Synthesis and Bioactivities of Naturally Occurring Anthraquinones:
Isochrysophanol, Isozyganein, ω-Hydroxyisochrysophanol and Morindaparvin

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Anthraquinones, Antibacterial Activity of Anthraquinones, Cytotoxicity of Anthraquinones

Isochrysophanol, isozyganein, ω-hydroxyisochrysophanol, and morindaparvin are naturally occurring substituted anthraquinones. We report the synthesis of these compounds as well as selected biological activities.

Introduction

There are numerous natural products in which the anthraquinone moiety is the prominent part of the bioactive principle. Among them are the anthracyclines used in chemotherapy of solid tumors of breast, lung and bladder. Several reports on the syntheses and their biological effects appeared in the literature during the last two decades [1]. Isochrysophanol (1), isozyganein (2), ω-hydroxyisochrysophanol (6), and morindaparvin (7) are naturally occurring anthraquinones, isolated from *Digitalis purpurea*, *Cassia occidentalis*, [2] *Ploclama pendula* Ait, *Tectona grandis*, *Cassia alata* Linn, [3] *Digitalis davissiana* [4] and *Morinda parvifolia*, [5] respectively. Several syntheses of isochrysophanol (1) were reported previously which include a ring-closing reaction of 2'-hydroxy-3'-methyl-2-benzoyl-3-hydroxybenzoic acid in presence of boric acid and fuming sulphuric acid at 90 °C, [6] by partial demethylation of 1,8-dihydroxyanthraquinone with HBr in acetic acid followed by reaction with alkaline formaldehyde and demethylation with HBr in acetic acid, [7] by electroreduction of 2-acetoxymethyl-1,8-dihydroxy-9,10-anthraquinone, [8] from the diazonium salt of 1,8-diamino-2-methylantraquinone reacting it with H2SO4 [9] or via Diels-Alder reactions [10]. Several reports on the syntheses of isozyganein (2) appeared in the literature using also similar methods as described for isochrysophanol (1) [6,9,10], except one, where it was synthesized by reacting 5,8-dichloro-1-hydroxy-2-methylanthraquinone with sodium methoxide in methanol and copper-bronze in a sealed tube at 100–105 °C [11]. Two syntheses of ω-hydroxyisochrysophanol (6) were reported: the first one uses the above mentioned electroreduction method, [8] while in the other one it was synthesized from 1,8-dihydroxyanthraquinone in the presence of sodium dithionite and formaldehyde [12]. Morindaparvin (7) was synthesized via methylation of 1,5-dihydroxyanthraquinone to its dimethyl ether followed by partial demethylation with boron trifluoride; aldol condensation with alkaline formaldehyde and sodium dithionite yielded 1-hydroxy-2-hydroxy-methyl-5-methoxyanthraquinone and demethylation of 5-OMe finally gave 7 [13].

Vincomycin B2, another natural product produced by *Streptomyces matensis* subsp. *v ineus* hav-
ing anthraquinone functionality, is active against several Gram-positive bacteria and sarcoma 180 solid tumours in mice [14], and as partial structure of vineomycin B2, a total synthesis of vineomycin B2 methyl ester (5) was carried out by us starting from commercially available inexpensive anthrarufin (4) [15] using Negishi's methodology [16]. As most of the reported synthetic approaches for 1, 2, 6, and 7 require multistep sequences we describe in this communication efficient access to these anthraquinone-based natural products starting from commercially available 1,8-dihydroxy-9,10-anthraquinone (3) and 1,5-dihydroxy-9,10-anthraquinone (anthrarufin 4) also, in order to evaluate selected biological activities.

