

Communication

## Total Synthesis, Cytotoxic Effects of Damnacanthal, Nordamnacanthal and Related Anthraquinone Analogues

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**Abstract:** Naturally occurring anthraquinones, damnacanthal (**1**) and nordamnacanthal (**2**) were synthesized with modified reaction steps and investigated for their cytotoxicity against the MCF-7 and K-562 cancer cell lines, respectively. Intermediate analogues 2-bromomethyl-1,3-dimethoxyanthraquinone (**5**,  $IC_{50} = 5.70 \pm 0.21$  and  $8.50 \pm 1.18$   $\mu\text{g/mL}$ ), 2-hydroxymethyl-1,3-dimethoxyanthraquinone (**6**,  $IC_{50} = 12.10 \pm 0.14$  and  $14.00 \pm 2.13$ ), 2-formyl-1,3-dimethoxyanthraquinone (**7**,  $IC_{50} = 13.10 \pm 1.02$  and  $14.80 \pm 0.74$ ), 1,3-dimethoxy-2-methylantraquinone (**4**,  $IC_{50} = 9.40 \pm 3.51$  and  $28.40 \pm 2.33$ ), and 1,3-dihydroxy-2-methylantraquinone (**3**,  $IC_{50} = 25.60 \pm 0.42$  and  $28.40 \pm 0.79$ ) also exhibited moderate cytotoxicity against MCF-7 and K-562 cancer cell lines, respectively. Other structurally related compounds like 1,3-dihydroxyanthraquinone (**13a**,  $IC_{50} = 19.70 \pm 0.35$  and  $14.50 \pm 1.28$ ), 1,3-dimethoxyanthraquinone (**13b**,  $IC_{50} = 6.50 \pm 0.66$  and  $5.90 \pm 0.95$ ) were also showed good cytotoxicity. The target compound damnacanthal (**1**) was found to be the most cytotoxic against the MCF-7 and K-562 cancer cell lines, with

IC<sub>50</sub> values of  $3.80 \pm 0.57$  and  $5.50 \pm 1.26$ , respectively. The structures of all compounds were elucidated with the help of detailed spectroscopic techniques.

**Keywords:** Anthraquinone; damnacanthal; nordamnacanthal; MCF-7; K-562; cytotoxic effects

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## 1. Introduction

Anthraquinone compounds, especially anthracyclines, have long been used as effective anticancer drugs. Depending on their chemical structure, anthraquinone drugs can kill tumor cells by diverse mechanisms, involving different initial intracellular targets that normally contribute to drug-induced toxicity [1–3]. Anthraquinones are known as “multipotent antioxidants”, as they are molecules that besides antioxidant activity possess additional pharmacological activities such as inhibition of platelet-aggregation or display antineoplastic and anticancer activities [4,5]. Many anthraquinones also display various biological activities such as antimicrobial, antifungal, hypotensive, analgesic, antimalarial [6–11], antileukemic, mutagenicity and anti-inflammatory properties [12–14]. Natural anthraquinones from *Damnacanthus subspinosus* and *Morinda parvifolia* have long been used in traditional medicine for the treatment of cancer [15]. The discovery of new compounds with antitumor activity has become one of the most important challenges in medicinal chemistry. The detail study on anthraquinones has revealed that a range of DNA-recognizing molecules that act as antitumor agents, including groove binders, alkylating and intercalator compounds. DNA intercalators have attracted particular attention because of their antitumor activity. For example, a number of acridine and anthracycline compounds are excellent DNA intercalators that are now on the market as chemotherapeutic agents [15,16]. Substituted anthraquinones such as rubiadin, subspinosin and morindaparvin are widely distributed in nature and are known to display various pharmacological activities [17–21]. Previously, we have reported the antitumor and anti-oxidant activities of anthraquinones isolated from *Morinda elliptica* [22]. Recently, we have also reported the cytotoxic and immunomodulatory effects of damnacanthal and nordamnacanthal against different cell lines [23,24]. Damnacanthal and nordamnacanthal were originally isolated from *Damnacanthus major* [25]. Previous studies on the synthesis of damnacanthal and nordamnacanthal were reported by Hirose, Roberts and Saha [26–28]. The current study describes the total synthesis of damnacanthal (**1**) and nordamnacanthal (**2**) with modified reaction steps, their cytotoxic activities of against MCF-7 and K-562 cancer cell lines and their structure activity relationships (SARs).

