A New Method for Thiomethylation of Hydroxy-1,4-naphthoquinones with *N*-Acetyl-L-cysteine; First Synthesis of Fibrostatins B, C, and D

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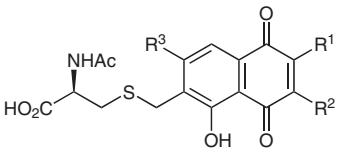
Abstract: A series of *N*-acetyl-*S*-[(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine conjugates were obtained in good yields by acid-catalyzed condensation of substituted 2-hydroxy-1,4-naphthoquinones with *N*-acetyl-L-cysteine and paraformaldehyde. Based on this reaction, a first synthesis of fibrostatins B, C, and D was developed.

Key words: quinones, thiols, natural products, fibrostatins, amino acids

1,4-Naphthoquinones are widely distributed in nature as metabolites of various living organisms.[1] These substances have wide spectrum of bioactivities, including anticancer,[2] antibacterial,[3] antiprotozoal,[4] antimalarial,[5] and other properties. Some 1,4-naphthoquinones are used in medicine. Quinones exert their actions through radical reactions: as prooxidants, they reduce oxygen to reactive oxygen species, and as antioxidants and as electrophiles, they form covalent bonds with tissue nucleophiles.[6] In most cases, however, naphthoquinones have low aqueous solubility that prevents their widespread application and biotesting. To improve the aqueous solubility of quinones, they can be conjugated with nontoxic carbohydrates[7] or amino acids.[8] These conjugates are more soluble and they retain the activity of the parent quinone.[9] In our opinion, the most interesting derivatives are the readily available conjugates of naphthoquinones with *N*-acetyl-L-cysteine.

Numerous *N*-acetyl-L-cysteine conjugates of secondary metabolites are found in nature. They are produced mainly by strains of *Streptomyceaceae* family.[10] In these natural products, the *N*-acetyl-L-cysteine moiety is directly attached to the polycyclic core or a side-chain substituent through a sulfur bond. Among the natural amino acid–quinone conjugates, fibrostatins A–F (**1**–**6**; Figure 1) are of particular interest. These were isolated from a culture broth of *Streptomyces catanulae*[11] and they are the first 1,4-naphthoquinones to be identified that contain an *N*-acetyl-L-cysteine group. These compounds showed inhibitory activity against prolylhydroxylase of chick embryos,[11] and they have been tested as nontoxic antifibrotic agents.[12] Despite their relative simplicity, fibrostatins A–D have not previously been synthesized. Earlier, Kwan and Moore reported a nine-step synthesis of the fibrostatin B quinone core,[13] involving a dibutyl squarate alkylation and a Hooker oxidation, but no procedure for thiomethylation of hydroxy-1,4-naphthoquinones by *N*-acetyl-L-cysteine has been reported.



1 fibrostatin A R^1 = H; R^2 = Me; R^3 = OMe
2 fibrostatin B R^1 = OMe; R^2 = Me; R^3 = OMe
3 fibrostatin C R^1 = OMe; R^2 = H; R^3 = OMe
4 fibrostatin D R^1 = OMe; R^2 = Me; R^3 = OH
5 fibrostatin E R^1 = H; R^2 = CH_2OH; R^3 = OMe
6 fibrostatin F R^1 = OMe; R^2 = CH_2OH; R^3 = OMe

Figure 1 Structures of fibrostatins

N-Acetyl-L-cysteine is a nontoxic water-soluble pharmaceutical, widely used as mucolytic agent,[14] as a detoxifier for overdosing by paracetamol,[15] and as a precursor of glutathione.[16] Therefore, conjugation of substituted 2-hydroxy-1,4-naphthoquinones with *N*-acetyl-L-cysteine should provide new *S*-amino acid derivatives with potential antifibrotic activity. It is well known that 2-hydroxy-1,4-naphthoquinone (**7a**; lawsone) and formaldehyde readily react to give an *ortho*-quinone methide that can be trapped by a diene to form α- and β-lapachones.[17] Sharma and co-workers synthesized a series of new (phenylsulfanyl)methyl-1,4-naphthoquinones by treating lawsone (**7a**) with appropriate aldehydes and various arenethiols in refluxing ethanol with microwave irradiation.[18] These new quinone derivatives showed moderate antimalarial activity when tested in vitro.

Here, we report the synthesis of a group of substituted 3-hydroxy-2-methylthiocysteinyl-1,4-naphthoquinone derivatives by an acid-catalyzed three-component condensation of various 2-hydroxynaphthoquinones with *N*-acetyl-L-cysteine and paraformaldehyde. On the basis of this reaction, we developed a first synthesis of fibrostatins B, C, and D and their analogues.

First, we condensed readily available 2-hydroxy-1,4-naphthoquinone (**7a**; lawsone) with *N*-acetyl-L-cysteine and paraformaldehyde. To avoid esterification of the carboxyl group of *N*-acetyl-L-cysteine, ethanol and other alcohols were excluded as solvents. We conducted our experiments in low-boiling acetone, which readily dissolves naphthoquinones, with formic acid as a catalyst. A mixture of lawsone (**7a**), *N*-acetyl-L-cysteine, and para-

