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Bioorganic & Medicinal Chemistry 13 (2005) 2325–2330
Bioorganic & Medicinal Chemistry

Antitumor agents 243. Syntheses and cytotoxicity of desmosdumotin C derivatives

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Received 13 October 2004; accepted 17 December 2004
Available online 22 January 2005

Abstract—New analogs of desmosdumotin C (**1**), in which other aromatic rings replaced the terminal phenyl group and the A-ring was modified, were synthesized. Compounds **2**–**9**, **13**, and **16** were evaluated in vitro against human tumor cell replication. The 4-bromophenyl analog (**2**) showed potent cytotoxic activity in four different tumor cell lines.

1. Introduction

Desmosdumotin C (**1**) was isolated as a novel compound in 2002 from the roots of *Desmos dumosus*, which has been used in Chinese folk medicine as an antimalarial, insecticidal, antirheumatic, antispasmodic, and analgesic agent.[1] NMR and X-ray analyses of **1** supported its unique chalcone structure, which bears an unusual A ring with a *gem*-dimethyl group on C-6, methyl group on C-4, and methoxy group on C-5. Interestingly, the ^{1}H NMR spectrum of **1** showed only one enolic tautomer, although three enolic tautomers are conceivable (Fig. 1). In fact, similar compounds, including safflomin C,[2] syzygiol,[3] and uliginosin A analog,[4] exist as a tautomeric mixture. Enolic forms **1a**–**c** would be preferred to keto form **1d**, because a strong hydrogen bond can be formed between the ketone and enolic hydroxy proton, as also indicated by the presence of a chelated OH proton at very low field (19.17 ppm) in the ^{1}H NMR of **1**. In addition, it might be postulated that form **1a**, which has extended conjugation from the terminal aromatic ring, could be more stable and populated than the other enolic forms **1b** and **c**.

Desmosdumotin C (**1**) showed significant and selective in vitro cytotoxicity with IC_{50} values of 4.0 and 3.5 μg/mL against 1A9 ovarian cancer and A549 human lung carcinoma, respectively. In addition, it was more active against vincristine-resistant KB cells than against the parent KB epidermoid nasopharyngeal carcinoma cell line.[5] Thus, **1** represents a promising new lead for further new antitumor analog development.

A literature search did not reveal any reported modifications of chalcones containing the unusual A ring found in **1**, although many modifications of chalcones with a normal completely aromatized A ring have been achieved.[6] Therefore, to develop structure–activity relationships (SAR) and more active derivative of this unique chalcone, several desmosdumotin C derivatives with substituted phenyl or hetero-aromatic rings (**2**–**9**) rather than the terminal phenyl ring and with modified A-ring (**13**, **16**) were synthesized. The resulting derivatives are evaluated in vitro against human tumor cell replications (1A9 ovarian cancer, A549 human lung carcinoma, KB human epidermoid carcinoma of the nasopharynx, and KB-V multi-drug resistant expressing P-glycoprotein). We report herein the synthesis and bioassay results.

2. Chemistry

The simple total synthesis of desmosdumotin C from 2,4,6-trihydroxyacetophenone (**10**), as shown in Scheme 1, was reported previously.[7] By using other aromatic aldehydes rather than benzaldehyde in the final aldol

Keywords: Desmosdumotin C analogs; Cytotoxicity; Antitumor.
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doi:10.1016/j.bmc.2004.12.040

