Tetrahedron 66 (2010) 3912–3916

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Reaction of dihydrolipoic acid with juglone and related naphthoquinones: unmasking of a spirocyclic 1,3-dithiane intermediate en route to naphtho[1,4]dithiepines

Giorgia Greco, Lucia Panzella, Alessandro Pezzella, Alessandra Napolitano *, Marco d'Ischia

Department of Organic Chemistry and Biochemistry, University of Naples 'Federico II', Via Cintia 4, I-80126 Naples, Italy

ARTICLE INFO

Article history:
Received 27 November 2009
Received in revised form 10 March 2010
Accepted 29 March 2010
Available online 2 April 2010

Keywords:
Juglone
Dihydrolipoic acid
Spiro 1,3-dithiane
Naphthodithiepine

ABSTRACT

The reaction of dihydrolipoic acid (DHLA) with 5-hydroxy-1,4-naphthoquinone (juglone) gives rise to the novel naphtho[1,4]dithiepine derivatives through ring expansion of an unstable spirocyclic 1,3-dithiane intermediate, which was isolated and completely characterized. Reported herein is also the characterization of novel reaction products of DHLA with other naphthoquinones and the extension of the study to the spirocyclic adduct formed by reaction with a representative 2-substituted naphthoquinone.

1. Introduction

The synthetic and biomedical relevance of thiols and quinones and the manifold chemical issues associated with their conjugation reactions have elicited unabated interest over the past decades.[1–7] Recently, studies of thiol/quinone coupling and the properties of their products have gained increasing importance in the search for new structural motifs, which can enhance the antioxidant and antinitrosating activities of phenols.[8] In this regard, the effect of adjacent sulfur substituents on the H-atom donor properties of phenolic groups has received considerable attention.[9]

Despite the vast literature on thiol/quinone coupling, surprisingly little is known about the reaction behavior of dihydrolipoic acid ((*R*)-6,8-dithiooctanoic acid, DHLA). DHLA and its disulfide, α-lipoic acid, provide a well-known prosthetic group in the α-keto acid dehydrogenase complexes of the mitochondria.[10] Lipoic acid is also a strong endogenous antioxidant[11] and is capable of inhibiting the conjugation of nonenzymatically oxidized catecholamines with cysteine and glutathione in vitro,[12] suggesting formation of adducts. So far, the chemical reactivity of DHLA with quinones has remained virtually unexplored in spite of the many synthetic opportunities offered by the 1,3-dithiopropane group. The only insights into this issue stem from recent papers showing that DHLA, but not the parent disulfide, reacts with L-DOPA in the presence of tyrosinase to form 5-*S*-lipoyl-DOPA.[13] As part of our studies on thiol/quinone coupling as a viable biomimetic access to novel bioactive compounds for practical applications in medicinal and food chemistry and in cosmetics, we recently focused on the reaction of DHLA with juglone (5-hydroxy-1,4-naphthoquinone), a characteristic constituent of walnut husks, which has received considerable attention as a useful scaffold for the synthesis of bioactive compounds, e.g., antioxidants,[14] cysteine protease inhibitors,[15] and antitubercular,[16] and antimelanoma agents.[17] Studies of this chemistry were further prompted by the growing interest in sulfur-substituted naphthoquinones as bioactive compounds.[18] During this study we came across unexpected mechanistic features of this reaction which led to the discovery of a novel class of spirocyclic DHLA derivatives of potential chemical and practical interest. Reported herein is the characterization of the novel reaction products of DHLA with juglone and other naphthoquinones, the expedient isolation of the spirocyclic juglone/DHLA adduct, and the extension of the study to the spirocyclic adduct formed by reaction with a representative 2-substituted naphthoquinone.

2. Results and discussion

α-Lipoic acid possesses a stereocenter at C-6 and the *R*-isomer, which is synthesized naturally, was used in this study. DHLA was prepared fresh before use by $NaBH_4$ reduction of the commercial α-lipoic acid followed by extraction into an organic solvent.[19]

---

* Corresponding author. Tel.: +39 81674133; fax: +39 81674393; e-mail address: alesnapo@unina.it (A. Napolitano).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.03.103

