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Original article

Synthesis and antitumor activity of 6- and 2-(1-substituted-thio-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-diones

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ABSTRACT

In an attempt to develop potent and selective antitumor agents, a series of 6- and 2-(1-substituted-thio-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-diones were designed and synthesized. The cytotoxicities of these compounds were evaluated in vitro against BEL-7402, HT-29 and SPC-A1 cell lines. The pharmacological results showed that most of the prepared compounds displayed the excellent selective cytotoxicity toward HT-29 cells. From the structure–activity relationships we may conclude that the introduction of a thioether functional group at the 1′-position in the side chain of shikonin is associated with an increase in cytotoxicity.

1. Introduction

Many clinically successful anticancer drugs are either natural products or have been developed from naturally occurring lead compounds, such as taxol, topotecan, irinotecan and vinblastine. Shikonin was isolated from the root of *Lithospermum erythrorhizon*. Closely related derivatives have been attracting much attention in view of their interesting antitumor activities [1–4]. A number of shikonin derivatives have been prepared and studied as to their tumor inhibitory potency. Most modifications to shikonin were focused on the hydroxyl group of side chain. For instance, Ahn et al. [5] and Plyta et al. [6] reported that acetylshikonin possessed more potent topoisomerase I inhibitory activity than shikonin. Lu et al. [7] synthesized a series of acylshikonin as antitumor agents and concluded that suitable modification of the side chain of shikonin would produce better telomerase inhibitors.

However, the shikonin derivatives prepared in the past were only some oxygen-containing acylshikonin derivatives. To this date there have been no reports on the sulfur-containing acylshikonin derivatives. To give a further insight into the antitumor activity, we introduced sulfur atom in place of oxygen atom into side chain of shikonin to synthesize sulfur-containing acylshikonin derivatives.

Thioether functional groups are known to contribute to the enhancement of the antitumor activity [8,9]. Combining the inherent antitumor activity of the shikonin and the functional group in one structure was expected to produce more active compounds.

Many of the existing results indicate that the dimethylation of naphthazarin ring will benefit the antitumor activities of quinones. For example, 5,8-dimethoxy-1,4-naphthoquinone was found to be less toxic and more active than naphthazarin ring [10,11]. So in this study, we designed and synthesized a series of 6- and 2-substituted-5,8-dimethoxy-1,4-naphthoquinone derivatives containing sulfur including thiol esters and thioethers to screen their antitumor activity against different cancer cell lines in vitro in order to get more potent and selective agents.

2. Chemistry

The preparation of target compounds is illustrated in Scheme 1. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-en-1-ol **2** was prepared using 1,4,5,8-tetramethoxynaphthalene-2-carbaldehyde **1** as starting material according to the procedure reported by our group previously [12]. Refluxing compound **2** with thiourea in ethanol in the presence of hydrobromic acid, followed by basic hydrolysis and neutralization with hydrochloric acid, subsequently formed thiol **3** [13].

Thereafter, alkylation of thiol **3** with various alkyl halides under basic conditions gave substituted thioethers **4**–**6**. The etherification by using alkyl halide and potassium carbonate was first carried out

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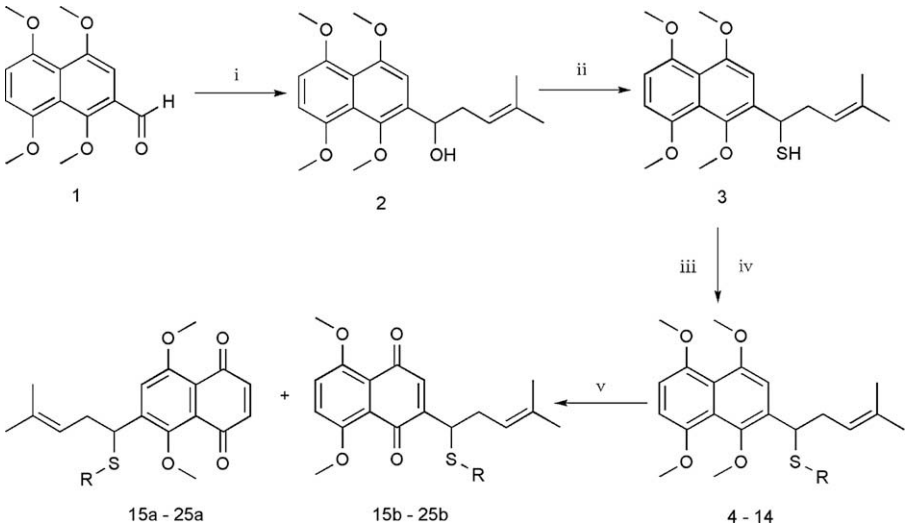