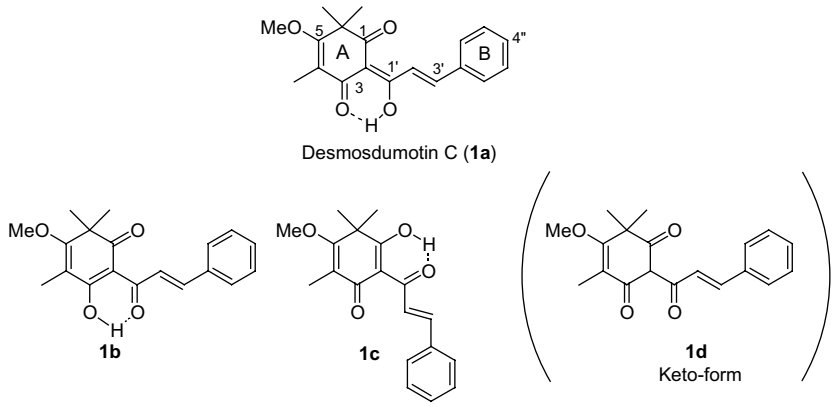
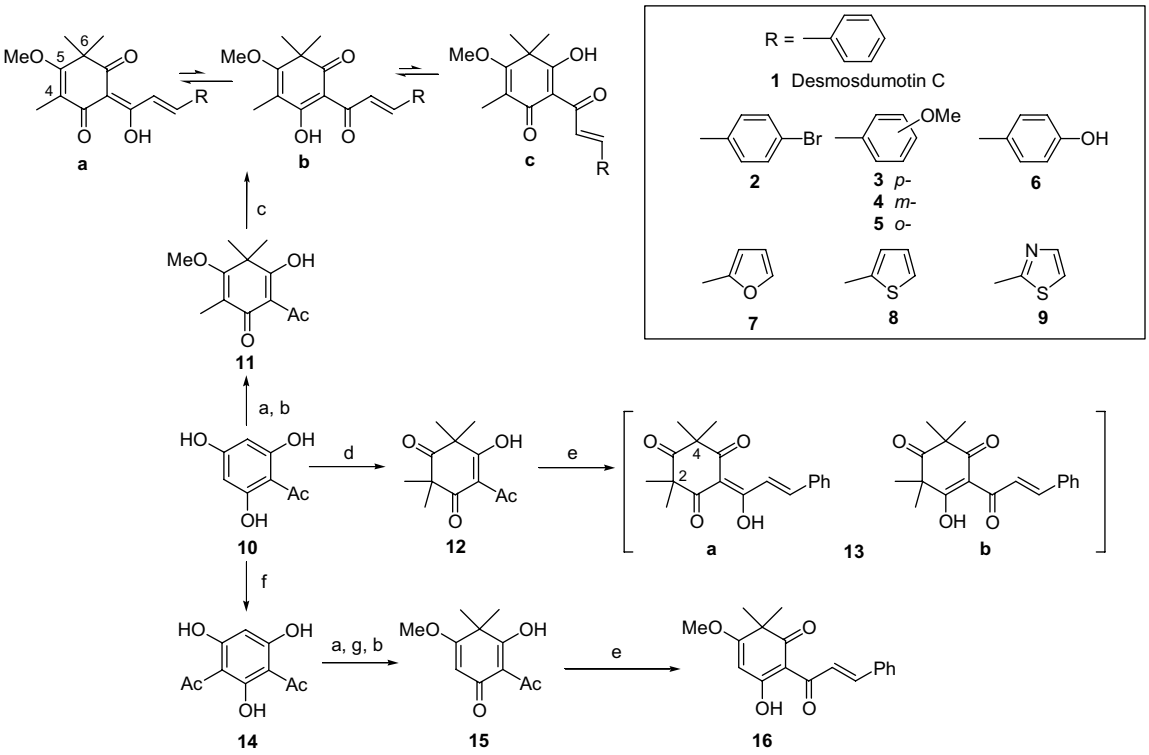


Figure 1. Desmosdumotin C (**1**) and possible tautomers.



Scheme 1. Reagents and conditions: (a) NaOMe (3.7 equiv mol), MeI (3.3 equiv mol) 56%; (b) $TMSCHN_2$, EtOAc/MeOH, 88% for **11**, 85% for **15**; (c) RCHO, base (see Table 1); (d) NaOMe, MeI (excess); (e) PhCHO, KOH/EtOH, 84% for **13**, 95% for **16**; (f) AcOH, Ac_2O, $BF_3{\cdot}OEt_2$, 92%; (g) 85% H_2SO_4, 56%.

