underline{C}H_2CH_3$), 1.24 (3H, t, $J = 7.0$ Hz, $–OCH_2C\underline{H}_3$); MS *m/z* (rel. int.) 312 (M^+, 2), 297 (42), 283 (56), 265 (100), 251 (50), 238 (66), 210 (45), 181 (52), 152 (37), 139 (38).

1,3-Dimethoxy-2-ethoxymethylanthraquinone ***(24)***

Compound **24** (240 mg, 76 %) was formed when **20** (349 mg, 0.97 mmol) was reacted with ethanol (37 mL) and 10 % aq. NaOH (5 mL) at room temperature.

1,3-Dimethoxy-2-ethoxymethylanthraquinone ***(24)***

Yellow crystals; mp 139–140 °C [lit. 141–142 °C, Roberts *et al.* (1997)]; UV ($CHCl_3$) λ_{max} 364, 336, 277, 245 nm; IR (KBr disc) *v* 2929, 2858, 1674 (C=O, unchelated), 1579 (C=C, aromatic), 1459, 1316, 1283, 1133, 1093, 979, 714 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.30–8.23 (2H, m, H-5 & H-8), 7.80–7.74 (2H, m, H-6 & H-7), 7.69 (1H, s, H-4), 4.66 (2H, s, $–CH_2O–$), 4.06 (3H, s, $–OCH_3$), 4.02 (3H, s, $–OCH_3$), 3.66 (2H, q, $J = 7.0$ Hz, $–C\underline{H}_2CH_3$), 1.26 (3H, t, $J = 7.0$ Hz, $–OCH_2C\underline{H}_3$); MS *m/z* (rel. int.) 326 (M^+, 3), 311 (88), 297 (44), 283 (100), 281 (100), 267 (68), 237

(43), 209 (30), 181 (42), 165 (34), 152 (45), 139 (30), 83 (14).

*3-Acetoxy-2-ethoxymethyl-1-methoxyanthraquinone (**25**)*

Acetylation of compound **23** (227 mg, 0.73 mmol) with acetic anhydride (0.7 mL, 7.3 mmol) and anhydrous potassium carbonate (402 mg, 2.92 mmol) yielded compound **25** (257 mg, 100 %).

*3-Acetoxy-2-ethoxymethyl-1-methoxyanthraquinone (**25**)* Yellow amorphous compound; mp 165–166 °C; UV ($CHCl_3$) λ_{max} 338, 273, 259, 237 nm; IR (KBr disc) ν 2976, 2931, 2893, 1764 (C=O, ester), 1666 (C=O, unchelated), 1583 (C=C, aromatic), 1327, 1214, 1079, 972, 717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.32–8.26 (2H, m, H-5 & H-8), 7.92 (1H, s, H-4), 7.84–7.77 (2H, m, H-6 & H-7), 4.65 (2H, s, $-CH_2O-$), 4.03 (3H, s, $-OCH_3$), 3.56 (2H, q, $J = 7.0$ Hz, $-O\underline{CH_2}CH_3$), 2.41 (3H, s, $-COCH_3$), 1.23 (3H, t, $J = 7.0$ Hz, $-OCH_2C\underline{H_3}$); MS *m/z* (rel. int.) 354 (M^+, 1), 339 (4), 312 (30), 297 (18), 283 (34), 265 (100), 238 (64), 210 (36), 181 (30), 252 (20), 139 (22).

*2-Formyl-3-hydroxy-1-methoxyanthraquinone or damnacanthal (**26**)*

Compound 25 (164 mg, 0.46 mmol) was brominated with *N*-bromosuccinimide (157 mg, 0.88 mmol) in the presence of benzoyl peroxide (16 mg) and the brominated product was washed with warm water and dissolved in 80 % aqueous acetic acid (40 mL). The reaction mixture was refluxed for 24 h and poured into crushed ice. The precipitates were collected by filtration and compound 26 (107 mg, 82 %) was found after purification by column chromatography.

