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Original article

Addition of thiols to *o*-quinone methide: New 2-hydroxy-3-phenylsulfanylmethyl [1,4]naphthoquinones and their activity against the human malaria parasite *Plasmodium falciparum* (3D7)

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ABSTRACT

A series of 36 new phenylsulfanylmethyl[1,4]naphthoquinones (**7**–**42**) were synthesized by a three-component reaction that involves lawsone, the appropriate aldehyde and thiols with variable substitution patterns. These reactions involve the *in situ* generation of *o*-quinone methides (*o*-QM) via Knoevenagel condensation and 1,4-nucleophilic addition under conventional heating or microwave irradiation. The new naphthoquinones obtained by this methodology were shown to have moderate to good *in vitro* antimalarial activity against *Plasmodium falciparum* (3D7).

1. Introduction

Malaria is an infection caused by a parasite from the genus *Plasmodium* [1] that represents a major public health issue due to the growing resistance to current antimalarial drugs. The World Health Organization (WHO) estimates that 300–500 million people are infected annually, and the number of deaths exceeds one million [2,3]

Plasmodium falciparum is a protozoan parasite with a complex life cycle, the intraerythrocytic cycle, which is comprised of well-studied stages: ring, trophozoite and schizonts [4–6]. Despite the worldwide effort to understand the molecular [7,8] and cellular mechanisms of infection and develop new lead natural [9–11] and synthetic compounds to fight the *P. falciparum* (the causative agent of human malaria), the disease is still devastating. Additionally, parasite resistance to classical antimalarial treatments raises the need for the development of new drugs. The antimalarial agents that are currently in use stem from six drug classes: aminoquinolines, arylaminoalcohols, artemisinins, antifolates, antibiotics and inhibitors of the respiratory chain [12]. It is important to highlight the problem of resistance, which further decreases the available therapeutic arsenal. Therefore, in the interest of public health, an urgent need remains for novel drug candidates.

The drug arsenals for fighting malaria include the synthetic naphthoquinone atovaquone (**1**) [13]. The molecular structure of compound **1** was inspired by lapachol (**2**), which is a natural naphthoquinone that belongs to a class of alkyl-1,4-naphthoquinones and a large group of important natural and synthetic compounds that present some biological activity. Lapachol was the first naphthoquinone discovered to possess antimalarial activity [14–16], and it was later discovered that its toxicity against the parasite was due to its interaction with the mitochondrial respiratory chain [17]. Indeed, this class of compounds possesses a broad spectrum of important biological activities, such as antiprotozoal, antibacterial, pesticidal, and antitumor [18], as well as activity against the snail *Biomphalaria glabrata* that is involved in the transmission of schistosomiasis [19,20], among others [21]. Lapachol (**1**) occurs as component found in various plant families, including *Bignoniaceae*, *Leguminosae*, *Sapotaceae*, *Scrophulariaceae*, *Verbenaceae*, *Malvaceae*, and *Proteaceae*, and exhibits an impressive

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list of biological activities [22]. Atovaquone (**2**) is a hydroxynaphthoquinone used in combination with proguanil (Malarone) for prophylaxis and therapy of uncomplicated tropical malaria [6]. Parvaquone (**3**) and buparvaquone (**4**) are additional important 3-substituted-2-hydroxy-1,4-naphthoquinones used as drugs for the treatment of *Pneumocystis pneumonia*, toxoplasmosis and malaria, which highlight the importance of this class of compounds (Fig. 1). Despite efforts toward the discovery of new bioactive compounds, the number of new cases of malaria continues to increase worldwide.

New biochemical targets and chemical entities are currently being researched as potential antimalarials with particular emphasis on those with low toxicity in normal cells. Based on what has been described about the chemotherapy of malaria and the importance of naphthoquinone derivatives, much remains to be studied in the search for new compounds that are more active and selective than compounds **1** and **2**. The literature data for compounds **1** and **2** show their potential as lead compounds for the development of new bioactive compounds.

The main objective of this work was the development of a new three-component reaction for the preparation of alkyl-and arylsulfanylmethyl[1,4]naphthoquinones via *o*-quinone methide (Fig. 2). The products of the reaction are structurally related to lapachol (**2**), and their biological evaluation against the human malaria parasite *Plasmodium falciparum* was studied.