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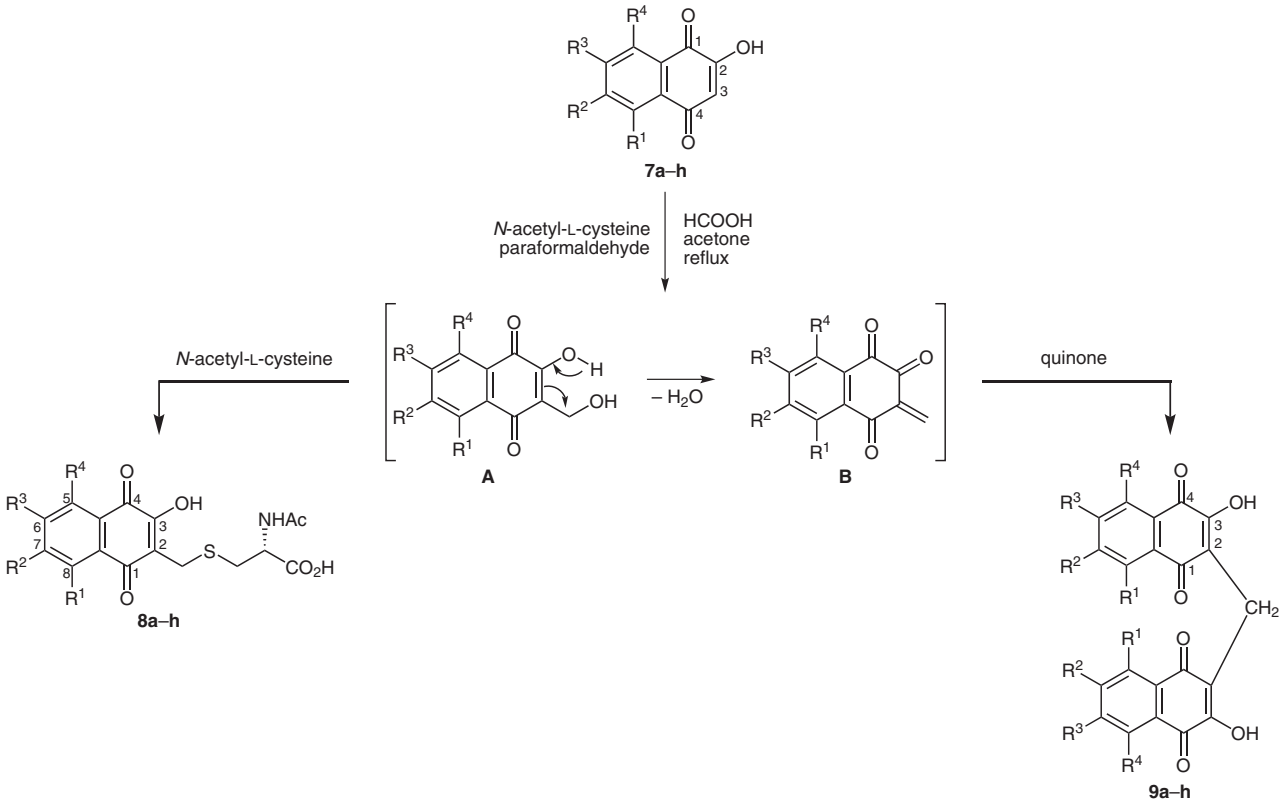
formaldehyde in a 1:1:1 molar ratio was refluxed for ten hours in acetone. Two new colored products were formed: the polar product **8a** in 60% yield and the nonpolar byproduct **9a** in ~2% yield (Table 1, entry 1); unreacted lawsone was also isolated (31%). The structures of **8a** and **9a** were determined by ^{1}H NMR and IR spectroscopy and mass spectrometry. Because formaldehyde is highly volatile, we next used a four molar excess of the reagent and we obtained conjugate **8a** in 80% yield, accompanied by a tarry material that was difficult to separate. To accelerate the formation of **8a**, we increased the amount of *N*-acetyl-L-cysteine to 1.5 equivalents. The optimal reaction time was then six to seven hours, because a longer reflux led to formation of tar and hampered the isolation of **8a**.

To verify the generality of the protocol, we examined the reactions of other substituted 2-hydroxy-1,4-naphthoquinones **7b–h**, bearing hydroxy, methoxy, methyl, or chloro substituents on the benzene ring. Under similar conditions, quinones **7b–h** gave the desired products **8b–h** in good yields accompanied by minor amounts of the corresponding byproducts **9a–h** (Table 1, entries 2–7). We suggest that under the reaction conditions, the 2-hydroxy-1,4-naphthoquinones **7a–h** react with formaldehyde to form unstable hydroxymethylquinones **A**, which readily lose water to form unstable *o*-quinone methides **B**; these undergo nucleophilic addition of *N*-acetyl-L-cysteine to form products **8a–h** and the dimeric byproducts **9a–h**. The structures of **9d–g** were determined by direct compar-

Table 1 Acid-Catalyzed Reaction of *N*-Acetyl-L-cysteine with Substituted 2-Hydroxy-1,4-naphthoquinones and Paraformaldehyde[a]

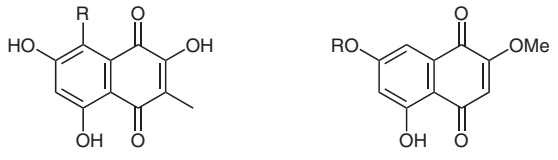


Entry	Substrate	R^1	R^2	R^3	R^4	Time (h)	Product	Yield (%)	Byproduct	Yield (%)
1	**7a**	H	H	H	H	6	**8a**	85	**9a**	3
2	**7b**	OH	H	H	H	4	**8b**	72	**9b**	5
3	**7c**	H	H	H	OH	4	**8c**	80	**9c**	3
4	**7d**	OH	H	H	OH	5	**8d**	74	**9d**	4
5	**7e**	OH	Cl	Cl	OH	8	**8e**	82	**9e**	5
6	**7f**	OH	Me	Me	OH	8	**8f**	83	**9f**	4
7	**7g**	OH	MeO	MeO	OH	8	**8g**	78	**9g**	6
8	**7h**	OH	H	OH	H	6	**8h**	80	**9h**	4

[a] Reactions were performed on a 0.25 mmol scale in triplicate.

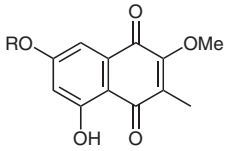
ison with authentic samples that we had in our laboratory.[19] The other 1,4-naphthoquinone derivatives **8b**–**h**, **9a**–**c**, and **9h** showed spectral patterns that were in good agreement with their proposed structures.

The next stage of our work was the synthesis of fibrostatins B, C, and D and related analogues. The starting 2,5,7-trihydroxynaphthoquinone (**7h**; flaviolin) was obtained in 50–55% yield by alkali fusion of commercially available disodium 4,5-dihydroxynaphthalene-2,7-disulfonate and subsequent air oxidation.[20] Radical methylation of flaviolin **7h** by diacetyl peroxide[21] in acetonitrile gave the methyl derivative **10** together with the dimethyl derivative **11** as a byproduct (Figure 2). Methylation of flaviolin **7h** by a solution of diazomethane gave the monomethoxylated flaviolin derivative **12**[22a] and dimethoxylated flaviolin derivative **13**;[22b] the monomethoxylated derivative **14** and dimethoxylated derivative **15** were similarly prepared from 3-methylflaviolin **10**. The alternative lengthy multistage approach adopted by Kwan and Moore[13] was rejected because it does not permit the synthesis of naphthoquinones with a hydroxy group in the 7-position.



10 R = H
11 R = Me

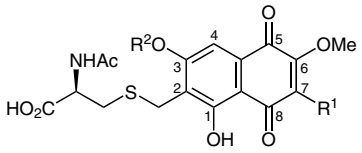
12 R = H
13 R = Me



14 R = H
15 R = Me

Figure 2 Structures of derivatives of flaviolin

Unfortunately, all four 2-methoxyflaviolin derivatives **12**–**15** were unreactive with *N*-acetyl-L-cysteine and paraformaldehyde in refluxing acetone–formic acid solution. However, when acetone and formic acid were replaced by high-boiling 1,4-dioxane and aqueous acetic acid, prolonged reflux (43 h) of the 2-methoxyflaviolin **12** gave the new *N*-acetyl-L-cysteine derivative **16** (Figure 3) in 55% yield. Under similar conditions, 2,7-dimethoxyflaviolin **13** was refluxed for 18 hours until half the starting quinone was consumed and two new colored products were formed: fibrostatin C (**3**; 47% yield) and the biscysteinyl conjugate **17** (7% yield). When the reaction time was extended to 23 hours, the yield of fibrostatin C (**3**) decreased to 32% and the formation of large amount of polar tar prevented the isolation of the bisconjugate **17**. Flaviolin derivatives **14** and **15** reacted with *N*-acetyl-L-cysteine and paraformaldehyde more slowly than did the corresponding derivatives **12** and **13**. Refluxing derivatives **14** and **15** with *N*-acetyl-L-cysteine and paraformaldehyde for 39 hours gave moderate yields of fibrostatin D **4** (57%) and fibrostatin B **2** (50%), respectively. The synthetic compounds **2**–**4** were identical in all respects to authentic samples of fibrostatins B, C, and D, respectively, isolated from *S. catanulae.*[11]



