Synthesis of Furanonaphthoquinones with Hydroxyamino Side Chains

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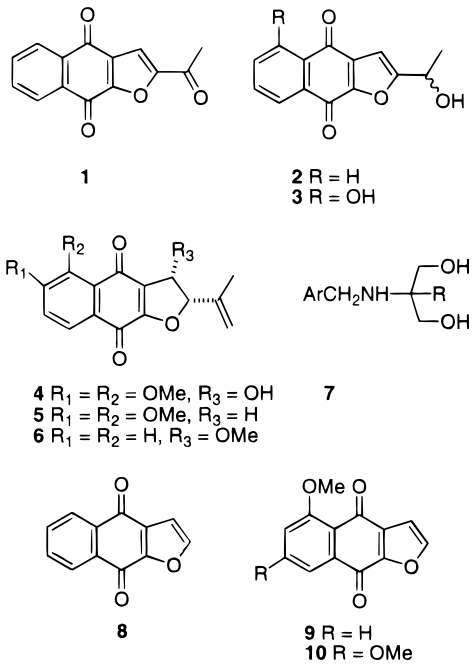
Several furanonaphthoquinones have shown useful activity in a yeast assay for DNA-damaging agents and cytotoxicity in mammalian cell culture assays. These results, together with the planar aromatic character of the furanonaphthoquinones, suggested that they might be acting as DNA intercalators. In an attempt to improve this activity, various analogues containing a hydroxyamino side chain have been synthesized. The analogues were prepared by standard methods, but some unexpected reactions were observed nonetheless. Thus, 8-formyl-5-methoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione (**24**) showed an unusual reactivity toward reductive amination, with the reaction proceeding further to give one of two different cyclized products, depending on the amination reagent used. Bioassay results indicated that only simple furanonaphthoquines showed activity in a yeast assay for DNA-damaging agents; compounds with a substituted hydroxyamino side chain were uniformly inactive in this assay. Most of the compounds with a substituted hydroxyamino side chain on the furan ring did, however, show cytotoxicity, although none of them was any more active than the simple aldehyde 2-formyl-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione (**14**). This evidence tends to suggest that the furanonaphthoquinones do not serve primarily as DNA intercalators, because if this were the case, they would have been expected to show an increased activity on conversion to their hydroxyamino side chain derivatives.

As a part of our ongoing search for naturally occurring compounds with anticancer activity, we isolated the first cytotoxic furanonaphthoquinones (**1**−**3**) from *Tabebuia cassinoides*,[1,2] and later the new quinones **4**−**6** from *Crescentia cujete*.[3] Other investigators have also been active in this area and have isolated a number of related compounds.[4] Quinones **1**−**6** all showed cytotoxic activity; in addition, compounds **2**−**6** showed selective DNA-damaging activity[3] against the repair-deficient *rad52* yeast strain.[5] These findings suggested that further development of the furanonaphthoquinone pharmacophore might well lead to compounds with even better activity.

Although the mechanism by which the natural furanonaphthoquinones cause DNA damage is unknown, it seems reasonable to suppose that it could involve intercalation into DNA. It is well-known that a number of drugs with planar aromatic systems can intercalate with DNA, although it has been stated that "a clear picture of the relationship among structure, DNA interaction, and chemotherapeutic activity does not exist."[6] Nevertheless, in view of the promising activity of the furanonaphthoquinone class of compounds, it seemed worthwhile to investigate them as potential DNA intercalators and to attempt to improve their activity on this basis.

DNA intercalators have been extensively studied because of their efficacy in cancer chemotherapy.[7] Biophysical studies on a large variety of carbocyclic polycyclic aromatic derivatives have shown that two structural elements contribute to a strong interaction of a molecule with DNA. The ring system that intercalates with DNA must contain at least three fused planar rings (a criterion met by the furanonaphthoquinones); a pendant side chain containing an amine group, which binds electrostatically to DNA, is also required.[6] The optimal nature of the amino side chain was investigated by Bair et al., who made a systematic study of the interactions between DNA and polycyclic aromatic derivatives containing polar side chains.[6] The best antitumor activity against a standard murine lymphocytic leukemia screen was seen for 2-[(arylmethyl)amino]propanediols (AMAPs) of the general structure **7**.