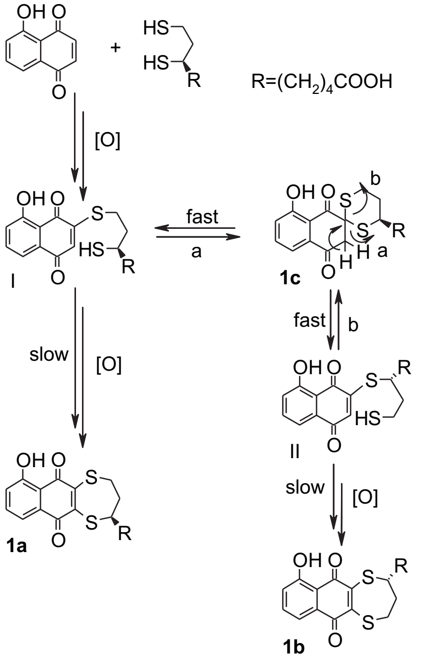
Addition of DHLA to an ethanol solution of juglone in the presence of triethylamine (TEA) as basic catalyst resulted in the fast development of a purple red coloration due to the formation of a main product ($[M+H]^+$ *m*/*z* 379), which was isolated and subjected to complete NMR spectroscopic characterization. The lack in the proton spectrum of the expected resonances for the H-2 and H-3 protons of juglone suggested a cyclic adduct in which both the SH functionalities of lipoic acid were linked to the 1,4-naphthoquinone unit forming a [1,4]dithiepine ring. Analysis of the ^{13}C NMR spectrum showed, unexpectedly, that some of the signals were present as a close pair, in particular those at δ 145.7/145.9 and δ 143.0/143.2, attributed to the C-2 and C-3 carbons of the naphthoquinone moiety, and those at δ 34.7/34.8 and 45.3/45.5, assigned to the C-5 and C-6 carbons of the lipoic acid unit. Based on this observation, it was concluded that the isolated product was in fact an intimate 1:1 mixture of two closely related species. Accordingly, the products were formulated as the two positional isomers 5-(2*H*-3,4-dihydro-7-hydroxy-6,11-dioxonaphtho[3,2-*b*][1,4]dithiepin-2-yl)pentanoic acid (**1a**) and 5-(2*H*-3,4-dihydro-7-hydroxy-6,11-dioxonaphtho[2,3-*b*][1,4]dithiepin-4-yl)pentanoic acid (**1b**) (overall isolated yield 70%, formation yield >95% as determined by HPLC). Unfortunately, all attempts at separating the compounds, e.g., by TLC and reverse phase HPLC under a variety of elutographic conditions, were defeated.

Compounds **1a**,**b** displayed a chromophore with λ_{max} at 416 and 533 nm (EtOH), which shifted to 497 and 547 (sh) in alkali (Fig. S1) and was stable both in the solid state and in solution. It was insensitive to hydrogen peroxide or thiols. Upon reduction with $NaBH_4$ it was changed into a finely structured chromophore (Fig. S2), which rapidly recovered the original features by reoxidation in air.

Formation of regioisomeric **1a**,**b** by the reaction of DHLA with juglone was of mechanistic interest. Previous studies of the reactions of juglone with thiols (e.g., thioglycolic acid, arylthiols) indicated the 3-position as the most reactive site.[20] This was attributed to electron delocalization from the OH group to the carbonyl group at position 4, decreasing the electrophilic character of the corresponding β position at C-2 and, hence, favoring attack at C-3. On this basis, and assuming a preferential reactivity of DHLA via the less hindered SH group at C-8,[13] isomer **1a** was predicted to be the main product via an initial chemo- and regioselective coupling. Though somewhat disappointing from a synthetic perspective, the unexpected lack of selectivity raised mechanistic issues that prompted further insights into this reaction. An explanation for this inconsistent result came eventually from careful HPLC analysis of the reaction course under higher dilution conditions. Kinetic monitoring showed the initial formation of a species (λ_{max} 356 nm) that gradually decayed concomitant to formation of the purple adducts. Initial attempts to isolate this unstable intermediate were unsuccessful. However after several trials it was found that conversion of this intermediate to products was blocked by acidification of the reaction mixture. Thus, acid quenching of a preparative scale mixture allowed the isolation of the labile intermediate. NMR spectroscopic analysis of this latter indicated a significant structural difference in the naphthoquinone moiety relative to **1a**,**b**. In particular, the resonances due to the C-2 and C-3 carbons (δ 145 and 143 in **1a**,**b**) were apparently replaced by a quaternary carbon signal at δ 53.1, suggestive of a dithioketal function, and a methylene resonance at δ 50.0 (HSQC cross peak with protons at δ 3.33). A down-field shift of the carbonyl resonances by ca. 10 ppm was also apparent, indicating loss of the conjugated double bond. Moreover, the proton signals at δ 3.33 showed long-range correlations with the quaternary carbon resonances at δ 133.6 and 53.1, as well as with the carbonyl signals (δ 191 and 196). Likewise, correlations were apparent between one of the C-8 protons of the lipoic acid unit (δ 2.8) and the carbon signal at δ 53.1.

Based on these extensive 2D-NMR data, the compound was identified as the spirocyclic 1,3-dithiane derivative **1c**. Inspection of the proton and carbon spectrum revealed that most signals were present as a close pair, indicating that the compound was in fact a mixture of two diastereoisomers that differed in the configuration of the spirocenter, and could not be separated under a variety of chromatographic conditions. Exposure of **1c** to TEA in ethanol or to phosphate buffer, pH 7.4, resulted in quantitative conversion to an equimolar mixture of **1a**,**b**, confirming its intermediacy in the route to naphthodithiepines.

On this basis, a plausible reaction mechanism for the reaction of DHLA with juglone can be proposed (Scheme 1).



Scheme 1. Proposed mechanism of reaction of juglone with DHLA.

In this scheme, selective addition of DHLA via the SH group at C-8 to the 3-position of juglone leads to adduct **I**, which then evolves via a fast intramolecular cyclization to give the spirocyclic 1,3-dithiane **1c**. This latter would be engaged in fast equilibria with the starting adduct **I** and the isomer **II**. Generation of products **1a**,**b** would then be the final outcome of slow, irreversible cyclization/oxidation processes from the ring-opened adducts **I** and **II**. The equimolar formation of **1a**,**b** is therefore the signature of the spirocyclic compound **1c**, which partitions equally between the ring opening routes a and b. Kinetic analysis of the reaction course (Fig. 1) demonstrates indeed the fast accumulation of **1c** and its slow decay concomitant to product formation.