Scheme 1. Reagents and conditions: (i) prenyl bromide/Zn/HMPA, 130 °C, 10 h, N_2; (ii) 48% HBr/thiourea/EtOH, reflux, 1 h, then hydrolyze with 40% NaOH, N_2; (iii) RBr/NaOH/EtOH, reflux, 4 h, N_2; (iv) DCC/DMAP/CH_2Cl_2/, rt, 12 h; (v) CAN/CH_3CN/H_2O, rt, 0.5 h.

in anhydrous DMF [14], however, invariably low yields of ether are obtained after refluxing for long periods. Nevertheless, the reaction proceeded smoothly until consumption of the starting material when using an ethanol/H_2O (1:1) mixture as solvent in the presence of sodium hydroxide.

Next, we turned our attention to the introduction of various carboxylic acids to compound **3**. Different experimental conditions were examined, that is, dicyclohexylcarbodiimide (DCC)/4-dimethylaminopyridine (DMAP) activation [15], triphenylphosphine/*N*-bromo succinimide activation [16] and direct condensation with acid chloride/triethylamine. The attempt using the triphenylphosphine/NBS activation failed to give any product, possibly due to the greater steric bulk of thiol and the intermediate triphenylphosphonium bromide preventing the nucleophilic attack. When treating compound **3** with acid chloride in the presence of triethylamine, the reaction occurred in very low yield. However, when it was performed in anhydrous dichloromethane using DCC/DMAP as the coupling agent, the coupling reaction occurred in very high yield. So substituted thiol esters **7**–**14** were synthesized by acylating with various carboxylic acids in the presence of DCC and DMAP at room temperature.

Finally, thioethers **4**–**6** were further transformed to 6-(1-(alkylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione **15a** and 2-(1-(alkylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione **15b** derivatives by oxidative demethylation with cerium(IV) ammonium nitrate (CAN) [17]. Meanwhile, the methyl group elimination of thiol esters **7**–**14** was accomplished using the same procedure and reagents as thioethers **4**–**6**.

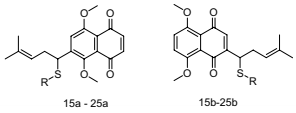
3. Results and discussion

The in vitro cytotoxicities of the shikonin derivatives against BEL-7402, HT-29 and SPC-A1 were evaluated by the standard MTT assay [18] using shikonin as a positive control. Antitumor potency of the compounds was indicated by IC_{50} values that were calculated by linear regression analysis of the concentration–response curves obtained for each compound. Data are reported in Table 1.

As shown in Table 1, while most of prepared compounds showed potent inhibitory effects on HT-29 cell lines, and the potencies of some compounds were better or comparative to the lead compound shikonin, some compounds showed moderate cytotoxicity against BEL-7402 and SPC-A1 cancer cell lines, although they were less potent than shikonin. It could be concluded that the cytotoxicity of the tested compound against HT-29 was higher than BEL-7402 and SPC-A1 cell lines, reflecting the excellent selectivity for a particular human colorectal cancer cell type.

The cytotoxicities of the resulting shikonin derivatives appeared to be related to the nature of the leaving group at the 1′-position in the side chain, which was in agreement with the cytotoxic mechanism reported [19]. It has been observed from Table 1 that most of the shikonin derivatives with thioether moieties have higher cytotoxicity than those with thiol ester groups. However, there were some exception, such as **17a** < **19a** in SPC-A1 cell line. Among **15a**–**17b** bearing thioether moieties, the IC_{50} of **15a** and **15b** against HT-29 is 8.4 μM and 6.9 μM, respectively, which is superior to

Table 1
Cytotoxicity of shikonin derivatives against BEL-7402, HT-29 and SPC-A1 cell lines