16 $R^1 = R^2 = H$
17 $R^1 = CH_2SCH_2CHNHAcCO_2H$; $R^2 = Me$

Figure 3 Structures of N-acetyl-L-cysteinyl derivatives of flaviolin

In conclusion, we have developed a new and efficient protocol for the preparation of derivatives of *N*-acetyl-*S*-[(1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine, and we have successfully applied this method in a first synthesis of the natural fibrostatins B, C, and D and their analogues. Our work provides a short route to the synthesis of new compounds of medical interest by simple conjugation of bioactive naphthoquinones with a known drug from the pharmacopoeia.

All reagents were obtained from commercial suppliers and were used without additional purification. All solvents were distilled before use. *N*-Acetyl-L-cysteine and lawsone (**7a**) were purchased from Alfa Aesar. Hydroxyjuglones **7b** and **7c** were prepared as described in the literature.[22a] Hydroxynaphthazarins **4d**–**h** were also prepared according to a previously described method.[19] Melting points were determined by using a Boetius apparatus and are uncorrected. IR spectra were recorded in KBr pellets or in $CHCl_3$ by using a Bruker Vector-22 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance-300 (300 MHz), a Bruker Avance III-500 HD (500 MHz), or a Bruker Avance III-700 (700 MHz) spectrometer with $CDCl_3$, DMSO-d_6, or acetone-d_6 as the solvent and TMS as the internal standard. ^{13}C NMR spectra were recorded on a Bruker Avance-300 spectrometer at 75 MHz, a Bruker Avance III HD-500 spectrometer at 125 MHz, or a Bruker Avance III-700 spectrometer at 176 MHz. EI mass spectra and high-resolution EI mass spectra were recorded on an AMD-604S instrument at 70 eV. ESI mass spectra were recorded on an Agilent 6510 Q-TOF LC/MS instrument. Optical rotations were determined by using a PerkinElmer 343 polarimeter. Elemental analyses were performed on a CHN-analyzer Flash EA-1112 instrument. Silufol UV-vis TLC plates treated with HCl vapor were used for analytical TLC. Preparative TLC was performed on silica gel 60 Merck (40–60 μm). TLC plates were developed in system A [hexane–benzene–acetone (2:1:1 v/v/v)], system B [benzene–EtOAc–MeOH (2:1:1 v/v/v)], or system C [hexane–benzene–acetone (2:1:2 v/v/v)].

Condensation of 2-Hydroxy-1,4-naphthoquinones 7a–h with Paraformaldehyde and *N*-Acetyl-L-cysteine; General Procedure

Powdered paraformaldehyde (30.3 mg, 1.00 mmol), *N*-acetyl-L-cysteine (60 mg, 0.37 mmol), and 85% aq HCO_2H (0.2 mL) were added to a solution of the appropriate 2-hydroxy-1,4-naphthoquinone **7a**–**h** (0.25 mmol) in acetone (15 mL). The mixture was gently refluxed with mixing for 4–8 h until the reaction was complete (TLC; system A). The mixture was concentrated in vacuo, and the resulting solid was purified by preparative TLC (silica gel, system C, two developments) to give two colored fractions. The polar colored fraction (R_f = 0.10–0.12) was the desired *N*-acetyl-L-cysteinyl

conjugate **8a–h** and the second colored fraction (R_f = 0.82–0.92) was the 2,2′-methylenebis(3-hydroxynaphthoquinone) **9a–h** by-product.

***N*-Acetyl-*S*-[(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8a)**
Yellow solid; yield: 75 mg (85%); mp 190–193 °C; $[\alpha]_D^{23}$ –81.0 (*c* 0.51, MeOH); R_f = 0.65 (system B).

IR (KBr): 3405, 2920, 1697, 1676, 1649, 1594, 1548, 1373, 1344, 1276, 1220, 1040, 1023, 944, 729 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.80 (s, 3 H, $COCH_3$,), 2.79 (dd, *J* = 8.7, 13.5 Hz, 1 H, SCH_2), 2.97 (dd, *J* = 5.1, 13.5 Hz, 1 H, SCH_2), 3.55 (d, *J* = 12.7 Hz, 1 H, $ArCH_2$), 3.64 (d, *J* = 12.7 Hz, 1 H, $ArCH_2$), 4.42 (ddd, *J* = 5.1, 8.2, 8.7 Hz, 1 H, CHN), 7.82 (m, 2 H, ArH), 7.99 (m, 2 H, ArH), 8.15 (d, *J* = 8.2 Hz, 1 H, NH), 11.46 (s, 1 H, β-OH), 12.62 (br s, 1 H, COOH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 22.4, 23.9, 33.8, 52.0, 121.1, 125.9 (2 C), 130.2, 131.9, 133.4, 134.7, 155.7, 169.3, 172.2, 180.9, 183.6.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{14}NO_6S$: 348.0547; found: 348.0566.

Anal. Calcd for $C_{16}H_{15}NO_6S$ (349.36): C, 55.01; H, 4.33; N, 4.01. Found: C, 55.22; H, 4.31; N, 3.92.

***N*-Acetyl-*S*-[(3,8-dihydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8b)**
Yellow solid; yield: 66 mg (72%); mp 207–209 °C; $[\alpha]_D^{23}$ –108 (*c* 0.12, MeOH); R_f = 0.59 (system B).

IR (KBr): 3344, 2980, 1719, 1705, 1646, 1620, 1536, 1457, 1379, 1323, 1270, 1185, 1166, 1069, 929, 918, 776, 763 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.80 (s, 3 H, $COCH_3$), 2.78 (dd, *J* = 8.8, 13.5 Hz, 1 H, SCH_2), 2.98 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 3.53 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.62 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 4.43 (ddd, *J* = 5.0, 8.2, 8.8 Hz, 1 H, CHN), 7.31 (dd, *J* = 0.8, 8.5 Hz, 1 H, ArH), 7.54 (dd, *J* = 0.8, 7.5 Hz, 1 H, ArH), 7.66 (dd, *J* = 7.5, 8.5 Hz, 1 H, ArH), 8.16 (d, *J* = 8.2 Hz, 1 H, NH), 12.39 (br s, 2 H, α-OH, COOH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 22.4, 23.2, 33.8, 51.9, 114.0, 118.8, 120.5, 124.9, 130.4, 135.5, 157.0, 160.1, 169.3, 172.2, 180.2, 189.8.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{14}NO_7S$: 364.0496; found: 364.0495.

Anal. Calcd for $C_{16}H_{15}NO_7S$ (365.36): C, 52.60; H, 4.14; N, 3.83. Found: C, 52.83; H, 4.16; N, 3.74.