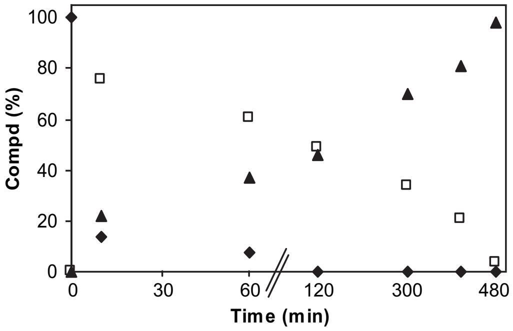
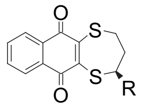
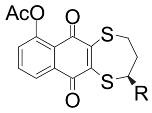


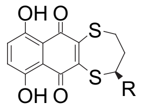
Figure 1. Kinetic profile of juglone decay (starting concn 0.5 mM) and product formation with DHLA; ◆: juglone, □: **1c**, ▲: **1a**,**b**.

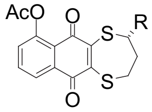
The reversible formation of the spirocyclic adduct raised the issue of what factors orient the initial ring closing attack of SH toward the *S*-substituted 3-position instead of the adjacent unsubstituted 2-position of **I**.

To address this issue, the reactions of DHLA with the parent 1,4-naphthoquinone, acetylated juglone and naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) were investigated. In all cases the corresponding naphtho[1,4]dithiepines **2**–**4** were obtained in 25–44% isolated yields.


2


3a


4


3b

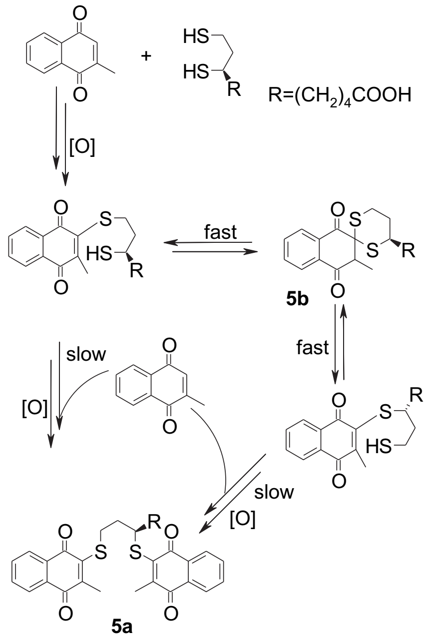
R= $(CH_2)_4COOH$

For these reactions, the mass balance was accounted for by side products, possibly ring-opened diadducts and polymeric species that were not investigated further. Notably, all attempts to identify or isolate the first formed adducts of the type **I** met with failure, on account of their marked tendency to cyclize or react further via the free SH group.

The generation of an equimolar mixture of regioisomers from acetylated juglone supported operation of the spirocyclic mechanism, which, however, could not be demonstrated despite several efforts. Nonetheless, the failure to isolate spirocyclic dithiane precursors to **2**–**4** under various concentration, pH, and temperature conditions does not rule out their transient generation in the mixtures. On this basis, it can be argued that the observed accumulation of the spirocyclic intermediate in the case of juglone reflects specifically remote electronic control exerted by the OH substituent disfavoring fast attack at C-2.

To address in more detail the generation and fate of spirocyclic dithianes, subsequent experiments were directed to investigate the reaction of DHLA with menadione (2-methyl-1,4-naphthoquinone). Product analysis showed the formation of the *S*,*S*′-bisnaphthoquinone adduct **5a** (16%) and of a spirocyclic 1,3-dithiane intermediate, which was isolated and identified as **5b**. For this latter product the presence of the spirodithiane ring at the 3-position of the menadione unit was deduced from distinct correlations of both the methyl protons at δ 1.5 and one of the C-8 protons of the lipoic acid unit (δ 2.77) with the dithioketal carbon resonating at δ 60 in the HMBC spectrum. Also in this case, most of the proton and carbon signals appeared as a couple, suggesting that this product, too, was a mixture of diastereoisomers. Thus, the presence of the methyl group on the 2-position inhibits formation of stable seven-membered intramolecular cyclization products, by preventing the irreversible oxidation step leading to the thermodynamic stable 1,4-naphthoquinone system. The ring-opened isomeric adducts deriving from the spiro compound can then evolve only by coupling of the SH group with a second molecule of quinone to give **5a** (Scheme 2).

The reaction behavior of the spirocyclic intermediates described herein is worthy of further attention. 1,3-Dithianes are valuable synthetic tools and have been employed in rearrangement reactions leading to ring expanded derivatives, but these reactions proceed usually in poor yields under conditions of acid or metal catalysis.[21] No report is available of fast, efficient, and mild, base-induced conversion of 1,3-dithianes to 1,4-dithiepines.



Scheme 2. Proposed mechanism of reaction of menadione with DHLA.