Results and Discussion

The syntheses of isochrysophanol (1) and \( \omega \)-hydroxyisochrysophanol (6) were started from commercially available 1,8-dihydroxy-9,10-anthraquinone (3). Accordingly, isozyganein (2) and morindaparvin (7) were synthesized from 1,5-di-

\[ \text{isozyganein (2)} \]

\[ \text{morindaparvin (7)} \]

\[ \text{Vineomycinone B2 methyl ester (5)} \]

\[ \text{\( \omega \)-Hydroxyisochrysophanol (6)} \]

\[ \text{Morindaparvin (7)} \]

hydroxy-9,10-anthraquinone (anthrarufin 4). In this sequence, 1,8-dihydroxy-9,10-anthraquinone (3) and 1,5-dihydroxy-9,10-anthraquinone (4) were converted to their bis-methoxymethyl derivatives 8 and 17, respectively, and reduced to the corresponding bis-methoxymethyl anthracenes 9 and 18, respectively. The \( \text{ortho} \)-directed metallation of these anthracenes, followed by stannyla-

\[ \text{isozyganein (2)} \]

\[ \text{morindaparvin (7)} \]

tion, produced the stable intermediates 10 and 19, which were converted into the \( \text{ortho} \)-idoanthracenes 11 and 20 in quantitative yields from which by reaction with methylzinc chloride in the presence of palladium catalyst [16], the \( \text{ortho} \)-methyl-substituted anthracenes [15] 12 and 21 were obtained. Oxidation of 12 and 21, respectively, by bis-

\[ \text{isozyganein (2)} \]

\[ \text{morindaparvin (7)} \]

pyridine silver permanganate [17] gave the anthraquinones 13 and 22; deprotection of the latter with a methanolic solution of acetyl chloride yielded the desired isochrysophanol (1) and isozyganein (2) (Scheme 1).

Metallation of the (methoxymethoxy)anthracenes 9 and 18 by \( n \)-butyllithium generated the corresponding lithio-anthracenes which were react with ethyl chloroformate to produce the \( \text{ortho} \)-substituted esters 14 and 23. 14 and 23 were reduced to the corresponding alcohols which were protected with ethyl vinyl ether (EVE) in dichlo-

\[ \text{isozyganein (2)} \]

\[ \text{morindaparvin (7)} \]

romethane in the presence of catalytic amounts of \( p \)-toluenesulphonic acid (PPTS) to the derivatives 15 and 24 and subsequently oxidized to the corresponding anthraquinones 16 and 25, respectively. Deprotection yielded \( \omega \)-hydroxyisochrysophanol (6) and morindaparvin (7) (Scheme 1).

All the synthesized compounds under study were screened for \textit{in vitro} antibacterial activity [18] against several Gram-positive and Gram-negative bacteria. The activity was determined via the growth inhibition of the microorganism \textit{i.e.} the zone of inhibition was measured in millimeters. Compounds with a zone of inhibition less than 10 millimeters were not considered as antibacterial agents. The results indicate that our compounds are generally weakly or moderately active. Isozyganein (2) was found to be slightly active against \textit{B. cereus}, \textit{S. aureus}, but showed significant activity against \textit{S. typhi}. Isochrysophanol (1) was active against four bacterial cultures: \textit{B. cereus}, \textit{K. pneumoniae}, \textit{S. typhi} and \textit{S. aureus}. \( \omega \)-Hydroxyisochrysophanol (6) and morindaparvin (7) showed moderate activity against \textit{B. aereus}, \textit{K. pneumoniae} and \textit{S. aureus}. 
The in vitro cytotoxic bioassays of compounds 1, 2, 6 and 7, using McLaughlin's brine shrimp lethality protocol, [19] showed no cytotoxic activity. An antioxidant assay of compounds 1, 2, 6 and 7 was also carried out which revealed that they act as weak 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavengers [20].

The present study suggests that ortho-substitution on simple 1,8-dihydroxy-9,10-anthraquinones and 1,5-dihydroxy-9,10-anthraquinones enhances...
the biological activity to a significant extent. This becomes obvious from the structure of vineomycin B2 with considerable antimicrobial activity, which has substituents at both neighbouring positions to the hydroxyl groups (2 and 6) of the anthraquinone moiety. Further modifications on these anthraquinone residues are under investigation to achieve structures with more pronounced activity.