step, various analogs of desmosdumotin C could be obtained. Therefore, **11** was treated with 4-bromophenylaldehyde, *o*-, *p*-, and *m*-anisaldehyde, 2-furaldehyde, 2-thiophenecarboxaldehyde, and 2-thiazolecarboxaldehyde in 50% aq KOH/EtOH solution (method I) to afford the corresponding compounds **2**–**5** and **7**–**9**. 4-Hydroxyphenyl derivative **6** was obtained by the treatment of **11** with 4-hydroxybenzaldehyde in piperidine (method II).[8] Treatment of **10** with excess MeI and NaOMe generated tetramethyl derivative **12**.[9] Compound **15** which lacks the C-4 methyl group of **11**, was synthesized using the following procedure. Acetylation of 2,4,6-trihydroxyacetophenone monohydrate using a modified Marchand's method[10] generated 1,5-diacetyl-2,4,6-trihydroxybenzene (**14**), which was converted to **15** according to a previously reported method.[11] The obtained **12** and **15** were treated with benzaldehyde under basic conditions (method I) to give **13** and ceroptene (**16**),[12] respectively. All aldol products, except for **2**, **13**, and **19**, existed as a mixture of two tautomers, enolic tautomers **a** and **b**,[13] in $CDCl_3$ solution; the third tautomer, enol **c**, was not observed. As mentioned in the introduction, desmosdumotin C (**1**) exists as enolic form **1a**, and accordingly, the major tautomer of **2**–**9** corresponded to enolic form **a** and the minor tautomer to enolic form **b**, based on ^1H NMR chemical shift data. However, the major tautomer of **13** was concluded to be the 3,5-diketo form **13b**. In the 1,3,5-trione form

Table 1. ^{1}H NMR chemical shifts of aldol products

Compd	Assignment										Ratio[a]
	6-*gem* diMe		4-Me		5- OMe		Olefin		Chelated-OH		
	a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a:b**
1	1.37	—	1.99	—	3.95		8.33, 7.92		19.17		
2	1.36	1.46	1.99	1.94	3.95	3.88	8.30, 7.83	8.51, 7.84	19.20	18.66	11:1
3	1.38		2.00		3.95		8.23, 7.94		19.19		
4	1.37	1.46	1.99	1.94	3.95	3.88	8.29, 7.89	8.50, 7.91	19.16	18.75	3.5:1
5	1.36	1.45	1.98	1.94	3.94	3.90	8.38, 8.31	8.54, 8.39	19.16	18.82	2.5:1
6	1.33	1.42	1.95	1.91	3.91	3.85	8.14, 7.89	8.32, 7.91	19.15	18.81	2:1
7	1.36	1.45	1.98	1.94	3.94	3.87	8.15, 7.68	8.35, 7.73	19.07	18.71	2:1
8	1.36	1.45	1.98	1.94	3.94	3.87	8.15, 8.05	8.34, 8.08	19.17	18.79	2.5:1
9	1.37	1.47	2.00	1.94	3.96	3.89	8.44, 8.00	8.65, 8.03	19.20	18.50	2.5:1
13		1.46, 1.41								18.17	
16	1.41		—		3. 84		8.34, 7.92		18.96		

[a] Ratio in $CDCl_3$.

13a, the *gem*-dimethyls on C-2 and C-4, as well as C-1, C-3, and C-5, would be equivalent spectroscopically.[13] However, the signals of the C-2 and C-4 *gem*-dimethyl groups occurred at different positions—1.46 and 1.41 ppm in the ^{1}H NMR spectrum and 24.1 and 23.9 ppm in the ^{13}C NMR spectrum. Furthermore, the C-1, C-3, and C-5 signals also appeared at different fields (210.0, 202.6, and 197.7 ppm). The ^{1}H NMR chemical shifts for the aldol products and the tautomer ratios in $CDCl_3$ solution are listed in Table 1.

3. Results and discussion

The new desmosdumotin C derivatives **2**–**9**, **13**, and **16**, together with synthetic desmosdumotin C (**1**), were evaluated for cytotoxic activity against replication of several human tumor cell lines, lung carcinoma A549, ovarian carcinoma 1A9, nasopharyngeal carcinoma KB, and KB-V, a multi-drug resistant (MDR) variant. The average IC_{50} values are shown in Table 2. All substituted phenyl derivatives **2**–**6** showed higher activity than the parent compound **1** against all cell lines. Especially, the 4-bromophenyl derivative (**2**) displayed ca. 4–6 fold enhanced activity against three different tumor cell lines. Compounds **7** and **8** which have hetero-aromatic five-membered rings, were less active compared with **1**, while thiazole derivative **9** was slightly more active against A549, 1A9, and KB cell lines. The modified A-ring derivative **13** was less potent against all cell lines. Ceroptene (**16**) was as active as **1** against 1A9, less active against A549, but more active against KB cells. Compounds **8**, **9**, **13**, and **16**, especially **8** and **13**, had statistically significant higher activity against the multi-drug resistant (KB-V) and non-resistant (KB) cell lines, while compounds **2**–**7**, as well as **1**, showed comparable sensitivity in the two cell lines.