As noted above, the cytotoxic furanonaphthoquinones possess three fused planar rings, suggesting that intercalation into DNA may be involved in the mechanism of cytotoxicity. It was thus decided to prepare a limited number of analogues with an aminopropanediol side chain in order to determine whether their activity could be

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improved by the addition of this side chain. Herein we report the synthesis of such derivatives.

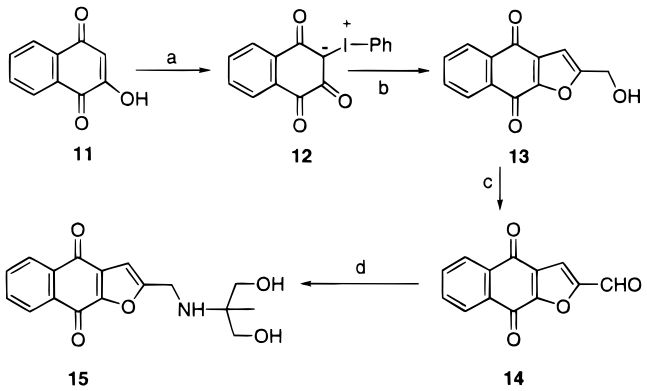
Results and Discussion

To evaluate the effect of a hydroxyamino side chain on the activity of furanonaphthoquinones, the three furanonaphthoquinone derivatives **8**−**10** were first synthesized as reference compounds, and their activities were determined in the yeast assay.[5] Compound **8** was prepared from 2-hydroxy-1,4-naphthoquinone and vinyl acetate by the procedure of Kobayashi et al.,[8] compound **9**, by the synthetic route of Perry et al.,[9] and compound **10** was also prepared as described by Perry et al.[10]

The synthetic scheme for the first target, 2-methyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionyl-methyl)-amino]-1,3-propanediol (**15**), began with 2-hydroxy-1,4-naphthoquinone (**11**), which was treated with iodobenzene diacetate to give 3-phenyliodonio-1,2,4-trioxo-1,2,3,4-tetrahydronaphthalenide (**12**).[11] Coupling of **12** with propargyl alcohol and subsequent ring closure afforded 2-hydroxymethyl-naphtho[2,3-*b*]furan-4,9-dione (**13**),[12] which was further oxidized to 2-formyl-4,9-dihydronaphtho[2,3-*b*]-furan-4,9-dione (**14**) by pyridinium chlorochromate (Scheme 1).

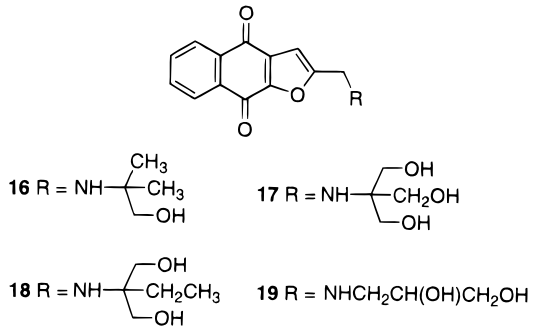
In the last step of this synthesis the hydroxyamino side chain was affixed to **14** via reductive amination. Treatment of **14** with 2-amino-2-methyl-1,3-propanediol in dichloromethane, using magnesium sulfate as the drying agent, followed by reduction with sodium cyanoborohydride afforded 2-methyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1,3-propanediol (**15**). The NMR spectra of **15** gave the expected side chain signals, its FABMS gave the expected protonated molecular ion $[MH]^+$ at *m*/*z* 316, and its EIMS showed significant peaks at *m*/*z* 226 $[M^+ - C_4H_9O_2]$ and 197 $[M^+ - C_5H_{12}NO_2]$.

Scheme 1[a]

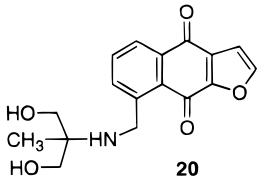


[a] (a) **11**, $PhI(OAc)_2$, $CHCl_3$, 5 h, 88%. (b) **12**, Propargyl alcohol, Cu_2O, 80 °C, 2 h, 25%. (c) **13**, PCC, CH_2Cl_2, rt, 20 h, 70%. (d) **14**, CH_2Cl_2, $MgSO_4$, 2-amino-2-methyl-1,3-propanediol, 24 h, then $NaBH_3CN$, MeOH, 61%.