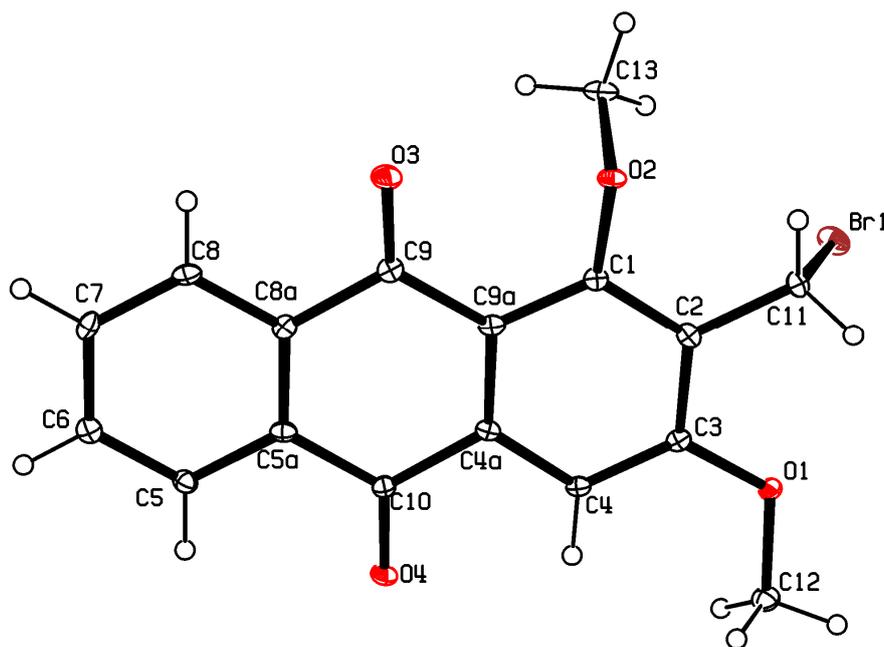
## 2. Results and Discussion

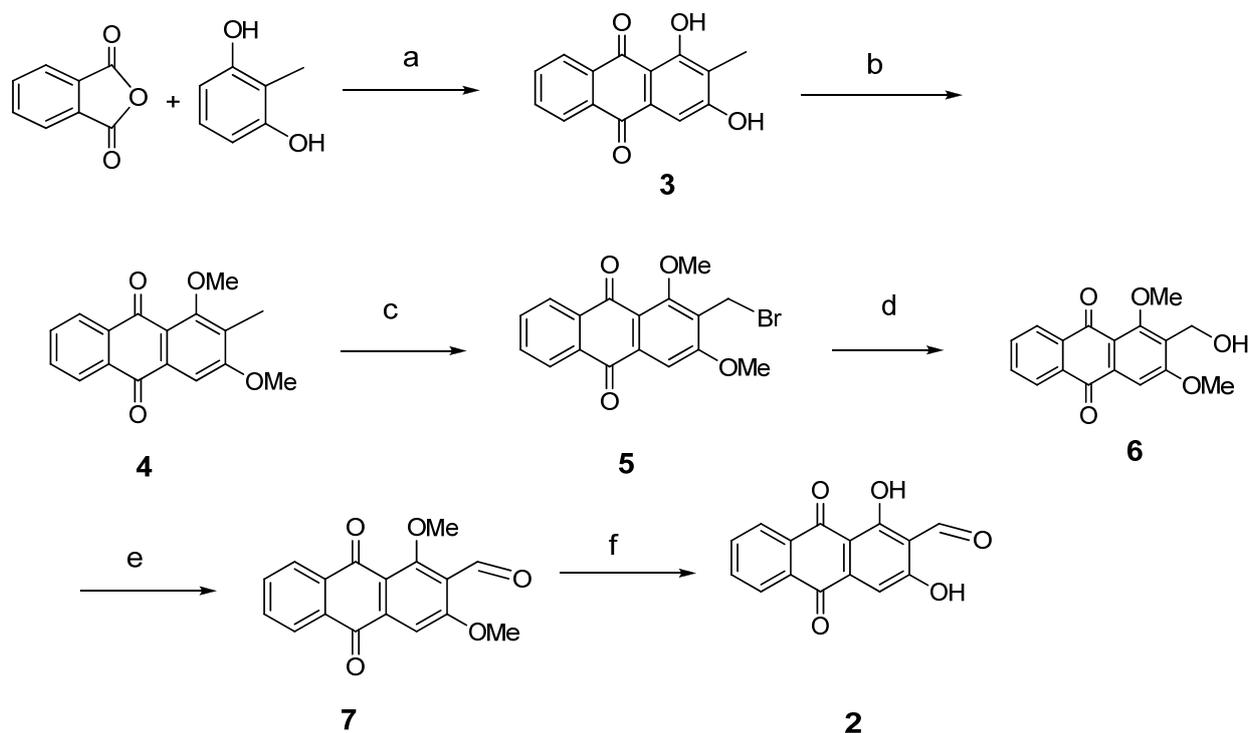
Anthraquinone skeletons are generally synthesized by Friedel-Crafts acylation condensation between phthalic anhydride and benzene derivatives [29]. 1,3-Dihydroxy-2-methylanthraquinone (**3**) was used as the common precursor for the synthesis of damnacanthal (**1**) and nordamnacanthal (**2**). This was synthesized by mixing of phthalic anhydride and 1,3-dihydroxy-2-methylbenzene in a molten mixture of AlCl<sub>3</sub>/NaCl [29,30].