3. Conclusions

We have disclosed herein the reaction of DHLA with representative 2,3-unsubstituted 1,4-naphthoquinones leading to an attractive, chemically robust yet virtually unexplored heterocyclic system of potential practical interest. Besides the novel naphtho[1,4]dithiepine derivatives, a significant outcome of this study is the identification and chemical investigation of labile spirocyclic 1,3-dithiane derivatives of DHLA. To the best of our knowledge, this is the first example of base-induced ring expansion of 1,3-dithianes to 1,4-dithiepines under mild conditions. Overall, these results fill a gap in the current knowledge of thiol/quinone reactions, and shed new light on the synthetic and practical opportunities offered by lipoic acid chemistry beyond the traditional boundaries of biomedicine.

4. Experimental

4.1. General methods and materials

1,4-Naphthoquinone, 5-hydroxy-1,4-naphthoquinone (juglone), 5,8-dihydroxy-1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone (menadione), (*R*)-(+)-1,2-dithiolane-3-pentanoic acid (lipoic acid), sodium acetate, acetic anhydride, sodium borohydride, and triethylamine (TEA) were commercially available and were used as obtained. DHLA was prepared according to a literature procedure.[19]

HR ESI$^+$/MS spectra were obtained in 0.5% TFA/methanol 1:1 v/v. UV/vis spectra were obtained on a diode array spectrophotometer. IR spectra were obtained on a FT spectrophotometer. ^{1}H NMR spectra were recorded at 200 or 400 MHz, ^{13}C NMR spectra at 50 or 100 MHz. ^{1}H, ^{1}H COSY, ^{1}H, ^{13}C DEPT HSQC, and ^{1}H, ^{13}C HMBC experiments were run at 400 MHz on an instrument equipped with a 5 mm ^{1}H/broadband gradient probe with inverse geometry using standard pulse programs. The HMBC experiments used a 100 ms long-range coupling delay. Chemical shifts are reported in δ values (ppm) downfield from TMS. Analytical and preparative TLC were carried out on silica gel plates (0.25 and 0.50 mm, respectively) using chloroform/methanol/acetic acid (90:10:1) (eluant A) or cyclohexane/ethyl acetate/acetic acid (70:30:1) (eluant B) as eluants. HPLC analysis of the reaction mixtures was carried out on an apparatus equipped with a UV detector set at 254 nm using a Sphereclone ODS (5 μm, 4.6×250 mm) column. 0.5% TFA (solvent a), acetonitrile (solvent b) were used as follows: 0–45 min 5–90% solvent b, 45–50 min 90% solvent b (gradient A), at a flow rate of 0.8 mL min^{-1}. Preparative HPLC was carried out on an instrument coupled with a UV detector set at 254 nm, using an Econosil C18 column (10 μm, 10×250 mm) (gradient A, flow rate 3 mL min^{-1}). Purity of isolated compounds was estimated by ^1H NMR analysis. Signals due to isomeric compounds are reported as δ/δ.

4.1.1. 5-Acetoxy-1,4-naphthoquinone. The title compound was prepared by acetylation of juglone according to a literature procedure with modifications.[22] A solution of 5-hydroxy-1,4-naphthoquinone (100 mg, 0.58 mmol) and sodium acetate (590 mg, 7.2 mmol) in acetic anhydride (2 mL) was stirred at 120 °C. After 30 min the mixture was diluted in 0.1 M phosphate buffer (pH 7.4) (5 mL) and extracted with chloroform (3×10 mL). The combined organic phases were dried over sodium sulfate, filtered, and taken to dryness in vacuo. The compound was isolated as a brown powder (R_f 0.44 (eluant B), 110 mg, 88% yield, purity >98%); UV (EtOH): λ_{max} 340 nm (log ε 3.41); IR($CHCl_3$): ν_{max} 1766, 1671, 1613, 1596 cm^{-1}; HR ESI^+/MS: found m/z 239.0332 ($[M+Na]^+$), calcd for $C_{12}H_8O_4Na$ m/z 239.0320; δ_H (200 MHz, $CDCl_3$) 2.43 (3H, s), 6.83 (1H, d, J=10.6 Hz), 6.93 (1H, d, J=10.6 Hz), 7.37 (1H, d, J=7.8 Hz), 7.74 (1H, t, J=7.8 Hz), 8.02 (1H, d, J=7.8 Hz); δ_C (50 MHz, $CDCl_3$) 21.0 (CH_3), 123.2 (C), 124.9 (CH), 129.7 (CH), 133.5 (C), 134.8 (CH), 137.3 (CH), 139.8 (CH), 149.4 (C), 169.3 (C), 183.6 (C), 184.1 (C).