2. Results

2.1. Chemistry

Compounds **7**–**42** were obtained by reaction of lawsone with the appropriate aldehyde to generate the intermediate *o*-quinone methide (**6**) *in situ*, followed by nucleophilic addition of a substituted thiol (Scheme 1). This reaction explores the *in situ* generation of *o*-quinone methides (*o*-QM) via the Knoevenagel condensation reaction between lawsone (**5**) and an appropriate aldehyde followed by 1,4-nucleophilic addition of thiol derivatives (Scheme 1) [23–25]

The reactions of an appropriate aldehyde, thiols (RSH) and lawsone (**5**) were carried out by using conventional heating (reflux) or microwave irradiation (150 °C, 20 min). The results are presented in Table 1. As shown in the data presented in Table 1, it is possible to use microwave irradiation to prepare compounds **7**–**18** in higher yields and with a drastic decrease in reaction time compared to conventional heating. After this improvement was observed, compounds **19**–**42** were prepared using only microwave irradiation and obtained as a racemic mixture (Table 1). After using either set of reaction conditions, the products were purified by column chromatography using silica gel and were characterized by spectroscopic techniques. This three-component reaction proved to be a good alternative for the synthesis of 2-hydroxy-3-alkyl[1,4]naphthoquinones or 3-arylsulfanylmethyl[1,4]naphthoquinones (**7**–**42**). These compounds were characterized using spectroscopic analyses where we can highlight for its unequivocal analysis the disappearance of the signal relative to the H-3 of **6** in ^{1}H NMR spectrum proving that the addition was in this site as well as the appearance of a singlet around 4 ppm for the CH_2 group (compounds **7**–**18**) or singlet around 6 ppm for the group CH (compounds **19**–**42**). In the 13C NMR spectrum we can easily note the presence of CH_2 groups around 28 ppm (compounds **7**–**18**) and around 50 ppm for the group CH (compounds **19**–**42**), as well as signs for the carbons from the thiols introduced.

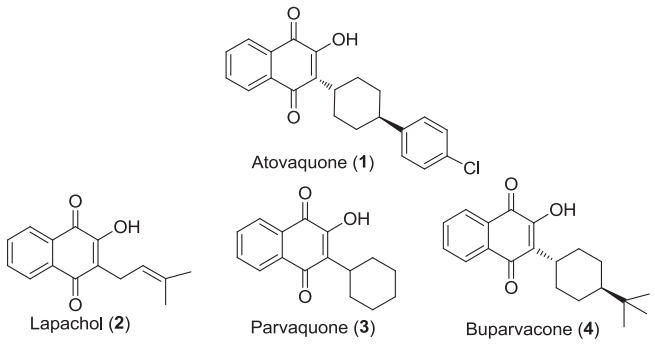


Fig. 1. Natural and synthetic bioactive naphthoquinones.

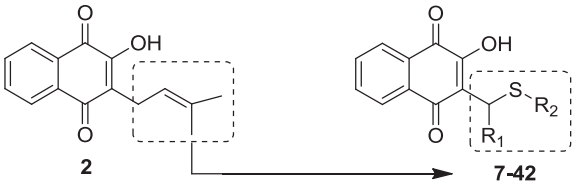


Fig. 2. Alkyl-and arylsulfanylmethyl[1,4]naphthoquinones structurally related to lapachol (**2**).

2.2. Biology

2.2.1. In vitro culture of Plasmodium falciparum (3D7)

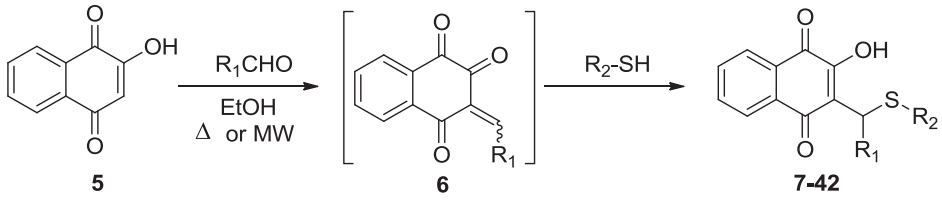
Parasites were cultured and synchronized as previously described [26]. In brief, parasites were maintained at 1–10% parasitemia and 2% hematocrit in RPMI 1640 culture medium supplemented with erythrocytes, 10% human serum, 0.16% glucose, 0.2 mM hypoxanthine, 2.1 mM L-glutamine and 22 mg/mL gentamycin. Cultures were incubated at 37 °C, 3% O_2, 3% CO_2 and 94% N_2. Synchronization of parasites in culture to ring stages was carried out by repetitive treatment with 5% (w/v) sorbitol. Parasite growth and parasitemia were monitored by assessing Giemsa-stained blood smears under the microscope.

2.2.2. Drug treatment

Drug treatment experiments were conducted in 96-well plates with varying concentrations (100, 20, 4, 0.8, 0.16, 0.032, 0.0064 and 0.00128 μM) of each drug in triplicate for each concentration. For this assay, 1% parasitemia and 2% hematocrit were set for each well, and 200 μL of RPMI with 10% human serum and drug were added. The parasites were exposed to the drug, and the 96-well plates were incubated for 48 h.