**Experimental**

**General**

All reactions were carried out in dried glass apparatus under a static nitrogen atmosphere. Thin-layer chromatography was performed on precoated plates Silica gel 60 F254 (E. Merck, Darmstadt, Germany). Column chromatography was performed on Silica gel 60, 70–230 mesh (E. Merck, Darmstadt, Germany). 1H NMR spectra were recorded on a Bruker AM-500 spectrometer (Rheinstetten-Forchheim, Germany), operating at 500 MHz for 1H nuclei. 1H chemical shifts are reported in δ (parts per million) and coupling constants in Hertz. Infrared spectra were recorded on a JASCO IRA-1 spectrometer (JASCO International Co. Ltd. Tokyo, Japan). The electron impact (EIMS) mass spectra were performed on a Finnigan MAT 311A (Bremen, Germany) spectrometer. Microanalyses were measured by a Yanaco CHN Corder MT-5 (Tokyo, Japan) apparatus.

**1,8-Bis(methoxymethoxy)-9,10-anthraquinone (8) and 1,5-bis(methoxymethoxy)-9,10-anthraquinone (17)**

Compounds 8 and 17 were prepared from 1,8-dihydroxyanthraquinone (3) and anthrarufin (4), respectively, using our previously reported method [6]. Yields, physical and spectral data were identical to those reported in the literature [15].

**1,8-Bis(methoxymethoxy)anthracene (9) and 1,5-bis(methoxymethoxy)anthracene (18)**

These compounds were synthesized by the reported protocol [15] from anthraquinones 8 and 17, respectively, and physical and spectral data were identical to the published ones [15].

1,8-Bis(methoxymethoxy)-2-iodoanthracene (11) and 1,5-bis(methoxymethoxy)-2-iodo-anthracene (20)

The anthracenes 9 and 18 were subjected to monometallation according to the published procedure [15] and the lithioanthracenes were quenched with tributyltin chloride. After purification, the stannanes 10 and 19 were obtained which were converted into the iodoanthracenes 11 and 20 in quantitative yield and physical and spectroscopic data was comparable as described in lit. [15].

1,8-Bis(methoxymethoxy)-2-methylanthracene (12) and 1,5-bis(methoxymethoxy)-2-methyl-anthracene (21)

Anhydrous ZnCl2 (700 mg, 5.13 mmol) was heated to 100 °C under vacuum for 1 h and then dissolved in 10 ml of anhydrous THF. The solution was cooled to −10 °C and (4.1 ml, 4.1 mmol) of a 1 M solution of methyl lithium was added slowly. The reaction mixture was allowed to stir at room temperature for 1 h. In a separate flask, Pd(PPh3)2Cl2 (45 mg, 0.06 mmol) was stirred in 1 ml of THF and treated with DIBAL in hexane until a dark brown coloration was obtained. The methylchlorozinc solution was transferred to the catalyst solution via a cannula, followed by the addition of a solution of iodoanthracene (11) (88 mg, 0.20 mmol) in 5 ml of THF. The reaction mixture was stirred for 5 h. Aqueous work up followed by silica gel column chromatography using as eluent hexane-EtOAc (98:2) yielded 12 as a light yellow liquid (50 mg, 77%). - IR (neat): ν = 2920, 1630, 1540 cm⁻¹. - 1H NMR (500 MHz, CDCl3): δ = 9.09 (s, 1H, 10-H), 8.33 (s, 1H, 9-H), 7.70 (dd, J = 9.0, 1.8 Hz, 1H, 5-H), 7.61 (dd, J = 9.0, 9.0 Hz, 1H, 6-H), 7.38 (d, J = 9.0 Hz, 1H, 3-H), 7.01 (dd, J = 9.0, 1.8 Hz, 1H, 7-H), 5.47 (s, 2H, CH2), 5.27 (s, 2H, CH2), 3.77 (s, 3H, CH3), 3.59 (s, 3H, CH3); 2,52 (s, 3H, CH3). - MS (EI, 70 eV): m/z = 312. - C19H20O4 (312.37): calcd. C 73.06, H 6.45; found C 72.97, H 6.40.