The synthesis of nordamnacanthal (**2**) was accomplished by first acetylating the precursor compound **3** with acetic anhydride and potassium carbonate to afford the monoacetylated intermediate **8**. Upon methylation of compound **8** with  $K_2CO_3/(CH_3)_2SO_4$  in dry acetone to afford 1,3-dimethoxy-2-methylathraquinone (**4**), which was then brominated with Wohl-Ziegler's reagent (*N*-bromosuccinimide) in dry  $CCl_4$  to yield 2-bromomethyl-1,3-dimethoxyathraquinone (**5**) [25,31,32] and structure was confirmed by single X-ray diffraction (Figure 1). It is noteworthy that the use of a catalytic amount of benzoyl peroxide in this reaction gave 2-dibromomethyl-1,3-dimethoxyanthraquinone [26]. Compound **5** was hydrolyzed by refluxing it in acetic acid-water (8:2) to give the desired 2-hydroxymethyl-1,3-dimethoxyanthraquinone (**6**) in quantitative yield [26,31]. Compound **6**, which contains a hydroxymethyl moiety, was converted into the corresponding aldehyde **7** in 92.3% yield using a mild oxidizing agent [pyridinium chlorochromate (PCC) in dry  $CH_2Cl_2$  at 20–25 °C]. The use of an excess amount (1.5 equiv.) of PCC also gave the undesired 1-hydroxy-3-methoxyanthraquinone-2-carboxylic acid. Upon treating compound **7** with  $AlCl_3/CH_2Cl_2$ , nordamnacanthal (**2**) was obtained in 28% yield. The detailed reaction conditions are shown in Scheme 1.

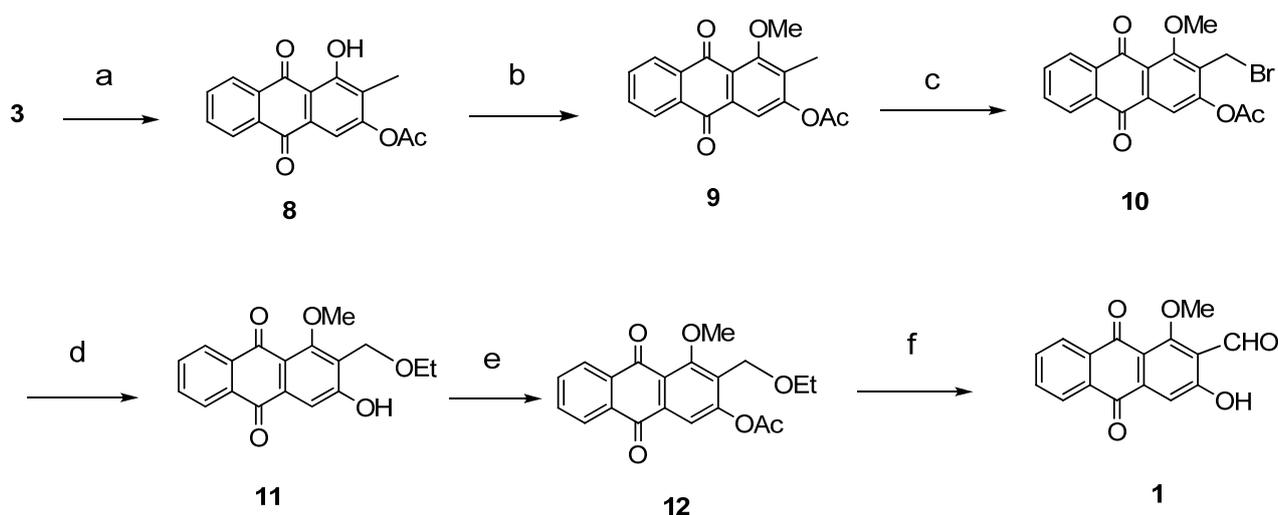
The synthesis of damnacanthal (**1**) was accomplished by first acetylated the precursor compound **3** with  $(CH_3)_2SO_4$  and potassium carbonate to afforded monoacetylated derivative **8**. Upon methylation of compound **8** with  $K_2CO_3/CH_3I$  compound **9** was obtained, which then brominated with NBS to yield 1-methoxy-3-aectoxy-2-bromomethyl-1-methoxyathraquinone (**10**) [25,32]. The bromo derivative **10** was converted into ethoxymethyl derivative **11** by dissolving it in a mixture of aq. NaOH/ethanol and followed by reflux in acidic media. Compound **11** was acetylated using acetic anhydride and  $K_2CO_3$  to afforded **12**, which on oxidation with PCC in  $CH_2Cl_2$  to give damnacanthal (**1**) in good yield. Thus the synthesis of damnacanthal (**1**) was achieved through modified steps as shown in Scheme 2.