These results indicated that modification of the terminal phenyl ring, regardless of the position and type of substituent, could enhance cytotoxicity of desmosdumotin C-type compounds against some cancer cell lines. Above all, the 4-bromophenyl derivative **2** was the most interesting compound. However, the introduction of hetero-aromatic five-membered rings rather than a phenyl ring was less favorable. Moreover, suitable modification

Table 2. Activities of desmosdumotin C analogs against human tumor cell line replication

Compound	Cell line/IC_{50} (μM)[a]			
	A549[b]	1A9[b]	KB[b]	KB-V[b]
1	12.82	12.82	20.83	17.94
2	3.57	2.80	4.33	4.84
3	8.74	8.16	10.49	8.45
4	8.16	8.16	9.62	6.99
5	6.99	7.28	9.62	8.16
6	9.48	8.86	11.62	11.62
7	33.00	26.07	32.67	29.04
8	14.10	12.53	17.24	10.65
9	10.93	10.93	13.43	9.37
13	>16.08	14.79	23.47	12.22
16	>16.78	12.08	13.76	10.07

[a] Cytotoxicity as IC_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to unreacted cells using the sulforhodamine B assay. The average value is from 2–4 independent determinations and variation (SEM) was no greater than 14%.

[b] Human lung carcinoma (A549), human ovarian carcinoma (1A9), human epidermoid carcinoma of the nasopharynx (KB), multi-drug resistant expressing P-glycoprotein (KB-V).

of the A-ring might confer high selectivity against cancer cell lines. Additional experiments are in progress.

4. Experimental section

All chemicals and solvents were used as purchased. All melting points were measured on a Fisher–Johns melting point apparatus without correction. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 (300 MHz) NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. NMR spectra were referenced to the residual solvent peak; chemical shifts δ in ppm; apparent scalar coupling constants J in Hz. Mass spectral data were obtained on a TRIO 1000 mass spectrometer. IR spectra were recorded on a Perkin–Elmer 1320 spectrophotometer. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). All target compounds were characterized by ^{1}H and IR spectral analyses and MS analyses.

4.1. General procedures for the aldol reactions

Method I: A solution of acetyl compound (**11**, **12**, or **15**) in EtOH–50% aq KOH (1:1, v/v) and an appropriate aldehyde (excess) was stirred at room temperature. After the reaction was complete by TLC analysis, the mixture was poured into ice-cold 1 N HCl, then extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc–hexane as eluent to afford the target compound, which was crystallized from CH_2Cl_2–hexane.

Method II: A solution of **11** and 4-hydroxybenzaldehyde (excess) in anhydrous piperidine (excess) was heated at reflux. The reaction was worked up following the procedure in method I.

4.1.1. 2-[1-Hydroxy-3-(4-bromophenyl)-allylidene]-5-methoxy-4,6,6-trimethylcyclohex-4-ene-1,3-dione (2). Method I: 61% yield (based on recovered starting material). mp 140–141 °C (CH_2Cl_2–hexane). IR (KBr): 2976, 2935, 1657, 1623, 1517, 1488, 1468, 1429 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 19.20 (s) and 18.66 (s) (11:1, 1H, chelated-O*H*), 8.51 (d) and 8.30 (d) (1:11, 1H, J = 15.9 Hz, *trans*-olefinic proton), 7.84 (d) and 7.83 (d) (1:11, 1H, J = 15.9 Hz, *trans*-olefinic proton), 7.56–7.48 (m, 4H, Ar-2″,3″,5″,6″-*H*), 3.95 (s) and 3.88 (s) (11:1, 3H, 5-OCH_3), 1.99 (s) and 1.94 (s) (11:1, 3H, 4-CH_3), 1.46 (s) and 1.36 (s) (1:11, 6H, 6-C$H_3 \times 2$). MS *m*/*z* 391 and 393 (M^+, 1:1). Anal. Calcd for $C_{19}H_{19}BrO_4 \cdot 1/4H_2O$: C, 57.66; H, 4.97; O, 17.18. Found: C, 57.42; H, 4.84.