In analogous manner, 1,5-bis(methoxymethoxy)-2-methylanthracene (21) was synthesized starting from iodoanthracene 20; yield (50 mg, 77%), light yellow liquid. - IR (neat): ν = 2960, 1630 cm⁻¹. - 1H NMR (500 MHz, CDCl3): δ = 8.82 (s, 1H, 9-H), 8.60 (s, 1H, 10-H), 7.79 (dd, J = 9.0, 1.8 Hz, 1H, 6-H), 7.68 (d, J = 9.0 Hz, 1H, 7-H), 7.39 (d, J = 9.0 Hz, 1H, 4-H), 7.27 (d, J = 9.0 Hz, 1H, 3-H), 7.03 (dd, J = 9.0, 1.8 Hz, 1H, 5-H), 5.47 (s, 2H, CH2), 5.25 (s, 2H, CH2), 3.73 (s, 3H, CH3), 3.59 (s, 3H, CH3), 2.53 (s, 3H, CH3). - MS
1.8-Bis(methoxymethoxy)-2-methyl-9,10-anthraquinone (13) and 1,5-bis(methoxymethoxy)-2-methyl-9,10-anthraquinone (22)

1.8-Bis(methoxymethoxy)-2-methylanthracene (12) was oxidized to the corresponding anthraquinone 13 using bis-pyridine silver permanganate as oxidizing agent [9]. Methylanthracene 12 (100 mg, 0.30 mM) was added to the permanganate reagent (810 mg, 2.0 mM), supported on 3.5 g silica gel 60, followed by addition of 15 ml of dried CH₂Cl₂. The reaction mixture was stirred for 5 h, filtered and the filter cake was washed thoroughly with CH₂Cl₂. The filtrate was evaporated and the residue was applied to a silica gel column and eluted with hexane-ETOAc (98:2) yielding methylquinone (22) as a light yellow liquid. - IR (neat): \( \nu = 3680, 3060, 1720, 1670, 1620, 1600, 1565 \text{ cm}^{-1} \). - \(^1\)H NMR (500 MHz, CDCl₃): \( \delta = 7.93 \) (dd, \( \mathbf{J} = 7.5, 1.5 \text{ Hz}, 1H, 5-H \)), 7.90 (d, \( \mathbf{J} = 7.5, 1 \text{ Hz}, 1H, 1-H \)), 7.61 (dd, \( \mathbf{J} = 7.5, 1.5 \text{ Hz}, 1H, 6-H \)), 7.53 (d, \( \mathbf{J} = 7.5, 1 \text{ Hz}, 1H, 3-H \)), 4.89 (dd, \( \mathbf{J} = 7.5, 1.5 \text{ Hz}, 1H, 7-H \)), 5.34 (s, 2H, CH₂), 5.17 (s, 2H, CH₂), 3.60 (s, 3H, CH₃), 3.55 (s, 3H, CH₃), 2.45 (s, 3H, CH₃). - MS ( EI, 70 eV): \( m/z = 342.35 \). - \( \text{C}_{19}\text{H}_{18}\text{O}_6 \) (342.35): calcd. C 68.10, H 5.99; found C 70.87, H 3.92.