*2-Formyl-3-hydroxy-1-methoxyanthraquinone or damnacanthal (**26**)* Pale yellow crystals; mp 211 °C [lit. 211–212 °C, Hirose (1960)]; UV ($CHCl_3$) λ_{max} 389, 289, 254 nm; IR (KBr disc) ν 3432 (OH), 2957, 2927, 1670 (C=O, unchelated), 1648 (C=O, chelated), 1566 (C=C, aromatic), 1344, 1260, 1132, 980, 716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.31 (1H, s, 3-OH), 10.49 (1H, s, –CHO), 8.32–8.26 (2H, m, H-5 & H-8), 7.87–7.78 (2H, m, H-6 & H-7), 7.69 (1H, s, H-4), 4.15 (3H, s, $-OCH_3$); MS *m/z* (rel. int.) 282 (M^+, 5), 267 (12), 254 (100), 237 (16), 225 (56), 208 (24), 197 (24), 180 (18), 168 (20), 152 (32), 139 (36).

*4-Bromo-2-formyl-3-hydroxy-1-methoxyanthraquinone (**27**)*

Bromination of compound **23** (227 mg, 0.73 mmol) with *N*-bromosuccinimide (247 mg, 1.38 mmol) followed by hydrolysis with 80 % aq. acetic acid (60 mL) gave compound **27** (228 mg) with 80 % yield.

*4-Bromo-2-formyl-3-hydroxy-1-methoxyanthraquinone (**27**)* Yellowish orange compound; mp 219–220 °C; UV ($CHCl_3$) λ_{max} 402, 290, 261 nm; IR (KBr disc) ν 3433 (OH), 2938, 1655 (C=O, unchelated), 1625 (C=O, chelated), 1535 (C=C, aromatic), 1334, 1254, 1193, 994, 726 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.28 (1H, s, 3-OH), 10.46 (1H, s, –CHO), 8.24–8.20 (2H, m, H-5 & H-8), 7.82–7.80 (2H, m, H-6 & H-7), 4.17 (3H, s, $-OCH_3$); MS *m/z* (rel. int.) 362 ($[M + 2]^+$, 5), 360 (M^+, 10), 333 (94), 331 (100), 304 (30), 302 (36), 252 (79), 224 (86), 196 (28), 178 (24), 150 (36), 138 (80), 82 (47).

*1,3-Dimethoxy-2-formylanthraquinone (**28**)*

Bromination of compound **24** (240 mg, 0.74 mmol) with *N*-bromosuccinimide (252 mg, 1.41 mmol) followed by hydrolysis with 80 % aq. acetic acid (60 mL) formed compound **28** (174 mg, 80 %).

*1,3-Dimethoxy-2-formylanthraquinone or damnacanthal 3-methyl ether (**28**)* Yellow amorphous solid; mp 183–184 °C [lit. 184–185 °C, Roberts *et al.* (1997)]; UV (MeOH) λ_{max} 366, 329, 274, 243 nm; IR (KBr disc) ν 2940, 1673 (C=O, unchelated), 1578 (C=C, aromatic), 1320, 1283, 1135, 978, 714 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.54 (1H, s, $-C\underline{H}O$), 8.32–8.26 (2H, m, H-5 &H-8), 7.86–7.77 (2H, m, H-6 & H-7), 7.75 (1H, s, H-4), 4.12 (3H, s, $-OCH_3$), 4.08 (3H, s, $-OCH_3$); MS *m/z* (rel. int.) 296 (M^+, 39), 281 (100), 267 (56), 239 (79), 209 (31), 181 (39), 152 (34), 139 (39), 126 (18), 75 (7).

*1,3-Dihydroxy-2-formylanthraquinone or nordamnacanthal (**29**)*

Demethylation of compound **28** (82 mg, 0.28 mmol) with anhydrous aluminium chloride (334 mg, 2.24 mmol) gave **29** (52 mg) with 70 % yield.

*1,3-Dihydroxy-2-formylanthraquinone or nordamnacanthal (**29**)* Orange crystals; mp 217–218 °C [lit. 220–221 °C, Hirose (1960)]; UV ($CHCl_3$) λ_{max} 422, 292, 263, 249, 234 nm; IR (KBr disc) ν 3436 (OH), 2929, 1630 (C=O), 1331, 1192, 1108, 786, 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 14.09 (1H, s, 1-OH), 12.72 (1H, s, 3-OH), 10.53 (1H, s, –CHO), 8.36–8.31 (2H, m, H-5 & H-8), 7.88–7.85 (2H, m, H-6 & H-7), 7.37 (1H, s, H-4); MS *m/z* (rel. int.) 268 (M^+, 10), 240 (100), 212 (34), 184 (34), 155 (8), 138 (12), 128 (21), 83 (7), 77 (6).