4.1.2. 2-[1-Hydroxy-3-(4-methoxyphenyl)-allylidene]-5-methoxy-4,6,6-trimethylcyclohex-4-ene-1,3-dione (3). Method I: 82% yield. mp 99–100 °C (CH_2Cl_2–hexane). IR (KBr): 2975, 2934, 1656, 1621, 1600, 1572, 1510, 1423, 1243, 1171 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 19.19 (s, 1H, chelated-O*H*), 8.23 (d, 1H, J = 15.5 Hz, *trans*-olefinic proton), 7.94 (d, 1H, J = 15.5 Hz, *trans*-olefinic proton), 7.68–7.61 (m, 2H, Ar-2″,6″-*H*), 6.95–6.87 (m, 2H, Ar-3″, 5″-*H*), 3.95 (s, 3H, 5-OCH_3), 3.85 (s, 3H, Ph–OCH_3), 2.00 (s, 3H, 4-CH_3), 1.38 (s, 6H, 6-C$H_3 \times 2$). MS *m*/*z* 343 (M^++1). Anal. Calcd for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48; O, 23.36. Found: C, 69.91; H, 6.60; O, 23.33.

4.1.3. 2-[1-Hydroxy-3-(3-methoxyphenyl)-allylidene]-5-methoxy-4,6,6-trimethyl-cyclohex-4-ene-1,3-dione (4). Method I: 66% yield. mp 72–73 °C (CH_2Cl_2–hexane). IR (KBr): 2976, 2938, 1656, 1624, 1580, 1514, 1450, 1425, 1256 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 19.16 (s) and 18.75 (s) (3.5:1, 1H, chelated-O*H*), 8.50 (d) and 8.29 (d) (1:3.5, 1H, J = 15.5 Hz, *trans*-olefinic proton), 7.91 (d) and 7.89 (d) (1:3.5, 1H, J = 15.5 Hz, *trans*-olefinic proton), 7.34–7.12 (m, 3H, Ar-2″,5″,6″-*H*), 6.98–6.90 (m, 2H, Ar-4″-*H*), 3.95 (s) and 3.88 (s) (3.5:1, 3H, 5-OCH_3), 3.84 (s, 3H, Ph-OCH_3), 1.99 (s) and 1.94 (s) (3.5:1, 3H, 4-CH_3), 1.46 (s) and 1.37 (s) (1:3.5, 6H, 6-C$H_3 \times 2$). MS *m*/*z* 343 (M^++1).

4.1.4. 2-[1-Hydroxy-3-(2-methoxyphenyl)-allylidene]-5-methoxy-4,6,6-trimethyl-cyclohex-4-ene-1,3-dione (5). Method I: 89% yield. mp 127–128 °C (CH_2Cl_2–hexane). IR (KBr): 2976, 2938, 1657, 1615, 1513, 1487, 1465, 1423, 1246 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 19.16 (s) and 18.82 (s) (2.5:1, 1H, chelated-O*H*), 8.54 (d) and 8.38 (d) (1:2.5, 1H, J = 15.5 Hz, *trans*-olefinic proton), 8.39 (d) and 8.31 (d) (1:2.5, 1H, J = 15.5 Hz, *trans*-olefinic proton), 7.79 (dd) and 7.76 (dd) (1:2.5, 1H, J = 6.9 and 1.2 Hz, Ar-6″-*H*), 7.37 (ddd) and 7.35 (ddd) (1:2.5, 1H, J = 8.2, 6.9 and 1.2 Hz, Ar-4″-*H*), 7.00–6.87 (m, 2H, Ar-3″,5″-*H*), 3.94 (s) and 3.90 (s) (2.5:1, 3H, 5-OCH_3), 3.89 (s) and 3.87 (s) (2.5:1, 3H, Ph–OCH_3), 1.98 (s) and 1.94 (s) (2.5:1, 3H, 4-CH_3), 1.45 (s) and 1.36 (s) (1:2.5, 6H, 6-C$H_3 \times 2$). MS *m*/*z* 343 (M^++1). Anal. Calcd for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48; O, 23.36. Found: C, 69.94; H, 6.46; O, 23.11.