**Figure 1.** The ORTEP diagram of 2-bromomethyl-1,3-dimethoxy-9,10-athraquinone (**5**).



**Scheme 1.** Reactions pathway for synthesis of nordamnacanthal (**2**).

*Reagents and conditions:* (a) anhydrous  $\text{AlCl}_3$ ,  $\text{NaCl}$ , 165–175 °C, 1 h; (b) Anhydrous  $\text{K}_2\text{CO}_3$  /  $(\text{CH}_3)_2\text{SO}_4$ , dry acetone, refluxed, 22 h; (c)  $\text{NBS}/\text{CCl}_4$  refluxed for 30 h; (d) 80% acetic acid refluxed 24 h; (e)  $\text{PCC}$ ,  $\text{CH}_2\text{Cl}_2$  stir. 2–4 h. rt; (f)  $\text{AlCl}_3$  /  $\text{CH}_2\text{Cl}_2$ ,  $\text{HCl}$ .

**Scheme 2.** Reactions pathway for synthesis of damnacanthal (**1**).

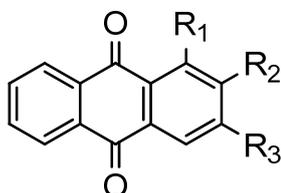
*Reagents and conditions:* (a) anhydrous  $\text{K}_2\text{CO}_3$  /  $\text{Ac}_2\text{O}$ , acetone; (b) anhydrous  $\text{K}_2\text{CO}_3$  /  $(\text{CH}_3)_2\text{SO}_4$  acetone, refluxed 22 h; (c)  $\text{NBS}/\text{CCl}_4$  refluxed 30 h; (d) 10% aq.  $\text{NaOH}/\text{EtOH}$ , aq.  $\text{HCl}$ ; (e)  $\text{Ac}_2\text{O}$  /  $\text{K}_2\text{CO}_3$  stirred for 24 h; (f)  $\text{PCC}$ ,  $\text{CH}_2\text{Cl}_2$  stir 2–4 h. rt.

For the SARs additional anthraquinone analogous **13a–h** were synthesized by Friedel-Crafts acylation of phthalic anhydride with resorcinol or catechol to yielded respective anthraquinones [29,30] as

shown in Figure 2. All compounds showed significant *in vitro* cytotoxicity against two cancer cell lines, indicating that anthraquinone is an interesting class of compounds for cancer therapy. The compound damnacanthal (**1**) contains methoxy, formyl and hydroxyl groups at the 1, 2 and 3 positions that might be important for the cytotoxicity since the standard drug doxorubicin [33] also possesses an anthraquinone moiety. However, nordamnacanthal (**2**), **3**, **8**, **13a**, **13c**, **13f** and **13h** exhibited less cytotoxic effects on both the MCF-7 and K-562 cell lines, suggesting that a protected OH group at position 1 and 3 increase the solubility and might make it easy for the compounds to diffuse across the cellular membrane as investigated by a theoretical study of 188 drug-like compounds by MI-QSAR analysis [34,35], while for poorly soluble drugs dissolution could be the rate limiting step in the absorption process. The methylated compound **13b** ( $IC_{50} = 24.25 \pm 2.46 \mu\text{M}$  and  $22.01 \pm 3.54 \mu\text{M}$ ) showed the stronger cytotoxicity than **13a** ( $IC_{50} = 82.08 \pm 1.46$  and  $60.42 \pm 5.33 \mu\text{M}$ ) and **13c** ( $IC_{50} = 87.80 \pm 2.01$  and  $64.96 \pm 1.57 \mu\text{M}$ ). Other derivatives **13e** ( $IC_{50} = 43.64 \pm 2.12$  and  $46.61 \pm 12.37$ ) and 2-hydroxymethylanthraquinone (**13d**) ( $IC_{50} = 49.58 \pm 12.61$  and  $50.84 \pm 14.12$ ) showed poor cytotoxicity as shown in Table 1. However, previously synthesized compounds **13f–h** with mono or dihydroxyl groups at the 1 and 2 positions exhibited moderate cytotoxic activity [36]. Further derivatives as exemplified by compounds **13c–h** indicated that this series of compounds only exhibited moderate activities not exceeding that of damnacanthal (**1**), suggesting that functional groups such as formyl at C-2, methoxy at C-1, hydroxyl groups at C-3 (damnacanthal) and bromomethyl at C-2 are important pharmacophores for the anticancer activity. However, the active compound **5** ( $IC_{50} = 15.83 \pm 0.58$  and  $23.61 \pm 3.28 \mu\text{M}$ ) might involve another mechanism like perfusion. The overall cytotoxic compounds (**1**, **5**, **13b**) showed more selectivity towards cancer cell lines because of their side chains and might fulfill the Lipinski rule-of-5 and drug-like properties.