Compounds	R	IC_{50} (μM)		
		BEL-7402	HT-29	SPC-A1
15a	Ethyl	24.2	8.4	19.5
15b	Ethyl	86.4	6.9	18.2
16a	Propyl	47.0	39.7	42.2
16b	Propyl	62.6	19.8	24.4
17a	Pentyl	61.7	11.8	>100
17b	Pentyl	19.4	16.8	71.2
18a	Acetyl	84.3	27.8	67.9
18b	Acetyl	64.6	43.2	17.4
19a	Propionyl	96.5	40.5	19.4
19b	Propionyl	85.2	29.3	35.2
20a	Butyryl	87.7	30.8	78.3
20b	Butyryl	>100	33.4	43.9
21a	(*E*)-But-2-enoyl	80.5	16.9	59.1
21b	(*E*)-But-2-enoyl	55.8	11.9	17.6
22a	Isobutyryl	59.8	23.9	39.8
22b	Isobutyryl	>100	88.3	51.4
23a	4-Methylpent-3-enoyl	97.9	23.2	>100
23b	4-Methylpent-3-enoyl	>100	15.3	>100
24a	Hexanoyl	>100	22.5	93.8
24b	Hexanoyl	>100	23.0	89.4
25a	2-Methoxybenzoyl	>100	82.4	93.2
25b	2-Methoxybenzoyl	>100	75.4	>100
Shikonin		54.5	16.7	18.1

Data represent the mean values of three independent determinations.

shikonin ($IC_{50} = 16.7$ μM). Within the series **18a**–**25b** bearing thiol ester moieties, the great majority of compounds displayed lower activity than shikonin. The results indicated that the introduction of thioether functional group at the 1′-position in the side chain of shikonin facilitates the increase of their cytotoxic activities, while thiol ester functional group was unfavorable.

For HT-29 cell line, most of the prepared compounds showed potent inhibitory activities with the IC_{50} values lower than 20 μM, which were slightly lower than those of shikonin ($IC_{50} = 16.7$ μM). While thiol esters with aliphatic acetic to butyric acid showed a slightly decreased cytotoxic activity compared with shikonin, thiol esters of aromatic acid faced considerable loss of cytotoxicity. A similar tendency was observed against BEL-7402 and SPC-A1 cells. For example, compounds **18a**–**20b** were approximately 2-fold less active with the IC_{50} values of 27.8, 43.2, 40.5, 29.3, 30.8, 33.4 μM than lead compound shikonin ($IC_{50} = 16.7$ μM) against HT-29. However, the aromatic thiol esters **25a** and **25b** were significantly less potent with the IC_{50} values of greater than 75.4 μM in all cell lines. The data indicated that the introduction of aromatic thiol esters in the side chain might result in the loss of cytotoxicity. Also, the size of the ester chain and whether it was saturated could affect the toxicity of target compounds. For instance, compound **21b** containing double bond ($IC_{50} = 11.9$ μM) was approximately 3-fold more cytotoxic than saturated compound **20b** ($IC_{50} = 33.4$ μM) against the HT-29 cell line. Similarly, the hexanoyl derivative **24b** was significantly less potent than acetyl derivative **18b** at killing human liver cancer cell ($IC_{50} > 100$ μM vs $IC_{50} = 64.6$ μM).

4. Conclusion

In summary, we have designed and synthesized twenty-two sulfur-containing shikonin derivatives, and evaluated their anti-tumor activities against three cancer cell lines. Most of the prepared compounds displayed the selective toxicity toward HT-29 cell line. From the structure–activity relationships we may conclude that the introduction of thioether functional group at the 1′-position in the side chain of shikonin is associated with enhanced cytotoxic activity. This study may provide valuable information for further designing more potent anticancer agents.

5. Experimental protocols

5.1. Chemistry

Reagents and solvents were obtained from commercial suppliers. Solvents were dried and purified using standard techniques. All reactions involving air or moisture sensitive reagents or intermediates were performed under nitrogen. ^{1}H NMR spectra were recorded on a Mercuryplus 300 spectrometer. Mass spectra were recorded on a Shimadzu LCMS-2010EV mass spectrometer.

5.2. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-en-1-ol (**2**)

1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-en-1-ol **2** was obtained as recently described procedure [12].