Similarly, isozyganein (2) was prepared from 1,5-bis(methoxymethoxy)-2-methyl-9,10-anthraquinone (22) in the same quantities; yield: 70 mg, (quantitative), orange powder; m. p. 188–189°C. - IR (neat): \( \nu = 3680, 2920, 1720, 1605, 1585 \text{ cm}^{-1} \). - \(^1\)H NMR (500 MHz, CDCl₃): \( \delta = 7.81 \) (dd, \( \mathbf{J} = 7.5, 1 \text{ Hz}, 1H, 8-H \)), 7.75 (d, \( \mathbf{J} = 7.5, 1 \text{ Hz}, 1H, 4-H \)), 7.63 (dd, \( \mathbf{J} = 7.5, 7.5 \text{ Hz}, 1H, 7-H \)), 7.52 (d, \( \mathbf{J} = 7.5, 1 \text{ Hz}, 1H, 3-H \)), 7.28 (dd, \( \mathbf{J} = 7.5, 1 \text{ Hz}, 1H, 6-H \)), 2.37 (s, 3H, CH₃). - MS ( EI, 70 eV): \( m/z = 354.35 \). - \( \text{C}_{15}\text{H}_{10}\text{O}_4 \) (354.24): calcd. C 70.86, H 3.96; found C 70.87, H 3.92.

In analogous manner, 1,5-bis(methoxymethoxy)-2-methylanthracene (21) was oxidized to the corresponding anthraquinone 22. Methylanthracene 21 (100 mg, 0.302 mM) was added to the permanganate reagent (810 mg, 2.0 mM), supported on 3.5 g silica gel 60 (15 ml). The reaction mixture was stirred for 5 h, filtered and the filter cake was washed thoroughly with CH₂Cl₂. The filtrate was evaporated and the residue purified by silica gel column chromatography using as eluent hexane-ETOAc (98:2) yielding methylanthraquinone (22) (100 mg, 90%) as light yellow liquid. - IR (neat): \( \nu = 2920, 1675, 1630, 1590 \text{ cm}^{-1} \). - \(^1\)H NMR (500 MHz, CDCl₃): \( \delta = 7.97 \) (dd, \( \mathbf{J} = 8.0, 1.0 \text{ Hz}, 1H, 8-H \)), 7.91 (d, \( \mathbf{J} = 8.0, 800 \text{ Hz}, 1H, 4-H \)), 7.65 (dd, \( \mathbf{J} = 8.0, 800 \text{ Hz}, 1H, 7-H \)), 7.57 (d, \( \mathbf{J} = 8.0 \text{ Hz}, 1H, 3-H \)), 7.51 (dd, \( \mathbf{J} = 8.0, 1 \text{ Hz}, 1H, 6-H \)), 5.38 (s, 2H, CH₂), 5.15 (s, 2H, CH₂), 3.62 (s, 3H, CH₃), 3.55 (s, 3H, CH₃), 2.45 (s, 3H, CH₃). - MS ( EI, 70 eV): \( m/z = 342.35 \). - \( \text{C}_{19}\text{H}_{18}\text{O}_6 \) (342.35): calcd. C 68.02, H 5.9. - MS ( EI, 70 eV): \( m/z = 370.41 \). - \( \text{C}_{19}\text{H}_{20}\text{O}_6 \) (370.41): calcd. C 68.02, H 5.99; found C 68.02, H 5.9.

Ethyl-1,8-bis(methoxymethoxy)anthracene-2-carboxylate (14) and ethyl-1,5-bis(methoxymethoxy)anthracene-2-carboxylate (23)