***N*-Acetyl-*S*-[(3,5-dihydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8c)**
Yellow solid; yield: 73 mg (80%); mp 200–203 °C; $[\alpha]_D^{23}$ –78.0 (*c* 0.51, MeOH); R_f = 0.64 (system B).

IR (KBr): 3384, 2850, 1692, 1645, 1638, 1597, 1536, 1478, 1383, 1293, 1217, 1161, 1062, 1038, 938, 815 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.81 (s, 3 H, $COCH_3$), 2.78 (dd, *J* = 8.9, 13.5 Hz, 1 H, SCH_2), 2.97 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 3.53 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.61 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 4.42 (ddd, *J* = 5.0, 8.2, 8.9 Hz, 1 H, CHN), 7.27 (dd, *J* = 0.8, 8.5 Hz, 1 H, ArH), 7.51 (dd, *J* = 0.8, 7.5 Hz, 1 H, ArH), 7.72 (dd, *J* = 7.5, 8.5 Hz, 1 H, ArH), 8.16 (d, *J* = 8.2 Hz, 1 H, NH), 11.34 (s, 1 H, α-OH), 11.50 (br s, 1 H, COOH), 12.66 (s, 1 H, β-OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 22.4, 23.8, 33.8, 51.9, 113.9, 118.4, 121.5, 123.0, 132.3, 137.0, 155.6, 160.1, 169.2, 172.2, 182.9, 184.5.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{14}NO_7S$: 364.0496; found: 364.0491.

Anal. Calcd for $C_{16}H_{15}NO_7S$ (365.36): C, 52.60; H, 4.14; N, 3.83. Found: C, 52.79; H, 4.10; N, 3.70.

***N*-Acetyl-*S*-[(3,5,8-trihydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8d)**
Red solid; yield: 70 mg (74%); mp 222–224 °C; R_f = 0.61 (system B).

IR (KBr): 3372, 2931, 1684, 1631, 1609, 1565, 1536, 1447, 1412, 1381, 1317, 1257, 1220, 1187, 1161, 1081, 973, 890, 825, 789 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.82 (s, 3 H, $COCH_3$), 2.78 (dd, *J* = 8.8, 13.5 Hz, 1 H, SCH_2), 2.98 (dd, *J* = 4.9, 13.5 Hz, 1 H, SCH_2), 3.55 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.63 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 4.43 (ddd, *J* = 4.9, 8.2, 8.8 Hz, 1 H, CHN), 7.29 (d, *J* = 9.5 Hz, 1 H, ArH), 7.36 (d, *J* = 9.5 Hz, 1 H, ArH), 8.16 (d, *J* = 8.2 Hz, 1 H, NH), 11.67 (s, 1 H, α-OH), 12.78 (br s, 2 H, α-OH, COOH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 22.4, 23.3, 33.8, 51.9, 110.7, 111.1, 121.5, 127.6, 130.2, 155.5, 156.3, 157.1, 169.2, 172.2, 182.8, 187.9.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{14}NO_8S$: 380.0446; found: 380.0444.

Anal. Calcd for $C_{16}H_{15}NO_8S$ (381.36): C, 50.39; H, 3.96; N, 3.67. Found: C, 50.64; H, 3.94; N, 3.55.

***N*-Acetyl-*S*-[(6,7-dichloro-3,5,8-trihydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8e)**
Red solid; yield: 92 mg (82%); mp 124–127 °C; R_f = 0.65 (system B).

IR (KBr): 3307, 2990, 1731, 1706, 1601, 1535, 1404, 1384, 1295, 1270, 1224, 1181, 1169, 1116, 1001, 910, 863 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.82 (s, 3 H, $COCH_3$), 2.77 (dd, *J* = 8.7, 13.5 Hz, 1 H, SCH_2), 2.97 (dd, *J* = 13.5, 4.9 Hz, 1 H, SCH_2), 3.55 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.64 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 4.43 (ddd, *J* = 4.9, 8.2, 8.7 Hz, 1 H, CHN), 8.18 (d, *J* = 8.2 Hz, 1 H, NH), 12.25 (br s, 2 H, α-OH, COOH), 13.75 (s, 1 H, α-OH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.7, 23.7, 34.0, 52.3, 110.4, 111.1, 121.1, 129.2, 131.9, 152.8, 152.9, 158.9, 169.6, 172.5, 182.4, 186.3.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{12}Cl_2NO_8S$: 447.9666; found: 447.9676.

Anal. Calcd for $C_{16}H_{13}Cl_2NO_8S$ (450.25): C, 42.68; H, 2.91; N, 3.11. Found: C, 42.81; H, 2.95; N, 3.06.

***N*-Acetyl-*S*-[(3,5,8-trihydroxy-6,7-dimethyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8f)**
Red solid; yield: 85 mg (83%); mp 216–219 °C; R_f = 0.64 (system B).

IR (KBr): 3379, 2951, 1733, 1619, 1598, 1549, 1428, 1383, 1322, 1189, 1036, 892, 818 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.82 (s, 3 H, $COCH_3$), 2.18 (s, 3 H, $ArCH_3$), 2.19 (s, 3 H, $ArCH_3$), 2.77 (dd, *J* = 8.9, 13.5 Hz, 1 H, SCH_2), 2.96 (dd, *J* = 4.9, 13.5 Hz, 1 H, SCH_2), 3.54 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.62 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 4.43 (ddd, *J* = 4.9, 8.2, 8.9 Hz, 1 H, CHN), 8.16 (d, *J* = 8.2 Hz, 1 H, NH), 12.49 (br s, 2 H, α-OH, COOH), 13.47 (br s, 1 H, α-OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 11.9, 12.4, 22.4, 23.3, 33.8, 52.0, 107.4, 108.3, 121.5, 135.9, 139.4, 156.4, 156.7, 157.4, 169.3, 172.2, 181.0, 186.2.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{18}H_{18}NO_8S$: 408.0759; found: 408.0759.

Anal. Calcd for $C_{18}H_{19}NO_8S$ (409.41): C, 52.81; H, 4.68; N, 3.42. Found: C, 52.98; H, 4.70; N, 3.31.

***N*-Acetyl-*S*-[(3,5,8-trihydroxy-6,7-dimethoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8g)**
Red solid; yield: 86 mg (78%); mp 138–141 °C; R_f = 0.61 (system B).

IR (KBr): 3365, 2940, 2854, 1688, 1620, 1601, 1549, 1469, 1419, 1333, 1293, 1211, 1071, 1029, 1002, 958, 813, 671 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 1.82 (s, 3 H, $COCH_3$), 2.76 (dd, *J* = 8.9, 13.5 Hz, 1 H, SCH_2), 2.95 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 3.56 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.66 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.95 (s, 3 H, OMe), 4.01 (s, 3 H, OMe), 4.43 (ddd, *J* = 5.0, 8.2, 8.9 Hz, 1 H, CHN), 8.19 (d, *J* = 8.2 Hz, 1 H, NH), 12.43 (br s, 1 H, β-OH), 12.74 (br s, 1 H, COOH), 13.43 (br s, 1 H, α-OH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.7, 23.5, 34.0, 52.2, 61.6 (2 C), 105.9, 108.0, 121.4, 146.7, 149.4, 156.8, 161.2, 162.6, 169.6, 172.4, 172.6, 179.1.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{18}H_{18}NO_{10}S$: 440.0657; found: 440.0665.