5.3. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-ene-1-thiol (**3**)

1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-en-1-ol **2** (0.87 g, 0.025 mol), thiourea (0.38 g, 0.005 mol), 48% hydrobromic acid (0.56 ml) and ethanol (30 ml) were refluxed for 1 h. After cooling to room temperature, the reaction mixture was concentrated. Ether (20 ml) and distilled water (20 ml) were poured into the residue. The aqueous layer was then brought to alkaline pH by careful addition of 40% NaOH (10 ml) under nitrogen and heated to reflux for 2 h. The reaction mixture was cooled to room temperature and acidified with 6 M hydrochloric acid and then extracted with CH_2Cl_2 (3 × 15 ml). The combined organic layer was washed with brine, dried over Mg_2SO_4 and concentrated *in vacuo*. The residual oil was purified by flash chromatography on silica gel (diethylether:hexane = 1:5) to give 1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-ene-1-thiol **3** (0.79 g, 87% yield) as brown yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.01 (s, 1H), 6.81 (d, 2H), 5.23 (t, $J = 7.2$ Hz, 1H), 4.54 (t, $J = 7.2$ Hz, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.91 (s, 3H), 3.76 (s, 3H), 2.62 (m, 2H), 1.71 (s, 3H), 1.68 (s, 3H).

5.4. General procedure for preparation of alkyl(1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl)sulfanes (**4**–**6**)

To a solution of 1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-ene-1-thiol **3** (0.50 mmol) in ethanol (10 ml) were added alkyl halide (0.60 mmol) and KI (0.05 mmol), followed by immediate addition of 40% aqueous NaOH (0.5 ml). The reaction mixture was refluxed for 4 h under nitrogen atmosphere, and then ethanol was removed under reduced pressure, extracted with CH_2Cl_2, dried over Mg_2SO_4 and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel to afford **4**–**6** as light yellow oil.

5.4.1. Ethyl(1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl)sulfane (**4**)

Yield 75%. ^{1}H NMR ($CDCl_3$) δ: 7.06 (s, 1H,), 6.83 (d, 2H), 5.14 (t, $J = 7.5$ Hz, 1H), 4.66 (t, $J = 7.5$ Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.72 (s, 3H), 2.58 (q, 2H), 2.35 (m, 2H), 1.61 (s, 3H), 1.57 (s, 3H), 1.13 (t, $J = 7.2$ Hz, 3H).

5.4.2. Propyl(1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl)sulfane (**5**)

Yield 81%. ^{1}H NMR ($CDCl_3$) δ: 7.08 (s, 1H), 6.83 (d, 2H), 5.14 (t, $J = 7.2$ Hz, 1H), 4.63 (t, $J = 6.9$ Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 3.72 (s, 3H), 2.58 (t, 2H), 2.31 (m, 2H), 1.61 (s, 3H), 1.56 (s, 3H), 1.47 (m, 2H), 0.86 (t, $J = 7.2$ Hz, 3H).

5.4.3. Phenyl(1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl)sulfane (**6**)

Yield 82%. ^{1}H NMR ($CDCl_3$) δ: 7.08 (s, 1H), 6.84 (d, 2H), 5.16 (t, $J = 7.5$ Hz, 1H), 4.64 (t, $J = 7.5$ Hz, 1H), 3.97 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 3.73 (s, 3H), 2.59 (m, 2H), 2.35 (m, 2H), 1.63 (s, 3H), 1.57 (s, 3H), 1.48 (m, 2H), 1.22 (m, 4H), 0.82 (t, $J = 7.2$ Hz, 3H).

5.5. General procedure for preparation of 1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl thioates (**7**–**14**)

To a solution of 1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-ene-1-thiol **3** (0.50 mmol) and carboxylic acid (0.60 mmol) in CH_2Cl_2 (10 ml) were added DCC (0.75 mmol) and DMAP (0.25 mmol). After stirring overnight at room temperature under nitrogen atmosphere, the reaction mixture was filtered, concentrated, redissolved in ether, filtered once more, and concentrated *in vacuo*. The residual oil was purified by flash chromatography to afford **7**–**14** as light yellow oil.

5.5.1. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl ethanethioate (**7**)

Yield 94%. ^{1}H NMR ($CDCl_3$) δ: 6.81 (m, 3H), 5.29 (t, $J = 7.2$ Hz, 1H), 5.05 (t, $J = 7.2$ Hz, 1H), 3.92 (s, 6H), 3.88 (s, 3H), 3.78 (s, 3H), 2.69 (m, 2H), 2.31 (s, 3H), 1.59 (s, 6H).