1,5-Bis(methoxymethoxy)anthracene (9) (587 mg, 1 mMol) was dissolved in dried THF (20 ml) and cooled to –10°C. n-BuLi (0.63 ml, 1.6 M solution) was added and the reaction mixture stirred for 1 h. The lithioanthracene was reacted with ethyl chloroformate. The solvent was evaporated and the residue chromatographed using as eluent hexane-ETOAc (98:2) yielding the ester 14 (74%) as pale yellow liquid. – IR (neat): \( \nu = 3020, 1720, 1665, 1645, 1640, 1595, 1575, 1560 \text{ cm}^{-1} \). – \(^1\)H NMR (500 MHz, CDCl₃): \( \delta = 8.94 \) (s, 1H, 10-H), 8.81 (s, 1H, 9-H), 7.80 (dd, \( \mathbf{J} = 8.5, 1.5 \text{ Hz}, 1H, 5-H \)), 7.71 (dd, \( \mathbf{J} = 8.5, 8.5 \text{ Hz}, 1H, 6-H \)), 7.41 (d, \( \mathbf{J} = 8.5 \text{ Hz}, 1H, 4-H \)), 7.37 (d, \( \mathbf{J} = 8.5 \text{ Hz}, 1H, 3-H \)), 7.08 (dd, \( \mathbf{J} = 8.5, 1.5 \text{ Hz}, 1H, 7-H \)), 5.46 (s, 3H, CH₂), 5.35 (s, 3H, CH₂), 4.44 (q, \( \mathbf{J} = 7.5 \text{ Hz}, 2H \)), 3.67 (s, 3H, CH₃), 3.58 (s, 3H, CH₃), 1.44 (t, \( \mathbf{J} = 7.5 \text{ Hz}, 3H \)). – MS ( EI, 70 eV): \( m/z = 370.34 \). – \( \text{C}_{21}\text{H}_{22}\text{O}_6 \) (370.41): calcd. C 68.10, H 5.99; found C 68.02, H 5.9.

The anthracene ester 23 was synthesized in analogous manner; yield (75%); pale yellow liquid. –
**1H NMR** (500 MHz, CDCl₃): δ = 9.11 (s, 1H, 9-H), 8.34 (s, 1H, 10-H), 7.78 (dd, J = 9.0, 1.8 Hz, 1H, 8-H), 7.65 (dd, J = 9.0, 9.0 Hz, 1H, 7-H), 7.35 (d, J = 9.0 Hz, 1H, 4-H), 7.26 (d, J = 9.0 Hz, 1H, 3-H), 7.01 (dd, J = 9.0, 1.8 Hz, 1H, 6-H), 5.45 (s, 2H, CH₂), 5.34 (s, 2H, CH₂), 4.42 (q, J = 7.5 Hz, 2H), 3.65 (s, 3H, CH₃), 3.58 (s, 3H, CH₃), 1.40 (t, J = 7.5 Hz, 3H). – MS (EI, 70 eV): m/z = 370. – C₂₁H₂₂O₆ (370.41): calcd. C 68.1, H 5.95.

**1,8-Bis(methoxymethoxy)-2-(ethoxyethoxy)methylanthracene** (15)

Ester 14 (370 mg, 1 mmol) was dissolved in THF and cooled to −10 °C. To this solution a 1 M solution of DIBAL in hexane (5 ml) was slowly added. After aqueous work-up, the crude product was dissolved in CH₂Cl₂, 50 mg of p-toluenesulphonic acid (PPTS) and then ethyl vinyl ether (1 ml) was added dropwise under stirring to the reaction mixture, monitoring the reaction by TLC. After completion within 45 min, the reaction was quenched with aqueous NaHCO₃ and worked up. The solvent was evaporated and the residue applied to a silica gel column and eluted with hexane-EtOAc (98:2); yield 180 mg (75%) as a pale yellow liquid. – IR (neat): v = 2880, 1682, 1660, 1560 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 9.13 (s, 1H, 10-H), 8.36 (s, 1H, 9-H), 7.79 (dd, J = 8.5, 1.6 Hz, 1H, 5-H), 7.62 (dd, J = 8.5, 8.5 Hz, 1H, 6-H), 7.53 (d, J = 8.5 Hz, 1H, 4-H), 7.39 (d, J = 8.5 Hz, 1H, 3-H), 7.02 (dd, 1H, J = 8.5, 1.6 Hz, 7-H), 5.47 (s, 2H, CH₂), 5.29 (s, 2H, CH₂), 4.91 (s, 2H, CH₂), 4.68–4.61 (4H, m), 4.41 (q, J = 7.0 Hz, 2H, OCH₂CH₃), 3.76 (s, 3H, CH₃), 3.59 (s, 3H, CH₃), 1.24 (t, J = 7.0 Hz, 3H, CH₃). – MS (EI, 70 eV): m/z = 400. – C₂₃H₂₈O₆ (400.48): calcd. C 68.98, H 7.05; found C 68.91, H 7.00.