Anal. Calcd for $C_{18}H_{19}NO_{10}S$ (441.41): C, 48.98; H, 4.34; N, 3.17. Found: C, 49.21; H, 4.29; N, 3.11.

***N*-Acetyl-*S*-[(3,6,8-trihydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl]-L-cysteine (8h)**
Red solid; yield: 76 mg (80%); mp 191–194 °C; $[\alpha]_D^{23}$ –72.1 (*c* 0.51, MeOH); R_f = 0.59 (system B).

IR (KBr): 3344, 2920, 1731, 1641, 1613, 1526, 1406, 1384, 1334, 1294, 1175, 1083, 1020, 928, 800, 761 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 1.81 (s, 3 H, $COCH_3$), 2.77 (dd, *J* = 8.9, 13.5 Hz, 1 H, SCH_2), 2.96 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 3.51 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.58 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 4.42 (ddd, *J* = 5.0, 8.2, 8.9 Hz, 1 H, CHN), 6.54 (d, *J* = 2.4 Hz, 1 H, ArH), 6.97 (d, *J* = 2.4 Hz, 1 H, ArH), 8.15 (d, *J* = 8.2 Hz, 1 H, NH), 10.98 (s, 1 H, β-OH), 11.50 (br s, 1 H, COOH), 12.48 (s, 1 H, α-OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 22.4, 23.2, 33.8, 52.0, 107.3, 108.1, 108.2, 120.3, 132.0, 156.0, 162.9, 163.7, 169.2, 172.3, 180.3, 188.5.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{16}H_{14}NO_8S$: 380.0446; found: 380.0440.

Anal. Calcd for $C_{16}H_{15}NO_8S$ (381.36): C, 50.39; H, 3.96; N, 3.67. Found: C, 50.59; H, 3.90; N, 3.54.

2,2′-Methylenebis(3-hydroxynaphthoquinone) (9a)
Yellow solid; yield: 2 mg (3%); mp 284–286 °C (dec) [Lit.[23] 249–251 °C (dec)]; R_f = 0.4 (system A).

IR (KBr): 3443, 3069, 1679, 1611, 1602, 1584, 1572, 1455, 1350, 1322, 1308, 1293, 1264, 1212, 986, 936, 767, 733 cm^{-1}.

^1H NMR (700 MHz, DMSO-d_6): δ = 3.75 (s, 2 H, CH_2), 7.77 (dt, *J* = 1.3, 7.5 Hz, 2 H, ArH), 7.82 (dt, *J* = 1.3, 7.5 Hz, 2 H, ArH), 7.97 (m, 4 H, ArH), 10.87 (s, 2 H, β-OH).

^{13}C NMR (176 MHz, DMSO-d_6): δ = 18.0, 122.0, 125.7, 125.9, 129.9, 132.0, 133.2, 134.6, 155.1, 180.8, 183.7.

MS (EI, 70 eV): *m/z* (%) = 360 (48) [M$^+$], 342 (93), 314 (100), 286 (20), 230 (22), 202 (34), 188 (30), 105 (72), 64 (77).

HRMS (ES): *m/z* [M – H]$^-$ calcd for $C_{21}H_{11}O_6$: 359.0561; found: 359.0569.

Anal. Calcd for $C_{21}H_{12}O_6$ (360.32): C, 70.00; H, 3.36. Found: C, 70.26; H, 3.39.

2,2′-Methylenebis(3,8-dihydroxynaphthoquinone) (9b)
Yellow solid; yield: 3 mg (6%); mp 260 °C (dec); R_f = 0.43 (system A).

IR (KBr): 3218, 2983, 2923, 1669, 1654, 1617, 1458, 1384, 1323, 1270, 1252, 1239, 1197, 1154, 1083, 1047, 990, 934, 904, 833, 766 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 3.70 (s, 2 H, $ArCH_2$), 7.30 (dd, *J* = 0.8, 8.3 Hz, 2 H, ArH), 7.52 (dt, *J* = 0.8, 7.5 Hz, 2 H, ArH), 7.65 (m, 2 H, ArH), 12.49 (s, 2 H, α-OH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 16.8, 114.2, 118.9, 121.3, 125.2, 130.5, 135.6, 157.1, 160.4, 180.5, 190.3.

MS (EI, 70 eV): *m/z* (%) = 392 (100) [M$^+$], 374 (46), 346 (66), 318 (22), 290 (19), 262 (13), 204 (18), 189 (22), 131 (16), 92 (43), 64 (28).

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{21}H_{11}O_8$: 391.0459; found: 391.0459.

Anal. Calcd for $C_{21}H_{12}O_8$ (392.32): C, 64.29; H, 3.08. Found: C, 64.52; H, 3.09.

2,2′-Methylenebis(3,5-dihydroxynaphthoquinone) (9c)
Yellow solid; yield: 2 mg (3%); mp 252 °C (dec); R_f = 0.41 (system A).

IR (KBr): 3333, 3050, 1642, 1622, 1594, 1575, 1485, 1385, 1326, 1265, 1204, 1156, 1076, 1026, 941, 907, 835, 779 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 3.70 (s, 2 H, $ArCH_2$), 7.26 (d, *J* = 8.2 Hz, 2 H, ArH), 7.48 (d, *J* = 7.9 Hz, 2 H, ArH), 7.70 (t, *J* = 7.9 Hz, 2 H, ArH), 10.90 (br s, 2 H, β-OH), 11.33 (s, 2 H, α-OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 18.1, 113.6, 118.5, 122.5, 122.9, 132.4, 137.0, 155.0, 160.0, 183.0, 184.5.

MS (EI, 70 eV): *m/z* (%) = 392 (87) [M$^+$], 374 (55), 346 (100), 318 (27), 290 (17), 245 (13), 204 (24), 187 (21), 131 (17), 121 (38), 64 (25).

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{21}H_{11}O_8$: 391.0459; found: 391.0481.

Anal. Calcd for $C_{21}H_{12}O_8$ (392.32): C, 64.29; H, 3.08. Found: C, 64.56; H, 3.03.

2,2′-Methylenebis(3,5,8-trihydroxynaphthoquinone) (9d)
Red solid; yield: 2 mg (4%); mp 310–312 °C (dec) (Lit.[17] 299–302 °C); R_f = 0.44 (system A).

2,2′-Methylenebis(6,7-dichloro-3,5,8-trihydroxynaphthoquinone) (9e)
Red solid; yield: 4 mg (5%); mp 305 °C (dec.) [Lit.[19a] 300 °C (dec)]; R_f = 0.49 (system A).

2,2′-Methylenebis(3,5,8-trihydroxy-6,7-dimethylnaphthoquinone) (9f)
Red solid; yield: 3 mg (4%); mp 292–295 °C (Lit.[19a] 290–293 °C); R_f = 0.51 (system A).