*5.5.2. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl propanethioate (**8**)*

Yield 92%. ^{1}H NMR ($CDCl_3$) δ: 6.80 (m, 3H), 5.30 (t, J = 7.2 Hz, 1H), 5.05 (t, J = 7.2 Hz, 1H), 3.92 (s, 6H), 3.88 (s, 3H), 3.78 (s, 3H,), 2.70 (m, 2H), 2.54 (q, J = 7.2 Hz, 2H), 1.58 (s, 6H), 1.15 (t, J = 7.2 Hz, 3H).

*5.5.3. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl butanethioate (**9**)*

Yield 92%. ^{1}H NMR ($CDCl_3$) δ: 6.82 (m, 3H), 5.32 (t, J = 6.9 Hz, 1H), 5.07 (t, J = 6.9 Hz, 1H), 3.92 (s, 6H), 3.88 (s, 3H), 3.79 (s, 3H), 2.68 (m, 2H), 2.51 (t, J = 7.2 Hz, 2H), 1.69 (q, J = 7.2 Hz, 2H), 1.59 (s, 6H), 0.93 (t, J = 7.2 Hz, 3H).

*5.5.4. (E)-1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl but-2-enethioate (**10**)*

Yield 87%. ^{1}H NMR ($CDCl_3$) δ: 6.91 (dd, J = 15.5 Hz, J = 6.9 Hz, 1H), 6.84 (s, 1H), 6.82 (s, 2H), 6.11 (dd, J = 15.5 Hz, J = 2.1 Hz, 1H), 5.38 (t, J = 6.9 Hz, 1H), 5.07 (t, J = 6.9 Hz, 1H), 3.92 (s, 6H), 3.88 (s, 3H), 3.79 (s, 3H), 2.72 (m, 2H), 1.85 (dd, J = 6.9 Hz, J = 2.1 Hz, 3H), 1.59 (s, 6H).

*5.5.5. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl 2-methylpropanethioate (**11**)*

Yield 90%. ^{1}H NMR ($CDCl_3$) δ: 6.82 (m, 3H), 5.29 (t, J = 6.9 Hz, 1H), 5.07 (t, J = 6.9 Hz, 1H), 3.93 (s, 6H), 3.89 (s, 3H), 3.76 (s, 3H), 2.70 (m, 2H), 1.19 (d, J = 6.6 Hz, 6H), 1.15 (d, J = 6.6 Hz, 3H).

*5.5.6. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl 4-methylpent-3-ene-thioate (**12**)*

Yield 89%. ^{1}H NMR ($CDCl_3$) δ: 6.81 (m, 3H), 5.28 (m, 2H), 5.05 (t, J = 6.0 Hz, 1H), 3.92 (s, 6H), 3.88 (s, 3H), 3.80 (s, 3H), 3.23 (d, J = 6.6 Hz, 2H), 2.68 (m, 2H), 1.73 (s, 3H), 1.63 (s, 3H), 1.59 (s, 6H).

*5.5.7. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl hexanethioate (**13**)*

Yield 88%. ^{1}H NMR ($CDCl_3$) δ: 6.82 (m, 3H), 5.31 (t, J = 7.2 Hz, 1H), 5.07 (t, J = 7.2 Hz, 1H), 3.93 (s, 6H), 3.89 (s, 3H), 3.79 (s, 3H), 2.68 (m, 2H, CH_2), 2.52 (t, J = 7.2 Hz, 2H), 1.61 (s, 6H), 1.32 (m, 6H), 0.90 (t, J = 7.2 Hz, 3H).

*5.5.8. 1-(1,4,5,8-Tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl 2-methoxybenzothioate (**14**)*

Yield 94%. ^{1}H NMR ($CDCl_3$) δ: 7.76 (d, J = 7.5 Hz, 1H), 7.44 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 7.5 Hz, 2H), 6.93 (s, 1H), 6.83 (s, 2H), 5.51 (t, J = 6.6 Hz, 1H), 5.13 (t, J = 6.3 Hz, 1H), 3.94 (s, 6H), 3.91 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H), 2.81 (m, 2H), 1.62 (s, 3H), 1.60 (s, 3H).

*5.6. General procedure for preparation of 6-(1-(alkylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-diones (**15a**–**17a**) and 2-(1-(alkylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-diones (**15b**–**17b**)*

To a solution of alkyl(1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl)sulfane **4**–**6** (0.20 mmol) in acetonitrile (3 ml) was added dropwise a solution of CAN (0.50 mmol) in water (2 ml). The reaction mixture was stirred at room temperature for 10 min, then diluted with water and extracted with CH_2Cl_2, washed with brine, dried over with Mg_2SO_4, and concentrated. The residual oil was purified by preparative TLC to afford **15a**–**17a** as yellow oil and **15b**–**17b** as red oil.