*4.1.2. 5-(2H-3,4-Dihydro-7-hydroxy-6,11-dioxonaphtho[3,2-b][1,4]dithiepin-2-yl)pentanoic acid (**1a**) and 5-(2H-3,4-dihydro-7-hydroxy-6,11-dioxonaphtho[2,3-b][1,4]dithiepin-4-yl)pentanoic acid (**1b**).* To a solution of juglone (50 mg, 0.3 mmol) in absolute ethanol (6 mL), TEA (42 μL, 0.3 mmol), and DHLA (62 mg, 0.3 mmol), previously dissolved in ethanol (600 μL), were added under vigorous stirring. After 10 min the reaction mixture was evaporated to dryness, and the residue obtained was dissolved in chloroform and fractionated by preparative TLC (eluant A) to afford **1a**,**b** as a purple red oil (R_f 0.59, 79 mg, 70% yield, purity >98%); UV (EtOH): λ_{max} 291, 416, 533 nm (log ε 3.92, 3.38, 3.15); IR($CHCl_3$): ν_{max} 3688, 3522, 1713, 1626, 1457 cm^{-1}; HR ESI^+/MS: found m/z 379.0658 ($[M+H]^+$), calcd for $C_{18}H_{19}O_5S_2$ m/z 379.0674; DHLA moiety δ_H (400 MHz, $CDCl_3$) 1.53 (1H, m, 4-H), 1.68 (5H, m, 3-H, 4-H, and 5-H), 1.94 (1H, m, 7-H), 2.23 (1H, m, 7-H), 2.38 (2H, m, 2-H), 2.97/2.99 (1H, m, 8-H), 4.19 (1H, m, 8-H), 4.37 (1H, m, 6-H); juglone moiety δ_H 7.20 (1H, d, J=8.0 Hz, 6-H), 7.55 (1H, t, J=8.0 Hz, 7-H), 7.60 (1H, m, 8-H), 11.8 (1H, br s); DHLA moiety δ_C (100 MHz, $CDCl_3$) 24.3 (CH_2, C-3), 26.3 (CH_2, C-4), 29.8 (CH_2, C-8), 33.5 (2×CH_2, C-2, and C-7), 34.7/34.8 (CH_2, C-5), 45.3/45.5 (CH, C-6), 178.5 (C, C-1); juglone moiety δ_C 114.2 (C, C-4a), 119.7/119.8 (CH, C-8), 124.3 (CH, C-6), 131.7 (C, C-8a) 136.1 (CH, C-6), 143.0/143.2 (C, C-3), 145.7/145.9 (C, C-2), 161.4 (C, C-5), 179.5 (C, C-1), 185.1 (C, C-4).

*4.1.3. 5-(2′,3′-Dihydro-spiro[1,3-dithiane-2,3′-[5]hydroxy[1,4]naphthoquinone-4-yl])pentanoic acid (**1c**).* To a solution of juglone (50 mg, 0.3 mmol) in absolute ethanol (600 mL), TEA (42 μL, 0.3 mmol), and DHLA (62 mg, 0.3 mmol), previously dissolved in ethanol (600 μL), were added under vigorous stirring. After 10 min the mixture was treated with TFA (6 mL) and concentrated in vacuo. The residue was purified by preparative HPLC to provide **1c** as a yellow oil (t_R 33.4 min, 50 mg, 44% yield, purity >98%); UV (EtOH): λ_{max} 232, 356 nm; IR($CHCl_3$): ν_{max} 3689, 3510, 1704, 1636, 1455 cm^{-1}; HR ESI^+/MS: found m/z 381.0845 ($[M+H]^+$), calcd for $C_{18}H_{21}O_5S_2$ m/z 381.0830; DHLA moiety δ_H (400 MHz, $CDCl_3$) 1.51 (4H, m, 3-H, and 5-H), 1.54 (1H, m, 7-H), 1.58 (2H, m, 4-H), 2.24/2.26 (1H, m, 7-H), 2.36 (2H, t, J=7.2 Hz, 2-H), 2.79/2.82 (1H, m, 8-H), 3.38 (1H, m, 8-H), 3.54 (m, 1H, 6-H); juglone moiety δ_H 3.33 (2H, s, 2-H), 7.28 (1H, d, J=9.2 Hz, 6-H), 7.50 (1H, d, J=7.2 Hz, 8-H), 7.60 (1H, m, 7-H), 11.95 (1H, s, OH); DHLA moiety δ_C (100 MHz, $CDCl_3$) 24.4 (CH_2, C-4), 25.4 (CH_2, C-3), 29.1 (CH_2, C-8), 31.0 (CH_2, C-7), 33.5 (CH_2, C-2), 35.5 (CH_2, C-5), 41.8 (CH, C-6), 178.2 (C, C-1); juglone moiety δ_C 50.0 (CH_2, C-2), 53.1 (C, C-3), 114.1 (C, C-4a), 117.7/117.8 (CH, C-8), 124.6/124.7 (CH, C-6), 133.6 (C, C-8a) 136.6/136.7 (CH, C-7), 162.8/163.1 (C, C-5), 191.4 (C, C-1), 196.3/196.4 (C, C-4).

4.2. General procedure for conjugation of 1,4-naphthoquinones with DHLA

The reaction was performed as described for **1a**,**b**. The reaction mixture was evaporated to dryness, and the residue obtained was dissolved in chloroform and fractionated by preparative TLC (eluant A) to afford **2**–**4** (reaction time: **2** and **4**, 10 min; **3**, 60 min).