To compare the effect of different side chains on the activity of the furanonaphthoquinones, some analogues of **15** with different hydroxyamino side chains were prepared. 2-Methyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1-propanol (**16**) was prepared in more than 80% yield by treating **14** with 2-amino-2-methylpropanol and magnesium sulfate in dichloromethane for 20 h, followed by reduction with sodium cyanoborohydride. The same procedure was also used to prepare 2-hydroxymethyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1,3-propanediol (**17**); 2-ethyl-2-[2′-(4′,9′-dihydro naphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1,3-propanediol (**18**); and 1-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-2,3-propanediol (**19**) from **14** in more than 80% yield.



The second target of interest was the quinone **20**, in which the amino side chain is attached to the naphthoquinone ring system rather than the furan ring. The

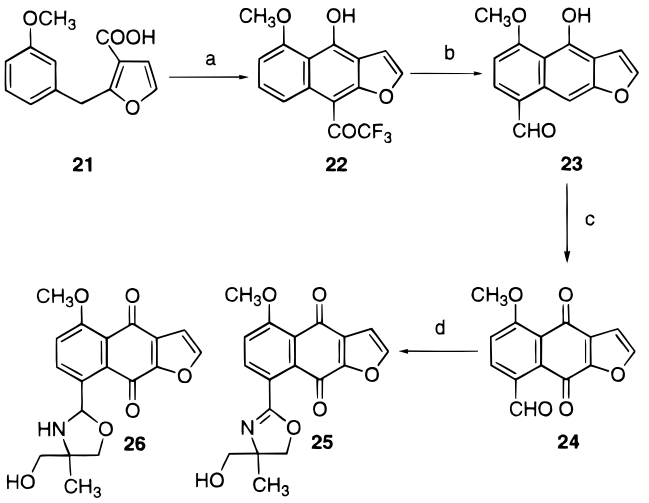


synthetic scheme for this target began with the formylation of 4-hydroxy-5-methoxy-9-(trifluoroacetyl)naphtho[2,3-*b*]furan (**22**), which was obtained from the Friedel−Crafts cyclization of **21**.[13] Compound **22** was treated with α,α-dichloromethyl methyl ether in dichloromethane in the presence of aluminum chloride[14] to give **23** as the major product after workup with aqueous sodium hydroxide solution and repeated chromatographic separation. Various attempts to oxidize phenol **23** with pyridinium dichromate,[15] pyridinium chlorochromate,[16] Fremy's salt[17] and Triton $B\text{-}O_2$[9] failed to give corresponding quinone **24**, but success was achieved by oxidizing **23** with salcomine-O_2,[18] and quinone **24** was obtained in 58% yield (Scheme 2).

With the quinone **24** in hand, conversion to the expected final product **20** by reductive amination was attempted. Treatment of **24** with 2-amino-2-methyl-1,3-propanediol and magnesium sulfate in dichloromethane for 20 h, followed by reduction with sodium cyanoborohydride as described previously afforded **25** as a yellow solid in 50% yield. The ^1H and ^{13}C NMR spectra of **25** showed signals for the hydroxyamino side chain, with signals for one methyl group (δ_H 1.43, δ_C 23.2) and two oxygen-bearing methylene groups (δ_H 3.56 and 3.72, δ_C 68.7; δ_H 4.17 and 4.55, δ_C 72.9), but the two methylene groups were obviously located in quite different chemical environments. Further evidence came from acetylation of **25**; the ^1H NMR spectrum of the acetate of **25** had only one acetate signal (δ_H 2.14, 3H), together with signals for the two oxygenated methylene groups. One of these sets of signals was at 4.24 and 4.51 ppm, close to the values of the unacetylated starting material, but the other set occurred at 4.20 and 4.27 ppm, 0.6 ppm downfield compared with those in **25**, indicating that **25** contained only one free hydroxy group. Because no signal was found for a methine proton on the benzylic carbon, one of the hydroxy groups in the side chain must be bound to that carbon. The HMBC spectrum of **25** showed correlations between H-2′ and C-1′, and H-7 and C-1′, indicating that the unprotonated C-1′ was indeed the carbon connecting the benzene ring and the side chain.