2,2′-Methylenebis(3,5,8-trihydroxy-6,7-dimethoxynaphthoquinone) (9g)
Red solid; yield: 4 mg (6%); mp 228–233 °C (Lit.[19b] 208–211 °C); R_f = 0.29 (system A).

2,2′-Methylenebis(3,6,8-trihydroxynaphthoquinone) (9h)
Red solid; yield: 2 mg (4%); mp 305 °C (dec); R_f = 0.16 (system A).

IR (KBr): 3376, 2923, 1697, 1672, 1647, 1607, 1489, 1388, 1353, 1269, 1251, 1171, 1098, 1171, 1097, 1029, 918 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 3.65 (s, 2 H, $ArCH_2$), 6.52 (d, *J* = 2.3 Hz, 2 H, 2ArH), 6.94 (d, *J* = 2.3 Hz, 2 H, ArH), 10.93 (s, 2 H, β-OH), 12.56 (s, 2 H, α-OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 16.5, 107.2, 107.8, 108.2, 121.0, 131.8, 155.6, 162.9, 163.5, 180.2, 188.8.

MS (EI, 70 eV): *m/z* (%) = 424 (25) [M$^+$], 378 (14), 364 (12), 294 (11), 281 (12), 220 (16), 203 (100), 150 (45), 137 (74), 69 (100), 65 (43).

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{21}H_{11}O_{10}$: 423.0358; found: 423.0380.

Anal. Calcd for $C_{21}H_{12}O_{10}$ (424.32): C, 59.44; H, 2.85. Found: C, 59.58; H, 2.79.

2,5,7-Trihydroxy-3-methylnaphthoquinone (10) and 3,6,8-Trihydroxy-2,5-dimethylnaphthoquinone (11)[21a]

A 0.34% solution of diacetyl peroxide[21b] in Et_2O (12 mL, 0.27 mmol) was added to a solution of flaviolin (**7h**; 150 mg, 0.72 mmol) in MeCN (20 mL), and the mixture was gently refluxed with mixing for 4.5 h. The mixture was concentrated in vacuo, and the resulting solid was purified by preparative TLC (silica gel, system A, two developments) to give three colored fractions. The polar fraction (55mg, 0.26 mmol; R_f = 0.23) was the starting flaviolin (**7h**), the second fraction (R_f = 0.36**)** was the 3-methyl derivative **10**, and the third fraction (R_f = 0.41) was the 2,5-dimethyl derivative **11**.

10

Red solid; yield: 64 mg (40%); mp 265 °C (dec); R_f = 0.36 (system A).

IR (KBr): 3542, 1651, 1613, 1574, 1462, 1385, 1316, 1169, 1107, 1038, 962, 874, 861, 768 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.88 (s, 3 H, $ArCH_3$), 6.50 (d, *J* = 2.3 Hz, 1 H, ArH), 6.94 (d, *J* = 2.3 Hz, 1 H, ArH), 10.91 (br s, 1 H, β-OH), 12.57 (s, 1 H, α-OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 7.9, 107.3, 107.9, 108.0, 119.3, 131.9, 156.7, 162.8, 163.5, 180.0, 189.7.

MS (EI, 70 eV): *m/z* (%) = 220 (33) [M$^+$], 192 (10), 167 (22), 149 (58), 137 (14), 122 (18), 83 (11), 71 (24), 57 (35), 43 (100), 32 (75).

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{11}H_7O_5$: 219.0299; found: 219.0301.

Anal. Calcd for $C_{11}H_8O_5$ (220.18): C, 60.01; H, 3.66. Found: C, 60.16; H, 3.69.

11

Red solid; yield: 6 mg (3%); mp 255–258 °C; R_f = 0.41 (system A).

IR ($CHCl_3$): 3579, 2929, 1730, 1616, 1392, 1343, 1302, 1190, 1146 cm^{-1}.

^{1}H NMR (500 MHz, acetone-d_6): δ = 1.97 (s, 3 H, $ArCH_3$), 2.50 (s, 3 H, $ArCH_3$), 6.66 (s, 1 H, ArH), 9.23 (br s, 1 H, β-OH), 9.88 (br s, 1 H, β-OH), 13.39 (s, 1 H, α-OH).

^{13}C NMR (125 MHz, acetone-d_6): δ = 7.0, 12.0, 107.9, 108.4, 117.6, 124.5, 128.0, 155.0, 162.2, 162.4, 181.9, 190.2.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{12}H_9O_5$: 233.0455; found: 233.0462.

Anal. Calcd for $C_{12}H_{10}O_5$ (234.21): C, 61.54; H, 4.30. Found: C, 61.78; H, 4.27.

5,7-Dihydroxy-2-methoxy-1,4-naphthoquinone (12)

A ~0.2 M solution of diazamethane in Et_2O was added dropwise to a solution of flaviolin **7h** (206 mg, 1.0 mmol) in 1,4-dioxane (50 mL), until TLC indicated the formation of dimethoxylated product with R_f = 0.65 (system A). The reaction mixture was evaporated in vacuo and the solid was recrystallized from chloroform. Yield: 165 mg (75%); orange solid; mp (sublimes above 225 °C) [Lit.[22a] mp 220–221 °C]; R_f = 0.43 (system A).

IR (KBr): 3388, 3084, 1640, 1602, 1492, 1449, 1403, 1358, 1317, 1240, 1172, 1117, 1092, 865 cm^{-1}.

^{1}H NMR (500 MHz, acetone-d_6): δ = 3.93 (s, 3 H, OCH_3), 6.15 (s, 1 H, ArH), 6.60 (d, *J* = 2.3 Hz, 1 H, ArH), 7.06 (d, *J* = 2.3 Hz, 1 H, ArH), 9.78 (s, 1 H, β-OH), 12.46 (s, 1 H, α-OH).

^{13}C NMR (125 MHz, acetone-d_6): δ = 56.9, 108.5, 108.7, 108.8, 109.9, 133.9, 161.8, 164.4, 164.5, 179.5, 190.4.

5-Hydroxy-2,7-dimethoxy-1,4-naphthoquinone (13)

A ~0.2 M solution of diazamethane in Et_2O was added dropwise to a solution of flaviolin **7h** (206 mg, 1.0 mmol) in 1,4-dioxane (50 mL), until TLC (system A) indicated the conversion of starting quinone **7h** (R_f = 0.30) into a single reaction product with R_f = 0.43. The reaction mixture was evaporated in vacuo to 3/4 volume and the product was precipitated by addition of hexane (10 mL) and cooling at 5 °C. Yield: 204 mg (87%); orange solid; mp 264–267 °C (Lit.[22b] 266–268 °C); R_f = 0.65 (system A).

IR ($CHCl_3$): 3416, 3054, 1686, 1625, 1592, 1444, 1433, 1385, 1354, 1286, 1242, 1215, 1166, 1120, 1092, 995, 954, 871 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.89 (s, 6 H, 2 × OCH_3), 6.04 (s, 1 H, ArH), 6.67 (d, *J* = 2.5 Hz, 1 H, ArH), 7. 22 (d, *J* = 2.5 Hz, 1 H, ArH), 12.38 (s, 1 H, α-OH).