**Figure 2.** Structure of compounds **13a** to **13h**.

	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<b>13a</b>	OH	H	OH
<b>13b</b>	OCH <sub>3</sub>	H	OCH <sub>3</sub>
<b>13c</b>	OH	H	OCH <sub>3</sub>
<b>13d</b>	H	CH <sub>2</sub> OH	H
<b>13e</b>	H	CHO	H
<b>13f</b>	OH	H	H
<b>13g</b>	OH	OH	H
<b>13h</b>	H	OH	H



**Table 1.** IC<sub>50</sub> values of compounds **1** to **13h** against MCF-7 and K-562 cell lines.

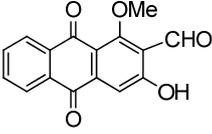
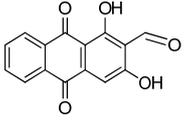
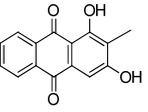
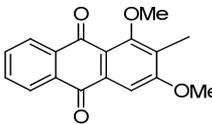
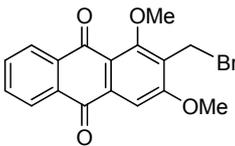
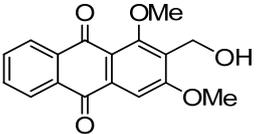
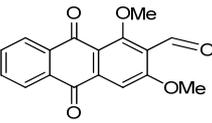
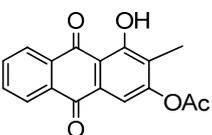
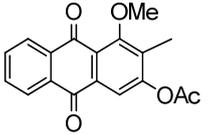
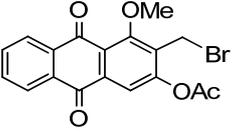
Compound No.	Chemical Structure	IC <sub>50</sub> value (μM)	
		MCF-7	K562
1		13.48 ± 2.02	19.50 ± 4.47
2		60.07 ± 6.46	32.46 ± 8.73
3		90.78 ± 1.49	100.71 ± 2.80
4		37.01 ± 13.82	87.81 ± 9.17
5		15.83 ± 0.58	23.61 ± 3.28
6		43.54 ± 0.48	47.62 ± 7.24
7		43.96 ± 3.42	49.66 ± 2.48
8		28.38 ± 7.94	69.59 ± 1.62
9		59.35 ± 8.23	46.77 ± 3.81
10		39.69 ± 6.24	54.90 ± 7.11

Table 1. Cont.

Compound No.	Chemical Structure	IC50 value ( $\mu\text{M}$ )	
		MCF-7	K562
11		59.13 $\pm$ 11.59	42.06 $\pm$ 6.39
12		43.62 $\pm$ 5.35	89.36 $\pm$ 3.83
13a	R <sub>1</sub> : OH, R <sub>2</sub> : H, R <sub>3</sub> : OH	82.08 $\pm$ 1.46	60.42 $\pm$ 5.33
13b	R <sub>1</sub> : OCH <sub>3</sub> , R <sub>2</sub> : H, R <sub>3</sub> : OCH <sub>3</sub>	24.25 $\pm$ 2.46	22.01 $\pm$ 3.54
13c	R <sub>1</sub> : OH, R <sub>2</sub> : H, R <sub>3</sub> : OCH <sub>3</sub>	87.80 $\pm$ 2.01	64.96 $\pm$ 1.57
13d	R <sub>1</sub> : H, R <sub>2</sub> : CH <sub>2</sub> OH, R <sub>3</sub> : H	49.58 $\pm$ 12.61	50.84 $\pm$ 14.12
13e	R <sub>1</sub> : H, R <sub>2</sub> : CHO, R <sub>3</sub> : H	43.64 $\pm$ 2.12	46.61 $\pm$ 12.37
13f	R <sub>1</sub> : OH, R <sub>2</sub> : H, R <sub>3</sub> : H	45.98 $\pm$ 2.95	107.14 $\pm$ 0.54
13g	R <sub>1</sub> : OH, R <sub>2</sub> : OH, R <sub>3</sub> : H	63.84 $\pm$ 12.46	70.54 $\pm$ 1.56
13h	R <sub>1</sub> : H, R <sub>2</sub> : OH, R <sub>3</sub> : H	52.50 $\pm$ 2.63	94.17 $\pm$ 1.88
Doxorubicin		0.94 $\pm$ 0.20	0.24 $\pm$ 0.07