*4.2.1. 5-(2H-3,4-Dihydro-6,11-dioxonaphtho[2,3-b][1,4]dithiepin-2-yl)-pentanoic acid (**2**).* Compound **2** was obtained as a purple red oil (R_f 0.62, 48 mg, 44% yield, purity >98%); UV (EtOH): λ_{max} 236, 293, 514 nm (log ε 4.07, 4.25, 3.32); IR($CHCl_3$): ν_{max} 3522, 1710, 1659, 1592 cm^{-1}; HR ESI^+/MS: found m/z 363.0710 ($[M+H]^+$), calcd for $C_{18}H_{19}O_4S_2$ m/z 363.0725; δ_H (400 MHz, $CDCl_3$) 1.51 (1H, m), 1.68 (5H, m), 1.91 (1H, m), 2.21 (1H, m), 2.36 (2H, t, J=6.8 Hz), 2.95 (1H, m), 4.18 (1H, m), 4.34 (1H, m), 7.66 (2H, m), 8.03 (2H, m); δ_C (100 MHz, $CDCl_3$) 24.3 (CH_2), 26.3 (CH_2), 29.8 (CH_2), 33.6 (CH_2), 33.7 (CH_2), 34.7 (CH_2), 45.3 (CH), 126.8 (CH), 126.9 (CH), 131.7 (C), 131.8 (C), 133.7 (2×CH), 144.2 (C), 144.4 (C), 178.8 (C), 180.2 (2×C).

*4.2.2. 5-(7-Acetoxy-2H-3,4-dihydro-6,11-dioxonaphtho[3,2-b][1,4]dithiepin-2-yl)pentanoic acid (**3a**) and 5-(7-acetoxy-2H-3,4-dihydro-6,11-dioxonaphtho[2,3-b][1,4]dithiepin-4-yl)pentanoic acid (**3b**).* Compound **3** was obtained as a purple red oil (R_f 0.79, 48 mg, 38% yield, purity >95%); UV (EtOH): λ_{max} 296, 520 nm (log ε 3.92, 2.97); IR($CHCl_3$): ν_{max} 3527, 1769, 1712, 1655, 1509 cm^{-1}; HR ESI^+/MS: found m/z 421.0793 ($[M+H]^+$), calcd for $C_{20}H_{21}O_6S_2$ m/z 421.0779; δ_H (400 MHz, $CDCl_3$) 1.49 (1H, m), 1.66 (5H, m), 1.89 (1H, m), 2.19 (1H, m), 2.35 (2H, m), 2.42/2.43 (3H, s), 2.93 (1H, m), 4.13 (1H, m), 4.31 (1H, m), 7.29 (1H, d, J=8.0 Hz), 7.67 (1H, t, J=8.0 Hz), 7.99/8.01 (1H, d, J=8.0 Hz); δ_C (100 MHz, $CDCl_3$) 21.1 (CH_3), 24.3 (CH_2), 26.3 (CH_2), 29.7/29.8 (CH_2), 33.5 (2×CH_2), 34.7 (CH_2), 45.2/45.3 (CH), 122.9/123.0 (C), 125.3/125.4 (CH), 129.5 (CH), 133.4/133.5 (C), 134.6 (CH), 143.1/143.3 (C), 145.4/145.6 (C), 149.6 (C), 169.4/169.5 (C), 178.5 (C), 178.6 (C), 179.4 (C).

*4.2.3. 5-(2H-3,4-Dihydro-7,10-dihydroxy-6,11-dioxonaphtho[2,3-b][1,4]dithiepin-2-yl)pentanoic acid (**4**).* Compound **4** was obtained as a purple red oil (R_f 0.67, 48 mg, 25% yield, purity >95%); UV (EtOH): λ_{max} 284, 507, 548 nm (log ε 4.14, 3.91, 3.74); IR($CHCl_3$): ν_{max} 3690, 3601, 3503, 1713, 1597, 1456 cm^{-1}; HR ESI^+/MS: found m/z 395.0606 ($[M+H]^+$), calcd for $C_{18}H_{19}O_6S_2$ m/z 395.0623; δ_H (400 MHz, $CDCl_3$) 1.52 (1H, m), 1.68 (5H, m), 1.94 (1H, m), 2.21 (1H, m), 2.35 (2H, m), 2.97 (1H, m), 4.18 (1H, m), 4.37 (1H, m), 7.18 (2H, s), 12.17 (1H, br s); δ_C (100 MHz, $CDCl_3$) 24.2 (CH_2), 26.2 (CH_2), 29.7 (CH_2), 33.4 (CH_2), 33.6 (CH_2), 34.7 (CH_2), 45.4 (CH), 110.8 (2×C), 129.1 (2×CH), 144.5 (C), 144.8 (C), 157.5(C), 157.6 (C), 178.9 (C), 183.1 (2×C).

4.3. Procedure for conjugation of menadione with DHLA

To a solution of menadione (52 mg, 0.3 mmol) in absolute ethanol (6 mL), TEA (42 μL, 0.3 mmol), and DHLA (62 mg, 0.3 mmol), previously dissolved in ethanol (600 μL), were added under vigorous stirring. After 2 h the reaction mixture was evaporated to dryness, and the residue obtained was dissolved in $CHCl_3$ and fractionated by preparative TLC (eluant A) to afford **5a** (yellow oil, R_f 0.79, 26 mg, 16% yield, purity >98%) and **5b** (light yellow oil, R_f 0.70, 23 mg, 20% yield, purity >95%).