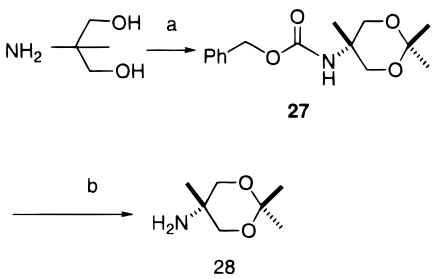
Based on these data, **25** was assigned the oxazoline structure shown. The cyclized product must be formed by

Scheme 2[a]



[a] (a) **21**, TFAA, CH_2Cl_2, 4 h, 0 °C, 32%. (b) **22**, $CH_3OCHCl_2/AlCl_3$, CH_2Cl_2, 0 °C, 30 min, 25%. (c) **23**, salcomine/O_2, 40 min, room temperature, 58%. (d) **24**, CH_2Cl_2, $MgSO_4$, 2-amino-2-methyl-1,3-propanediol, 24 h, then $NaBH_3CN$, MeOH, 50%.

Scheme 3[a]



[a] (a) CbzCl, DMF, 4 h, room temperature, then $Me_2C(OMe)_2$, PPTS, 20 h, 75%. (b) H_2, Pd-C, 2 h, room temperature, 98%

a nucleophilic attack of one of the side chain hydroxy groups onto the highly electrophilic −C=N− group to give the intermediate **26**. Transfer of hydrogen from this intermediate to another mole of the imine[19] would then result in the formation of the stable oxazoline product **25**.

In an attempt to prevent such a cyclization reaction from occurring, the side chain was protected before the amination reaction (Scheme 3). Thus 2-amino-2-methyl-1,3-propanediol was treated with benzyl chloroformate in *N*,*N*-dimethylformamide for 4 h with stirring, followed by treatment with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate with stirring for 20 h to give the N,O-diprotected substrate **27** in 70% yield. Deprotection of the NH_2 group of **27** was carried out by hydrogenolysis with Pd−C as catalyst to give the protected side chain amine **28** in 98% yield.

Reductive amination of **24** under argon with the protected side chain **28** was repeated by the procedure described. Usual workup and purification by preparative TLC gave the amination product **29**. The NMR spectra of **29** showed the expected signals for the protected side chain: three methyl groups (δ_H 1.25, 1.58, 1.76, δ_C 19.4, 21.4, 28.6), and two methylene groups (δ_H 4.45, 4.69, δ_C 67.7). However, no signal was found for the benzylic methylene group in the expected structure **30**. Instead, the ^1H NMR spectrum contained a sharp singlet at 8.38 ppm in addition to the signals of the two furan protons (δ_H 7.06, 7.66) and the two protons characteristic of a 1,2,3,4-tetrasubstituted benzene ring. The HMBC spectrum of **29** revealed correlations between this downfield singlet proton and C-8, C-9, and C-3′, indicating that it must be assigned

Table 1. Biological Activity of **8** and Its Analogues

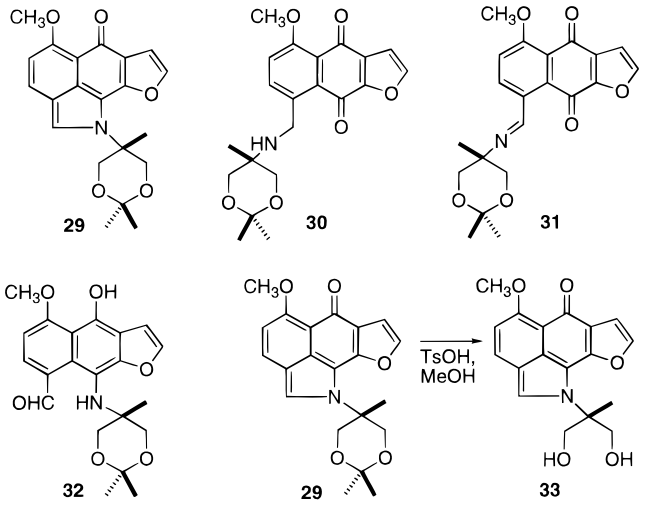
compound	yeast strain rad52.top1[a]	yeast strain rad52[a]	yeast strain RAD+	cytotoxicity to rat hepatoma cells[b]
2	N.T.	14	180	15.3[c]
8	17	83	78, 30	5, 3
9	44	152	135, 183	7, 5
10	125[d]	>8000	>1000	5, 7
14	44	160	185	2, 0.8
15	240	>500	>1000	2, 3
16	210	>500	>1000	4, 4
17	>250	>500	>1000	>20
18	>500	>500	>1000	5, 4
19	220	>500	>1000	2, 2
33	>500	>500	>1000	>20