### 3. Experimental

#### 3.1. General

Melting points were determined on a XSP-12 500X hot-stage melting point apparatus, and are uncorrected. UV spectra were recorded on a CARY 100 Conc. UV-visible spectrophotometer in MeOH. IR spectra were recorded on a Perkin-Elmer RXI Fourier Transform Infrared spectrometer (FTIR) as KBr discs. Mass spectra were measured on a Finnigan Mat SSQ 710 Spectrometer with ionization induced by electron impact at 70 eV. NMR spectra were recorded in CDCl<sub>3</sub> and acetone-*d*<sub>6</sub> using a Varian 500 MHz NMR spectrometer. Column chromatography was performed on silica gel (60 Merck 9385, 230–400 mesh, ASTM). The molecular structure of the intermediate 1,3-dimethoxy-2-

bromomethylantraquinone (**5**) was determined by X-ray crystallography. The data were collected at 100K using a Bruker APEXII with a CCD area-detector X-ray diffractometer. The structure was solved by direct method with SHELXS97 program and refined on F2 by full-matrix least-squares methods with anisotropic non-hydrogen atoms. The compound crystallizes in the monoclinic P2(1)/c space group with the crystallographic detail given in Table 2. The molecule exists as a planar molecule with r.m.s deviation of 0.018Å (Figure 1).

**Table 2.** Crystal data and structure refinement for 2-bromomethyl-1,3-dimethoxyanthraquinone (**5**).

Empirical formula	C <sub>17</sub> H <sub>13</sub> O <sub>4</sub> Br
Formula weight	361.18
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)/c
Unit cell dimensions	$a = 4.7401(8)\text{Å}$ , $\alpha = 90^\circ$ $b = 17.364(3)\text{Å}$ , $\beta = 94.543(2)^\circ$ $c = 16.825(3)\text{Å}$ , $\gamma = 90^\circ$
Volume	1380.5(4) Å <sup>3</sup>
Z	4
Calculated density	1.738 Mgm <sup>-3</sup>
Absorption coefficient	2.994 mm <sup>-1</sup>
F(000)	728
Crystal size	0.20 × 0.10 × 0.10 mm
θ range for data collection	1.69–28.26°
Limiting indices	−6 ≤ h ≤ 6, −14 ≤ k ≤ 23, −22 ≤ l ≤ 16
Reflections collected	3404
Independent reflections	2522 [R (int) = 0.0494]
Completeness to θ = 26.00°	99.40%
Absorption correction	Multi-scan
Max. and min. transmission	0.5973 and 0.7457
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	3404/0/201
Goodness-of-fit on F <sup>2</sup>	1.038
Final R indices [I > 2σ(I)]	R1 = 0.0417, wR2 = 0.0984
R indices (all data)	R1 = 0.0633, wR2 = 0.1124
Largest difference peak and hole	0.521 and −0.782 e.Å <sup>-3</sup>

### 3.2. General Procedure for Anthraquinone Synthesis

A mixture of anhydrous aluminium chloride (30 g, 225.0 mmol) and sodium chloride (12 g, 205.0 mmole) were melted at 125–130 °C. Phthalic anhydride (6.7 g, 45.0 mmole) and 2-methyl resorcinol (40.5 mmole) were mixed well and added slowly into the molten mixture of aluminum chloride and sodium chloride. The reaction temperature was raised till to 165–175 °C and maintained for 45–60 min. Another portion of anhydrous aluminum chloride (30 g, 225.0 mmole) was added into the hot mixture of the reactants [28]. After being cooled, the deep red solid product was decomposed

by adding a mixture of ice water (250 mL) and conc. hydrochloric acid (250 mL). The crude mixture was dissolved in distilled water and organic layer was extracted with ethyl acetate, washed with brine and dried over anhydrous sodium sulfate. The crude products were purified by flash silica gel column chromatography with elution of the ethyl acetate /hexane as yellow needles.

*1,3-Dihydroxy-2-methylantraquinone (3)*. Yellow needles: 65% yield: m.p. 280–282 °C; {lit. [27] 280–283 °C}. UV (nm) in MeOH: 408, 279, 251. IR (cm<sup>-1</sup>): 3402 (OH), 2930, (C-H), 1661 (C=O non-chelated), 1628, 1591 (C=C< aromatic), 1338, 1310, 1122, 712. <sup>1</sup>H-NMR (acetone-d<sub>6</sub>): δ 13.22 (s, 1H, 1-OH), 8.13 (dd, 1H, *J* = 1.5, 7.5 Hz, H-8), 8.23 (dd, 1H, *J* = 1.5, 7.5 Hz, H-5), 7.95–7.90 (m, 2H, H-6 & H-7), 7.37 (1H, s, H-4), 2.19 (3H, s, CH<sub>3</sub>). MS *m/z* (rel.int.): 254 (M<sup>+</sup>, 100), 226, 152 (10), 128 (21), 105 (11).