*4.3.1. S,S′-Bis(2-methyl-1,4-naphthoquinon-3-yl)-6,8-dithiooctanoic acid (**5a**).* UV (EtOH): λ_{max} 261, 319, 425 nm; IR ($CHCl_3$): ν_{max} 3522, 1702, 1663, 1593, 1465 cm^{-1}; HR ESI$^+$/MS: found m/z 549.1432 ($[M+H]^+$), calcd for $C_{30}H_{29}O_6S_2$ m/z 549.1425; δ_H (400 MHz, $CDCl_3$) 1.60 (2H, m), 1.63 (2H, m), 1.68 (2H, m), 1.92 (2H, m), 2.25 (3H, s), 2.33 (3H, s), 2.37 (2H, m), 3.38 (2H, m), 4.07 (1H, m), 7.64 (4H, m), 7.96 (4H, m); δ_C (100 MHz, $CDCl_3$) 15.1 (CH_3), 15.4 (CH_3), 24.3 (CH_2), 26.1 (CH_2), 31.4 (CH_2), 33.4 (CH_2), 35.2 (CH_2), 38.9 (CH_2), 47.1 (CH), 126.4 (CH), 126.5 (CH), 126.6 (CH), 126.7 (CH), 133.2 (CH), 133.3 (CH), 133.5 (CH), 133.7 (CH), 131.8 (C), 132.7 (C), 134.4 (2×C), 146.4 (2×C), 147.7 (2×C), 178.0 (C), 181.0 (C), 181.1 (C), 181.8 (C), 182.0 (C).

*4.3.2. 5-(2′,3′-Dihydro-spiro[1,3-dithiane-2,3′-[2]methyl[1,4]naphthoquinon-4-yl])pentanoic acid (**5b**).* UV (EtOH): λ_{max} 253, 301 nm; IR ($CHCl_3$): ν_{max} 3523, 1697, 1593, 1459 cm^{-1}; HR ESI$^+$/MS: found m/z 379.1022 ($[M+H]^+$), calcd for $C_{19}H_{23}O_4S_2$ m/z 379.1038; DHLA moiety δ_H (400 MHz, $CDCl_3$) 1.44 (2H, m, 3-H), 1.50 (2H, m, 5-H), 1.56 (1H, m, 7-H), 1.58 (2H, m, 4-H), 2.14/2.18 (1H, m, 7-H), 2.30/2.35 (2H, m, 2-H), 2.77 (1H, m, 8-H), 3.16/3.26 (1H, m, 8-H), 3.43 (1H, m, 6-H); menadione moiety δ_H 1.48/1.51(3H, d, J=7.0 Hz, CH_3), 3.35 (1H, m, 2-H), 7.71 (1H, m, 6-H), 7.75 (1H, m, 7-H), 7.96 (1H, d, J=7.2 Hz, 8-H), 8.12 (1H, d, J=7.2 Hz, 5-H); DHLA moiety δ_C (100 MHz, $CDCl_3$) 24.3 (CH_2, C-4), 25.4 (CH_2, C-3), 28.2/29.1 (CH_2, C-8), 31.5 (CH_2, C-7), 33.6 (CH_2, C-2), 35.5/35.7 (CH_2, C-5), 41.1/41.8 (CH, C-6), 178.7 (C, C-1); menadione moiety δ_C 12.8/13.4 (CH_3), 53.0/53.6 (CH, C-2), 60.4/60.7 (C, C-3), 126.3/126.4 (CH, C-8), 128.0/128.1 (CH, C-5), 132.2 (C, C-4a or C-8a), 132.4 (C, C-8a or C-4a), 134.0 (CH, C-7), 134.4 (CH, C-6), 189.1 (C, C-4), 195.6/195.9 (C, C-1).

Acknowledgements

This work was supported in part by a grant from Regione Campania (L5 2006 project). We thank the Centro Interdipartimentale di Metodologie Chimico-fisiche (CIMCF) of Naples University 'Federico II' for spectral facilities.

Supplementary data

NMR spectra of **1**-**5** and acetylated juglone and UV/vis spectra of **1a**,**b** in EtOH, in alkali and after reduction with $NaBH_4$ are provided. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.03.103.