[a] Results are expressed as IC_{12} values in μg/mL (concentration required to produce an inhibition zone of 12 mm around a 100-μLwell in an agar layer overlayered with the yeast strain). [b] Results are expressed as IC_{50} values in μM (concentration that produces 50% inhibition of growth); two assays were carried out for active compounds, and the results of both assays are given. [c] Cytotoxicity data for compound **2** are for cytotoxicity to Vero cells, from Heltzel et al.[3] [d] The zones observed for compound **10** were not completely clear.

to a methine group between the benzene ring and the side chain. The imine structure **31** was excluded by its mass spectrum, which indicated its composition to be $C_{21}H_{21}NO_5$, and by its ^{13}C NMR spectrum, which showed the presence of only one carbonyl carbon and not two as in **31**. These data thus indicate that compound **29** has the structure shown.

The formation of **29** presumably occurs through the initial formation of the reductive amination product **30** or **32**. This intermediate could then react further by an intramolecular attack of the secondary amine on the quinone or formyl carbonyl group, followed by loss of water, to yield the stable enamine **29**; at least part of the stability of **29** may be attributed to the fact that it is a vinylogous amide. Hydrolysis of **29** with *p*-toluenesulfonic acid in methanol overnight gave compound **33**.

Although this work did not yield the desired compound **20**, it did reveal some interesting and unexpected reactivity of the 8-formyl-5-methoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione system (**24**) toward reductive amination, with the reaction proceeding further to give the cyclized products **25** or **29**, depending on the amination reagent used.



The target compounds were tested in our yeast bioassays and also for cytotoxicity against H4IIE rat hepatoma cells; the results are given in Table 1. Consistent with earlier results,[3] the simple furanonaphthoquinone derivatives

8−**14** showed modest but reproducible activity against the repair-deficient *rad52.top1* yeast strain, suggesting that they act as DNA-damaging agents, although possibly as topoisomerase 2 inhibitors rather than as intercalators inasmuch as the activity was greater against the Δrad52Δtop1 call line than against the Δrad52 line. These compounds also showed reproducible activity against H4IIE rat hepatoma cells, with the aldehyde **14** having the best activity.

Compounds **15**−**19** with different amino side chains surprisingly had a much weaker or no activity against the topoisomerase 1 and repair-deficient yeast strain *rad52.top1* and essentially no activity against the repair-deficient strain *rad52*. Because the parent compounds showed activity against both strains in this assay, it seems at least possible that the lack of activity of the hydroxyamino side chain derivatives is due to a physical effect, such as their failure to be transported through the cell membrane, rather than to any inherent lack of activity. All of them except **17**, however, showed cytotoxicity against H4IIE rat hepatoma cells, and compounds **15** and **19** gave better activity than their parent compound **8** or the side chain derivative **2** and activity similar to the aldehyde **14**. The different side chains tested did not make significant differences to the activity of the test compounds, except that the triol **17** was not active, possibly due to its higher polarity and consequent greater difficulty in entering the cell. Compound **33** did not show activity in either the yeast bioassay or in the cytotoxicity assay.

This evidence tends to suggest that the furanophthoquinones do not serve primarily as DNA intercalators. If this had been the case, they would have been expected to show a significantly increased activity on conversion to their aminopropane derivatives.[6] They do appear to function as weak topoisomerase 2 inhibitors, but, regrettably, this activity is too weak to warrant further investigation in this area.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively, in $CDCl_3$, unless otherwise specified. ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR, HMQC, and HMBC NMR experiments were performed on the same spectrometer, using standard Varian pulse sequences. Mass spectra were obtained on a VG 7070 E-HF instrument. Chromatography was performed using Si gel Merck G60 (230−400 mesh), preparative TLC with Si gel GF_{254} plates (Analtech, 500 μm, 20 × 20 cm), and reversed-phase preparative TLC with Whatman PLKC18F linear K reversed-phase (500 mm, 20 × 20 cm) plates.