2.2.3. Flow cytometry analysis

Flow cytometry analysis was performed according the procedure already published [27]. In brief, after 48 h incubation, the plates were centrifuged at 3000 rpm for 5 min, and RPMI was removed. Any traces of drug were washed away with PBS (pH 7.2–7.4). The sample was incubated with 2% formaldehyde in PBS for 24 h to fix the parasite. After fixation, the samples were washed with PBS again. Permeabilization and staining was performed with 0.1% Triton-X100 and 5 nM YoYo-1 dye (Molecular Probes) [28] by incubating the sample reaction mixtures at 37 °C for 30 min. Parasitemia and the proportions of parasites at each drug concentration for all drugs tested and control samples (without drug treatment) were determined from dot plots [side scatter (SSC) versus fluorescence] of 10^5 cells acquired on a FACS Calibur flow cytometer using BD CellQuest Pro software (Becton & Dickinson Inc.). YOYO-1 was excited with a 488 nm Argon laser, and fluorescence emission was collected at 520–530 nm. Parameters subject to adjustment of the FACS Calibur flow cytometer were forward scatter (FSC) (log scale, E–1), SSC (log scale, 269), and FL-1 (log



Scheme 1. General scheme for preparing the 2-hydroxy-3-alkyl[1,4]naphthoquinones or 3-arylsulfanylmethyl[1,4]naphthoquinones under conventional heating or microwave irradiation.

scale, 530), and the compensation parameters were FL1-0.8% FL2 and FL1-23.6% FL2.

2.2.4. Statistical analysis

GraphPad Prism (GraphPad Software) software was used for statistical analysis to calculate IC_{50} values. At least three independent experiments were performed for each experimental condition.

3. Results

3.1. Rationale

Taking into consideration that the naphthoquinone core remains the same in all the assayed compounds, we analyzed them using a comparative structural. All compounds proved active against *Plasmodium falciparum* (3D7); the vast majority revealed IC_{50} values <20 μM, and the compounds with two phenyl groups at R_1 and R_2 were the most active. Among the ten most active compounds (IC_{50} < 10 μM), 40% displayed a *p*-nitro group on the phenyl ring as a substituent at R_1. The most active compound (**34**) has an electron withdrawing substituent at R_1 and an electron donating substituent at R_2. The less active compound (**38**) has two nitro groups on the phenyl rings at R_1 and R_2. The results show that a strongly electron withdrawing substituent at R_1 and an electron donor at R_2 are necessary for better antimalarial activity. This same trend can be observed in the 4-MeO series (**34**, **10** and **22**), which is more active than 4-MeS series (**35**, **23** and **11**).

Table 1
2-Hydroxy-3-alkyl or 3-arylsulfanylmethyl[1,4]naphthoquinones **7**–**42** prepared by conventional heating (**7**–**18**) and microwave irradiation (**7**–**42**).

Compound	R_1	R_2	Heathing (%)	Microwave[c] (%)	IC_{50} values (μM)
34	4-$NO_2C_6H_4$	4-$CH_3OC_6H_4$	–	46	3.1
16	H	3-$CH_3C_6H_4$	71[a]	84	5.4
39	4-$NO_2C_6H_4$	2-$CH_3C_6H_4$	–	77	6.6
12	H	4-$CH_3C_6H_4$	73	84	6.8
9	H	4-FC_6H_4	62[a]	71	7.0
37	4-$NO_2C_6H_4$	4-HOC_6H_4	–	65	7.5
32	4-$NO_2C_6H_4$	4-ClC_6H_4	–	79	7.5
21	$-C_6H_5$	4-FC_6H_4	–	58	8.7
10	H	4-$CH_3OC_6H_4$	78[b]	83	9.3
15	H	2-$CH_3C_6H_4$	65[a]	77	9.7
40	4-$NO_2C_6H_4$	3-$CH_3C_6H_4$	–	54	10.7
19	$-C_6H_5$	$-C_6H_5$	–	60	11.2
22	$-C_6H_5$	4-$CH_3OC_6H_4$	–	78	13.6
30	$-C_6H_5$	Propyl	–	71	13.8
26	$-C_6H_5$	4-$NO_2C_6H_4$	–	33	13.9
35	4-$NO_2C_6H_4$	4-$CH_3SC_6H_4$	–	52	14.3
33	4-$NO_2C_6H_4$	4-FC_6H_4	–	45	14.3
14	H	4-$NO_2C_6H_4$	71[b]	83	14.4
17	H	2-Naphthyl	33	49	14.5
31	4-$NO_2C_6H_4$	$-C_6H_5$	–	52	16.8
27	$-C_6H_5$	2-$CH_3C_6H_4$	–	60	17.6
23	$-C_6H_5$	4-$CH_3SC_6H_4$	–	64	18.2
24	$-C_6H_5$	4-MeC_6H_4	–	79	18.4
41	4-$NO_2C_6H_4$	2-Naphthyl	–	63	19.9
8	H	4-ClC_6H_4	57[a]	78	20.0
11	H	4-$CH_3C_6H_4$	49[b]	68	22.3
25	$-C_6H_5$	4-HOC_6H_4	–	60	25.0
29	$-C_6H_5$	2-Naphthyl	–	49	26.1
36	4-NO_2Ph	4-$CH_3C_6H_4$	–	76	27.0
7	H	$-C_6H_5$	52[a]	85	27.1
42	4-$NO_2C_6H_4$	Propyl	–	44	27.8
20	$-C_6H_5$	4-ClC_6H_4	–	40	31.6
18	$-C_6H_5$	Propyl	63[a]	89	31.8
28	$-C_6H_5$	3-$CH_3C_6H_4$	–	72	34.2
13	H	4-HOC_6H_4	72[b]	81	49.9
38	4-$NO_2C_6H_4$	4-$NO_2C_6H_4$	–	53	56.9