^{13}C NMR (176 MHz, $CDCl_3$): δ = 56.0, 56.5, 107.2, 108.0, 109.6, 121.6, 132.5, 160.7, 163.7, 165.4, 179.4, 189.3.

5,7-Dihydroxy-2-methoxy-3-methylnaphthoquinone (14) and 5-Hydroxy-2,7-dimethoxy-3-methylnaphthoquinone (15)

A ~0.2 M solution of diazomethane in Et_2O was added dropwise to a solution of naphthoquinone **10** (220 mg, 1.0 mmol) in 1,4-dioxane (30 mL) until the starting quinone was consumed (TLC, system A). The mixture was concentrated in vacuo, and the residue was purified by preparative TLC (silica gel, system A, two developments) to give the methoxy derivatives **14** and **15**.

14

Orange solid; yield: 136 mg (58%); mp 220–224 °C; R_f = 0.54 (system A).

IR (KBr): 3427, 1663, 1634, 1610, 1450, 1410, 1383, 1332, 1310, 1258, 1156, 1108, 1048, 947, 856, 778 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.94 (s, 3 H, $ArCH_3$), 3.99 (s, 3 H, OCH_3), 6.50 (d, *J* = 2.3 Hz, 1 H, ArH), 6.92 (d, *J* = 2.3 Hz, 1 H, ArH), 11.05 (br s, 1 H, β-OH), 12.28 (s, 1 H, α-OH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 8.6, 60.7, 107.4, 107.5, 108.1, 130.4, 133.2, 157.7, 162.9, 164.2, 179.9, 189.1.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{12}H_9O_5$: 233.0455; found: 233.0453.

Anal. Calcd for $C_{12}H_{10}O_5$ (234.21): C, 61.54; H, 4.30. Found: C, 61.68; H, 4.30.

5-Hydroxy-2,7-dimethoxy-3-methylnaphthalene-1,4-dione (15)

Orange solid; yield: 92 mg (37%); mp 114–118 °C; R_f = 0.74 (system A).

IR (KBr): 1669, 1632, 1606, 1577, 1441, 1400, 1385, 1295, 1258, 1207, 1160, 1102, 947, 839, 764 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.06 (s, 3 H, $ArCH_3$), 3.89 (s, 3 H, OCH_3), 4.10 (s, 3 H, OCH_3), 6.62 (d, *J* = 2.3 Hz, 1 H, ArH), 7.13 (d, *J* = 2.3 Hz, 1 H, ArH), 12.45 (s, 1 H, α-OH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 8.6, 55.8, 60.9, 106.2, 107.6, 108.7, 131.5, 132.9, 157.9, 163.5, 165.2, 180.4, 189.6.

HRMS (ESI): *m/z* [M + H]$^{+\bullet}$ calcd for $C_{13}H_{13}O_5$: 249.0757; found: 249.0737.

Anal. Calcd for $C_{13}H_{12}O_5$ (248.24): C, 62.90; H, 4.87. Found: C, 63.22; H, 4.79.

***N*-Acetyl-*S*-[(1,3-dihydroxy-6-methoxy-5,8-dioxo-5,8-dihydronaphthalen-2-yl)methyl]-L-cysteine (16)**

Powdered paraformaldehyde (30.3 mg, 1.00 mmol), *N*-acetyl-L-cysteine (61 mg, 0.37 mmol), H_2O (3 mL), and AcOH (0.5 mL) were added to a solution of the methoxy derivative **12** (55 mg, 0.25 mmol) in 1,4-dioxane (15 mL), and the mixture was gently refluxed with mixing for 43 h. The mixture was concentrated in vacuo to give a solid residue that was purified by preparative TLC (silica gel, system C, two developments) to give the starting quinone **12** [yield: 12

mg (22%)] and a new orange product; yield: 55 mg (55%); mp 229 °C (dec); $[\alpha]_D^{23}$ –81 (*c* 0.13, MeOH); R_f = 0.59 (system B).

IR (KBr): 3378, 3310, 1755, 1742, 1686, 1626, 1593, 1542, 1449, 1433, 1408, 1382, 1312, 1297, 1245, 1191, 1117, 1073, 994, 873 cm^{-1}.

^1H NMR (700 MHz, DMSO-d_6): δ = 1.84 (s, 3 H, $COCH_3$), 2.75 (dd, *J* = 8.8, 13.5 Hz, 1 H, SCH_2), 2.94 (dd, *J* = 4.8, 13.5 Hz, 1 H, SCH_2), 3.65 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.75 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.85 (s, 3 H, OCH_3), 4.47 (ddd, *J* = 4.8, 8.2, 8.8 Hz, 1 H, CHN), 6.21 (s, 1 H, ArH), 7.07 (s, 1 H, ArH), 8.16 (d, *J* = 8.2 Hz, 1 H, NH), 11.28 (s, 1 H, β-OH), 12.87 (s, 1 H, α-OH).

^{13}C NMR (176 MHz, DMSO-d_6): δ = 22.4, 23.0, 33.5, 51.9, 56.8, 107.0, 107.2, 109.4, 119.3, 130.5, 160.5, 160.7, 161.3, 169.3, 172.3, 178.8, 189.8.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{17}H_{16}NO_8S$: 394.0602; found: 394.0607.

Anal. Calcd for $C_{17}H_{17}NO_8S$ (395.38): C, 51.64; H, 4.33; N, 3.54. Found: C, 51.69; H, 4.39; N, 3.50.

Fibrostatin C (3) and (2*R*,2′*R*)-3,3′-[(8-Hydroxy-3,6-dimethoxy-1,4-dioxo-1,4-dihydronaphthalene-2,7-diyl)bis(methylenesulfanediyl)]bis[2-(acetylamino)propanoic acid] (17)

Powdered paraformaldehyde (30.3 mg, 1.00 mmol), *N*-acetyl-L-cysteine (61 mg, 0.37 mmol), H_2O (3 mL), and AcOH (0.5 mL) were added to a solution of the quinone **13** (59 mg, 0.25 mmol) in 1,4-dioxane (15 mL), and the mixture was gently refluxed with mixing for 18 h until TLC (system A) indicated that about half the starting quinone **13** had been converted into two new colored products. The mixture was concentrated in vacuo, and the solid residue was purified by preparative TLC (silica gel, system C, two developments) to give the unreacted quinone **13** [yield: 25 mg, (42%)] and two new colored products: fibrostatin C (**3**; R_f = 0.51) and the bis-conjugate **17** (R_f = 0.59).

3

Yellow solid; yield: 47 mg (46%); mp 184–186 °C (Lit.[11] 187–190 °C); $[\alpha]_D^{23}$ –97.0 (*c* 0.51, MeOH) [Lit.[11] –93 (*c* 0.51, MeOH)]; R_f = 0.59 (system B).