4.1.5. 2-[1-Hydroxy-3-(4-hydroxyphenyl)-allylidene]-5-methoxy-4,6,6-trimethyl-cyclohex-4-ene-1,3-dione (6). Method II: 35% yield (based on recovered starting material). mp 234–235 °C (CH_2Cl_2–MeOH, lit. 215–216 °C).[11b] IR (KBr): 2359, 2331, 1647, 1619, 1600, 1518, 1446, 1415, 1148, 830, 771 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 19.15 (s) and 18.81 (s) (2:1, 1H, chelated-O*H*), 8.32 (d) and 8.14 (d) (1:2, 1H, J = 15.7 Hz, *trans*-olefinic proton), 7.91 (d) and 7.89 (d) (1:2, 1H, J = 15.7 Hz, *trans*-olefinic proton), 7.54 (d) and 7.53 (d) (1:2, 2H, J = 8.6 Hz, Ar-2″,6″-*H*), 6.82 (d) and 6.81 (d) (1:2, 2H, J = 8.6 Hz, Ar-3″,5″-*H*), 3.91 (s) and 3.85 (s) (2:1, 3H, 5-OCH_3), 2.36 (br s, 1H, Ph-O*H*), 1.95 (s) and 1.91 (s) (2:1, 3H, 4-CH_3), 1.42 (s) and 1.33 (s) (1:2, 6H, 6-C$H_3 \times 2$). MS *m*/*z* 327 (M^+-1). Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.18; O, 24.36. Found: C, 69.50; H, 6.18; O, 24.36.

4.1.6. 2-(3′-Furan-2″-yl-1′-hydroxy-allylidene)-5-methoxy-4,6,6-trimethyl-cyclohex-4-ene-1,3-dione (7). Method I: 74% yield (based on recovered starting material). mp 119–120 °C (CH_2Cl_2–hexane). IR (KBr): 3120, 2986, 2945, 1654, 1626, 1558, 1502, 1442, 1414 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 19.07 (s) and 18.71 (s) (2.2:1, 1H,

chelated-O*H*), 8.35 (d) and 8.15 (d) (1:2.2, 1H, $J = 15.7$ Hz, *trans*-olefinic proton), 7.73 (d) and 7.68 (d) (1:2.2, 1H, $J = 15.7$ Hz, *trans*-olefinic proton), 7.54 (d) and 7.52 (d) (1:2.2, 1H, $J = 0.8$ Hz, Ar-5″-*H*), 6.75 (d) and 6.72 (d) (1:2.2, 1H, $J = 3.1$ Hz, Ar-3″-*H*), 6.50 (dd) and 6.48 (dd) (1:2.2, 1H, $J = 3.1$ and 0.8 Hz, Ar-4″-*H*), 3.94 (s) and 3.87 (s) (2.2:1, 3H, 5-OCH_3), 1.98 (s) and 1.94 (s) (2.2:1, 3H, 4-CH_3), 1.45 (s) and 1.36 (s) (1:2.2, 6H, 6-$CH_3 \times 2$). MS *m*/*z* 303 (M^++1).

4.1.7. 2-(1′-Hydroxy-3′-thiophen-2″-yl-allylidene)-5-methoxy-4,6,6-trimethyl-cyclohex-4-ene-1,3-dione (8). Method I: 61% yield (based on recovered starting material). mp 113–114 °C (CH_2Cl_2–hexane). IR (KBr): 3083, 2978, 2934, 1655, 1607, 1521, 1501, 1467, 1447, 1408, 1199, 1150 cm^{-1}. ^1H NMR ($CDCl_3$): δ 19.17 (s) and 18.79 (s) (2.5:1, 1H, chelated-O*H*), 8.34 (d) and 8.15 (d) (1:2.5, 1H, $J = 15.6$ Hz, *trans*-olefinic proton), 8.08 (d) and 8.05 (d) (1:2.5, 1H, $J = 15.6$ Hz, *trans*-olefinic proton), 7.49–7.34 (m, 2H, Ar-3″,5″-*H*), 7.11–7.04 (m, 1H, Ar-4″-*H*), 3.94 (s) and 3.87 (s) (2.5:1, 3H, 5-OCH_3), 1.98 (s) and 1.94 (s) (2.5:1, 3H, 4-CH_3), 1.45 (s) and 1.36 (s) (1:2.5, 6H, 6–$CH_3 \times 2$). MS *m*/*z* 319 (M^++1).