References and notes

1. Nkpa, N. N.; Chedekel, M. R. *J. Org. Chem.* **1981**, *46*, 213–215.
2. (a) Prota, G. *Melanins and Melanogenesis*; Academic: San Diego, CA, 1992; (b) Ito, S. *Pigment Cell Res.* **2003**, *16*, 230–236; (c) Ito, S.; Wakamatsu, K. *Photochem. Photobiol.* **2008**, *84*, 582–592.
3. Ito, S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 14647–14648.
4. Okada, K.; Iio, H.; Goto, T. *J. Chem. Soc., Chem. Commun.* **1976**, 32.
5. (a) Bisaglia, M.; Mammi, S.; Bubacco, L. *J. Biol. Chem.* **2007**, *282*, 15597–15605; (b) Nicolis, S.; Zucchelli, M.; Monzani, E.; Casella, L. *Chem.—Eur. J.* **2008**, *14*, 8661–8673; (c) Kato, T.; Ito, S.; Fujita, K. *Biochim. Biophys. Acta* **1986**, *881*, 415–421.
6. (a) Chandrasena, R. E. P.; Edirisinghe, P. D.; Bolton, J. L.; Thatcher, G. R. J. *Chem. Res. Toxicol.* **2008**, *21*, 1324–1329; (b) Watanabe, N.; Dickinson, D. A.; Liu, R.-M.; Forman, H. J. *Methods Enzymol.* **2004**, *378*, 319–340.
7. Greco, G.; Panzella, L.; Napolitano, A.; d'Ischia, M. *Tetrahedron Lett.* **2009**, *50*, 3095–3097.
8. De Lucia, M.; Panzella, L.; Pezzella, A.; Napolitano, A.; d'Ischia, M. *Chem. Res. Toxicol.* **2008**, *21*, 2407–2413.
9. Amorati, R.; Fumo, M. G.; Menichetti, S.; Mugnaini, V.; Pedulli, G. F. *J. Org. Chem.* **2006**, *71*, 6325–6332.
10. Bustamante, J.; Lodge, J. K.; Marcocci, L.; Tritschler, H. J.; Packer, L.; Rihn, B. H. *Free Radical Biol. Med.* **1998**, *24*, 1023–1039.
11. (a) Smith, A. R.; Shenvi, S. V.; Widlansky, M.; Suh, J. H.; Hagen, T. M. *Curr. Med. Chem.* **2004**, *11*, 1135–1146; (b) Li, Y.; Zhao, Y.; Yu, W.; Jiang, S. *Food Chem.* **2004**, *84*, 563–567; (c) Packer, L.; Witt, E. H.; Tritschler, H. J. *Free Radical Biol. Med.* **1995**, *19*, 227–250.
12. Spencer, J. P.; Jenner, P.; Daniel, S. E.; Lees, A. J.; Marsden, D. C.; Halliwell, B. *J. Neurochem.* **1998**, *71*, 2112–2122.
13. (a) Tsuji-Naito, K.; Hatani, T.; Okada, T.; Tehara, T. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 15–20; (b) Tsuji-Naito, K.; Hatani, T.; Okada, T.; Tehara, T. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 1967–1975.
14. Foti, M. C.; Johnson, E. R.; Vinqvist, M. R.; Wright, J. S.; Barclay, L. R. C.; Ingold, K. U. *J. Org. Chem.* **2002**, *67*, 5190–5196.
15. Valente, C.; Moreira, R.; Guedes, R. C.; Iley, J.; Jaffar, M.; Douglas, K. T. *Bioorg. Med. Chem.* **2007**, *15*, 5340–5350.
16. Sharma, S.; Sharma, B. K.; Prabhakar, Y. S. *Eur. J. Med. Chem.* **2009**, *44*, 2847–2853.
17. Kiran Aithal, B.; Sunil Kumar, M. R.; Nageshwar Rao, B.; Udupa, N.; Satish Rao, B. S. *Cell Biol. Int.* **2009**, *33*, 1039–1049.
18. (a) Tandon, V. K.; Maurya, H. K.; Mishra, N. N.; Shukla, P. K. *Eur. J. Med. Chem.* **2009**, *44*, 3130–3137; (b) Tandon, V. K.; Maurya, H. K.; Yadav, D. B.; Tripathi, A.; Kumar, M.; Shukla, P. K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5883–5887; (c) Mecklenburg, S.; Shaaban, S.; Ba, L. A.; Burkholz, T.; Schneider, T.; Diesel, B.; Kiemer, A. K.; Röseler, A.; Becker, K.; Reichrath, J.; Stark, A.; Tilgen, W.; Abbas, M.; Wessjohann, L. A.; Sasse, F.; Jacob, C. *Org. Biomol. Chem.* **2009**, *7*, 4753–4762; (d) Braud, E.; Goddard, M.; Kolb, S.; Brun, M.; Mondésert, O.; Quaranta, M.; Gresh, N.; Ducommun, B.; Garbay, C. *Bioorg. Med. Chem.* **2008**, *16*, 9040–9049.
19. Gunsalus, I. C.; Barton, L. S.; Gruber, W. *J. Am. Chem. Soc.* **1956**, *78*, 1763–1766.
20. (a) Thomson, R. H. *J. Org. Chem.* **1951**, *16*, 1082–1090; (b) Ryu, C.-K.; Shim, J.-Y.; Chae, M. J.; Choi, I. H.; Han, J.-Y.; Jung, O.-J.; Lee, J. Y.; Jeong, S. K. *Eur. J. Med. Chem.* **2005**, *40*, 438–444.
21. (a) Ong, C. W.; Yu, C. Y. *Tetrahedron* **2003**, *59*, 9677–9682; (b) Waddell, S. T.; Blizzard, T. A.; Doss, G. A. *Heterocycles* **1993**, *36*, 1213–1215; (c) Saigo, K.; Hashimoto, Y.; Fang, L.; Hasegawa, M. *Heterocycles* **1989**, *29*, 2079–2082; (d) Zhao, X.; Zhong, Z.; Peng, L.; Zhang, W.; Wang, J. *Chem. Commun.* **2009**, 2535–2537.
22. Panzella, L.; De Lucia, M.; Napolitano, A.; d'Ischia, M. *Tetrahedron Lett.* **2007**, *48*, 7650–7652.