[a] Reaction time 24 h.
[b] Reaction time 48 h.
[c] Internal temperature 150 °C, reaction time 20 min. Atovaquone (IC_{50} value was 3 nM for 3D7) and Chloroquine (IC_{50} value was 100 nM for 3D7) were used as positive controls for the present study.

3.2. Drug screening

The antimalarial activities of the new synthetic compounds were evaluated against *P. falciparum* (3D7)-infected RBC cells in culture. Drug treatment experiments were conducted in 96-well plates with varying concentrations of each drug, which were tested in triplicate for each concentration. For this assay, 1% parasitemia and 2% hematocrit were set for each well, and 200 μL of RPMI with 10% human serum and drug was added. The parasites were exposed to drug for 48 h. After fixation in 2% formaldehyde, permeabilization and staining were performed by adding Triton X-100 and YoYo-1 dye. The samples were then subjected to FACS analysis, and IC_{50} values were calculated. Among all of the screened drug compounds, **34** (IC_{50} = 3.1 μM) was found to be most effective against the parasite (with IC_{50} < 5 μM). Other drugs also showed good activity against the malaria parasite (Table 1).

4. Conclusion

A new three-component method for the synthesis of 2-hydroxy-3-phenylsulfanylmethyl[1,4]naphthoquinones was developed and used to synthesize a series of 36 new phenylsulfanylmethyl [1,4]naphthoquinones. This three-component reaction explored the *in situ* the generation of *o*-quinone methides (*o*-QM) via the Knoevenagel condensation between lawsone and the appropriate aldehyde followed by nucleophilic addition of a thiol. The reactions were evaluated under both conventional heating and microwave irradiation. The microwave conditions produced several compounds with higher yields and shorter reaction times. The new naphthoquinones obtained by this methodology were evaluated for their *in vitro* antimalarial activity against *P. falciparum* (3D7). Most were active, although none of them showed a biological profile better than the control drugs. The most active compounds from this study (IC_{50} < 10 μM) have a *p*-nitro phenyl group as a substituent at R_1.

5. Experimental section

Melting points were obtained on a Fisher Johns apparatus and are reported as uncorrected values. Analytical grade solvents were

used. Column chromatography was performed on silica gel (Acros Organics 0.035–0.070 mm, pore diameter of approximately 6 nm). Infrared spectra were recorded on an ABB FTLA2000-100 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at room temperature using a Varian VNMRS 300 MHz instrument in the solvents indicated in their monographs with TMS as the internal standard. Chemical shifts (δ) are given in ppm, and coupling constants (J) are reported in Hertz. High-resolution mass spectra (electrospray ionization) were obtained using a QTOF Micro (Waters, Manchester, UK) mass spectrometer (HRESIMS). The ions in the mass spectra were described as mass-to-charge ratio (m/z) and the relative abundance in percentage of the base peak intensity. All the compounds were drawn using CS ChemDraw Ultra version 10.0. Compounds **7**–**42** were prepared using an Anton Paar monowave 300 microwave reactor.

5.1. Chemistry

5.1.1. General procedure for preparing 7–18 under conventional heating

Compound **5** (500 mg, 2.9 mmol), the appropriate aldehyde (5.8 mmol, 2 eq.) and the alkyl-or arylthiol (R–SH) (5.8 mmol, 2 eq.) dissolved in ethanol (15 mL) were added to a 25 mL round-bottom flask equipped with a reflux condenser. The mixture was refluxed for 24–48 h (see Table 1), and the solvent was subsequently evaporated under reduced pressure. The residual solid was purified by column chromatography on silica gel and eluted with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1 to 7/3).

5.1.2. General procedure for preparing **7**–**42** under microwave irradiation

A 10 mL microwave tube was loaded with **5** (500 mg, 2.9 mmol), the appropriate aldehyde (5.8 mmol, 2 eq.), the alkyl-or arylthiol (5.8 mmol, 2 eq.) and ethanol (5 mL). The mixture was irradiated for 20 min at 150 °C (internal temperature), and the solvent was then evaporated under reduced pressure. The residual solid was purified by column chromatography on silica gel and eluted with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1 to 7/3).