4.1.8. 2-[(1′-Hydroxy-3′-thiazol-2-yl-allylidene)-5-methoxy-4,6,6-trimethyl-1,3-cyclohex-4-ene-1,3-dione (9). Method I: 50% yield (based on recovered starting material). mp 111–113 °C (CH_2Cl_2–hexane). IR (KBr): 3078, 2976, 2934, 1655, 1621, 1517, 1470, 1448, 1433, 1387, 1199, 1136, 974, 942 cm^{-1}. ^1H NMR ($CDCl_3$): δ 19.02 (s) and 18.50 (s) (2.5:1, 1H, chelated-O*H*), 8.65 (d) and 8.44 (d) (1:2.5, 1H, $J = 15.6$ Hz, *trans*-olefinic proton), 8.03 (d) and 8.00 (d) (1:2.5, 1H, $J = 15.6$ Hz, *trans*-olefinic proton), 7.98–7.92 (m, 1H, Ar-4″-*H*), 7.48–7.42 (m, 1H, Ar-5″-*H*), 3.96 (s) and 3.89 (s) (2.5:1, 3H, 5-OCH_3), 2.00 (s) and 1.94 (s) (2.5:1, 3H, 4-CH_3), 1.47 (s) and 1.37 (s) (1:2.5, 6H, 6-$CH_3 \times 2$). MS *m*/*z* 320 (M^++1).

4.1.9. 5-Hydroxy-2,2,6,6-tetramethyl-4-(3-phenylacryloyl)cyclohex-4-ene-1,3-dione (13). Method I: 84% yield. mp 90–91 °C (CH_2Cl_2–hexane). IR (KBr): 2981, 1720, 1666, 1622, 1576, 1521, 1449, 1413, 1047, 971 cm^{-1}. ^1H NMR ($CDCl_3$): δ 18.17 (s, 1H, chelated-O*H*), 8.02 (s, 2H, *trans*-olefinic proton), 7.72–7.64 (m, 2H, *trans*-olefinic proton), 7.47–7.40 (m, 3H, Ar-4″-*H*), 1.46 (s, 6H, $CH_3 \times 2$), 1.41 (s, 6H, $CH_3 \times 2$). ^{13}C NMR ($CDCl_3$): δ 210.0 (C1), 202.6 (C3), 197.6 (C5), 186.1 (C1′), 146.8 (C3′), 134.7 (C1″), 131.3 (C4″), 129.1 and 129.0 (C2″, 3″, 5″, 6″), 121.0 (C2′), 108.3 (C4), 57.1 (C2), 53,9 (C6), 24.1 (2-$CH_3 \times 2$), 23.9 (6-$CH_3 \times 2$). MS *m*/*z* 311 (M^+-1). Anal. Calcd for $C_{19}H_{20}O_4$: C, 73.06; H, 6.45; O, 20.49. Found: C, 73.10; H, 6.50; O, 20.25.

4.1.10. 1,5-Diacetyl-2,4,6-trihydroxybenzene (14). A solution of **10** (monohydrate, 3.03 g, 18 mmol) in HOAc (100 mL), Ac_2O (10 mL), and BF_3OEt_2 (6.5 mL) was refluxed for 21 h. The reaction mixture was cooled to 0 °C and 2 N NaOH was added to adjust the pH to 4. The mixture was repeatedly extracted with 5% MeOH/AcOEt until TLC analysis did not show the desired compound in the water phase. The combined organic phases were concentrated in vacuo and the residue was resolved in MeOH (50 mL). After the addition of 2 N NaOH (50 mL), the mixture was stirred for 5 h at room temperature. After removal of volatile solvent in vacuo, the remaining mixture was acidified with 3 N HCl, then extracted with 5% MeOH/EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 (EtOAc–hexane 1:4) to provide **14** (3.48 g, 92%). All spectra data were identical with those previously reported.[10]

4.2. Biological assay

The in vitro cytotoxicity assay was carried out according to procedures described in Rubinstein et al.[14] Drug stock solutions were prepared in DMSO, and the final solvent concentration was <2% DMSO (v/v), a concentration without effect on cell replication. The human tumor cell line panel consisted of epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), and ovarian cancer (1A9). The drug resistant cell line panel consisted of KB-VCR. The property of this cell is described elsewhere.[15,16] Cells were cultured at 37 °C in RPMI-1640 with 100 μg/mL kanamycin and 10% (v/v) fetal bovine serum in a humidified atmosphere containing 5% CO_2. Initial seeding densities varied among the cell lines to ensure a final absorbance of 1–2.5 A_{562} units. Drug exposure was for 3 days, and the ED_{50} value, the drug concentration that reduced the absorbance by 50%, was interpolated from does–response data. Each test was performed in triplicate, and absorbance reading varied no more than 5%.

Acknowledgements

This study was supported by grant CA17625 from the National Cancer Institute, NIH awarded to K.H.L.

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