*2-Bromomethyl-1,3-dimethoxyanthraquinone (5)*. To a solution of compound **4** (400 mg, 1.4 mmole) in dry CCl<sub>4</sub> (40 mL), *N*-bromosuccinimide (500 mg, 3.0 mmole) was added portionwise. The whole mixture was stirred for 30 h. Compound **5** was extracted with ethyl acetate, filtered and purified by passing through a short column containing anhydrous sodium sulfate. The compound was recrystallized in a mixture of EtOAc and drops of MeOH as yellow crystals. Yield 90%; m.p.: 154–155 °C; {lit [27] 159–160 °C}. IR (cm<sup>-1</sup>): 2938 (CH), 1670 (C=O non-chelated), 1589 (C=C< aromatic), 1330, 1288, 1231, 1161, 730. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 8.30 (d, 1H, *J* = 8.0 Hz, H-8), 8.25 (d, 1H, *J* = 8.0 Hz, H-5), 7.80–7.75 (m, 2H, H-6 & H-7), 7.26 (s, 1H, H-4), 4.76 (s, 2H, -CH<sub>2</sub>Br), 4.05 (s, 3H, OCH<sub>3</sub>), 3.97 (s, 3H, OCH<sub>3</sub>). MS *m/z* (rel.int.): 361 (M<sup>+</sup>+H, 38) 362 (M<sup>+</sup>+2, 37), 281 (100), 267 (35), 253 (36), 236 (42), 221 (7), 105 (11), 223 (41), 77 (21).

*1,3-Dihydroxy-2-formylantraquinone (nordamnacanthal) (2)*. Compound **7** (150 mg, 0.5 mmole) was suspended in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL), stirred for 10 min. at rt and anhydrous AlCl<sub>3</sub> (500 mg, 3.7 mmole) was added and stirred for 1h and then reflux for 4–6 h. The reaction mixture was cooled and added mixture of ice HCl (250 mL) and water (250 mL). The products was extracted by EtOAc and purified by CC and followed by recrystallized from a mixture of MeOH/EtOAc as orange crystals. Yield 28%; m.p.: 217–218 °C; {lit. [25] 220–221 °C}. UV (nm) in CHCl<sub>3</sub>: 422, 292, 263, 250. IR (cm<sup>-1</sup>): 3436 (OH), 2928, 2927, (CH), 1630 (C=O), 1560 (>C=C< aromatic), 1331, 1108, 786, 715. <sup>1</sup>H-NMR (MeOD): δ 10.34 (s, 1H, CHO), 8.29 (d, 1H, *J* = 7.0 Hz, H-8), 8.25 (d, 1H, *J* = 7.0 Hz, H-5), 7.85–7.82 (m, 2H, H-6 & H-7), 7.29 (s, 1H, H-4). MS *m/z* (rel.int.): 268 (M<sup>+</sup>, 65) (10), 281 (100), 249 (100), 212 (34), 184 (32), 1197(24), 155 (7), 138 (12), 128 (21), 77 (12).

*2-Formyl-3-hydroxy-1-methoxyanthraquinone (1)*. Compound **12** (200 mg 0.56 mmole) was dissolved in dry dichloromethane (30 mL) and PCC (125 × 2 mg, 1.39 mmole) was introduced slowly in a stirred solution. The reaction mixture was stirred at room temperature for 4–6 h. A black brown solid was obtained, dissolved in aqueous solution and extracted with EtOAc, solvent removed *in vacuo* on a rotary evaporator and dried over anhydrous sodium sulfate. The product was purified by column chromatography using EtOAc/hexane as solvent system. The product was obtained as orange crystals. Yield 64.5%; m.p.: 209–211 °C; (lit. (25) 211–212 °C. IR (cm<sup>-1</sup>): 3434 (OH), 2957, 2927, (CH), 1670 (non-chelated C=O), 1648 (C=O non-chelated), 1566 (C=C< aromatic), 1344, 1260, 1231, 1132, 7310. UV (nm) in CHCl<sub>3</sub>: 412, 284, 264, 254. <sup>1</sup>H-NMR (acetone-d<sub>6</sub>): δ 10.45 (s, 1H, CHO), 8.32 (dd, 1H,

$J = 1.5, 7.5$  Hz, H-8), 8.26 (dd, 1H,  $J = 1.5, 7.5$  Hz, H-5), 7.87–7.78 (m, 2H, H-6 & H-7) 7.69 (s, 1H, H-4), 4.11 (s, 3H, OCH<sub>3</sub>). MS  $m/z$  (rel.int.): 282 (M<sup>+</sup>, 100) 267 (35), 254 (100), 237 (16), 225 (7), 197 (24), 180 (31), 168 (6) 152 (9), 139 (41).