*5.1.2.1. 2-Hydroxy-3-((phenylthio)methyl)naphthalene-1,4-dione (**7**).* The reaction produced compound **7** in 85% as a orange solid. m.p. 125 °C. IR (KBr) ν_{max} (cm^{-1}) 1588 (C=C), 1639 (C=O), 3330 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (J in Hz): 8.14 (d, 1H, J = 7.8, H-8), 8.08 (m, 1H, J = 7.8, H-5), 7.77 (ddd, 1H, J = 7.8, 7.8, 1.5, H-6), 7.70 (ddd, 1H, J = 7.8, 7.8, 1.5, H-7), 7.48–7.47 (m, 2H, H-*ortho*-aryl), 7.27–7.24 (m, 2H, H-meta-aryl), 7.22–7.19 (m, 1H, H-*para*-aryl), 4.11 (s, 2H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.3 (C-4), 181.1 (C-1), 153.1 (C-2), 135.6 (C-1′-aryl), 135.1 (C-7), 133.1 (C-6), 132.6 (C-8a), 131.1 (C-2′ and C-6′-aryl), 129.3 (C-4a), 128.7 (C-3′ and C-5′-aryl), 126.9 (C-5), 126.8 (C-4′-aryl), 126.2 (C-8), 120.1 (C-3), 27.3 (CH_2) ppm. Anal. Calcd. for $C_{17}H_{12}O_3S$: C, 68.90; H, 4.08 (Exp. C, 68.94; H, 4.24).

*5.1.2.2. 2-Hydroxy-3-(((4-chlorophenyl)thio)methyl)naphthalene-1,4-dione (**8**).* The reaction produced compound **8** in 78% as a orange solid. m.p. 177 °C. IR (KBr) ν_{max} (cm^{-1}) 1589 (C=C), 1637 (C=O), 3265 (OH). ^{1}H NMR (DMSO-d6, 300 MHz) δ (J in Hz): 8.13 (dd, 1H, J = 7.6, 1.3, H-8), 8.08 (dd, 1H, J = 7.6, 1.3, H-5), 7.78 (ddd, 1H, J = 7.6, 7.6, 1.5, H-6), 7.70 (ddd, 1H, J = 7.6, 7.6, 1.5, H-7), 7.40 (ddd, 2H, J = 8.6, 2.6, 2.0, H-*ortho*-aryl), 7.22 (ddd, 2H, J = 8.6, 2.6, 2.0, H-*meta*-aryl), 4.08 (s, 2H, CH_2) ppm. ^{13}C NMR (DMSO-d6, 75 MHz): 183.0 (C-4), 180.6 (C-1), 156.2 (C-2), 135.4 (C-1′-aryl), 134.6 (C-7), 133.2 (C-6), 131.6 (C-8a), 131.0 (C-2′ and C-6′-aryl), 130.9 (C-4′-aryl), 129.9 (C-4a), 128.7 (C-3′ and C-5′-aryl), 125.7 (C-5), 125.7 (C-8), 119.2 (C-3), 26.4 (CH_2) ppm. Anal. Calcd. for $C_{17}H_{11}ClO_3S$: C, 61.73; H, 3.35 (Exp. C, 61.78; H, 3.33).

*5.1.2.3. 2-Hydroxy-3-(((4-fluorophenyl)thio)methyl)naphthalene-1,4-dione (**9**).* The reaction produced compound **9** in 71% as a light brown solid. m.p. 105 °C. IR (KBr) ν_{max} (cm^{-1}) 1583 (C=C), 1643 (C=O), 3353 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (J in Hz): 8.12 (ddd, 1H, J = 7.6, 1.5, 0.5, H-8), 8.08 (ddd, 1H, J = 7.6, 1.5, 0.5 H-5), 7.78 (ddd, 1H, J = 7.6, 7.4, 1.5, H-6), 7.70 (ddd, 1H, J = 7.6, 7.4, 1.5, H-7), 7.47 (ddd, 2H, J = 8.8, 2.3, 2.0, H-*ortho*-aryl), 6.95 (ddd, 2H, J = 8.8, 2.3, 2.0, H-*meta*-aryl), 4.04 (s, 2H, CH_2) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.2 (C-4), 181.1 (C-1), 162.4 (C-4′-aryl), 153.1 (C-2), 135.1 (C-7), 134.4 (C-2′ and C-6′-aryl), 133.1 (C-6), 132.7 (C-1′-aryl), 132.7 (C-8a), 115.7 (C-3′ and C-5′-aryl), 129.3 (C-4a), 127.0 (C-5), 126.3 (C-8), 120.1 (C-3), 28.5 (CH_2). Anal. Calcd. for $C_{17}H_{11}FO_3S$: C, 64.96; H, 3.53, (Exp. C, 64.97; H, 3.51).