**1,5-Bis(methoxymethoxy)-2-(ethoxyethoxy)methylanthracene** (24)

24 was prepared from 23 in an analogous way as described for 15 as pale yellow liquid (180 mg, 75%). – IR (neat): v = 2920, 2880, 1660, 1630, 1540 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 8.83 (s, 1H, 9-H), 8.64 (s, 1H, 10-H), 7.85 (dd, J = 9.0, 1.7 Hz, 1H, 8-H), 7.67 (dd, J = 9.0, 9.0 Hz, 1H, 7-H), 7.36 (d, J = 9.0 Hz, 1H, 4-H), 7.25 (d, J = 9.0 Hz, 1H, 3-H), 7.03 (dd, J = 9.0, 1.7 Hz, 1H, 6-H) 5.46 (s, 2H, CH₂), 5.27 (s, 2H, CH₂), 4.95 (s, 2H, CH₂), 4.84–4.75 (m, 4H), 4.45 (q, J = 7.5 Hz, 2H, OCH₂CH₃), 3.72 (s, 3H, CH₃), 3.59 (s, 3H, CH₃), 1.24 (t, J = 7.5 Hz, 3H, CH₃). – MS (EI, 70 eV): m/z = 400. – C₂₃H₂₈O₆ (400.48): calcd. C 68.98, H 7.05; found C 68.98, H 7.03.

**1,8-Bis(methoxymethoxy)-2-(ethoxyethoxy)methyl-9,10-anthraquinone** (16) and **1,5-bis(methoxymethoxy)-2-(ethoxyethoxy)methyl-9,10-anthraquinone** (25)

The anthraquinones 16 and 25 were obtained by oxidation of the anthracenes 15 and 24, respectively, using the procedure for the synthesis of anthraquinones 13 and 22.

**1,8-Bis(methoxymethoxy)-2-(ethoxyethoxy)methyl-9,10-anthraquinone** (16)

Thick yellow liquid (quantitative yield). – IR (neat): v = 2900, 1720, 1670, 1630, 1585 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 8.05 (dd, J = 8.0, 1.0 Hz, 1H, 5-H), 7.92 (d, J = 8.0 Hz, 1H, 4-H), 7.86 (dd, J = 8.0, 8.0 Hz, 1H, 6-H), 7.63 (d, J = 8.0 Hz, 1H, 3-H), 7.51 (dd, J = 8.0, 1.0 Hz, 1H, 7-H), 5.35 (s, 2H, CH₂), 5.20 (s, 2H, CH₂), 4.93 (s, 2H, CH₂), 4.84–4.78 (m, 4H), 4.46 (q, 2H, J = 7.5 Hz, OCH₂CH₃), 3.60 (s, 3H, CH₃), 3.56 (s, 3H, CH₃), 1.22 (t, J = 7.5 Hz, 3H, CH₃). – MS (EI, 70 eV): m/z = 430. – C₂₃H₂₈O₈ (430.46): calcd. C 64.18, H 6.09; found C 64.10, H 6.11.

**1,5-Bis(methoxymethoxy)-2-(ethoxyethoxy)methyl-9,10-anthraquinone** (25)

Thick yellow liquid (quantitative yield). – IR (neat): v = 2920, 2880, 1720, 1665, 1585 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 7.96 (dd, J = 8.0, 1.0 Hz, 1H, 8-H), 7.94 (d, J = 8.0 Hz, 1H, 4-H), 7.66 (dd, J = 8.0, 8.0 Hz, 1H, 7-H), 7.53 (d, J = 8.0 Hz, 1H, 3-H), 7.50 (dd, J = 8.0, 1.0 Hz, 1H, 6-H), 5.50 (s, 2H, CH₂), 5.35 (s, 2H, CH₂), 5.04 (s, 2H, CH₂), 4.84–4.78 (4H, m), 4.47 (q, J = 6.5 Hz, 2H, OCH₂CH₃), 3.82 (s, 3H, CH₃), 3.63 (s, 3H, CH₃), 1.27 (t, J = 6.5 Hz, 3H, CH₃). – MS (EI, 70 eV): m/z = 430. – C₂₃H₂₈O₈ (430.46): calcd. C 64.18, H 6.09; found C 64.09, H 6.06.