^1H NMR (500 MHz, DMSO-d_6): δ = 1.84 (s, 3 H, $COCH_3$), 2.71 (dd, *J* = 8.7, 13.5 Hz, 1 H, SCH_2), 2.91 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 3.68 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.78 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.87 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 4.46 (ddd, *J* = 5.0, 8.2, 8.7 Hz, 1 H), 6.27 (s, 1 H, ArH), 7.16 (s, 1 H, ArH), 8.16 (d, *J* = 8.2 Hz, 1 H, NH), 12.69 (br s, 1 H, COOH), 12.73 (s, 1 H, α-OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 22.4, 22.8, 33.5, 51.9, 56.5, 56.9, 102.3, 108.5, 109.2, 121.1, 130.8, 159.4, 160.9, 161.8, 169.3, 172.3, 178.6, 190.1.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{18}H_{18}NO_8S$: 408.0759; found: 408.0758.

Anal. Calcd for $C_{18}H_{19}NO_8S$ (409.41): C, 52.81; H, 4.68; N, 3.42. Found: C, 52.98; H, 4.75; N, 3.30.

17

Yellow solid; yield: 10 mg (7%); mp 219–222 °C; R_f = 0.51 (system B).

IR (KBr): 3285, 1718, 1660, 1656, 1625, 1587, 1526, 1487, 1457, 1419, 1382, 1305, 1273, 1223, 1209, 1124, 1093, 1033, 1019 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 1.83 (s, 3 H, $COCH_3$), 1.86 (s, 3 H, $COCH_3$), 2.70 (dd, *J* = 8.8, 13.5 Hz, 1 H, SCH_2), 2.76 (dd, *J* = 8.8, 13.5 Hz, 1 H, SCH_2), 2.90 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 2.97 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 3.53 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.62 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.68 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.80 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.98 (s, 3 H, OCH_3), 4.11 (s, 3 H, OCH_3), 4.47 (m, 2 H, CHN), 7.18 (s, 1 H, ArH), 8.21 (d, *J* = 8.2, 1 H, NH), 8.24 (d, *J* = 8.2, 1 H, NH), 12.55 (s, 1 H, α-OH), 12.75 (br s, 2 H, 2 × COOH).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 22.4, 22.5, 22.8, 23.1, 33.4, 33.6, 51.8, 51.9, 56.6, 61.4, 102.6, 108.7, 120.6, 129.6, 131.6, 157.9, 159.3, 162.1, 169.3, 169.4, 172.3, 172.4, 180.0, 188.7.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{24}H_{27}N_2O_{11}S_2$: 583.1062; found: 583.1069.

Anal. Calcd for $C_{24}H_{28}N_2O_{11}S_2$ (584.61): C, 49.31; H, 4.83; N, 4.79. Found: C, 49.56; H, 4.82; N, 4.69.

Fibrostatin D (4) and Fibrostatin B (2); General Procedure

Powdered paraformaldehyde (30.3 mg, 1.00 mmol), *N*-acetyl-L-cysteine (61 mg, 0.37 mmol), H_2O (3 mL), and AcOH (0.5 mL) were added to a solution of the appropriate 2-methoxy 3-methyl derivative **14** or **15** (0.25 mmol) in 1,4-dioxane (15 mL), and mixture was gently refluxed with mixing for 39 h until TLC (system A) indicated that the initial quinone had been consumed. The mixture was concentrated in vacuo and the solid residue was purified by preparative TLC (silica gel, system C, two developments).

Fibrostatin D (4)

Orange solid; yield: 58 mg (57%); mp 203–205 °C (Lit.[11] mp 205–207 °C); $[\alpha]_D^{23}$ –58.0 (*c* 0.51, MeOH) [Lit.[11] –62 (*c* 0.51, MeOH)]; R_f = 0.59 (system B).

IR (KBr): 3480, 2900, 1685, 1666, 1627, 1551, 1445, 1411, 1383, 1326, 1298, 1203, 1126, 1091, 1057, 994 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 1.84 (s, 3 H, CO–CH_3), 1.94 (s, 3 H, $ArCH_3$), 2.74 (dd, *J* = 8.8, 13.5 Hz, 1 H, SH_2), 2.93 (dd, *J* = 5.0, 13.5 Hz, 1 H, SH_2), 3.65 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.75 (d, *J* = 12.9 Hz, 1 H, $ArCH_2$), 3.99 (s, 3 H, OCH_3), 4.47 (ddd, *J* = 5.0, 8.2, 8.8 Hz, 1 H, CHN), 7.04 (s, 1 H, ArH), 8.15 (d, *J* = 8.2 Hz, 1 H, NH), 11.28 (br s, 1 H, β-OH), 12.65 (br s, 1 H, COOH), 12.77 (s, 1 H, α OH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 8.6, 22.4, 23.0, 33.5, 51.9, 60.7, 107.3, 107.3, 118.5, 130.2, 131.0, 157.8, 160.5, 161.5, 169.3, 172.3, 179.8, 189.5.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{18}H_{18}NO_8S$: 408.0759; found: 408.0766.

Anal. Calcd for $C_{18}H_{19}NO_8S$ (409.41): C, 52.82; H, 4.68; N, 3.42. Found: C, 53.02; H, 4.67; N, 3.34.

Fibrostatin B (2)

Yellow solid; yield: 53 mg (50%); mp 193–195 °C (Lit.[11] 200–202 °C); $[\alpha]_D^{23}$ –88 (*c* 0.51, MeOH) [Lit.[11] –90 (*c* 0.51, MeOH)]; R_f = 0.60 (system A).

IR (KBr): 3490, 3288, 1729, 1667, 1625, 1594, 1528, 1487, 1459, 1434, 1417, 1376, 1332, 1300, 1280, 1211, 1123, 1101, 1018 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 1.84 (s, 3 H, $COCH_3$), 1.96 (s, 3 H, $ArCH_3$), 2.70 (dd, *J* = 8.7, 13.5 Hz, 1 H, SH_2), 2.89 (dd, *J* = 5.0, 13.5 Hz, 1 H, SCH_2), 3.67 (d, *J* = 13.0 Hz, 1 H, $ArCH_2$), 3.78 (d, *J* = 13.0 Hz, 1 H, $ArCH_2$), 3.96 (s, 3 H, OCH_3), 4.03 (s, 3 H, OCH_3), 4.46 (ddd, *J* = 5.0, 8.2, 8.7 Hz, 1 H, CHN), 7.15 (s, 1 H, ArH), 8.16 (d, *J* = 8.2, 1 H, NH), 12.62 (s, 1 H, α-OH), 12.67 (br s, 1 H, COOH).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 8.6, 22.4, 22.8, 33.4, 51.8, 56.5, 60.8, 102.3, 108.7, 120.3, 129.9, 131.3, 158.0, 159.3, 162.0, 169.2, 172.3, 179.7, 190.0.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{19}H_{20}NO_8S$: 422.0915; found: 422.0912.

Anal. Calcd for $C_{19}H_{21}NO_8S$ (423.44): C, 53.89; H, 5.00; N, 3.31. Found: C, 54.12; H, 4.97; N, 3.24.

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