*5.1.2.4. 2-Hydroxy-3-(((4-methoxyphenyl)thio)methyl)naphthalene-1,4-dione (**10**).* The reaction produced the compound **10** in 83% as a brown solid. m.p. 127 °C. IR (KBr) ν_{max} (cm^{-1}) 1586 (C=C), 1644 (C=O), 3343 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (J in Hz): 8.12 (dd, 1H, J = 7.8, 1.0, H-8), 8.07 (dd, 1H, J = 7.8, 1.0, H-5), 7.77 (ddd, 1H, J = 7.3, 7.3, 1.0, H-6), 7.69 (ddd, 1H, J = 7.3, 7.3, 1.0, H-7), 7.42 (d, 2H, J = 8.8, H-*ortho*-aryl), 6.78 (d, 2H, J = 8.8, H-*meta*-aryl), 3.99 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.2 (C-4), 181.1 (C-1), 159.5 (C-4′-aryl), 153.0 (C-2), 135.0 (C-7), 135.0 (C-2′ and C-6′-aryl), 133.0 (C-6), 132.6 (C-8a), 129.3 (C-4a), 126.9 (C-5), 126.2 (C-8), 126.2 (C-1′-aryl), 120.4 (C-3), 114.3 (C-3′ and C-5′-aryl), 55.2 (OCH_3), 29.1 (CH_2) ppm. Anal. Calcd. for $C_{18}H_{14}O_4S$: C, 66.24; H, 4.32 (Exp. C, 66.46; H, 4.36).

*5.1.2.5. 2-Hydroxy-3-(((4-(methylthio)phenyl)thio)methyl)naphthalene-1,4-dione (**11**).* The reaction produced the compound **11** in 68% as a orange solid. m.p. 120 °C. IR (KBr) ν_{max} (cm^{-1}) 1585 (C=C), 1641 (C=O), 3338 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (J in Hz): 8.13 (d, 1H, J = 7.8, H-8), 8.08 (dd, 1H, J = 7.8, H-5), 7.77 (dd, 1H, J = 7.3, 7.3, H-6), 7.70 (dd, 1H, J = 7.3, 7.3, H-7), 7.39 (d, 2H, J = 8.3, H-*ortho*-aryl), 7.13 (d, 2H, J = 8.3, H-*meta*-aryl), 4.06 (s, 2H, CH_2), 2.45 (s, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.3 (C-4), 181.0 (C-1), 153.1 (C-2), 137.8 (C-4′-aryl), 135.1 (C-7), 133.1 (C-6), 132.6 (C-8a), 132.2 (C-2′ and C-6′-aryl), 131.8 (C-1′-aryl), 129.3 (C-4a), 127.0 (C-3′ and C-5′-aryl), 126.7 (C-5), 126.2 (C-8), 120.1 (C-3), 27.9 (CH_2), 15.3 (CH_3) ppm. Anal. Calcd. for $C_{18}H_{14}O_3S_2$: C, 63.13; H, 4.12 (Exp. C, 63.05; H, 4.07).

*5.1.2.6. 2-Hydroxy-3-((p-tolylthio)methyl)naphthalene-1,4-dione (**12**).* The reaction produced the compound **12** in 84% as a orange solid. m.p. 122 °C. IR (KBr) ν_{max} (cm^{-1}) 1593 (C=C), 1637 (C=O), 3309 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (J in Hz): 8.13 (dd, 1H, J = 7.8, 1.0, H-8), 8.07 (dd, 1H, J = 7.8, 1.0, H-5), 7.77 (ddd, 1H, J = 7.3, 7.3, 1.0, H-6), 7.70 (ddd, 1H, J = 7.3, 7.3, 1.0, H-7), 7.37 (d, 2H, J = 8.3, H-*ortho*-aryl), 7.06 (d, 2H, J = 8.3, H-*meta*-aryl), 4.06 (s, 2H, CH_2), 2.30 (s, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.3 (C-4), 181.1 (C-1), 153.0 (C-2), 137.1 (C-1′-aryl), 135.1 (C-7), 133.1 (C-6), 132.7 (C-8a), 131.9 (C-2′ and C-6′-aryl), 131.8 (C-4′-aryl), 129.5 (C-3′ and C-5′-aryl), 129.3 (C-4a), 127.0 (C-5), 126.2 (C-8), 120.3 (C-3), 28.0 (CH_2), 21.1 (CH_3) ppm. Anal. Calcd. for $C_{18}H_{14}O_3S$: C, 69.66; H, 4.55 (Exp. C, 69.70; H, 4.48).