**ω-Hydroxyisochrysophanol** (6) and **morindaparvin** (7)

**ω-Hydroxyisochrysophanol** (6) and **morindaparvin** (7) were obtained via deprotection of anthraquinones 16 and 25 by stirring in methanol (10 ml) containing five drops of freshly distilled acetyl chloride for 1 h. The reaction was quenched with 1 ml of triethylamine. Methanol was evaporated and pure samples of **ω-hydroxyisochryso-**
phanol (6) and morindaparvin (7) were obtained after silica gel column chromatography with hexane-EtOAc (93:7) as an eluent.

6: 70 mg (quantitative yield) as yellow powder; m.p. 245–245 °C. – IR (neat): ν = 3640, 3320–3000 (broad), 1720, 1630, 1605, 1580 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 7.88 (dd, J = 7.5, 1.0 Hz, 1H, 5-H), 7.69 (dd, J = 7.5, 7.0 Hz, 1H, 6-H), 7.53 (d, J = 7.5 Hz, 1H, 3-H), 7.32 (dd, 1H, J = 7.5, 1.0 Hz, 7-H), 4.87 (s, 2H, CH₂). – MS (EI, 70 eV): m/z = 270. – C₁₅H₁₀O₅ (270.24): calcd. C 66.67, H 3.73; found C 66.65, H 3.67.

7: 70 mg (quantitative yield) of an orange yellow powder; m.p. 208–210 °C. – IR (neat): ν = 3660, 3520, 2920, 2870, 1720, 1650, 1635, 1595 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 8.02 (dd, J = 8.0, 1.0 Hz, 1H, 8-H), 7.80 (d, J = 8.0 Hz, 1H, 4-H), 7.68 (dd, J = 8.0, 8.0 Hz, 1H, 7-H), 7.54 (d, J = 8.0 Hz, 1H, 3-H), 7.25 (dd, 1H, J = 8.0, 1.0 Hz, 6-H), 4.78 (s, 2H, CH₂). – MS (EI, 70 eV): m/z = 270. – C₁₅H₁₀O₅ (270.24): calcd. C 66.67, H 3.73; found C 66.65, H 3.71.

Bioassays

Antibacterial activity

The disc diffusion technique was adopted to determine the antibacterial activity of the test compounds [18]. Sterile discs containing 500 mg of compound/disc were prepared. The sensitest agar (Oxoid, Hampshire, England) plates were seeded with 24 h old cultures, grown in trypticase soya broth (TSB; Oxoid) containing 10⁷ cfu ml⁻¹ (cfu: colony forming units), using sterile cotton swabs to obtain a confluent lawn. The prepared discs were placed on the surface at different positions and plates were incubated at 37 °C for 24 h. The results were recorded by measuring the zones of inhibition in mm against each compound.

Brine shrimp bioassay

Brine shrimp (Artemia salina leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial sea water, prepared from a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, Ohio, USA) and double-distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the smaller compartment was opened to ordinary light. After two days, nauplii from the lighted side were collected with a pipette. A sample of the test compound was prepared by dissolving 20 mg of each compound in methanol (2 ml). From this stock solution, 500, 50 and 5 mg/ml in methanol were transferred to 9 vials, three for each dilution, and one vial was kept as control having 2 ml of methanol only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 ml of sea water was added to each vial and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 ml per vial. After 24 h, the number of survivors was counted. Data were analyzed by a Finney computer program to determine the LD₅₀ [19].

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