*5.6.1. 6-(1-(Ethylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione (**15a**) and 2-(1-(ethylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione (**15b**)*

Compound **15a**. Yield 18%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.54 (s, 1H, benzene ring H), 6.77 (s, 2H, quinone ring H), 5.08 (t, J = 6.0 Hz, 1H), 4.51 (t, J = 7.5 Hz, 1H), 3.95 (s, 3H), 3.79 (s, 3H), 2.53 (m, 2H), 2.39 (q, 2H), 1.69 (s, 3H), 1.54 (s, 3H), 1.20 (t, 3H). ESI-MS: 383.10 $(M + Na)^+$.

Compound **15b**. Yield 35%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.30 (s, 2H, benzene ring H), 6.79 (s, 1H, quinone ring H), 5.10 (t, J = 6.6 Hz, 1H), 4.18 (t, J = 6.9 Hz, 1H), 3.95 (s, 6H), 2.60 (m, 2H), 2.48 (q, 2H), 1.64 (s, 3H), 1.57 (s, 3H), 1.20 (t, 3H).

*5.6.2. 6-(1-(Propylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione (**16a**) and 2-(1-(propylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione (**16b**)*

Compound **16a**. Yield 20%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.57 (s, 1H, benzene ring H), 6.78 (s, 2H, quinone ring H), 5.10 (t, J = 7.5 Hz, 1H), 4.48 (t, J = 6.9 Hz, 1H), 3.99 (s, 3H), 3.82 (s, 3H), 2.55 (t, 2H), 2.38 (m, 2H), 1.73 (m, 2H), 1.64 (s, 3H), 1.54 (s, 3H), 0.92 (t, J = 7.2 Hz, 3H).

Compound **16b**. Yield 41%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.30 (s, 2H, benzene ring H), 6.79 (s, 1H, quinone ring H), 5.10 (t, J = 7.2 Hz, 1H), 4.16 (t, J = 7.2 Hz, 1H), 3.96 (s, 6H), 2.45 (m, 4H), 1.64 (s, 3H), 1.55 (s, 3H), 0.92 (t, J = 7.2 Hz, 3H). ESI-MS: 397.10 $(M + Na)^+$.

*5.6.3. 6-(1-(Pentylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione (**17a**) and 2-(1-(pentylthio)-4-methylpent-3-enyl)-5,8-dimethoxynaphthalene-1,4-dione (**17b**)*

Compound **17a**. Yield 19%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.56 (s, 1H, benzene ring H), 6.78 (s, 2H, quinone ring H), 5.10 (t, J = 7.5 Hz, 1H), 4.48 (t, J = 7.5 Hz, 1H), 3.99 (s, 3H), 3.82 (s, 3H), 2.54 (t, 2H), 2.39 (m, 2H), 1.64 (s, 3H), 1.54 (s, 3H), 1.28 (m, 6H), 0.84 (t, J = 7.2 Hz, 3H). ESI-MS: 417.05 $(M + Na)^+$.

Compound **17b**. Yield 33%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.30 (s, 2H, benzene ring H), 6.79 (s, 1H, quinone ring H), 5.11 (t, J = 6.9 Hz, 1H), 4.16 (t, J = 7.2 Hz, 1H), 3.95 (s, 6H), 2.46 (m, 4H), 1.64 (s, 3H), 1.57 (s, 3H), 1.26 (m, 6H), 0.84 (t, J = 7.2 Hz, 3H).

*5.7. General procedure for preparation of 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl thioates (**18a**–**25a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl thioates (**18b**–**25b**)*

Compounds **18a**–**25a** and **18b**–**25b** were synthesized with the same method used for the preparation of compounds **15a**–**17a** and **15b**–**15b** using 1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl thioate **7**–**14** instead of alkyl(1-(1,4,5,8-tetramethoxynaphthalen-7-yl)-4-methylpent-3-enyl)sulfane **4**–**6**.