*5.1.2.7. 2-Hydroxy-3-(((4-hydroxyphenyl)thio)methyl)naphthalene-1,4-dione (**13**).* The reaction produced the compound **13** in 78% as a dark red solid. m.p. 177 °C. IR (KBr) ν_{max} (cm^{-1}) 1579 (C=C), 1634 (C=O), 3320 (OH). ^{1}H NMR (DMSO-D_6, 300 MHz) δ (J in Hz): 7.97 (dd, 1H, J = 7.5, 1.1, H-8), 7.94 (dd, 1H, J = 7.5, 1.1, H-5), 7.83 (ddd, 1H, J = 7.5, 7.5, 1.7, H-6), 7.78 (ddd, 1H, J = 7.5, 7.5, 1.4, H-7), 7.22 (ddd, 2H, J = 8.8, 3.0, 2.2, H-*ortho*-aryl), 6,66 (ddd, 2H, J = 8.8, 3.0, 2.2, H-*meta*-aryl), 3.86 (s, 2H, CH_2) ppm. ^{13}C NMR (DMSO-d6, 75 MHz): 183.2 (C-4), 180.9 (C-1), 157.2 (C-4′-aryl), 155.8 (C-2), 134.8 (C-7), 134.2 (C-2′ and C-6′-aryl), 133.4 (C-6), 131.8 (C-1′-aryl), 131.8 (C-8a),

125.8 (C-3′ and C-5′-aryl), 120.3 (C-4a), 120.3 (C-3), 116.4 (C-5), 116.0 (C-8), 28.7 (CH_2) ppm. m.p. 144 °C, Anal. Calcd. for $C_{17}H_{12}O_4S$: C, 65.37; H, 3.87 (Exp. C, 64.82; H, 3.80).

*5.1.2.8. 2-Hydroxy-3-(((4-nitrophenyl)thio)methyl)naphthalene-1,4-dione (**14**).* The reaction produced the compound **14** in 83% as a light brown solid. m.p. 189 °C. IR (KBr) ν_{max} (cm^{-1}) 1503 (N=O), 1578 (C=C), 1643 (C=O), 3307 (OH). ^{1}H NMR (DMSO-D_6, 300 MHz) δ (*J* in Hz): 8.10 (ddd, 2H, *J* = 9.2, 2.6, 2.2, H-*meta*-aryl), 7.97 (ddd, 1H, *J* = 7.6, 1.5, 0.4, H-8), 7.93 (ddd, 1H, *J* = 7.6, 1.5, 0.4, H-5), 7.80 (ddd, 1H, *J* = 7.5, 7.5, 1.5, H-6), 7.70 (ddd, 2H, *J* = 7.5, 7.5, 1.5, H-7), 7.62 (ddd, 2H, *J* = 9.2, 2.6, 2.2, H-*ortho*-aryl), 4.19 (s, 2H, CH_2) ppm. ^{13}C NMR (DMSO-d6, 75 MHz): 181.5 (C-4), 179.8 (C-1), 149.1 (C-2), 144.0 (C-4′-aryl and C-1′-aryl), 133.0 (C-8a), 134.5 (C-7), 132.2 (C-6), 130.2 (C-4a), 126.3 (C-2′ and C-6′-aryl), 125.6(C-5), 125.5 (C-8), 123.6 (C-3′ and C-5′-aryl), 115.6 (C-3), 26.6 (CH_2) ppm. Anal. Calcd. for $C_{17}H_{11}NO_5S$: C, 59.82; H, 3.25; N, 4.10, (Exp. C, 59.92; H, 3.35; N, 4.22).

*5.1.2.9. 2-Hydroxy-3-((o-tolylthio)methyl)naphthalene-1,4-dione (**15**).* The reaction produced the compound **15** in 77% as a brown solid. m.p. 97–99 °C. IR (KBr) ν_{max} (cm^{-1}) 1583 (C=C), 1643 (C=O), 3361 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (*J* in Hz): 8.13 (ddd, 1H, *J* = 7.6, 1.5, 0.6, H-8), 8.08 (ddd, 1H, *J* = 7.6, 1.5, 0.5, H-5), 7.77 (ddd, 1H, *J* = 7.6, 7.6, 1.5, H-6), 7.70 (ddd, 1H, *J* = 7.4, 7.4, 1.5, H-7), 7.48–7.35 (m, 2H, H-3′ and H-4′-aryl), 7.18–7.07 (m, 2H, H-5′ and H-6′-aryl), 4.06 (s, 2H, CH_2), 2.43 (s, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.2 (C-4), 181.0 (C-1), 153.2 (C-2), 139.3 (C-1′-aryl), 135.1 (C-7), 134.7 (C-2′-aryl), 133.0 (C-6), 132.6 (C-8a), 131.4 (C-3′-aryl), 130.0 (C-6′-aryl), 129.2 (C-4a), 127.0 (C-5), 126.9 (C-5′-aryl), 126.2 (C-8), 126.2 (C-4′-aryl), 119.8 (C-3), 26.4 (CH_2), 20.5 (CH_3) ppm. Anal. Calcd. for $C_{18}H_{14}O_3S$: C, 69.66; H, 4.55 (Exp. C, 69.62; H, 4.58).