### 3.3. X-ray Crystallography of Compound 5

A single crystal of compound **5** was obtained by slow evaporation at room temperature from a mixture of ethyl acetate-MeOH (drops). The crystal structure was solved by direct method with SHELXS97 programme and refined on F<sup>2</sup> by full-matrix least-squares methods with anisotropic non-hydrogen bond atoms. The compound crystallized as a monoclinic  $P2(1)/c$  space group with the crystallographic data is given in Table 2. The molecule exists as a planar molecule with r.m.s deviation of 0.018 Å (Figure 1). There is no evidence of any hydrogen bonding interaction or  $\pi$ - $\pi$  stacking present in the crystal packing of the compound. Full crystallographic data for structures **5** has been deposited at the Cambridge Crystallographic Data Center (CCDC) as supplementary publication number CCDC870566. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: þ44 1223 336033. email: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk].

## 4. Cytotoxicity Assay

### 4.1. Preparation of Compounds

The compounds were dissolved in dimethylsulfoxide (DMSO, Sigma, St. Louis., MO, USA) to get a stock solution of 1 mg/mL and stored at 4 °C.

### 4.2. Preparation of Cell Lines

Adherent human estrogen dependent breast carcinoma MCF-7 and suspension human chronic myelogenic leukemia K-562 cell lines were obtained from ATCC, USA. Both MCF-7 and K562 were cultured in RPMI-1640 supplemented with 10% FBS and incubated at 37 °C, 5% CO<sub>2</sub> and 90% humidity throughout the study. Cell viability was assessed by trypan blue exclusion method where both MCF-7 and K562 cell lines were collected, washed with PBS and mixed with 1:1 ratio of trypan blue dye and the cell number and viability were evaluated using haemocytometer under inverted light microscope. Only cell with viability higher than 95% will be subjected to MTT cell viability assay.

### 4.3. Cell Viability Assay

The effect of all compounds on cell viability of MCF-7 and K-562 cell lines were determined using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [37]. MCF-7 cell were seeded on a 96 well microtiter plate with each well containing 200 µL of culture media with 10% of FBS per well at a density of  $1 \times 10^5$  cells/mL and incubated overnight at 37 °C, 5% CO<sub>2</sub> at humidified atmosphere of 95% air. When cells were reached 70% confluence then subjected to the cell viability assay. Medium containing the seeded MCF-7 cell was discarded. Treatment of both cultures were carried out by first adding 100 µL of RPMI-1640 medium

supplemented with 10% of FBS into all wells except row A of the 96 well plates (TPP, Zollstrasse, Trasadingen, Switzerland). Then, 100  $\mu$ L of each diluted compound (60  $\mu$ g/mL) was added into row A and row B. A series of two-fold dilutions of compounds were carried out from row B to row G to give concentrations of 30, 15, 7.5, 3.75, 1.375 and 0.687  $\mu$ g/mL, while row H was left as untreated control cell. For K-562 culture plate, 100  $\mu$ L of cells ( $2 \times 10^5$  cells/mL) was added into all wells. For MCF-7 culture plate, 100  $\mu$ L of RPMI-1640 medium was added into all wells. The plates were incubated in 37  $^{\circ}$ C, 5% CO<sub>2</sub> and 90% humidity incubator for 72 h. After incubation period, 20  $\mu$ L of MTT (Sigma, 5 mg/mL) was added into all wells and the plates were further incubated for 4 h. Then, 170  $\mu$ L of supernatant was aspirated from every well plate. The plates containing K-562 culture were centrifuged at  $200 \times g$  for 5 min prior to medium aspiration. The resulting formazan crystals in each well were solubilized by 100  $\mu$ L of DMSO (Fisher Scientific, Waltham, MA, USA) followed by incubation for 20 min. Finally, the plates were read at 570 nm and 630 nm as reference wavelength by using  $\mu$ Quant ELISA Reader (Bio-tech Instruments, Winooski, VT, USA). The results of the compound-treated cells were compared with the standard doxorubicin. Each compound and control was assayed in triplicates in three independent experiments. The percentage of inhibitions were calculated by using Graph pad and expressed in  $\mu$ g/mL or  $\mu$ M and listed in Table 1.

## 5. Conclusions

The synthesis of damnacanthal (**1**) and nordamnacanthal (**2**) was achieved. The key difference from the previously synthesis was the conversion of hydroxymethylantraquinones **7** or **12** into the corresponding aldehyde, which was achieved by the use of optimized mild conditions with PCC as oxidant. The synthesis in good yield of 2-ethoxymethyl-3-hydroxy-1-methoxyanthraquinone (**11**) was achieved through a short sequence. The structure activity relationship suggested that methoxy, formyl, bromomethyl and hydroxyl groups are important for the cytotoxicity and selectivity in these substituted anthraquinones. These side chain modifications give an idea for further efforts to increase the therapeutic potential of this class of compounds.

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## Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability:* Samples of the compounds **1–13h** are available from the authors.

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