*5.7.1. 1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl ethanethioate (**18a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl ethanethioate (**18b**)*

Compound **18a**. Yield 35%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.30 (s, 1H, benzene ring H), 6.77 (s, 2H, quinone ring H), 5.03 (m, 2H), 3.96 (s, 3H), 3.90 (s, 3H), 2.61 (t, 2H), 2.31 (s, 3H), 1.63 (s, 3H), 1.55 (s, 3H). ESI-MS: 397.05 $(M + Na)^+$.

Compound **18b**. Yield 30%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.28 (s, 2H, benzene ring H), 6.75 (s, 1H, quinone ring H), 5.04 (t, J = 7.2 Hz, 1H), 4.67 (t, J = 7.2 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 2.57 (m, 2H), 2.28 (s, 3H), 1.64 (s, 3H), 1.57 (s, 3H).

*5.7.2. 1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl propanethioate (**19a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl propanethioate (**19b**)*

Compound **19a**. Yield 36%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.31 (s, 1H, benzene ring H), 6.78 (s, 2H, quinone ring H), 5.05 (m, 2H), 3.96 (s, 3H), 3.91 (s, 3H), 2.59 (m, 4H), 1.64 (s, 3H), 1.55 (s, 3H), 1.15 (t, J = 7.2 Hz, 3H). ESI-MS: 411.10 $(M + Na)^+$.

Compound **19b**. Yield 28%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.29 (s, 2H, benzene ring H), 6.75 (s, 1H, quinone ring H), 5.04 (t, J = 7.2 Hz, 1H), 4.69 (t, J = 7.2 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 2.53 (m, 4H), 1.63 (s, 3H), 1.57 (s, 3H), 1.13 (t, J = 7.2 Hz, 3H).

*5.7.3. 1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl butanethioate (**20a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl butanethioate (**20b**)*

Compound **20a**. Yield 37%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.31 (s, 1H, benzene ring H), 6.77 (s, 2H, quinone ring H), 5.06 (m, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 2.60 (m, 2H), 2.51 (t, J = 6.6 Hz, 2H), 2.60 (m, 2H), 1.67 (m, 5H), 1.54 (s, 3H), 0.92 (t, J = 7.2 Hz, 3H). ESI-MS: 425.05 $(M + Na)^+$.

Compound **20b**. Yield 28%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.29 (s, 2H, benzene ring H), 6.75 (s, 1H, quinone ring H), 5.04 (t, J = 7.5 Hz, 1H), 4.69 (t, J = 7.2 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 2.50 (m, 2H), 2.47 (t, J = 6.9 Hz, 2H), 1.65 (m, 5H), 1.56 (s, 3H), 0.90 (t, J = 7.5 Hz, 3H).

*5.7.4. (2E)-1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl but-2-enethioate (**21a**) and (2E)-1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl but-2-enethioate (**21b**)*

Compound **21a**. Yield 43%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.34 (s, 1H, benzene ring H), 6.90 (dd, J = 15.5 Hz, J = 6.9 Hz, 1H), 6.77 (s, 2H, quinone ring H), 6.09 (dd, J = 15.5 Hz, J = 1.8 Hz, 1H), 5.13 (t, J = 7.2 Hz, 1H), 5.05 (t, J = 7.2 Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H), 2.63 (m, 2H), 1.87 (dd, J = 7.2 Hz, J = 1.8 Hz, 3H), 1.63 (s, 3H), 1.55 (s, 3H). ESI-MS: 423.05 $(M + Na)^+$.

Compound **21b**. Yield 30%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.29 (s, 2H, benzene ring H), 6.87 (dd, J = 15.3 Hz, J = 6.9 Hz, 1H), 6.77 (s, 1H, quinone ring H), 6.06 (dd, J = 15.3 Hz, J = 1.8 Hz, 1H), 5.05 (t, J = 7.2 Hz, 1H), 4.74 (t, J = 7.2 Hz, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 2.61 (m, 2H), 1.84 (dd, J = 7.2 Hz, J = 1.8 Hz, 3H), 1.63 (s, 3H), 1.56 (s, 3H).

*5.7.5. 1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl 2-methylpropanethioate (**22a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-methylpropanethioate (**22b**)*

Compound **22a**. Yield 39%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.32 (s, 1H, benzene ring H), 6.77 (s, 2H, quinone ring H), 5.05 (m, 2H), 3.96 (s, 3H), 3.91 (s, 3H), 2.72 (m, 1H), 2.60 (m, 2H), 1.63 (s, 3H), 1.55 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H), 1.15 (d, J = 7.2 Hz, 3H).