*5.1.2.10. 2-Hydroxy-3-((m-tolylthio)methyl)naphthalene-1,4-dione (**16**).* The reaction produced the compound **16** in 84% as a orange solid. m.p.118–120 °C. IR (KBr) ν_{max} (cm^{-1}) 1586 (C=C), 1639 (C=O), 3350 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (*J* in Hz): 8.14 (ddd, 1H, *J* = 7.6, 1.6, 0.6, H-8), 8.07 (dd, 1H, *J* = 7.6, 1.6, 0.6, H-5), 7.77 (ddd, 1H, *J* = 7.6, 7.6, 1.3, H-6), 7.70 (ddd, 1H, *J* = 7.6, 7.6, 1.3, H-7), 7.37–7.28 (m, 2H, H-2′ and H-5′-aryl), 7.17–7.12 (m, 1H, H-6′-aryl), 7.02–6.99 (m, 1H, H-4′-aryl), 4.11 (s, 2H, CH_2), 2.29 (s, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.3 (C-4), 181.1 (C-1), 153.1 (C-2), 138.5 (C-1′-aryl), 135.4 (C-3′-aryl), 135.1 (C-7), 133.0 (C-6), 132.7 (C-8a), 131.5 (C-5′-aryl), 129.2 (C-4a), 128.6 (C-2′-aryl), 127.9 (C-5), 127.6 (C-6′-aryl), 127.0 (C-8), 126.2 (C-4′-aryl), 120.1 (C-3), 27.2 (CH_2), 21.2 (CH_3) ppm. Anal. Calcd. for $C_{18}H_{14}O_3S$: C, 69.66; H, 4.55 (Exp. C, 69.23; H, 4.44).

*5.1.2.11. 2-Hydroxy-3-((naphthalen-2-ylthio)methyl)naphthalene-1,4-dione (**17**).* The reaction produced the compound **17** in 49% as a orange solid. m.p. 139–141 °C. IR (KBr) ν_{max} (cm^{-1}) 1589 (C=C), 1639 (C=O), 3282 (OH). ^{1}H NMR (DMSO-D_6, 300 MHz) δ (*J* in Hz): 7.99–7.94 (m, 2H, H-8 and H-5), 7.91–7.90 (m, 1H, H-6), 7.85–7.84 (m, 1H, H-7), 7.87–7.82 (m, 4H, naphthyl), 7.51–7.41 (m, 3H, naphthyl), 4.14 (s, 2H, CH_2) ppm. ^{13}C NMR (DMSO-D_6, 75 MHz): 183.3 (C-4), 180.8 (C-1), 156.5 (C-2), 134.8 (C-7), 133.4 (C-6), 134.4 (C-2′-naphthyl), 133.4 (C-8a'-naphthyl), 131.9 (C-8a), 131.3 (C-4a), 130.0 (C-4a′-naphthyl), 128.3 (C-1′-naphthyl), 127.7 (C-4′-naphthyl), 127.2 (C-8′-naphthyl), 127.1 (C-5′-naphthyl), 126.7 (C-6′ and C-7′-naphthyl), 126.4 (C-5), 125.9 (C-8), 125.8 (C-3′-naphthyl), 119.5 (C-3), 26.2 (CH_2) ppm. Anal. Calcd. for $C_{21}H_{14}O_3S$: C, 72.81; H, 4.07 (Exp. C, 72.52; H, 4.09).

*5.1.2.12. 2-Hydroxy-3-((propylthio)methyl)naphthalene-1,4-dione (**18**).* The reaction produced the compound **18** in 89% as a yellow solid. m.p. 102 °C. IR (KBr) ν_{max} (cm^{-1}) 1585 (C=C), 1637 (C=O), 3336 (OH). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (*J* in Hz): 8.14 (dd, 1H, *J* = 7.8, 1.0, H-8), 8.09 (dd, 1H, *J* = 7.8, 1.0, H-5), 7.77 (ddd, 1H, *J* = 7.4, 7.4, 1.0, H-6), 7.70 (dd, 1H, *J* = 7.4, 7.4, 1.0, H-7), 3.70 (s, 2H, CH_2), 2.58 (t, 2H, *J* = 7.4, $C\underline{H}_2CH_2CH_3$), 1.66 (st, 2H, *J* = 7.4, $CH_2C\underline{H}_2CH_3$), 0.98 (t, 3H, *J* = 7.4, $CH_2CH_2C\underline{H}_3$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): 183.7 (C-4), 181.3 (C-1), 152.9 (C-2), 135.0 (C-7), 133.1 (C-6), 132.7 (C-8a), 129.3 (C-4a), 126.9 (C-5), 126.2 (C-8), 121.8 (C-3), 34.7 ($\underline{C}H_2CH_2CH_3$), 23.4 ($CH_2\underline{C}H_2CH_3$), 22.8 (CH_2), 13.5 ($CH_2CH_2\underline{C}H_3$) ppm. Anal. Calcd. for $C_{14}H_{14}O_3S$: C, 64.10; H, 5.38 (Exp. C, 64.07; H, 5.39).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.10.052.

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