Cytotoxic assay

Cell culture

The cancer cell lines were cultured in incubator with a 95 % humidified atmosphere containing 5 % CO_2 at 37 °C. Once cells reached 80 % confluency, the medium was removed and 1 mL of trypsin–EDTA (concentrated) was added to detach the cells from the flask. The cells were collected in a fresh medium as subculture. This subculturing procedure was done approximately every 3–4 days at a density of 0.64×10^6 cells/ml in a 25 cm^2 flask. Cell cultures were routinely checked for mycoplasmal contamination.

Assay procedure

Rapidly growing cells were harvested by mild trypsinization to detach them from the substratum of the culture flask and fresh medium was added to prepare suspensions of single cell. The cells were counted using the improved Newbauer haemacytometer. 4,000–5,000 cells in 180 μL media were transferred into each well of 96-well plates. The microtiter plates were incubated overnight for cell attachment. Subsequently, each well was added with 20 μL of sample solution diluted in RPMI 1640 medium. Final concentrations 0.1–100 μM for compounds were used with tenfold serial dilution. The DMSO concentration was maintained at 0.1 %. 20 μl of fresh medium alone was added to control wells to make the final volume of 200 μl and medium alone (without cells and sample) was used as blank. Each concentration of the sample was assayed in quadruplicate and the plate was incubated for 96 h. The fraction of surviving cells was determined relative to the untreated cell population by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method (Mosmann, 1983). 50 μl of 2 mg/mL MTT was added to each well after discarding the supernatant and the plates were incubated for 4 h at 37 °C in 95 % air and 5 % CO_2 for the formation of insoluble purple formazan. Then, the medium with MTT was aspirated and this was followed by the addition of 100 μL of DMSO to dissolve the insoluble formazan. The plates were then shaken for 10 min. The absorbance of the formazan solution was read at 550 nm and the percentage of cell viability is calculated using the formula:

$$\%\ \text{of Viability} = \frac{\text{OD of Sample} - \text{OD of Blank (media only)}}{\text{OD of Control} - \text{OD of Blank (media only)}} \times 100$$

For analysis of the results, 50 % inhibitory concentration (IC_{50}) was determined from the dose–response cytotoxic curves.

Antioxidant assays

Ferric thiocyanate (FTC) method

A screw-cap vial (ϕ 38 × 75 mm) containing a mixture of 2 mg of sample in 4 mL of 99.5 % ethanol, 4.1 mL of 2.51 % linoleic acid in 99.5 % ethanol, 8.0 mL of 0.02 M phosphate buffer (pH 7.0) and 3.9 mL of water was placed in an oven at 40 °C in the dark (Kikuzaki and Nakatani, 1993). 0.1 mL of this mixture in a test tube (ϕ 1.5 × 14.5 cm), 9.7 mL of 75 % (v/v) ethanol, 0.1 mL 30 % ammonium thiocyanate and finally, 0.1 mL of 2×10^{-2} M ferrous chloride in 3.5 % hydrochloric acid were added to the reaction mixture. Three minutes after the addition of ferrous chloride, the absorbance was measured at 500 nm. This step was repeated every 24 h until 1 day after the control reached its maximum absorbance value.

Thiobarbituric acid (TBA) method

The samples prepared for FTC method were used for this assay. To 1 mL of 20 % aqueous trichloroacetic acid and 2 mL of 0.67 % aqueous thiobarbituric acid (Mackeen *et al.*, 2000), 2 mL of the sample solution in a 10 mL centrifuge tube was added. The mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3,000 rpm for 30 min. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance of the final day of FTC assay.

All the assays were carried out in triplicate and the readings were averaged.

Physicochemical calculations

Evaluation on the lipophilicity, solubility, polar surface area and other 'rule-of-five' parameters on the anthraquinones was performed using the online tools Molsinspiration and ALOGPS2.1 (Tetko *et al.*, 2005; Veber *et al.*, 2002).

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