Compound **22b**. Yield 28%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.29 (s, 2H, benzene ring H), 6.75 (s, 1H, quinone ring H), 5.04 (t, J = 7.5 Hz, 1H), 4.67 (t, J = 7.2 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 2.68 (m, 1H), 2.56 (m, 2H), 1.63 (s, 3H), 1.56 (s, 3H), 1.15 (d, J = 7.2 Hz, 3H), 1.13 (d, J = 7.2 Hz, 3H). ESI-MS: 425.05 $(M + Na)^+$.

*5.7.6. 1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl 4-methylpent-3-enethioate (**23a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 4-methylpent-3-enethioate (**23b**)*

Compound **23a**. Yield 36%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.30 (s, 1H, benzene ring H), 6.76 (s, 2H, quinone ring H), 5.23 (t, J = 7.5 Hz, 1H), 5.03 (m, 2H), 3.95 (s, 3H), 3.89 (s, 3H), 3.21 (d, J = 7.5 Hz, 2H), 2.60 (m, 2H), 1.74 (s, 3H), 1.63 (s, 6H), 1.54 (s, 3H). ESI-MS: 451.10 $(M + Na)^+$.

Compound **23b**. Yield 27%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.29 (s, 2H, benzene ring H), 6.74 (s, 1H, quinone ring H), 5.23 (t, J = 7.2 Hz, 1H), 5.03 (t, J = 7.2 Hz, 1H), 4.66 (t, J = 7.2 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.18 (d, J = 7.5 Hz, 2H), 2.56 (m, 2H), 1.73 (s, 3H), 1.62 (s, 6H), 1.56 (s, 3H).

*5.7.7. 1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl hexanethioate (**24a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl hexanethioate (**24b**)*

Compound **24a**. Yield 39%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.31 (s, 1H, benzene ring H), 6.77 (s, 2H, quinone ring H), 5.05 (m, 2H), 3.96 (s, 3H), 3.91 (s, 3H), 2.60 (m, 2H), 2.52 (t, J = 7.2 Hz, 2H), 1.64 (s, 3H), 1.55 (s, 3H), 1.31 (m, 6H), 0.89 (t, J = 7.2 Hz, 3H). ESI-MS: 453.10 $(M + Na)^+$.

Compound **24b**. Yield 23%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.30 (s, 2H, benzene ring H), 6.75 (s, 1H, quinone ring H), 5.04 (t, J = 7.5 Hz, 1H), 4.68 (t, J = 7.5 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 2.55 (m, 2H), 2.48 (t, J = 7.2 Hz, 2H), 1.64 (s, 3H), 1.56 (s, 3H), 1.26 (m, 6H), 0.86 (t, J = 7.2 Hz, 3H).

*5.7.8. 1-(1,4-Dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-6-yl)-4-methylpent-3-enyl 2-methoxybenzothioate (**25a**) and 1-(1,4-dihydro-5,8-dimethoxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-methoxybenzothioate (**25b**)*

Compound **25a**. Yield 36%, yellow oil. ^{1}H NMR ($CDCl_3$) δ: 7.74 (d, J = 7.8 Hz, 1H), 7.46 (t, J = 7.8 Hz, 1H), 7.43 (s, 1H, benzene ring H), 6.98 (t, J = 7.8 Hz, 2H), 6.77 (s, 2H, quinone ring H), 5.25 (t, J = 7.2 Hz, 1H), 5.10 (t, J = 7.2 Hz, 1H), 3.96 (s, 6H), 3.90 (s, 3H), 2.71 (m, 2H), 1.64 (s, 3H), 1.58 (s, 3H).

Compound **25b**. Yield 27%, red oil. ^{1}H NMR ($CDCl_3$) δ: 7.74 (d, J = 7.5 Hz, 1H), 7.44 (t, J = 7.5 Hz, 1H), 7.28 (s, 2H, benzene ring H), 6.95 (t, J = 7.5 Hz, 2H), 6.87 (s, 1H, quinone ring H), 5.12 (t, J = 6.9 Hz, 1H), 4.90 (t, J = 6.9 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 2.67 (m, 2H), 1.64 (s, 3H), 1.59 (s, 3H). ESI-MS: 489.10 $(M + Na)^+$.

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