2-Acetoxy-2,3-dihydro-naphtho[2,3-*b*]furan-4,9-dione and 2-acetoxy-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione. To a solution of 2-hydroxy-1,4-naphthoquinone (1.0 g, 5.8 mmol) and vinyl acetate (5.34 mL, 58 mmol) in MeCN (20 mL) at 0 °C was added ceric ammonium nitrate (3.0 g, 5.5 mmol) with stirring. The mixture was stirred at 0 °C for 1 h. Then the reaction solution was diluted with H_2O and extracted with EtOAc three times. The combined extract was washed with H_2O, and dried over sodium sulfate. Evaporation followed by repeated column chromatography on Si gel with $CHCl_3$−MeOH afforded pure compounds 2-acetoxy-2,3-dihydro-naphtho[2,3-*b*]furan-4,9-dione and 2-acetoxy-2,3-dihydro-naphtho[1,2-*b*]-furan-4,5-dione. Data for 2-acetoxy-2,3-dihydro-naphtho[2,3-*b*]furan-4,9-dione: orange crystals (364 mg, 24.3%), mp 189−191 °C; ^{1}H NMR δ 2.15 (3H, s), 3.11 (1H, dd, J = 17.2, 2.8), 3.39 (1H, dd, J = 17.2, 7.6), 7.01 (1H, dd, J = 7.6, 2.8), 7.60 (1H, m), 7.66 (2H, m), 8.08 (1H, m); ^{13}C NMR δ 20.8, 32.6, 99.4, 113.8, 124.8, 126.9, 129.8, 130.6, 132.2, 134.8, 168.0, 169.0, 175.2, 180.3; EIMS m/z 258 [$M^{\bullet+}$], 216, 198, 188, 170, 159. Data for 2-acetoxy-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione: yellow crystals (526 mg, 35.2%), mp 187−189 °C; ^{1}H NMR δ 2.12 (3H, s), 3.17 (1H, dd, J = 18.4, 2.4), 3.45 (1H, dd, J = 18.4, 7.6), 6.99 (1H, dd, J = 7.6, 2.4), 7.72 (2H, m), 8.09 (2H, m); ^{13}C NMR δ 20.8, 33.6, 98.3, 123.3, 126.2, 126.5, 131.5, 132.7, 133.3, 134.2, 158.3, 168.7, 176.7, 181.7.

Naphtho[2,3-*b*]furan-4,9-dione (8). To a solution of 2-acetoxy-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (200 mg, 0.77 mmol) in THF (5 mL) lithium bis(trimethylsilyl) amide (1.6 mL of a 1.0 mol dm^{-3} solution in THF, 1.6 mmol) was added at −78 °C with stirring. After 15 min the reaction solution was diluted with H_2O and extracted with EtOAc three times. The EtOAc was collected, washed with H_2O, and dried over Na_2SO_4. Evaporation and column chromatography on Si gel with hexane−EtOAc gave compound **8** as a yellow solid (50 mg, 64.7%): mp 225−226 °C (lit.[20] 225−225.5 °C); ^{1}H NMR δ 7.01 (1H, d, J = 1.6), 7.76 (2H, m), 7.78 (1H, d, J = 1.6), 8.22 (2H, m); ^{13}C NMR δ 108.6, 126.9, 127.1, 130.5, 132.5, 133.2, 133.8, 133.9, 148.6, 152.7, 173.6, 180.5; EIMS m/z 198 [$M^{\bullet+}$], 170, 142, 114.

3-Phenyliodonio-1,2,4-trioxo-1,2,3,4-tetrahydronaphthalenide (12). To a stirred solution of 2-hydroxy-1,4-naphthoquinone (2.06 g, 11.8 mmol) in $CHCl_3$ (40 mL) was added a solution of (diacetoxyiodo)benzene (3.8 g) dissolved in $CHCl_3$ (25 mL) at 0 °C. The mixture was stirred at this temperature for 1 h and then for 4 h further at room temperature. Filtration gave an orange precipitate that was washed with $CHCl_3$ and dried to afford compound **7** (3.9 g, 88%) as orange crystals: mp >320 °C; ^{1}H NMR (DMSO) δ 7.38 (2H, m), 7.50 (1H, m), 7.70 (1H, m), 7.79 (1H, m), 7.84 (2H, m), 7.95 (1H, m), 8.03 (1H, m); ^{13}C NMR (DMSO) δ 103.0, 113.9, 126.8, 126.9, 130.6, 130.8, 131.1, 132.2, 133.1, 133.4, 134.6, 169.9, 174.8, 181.0; FABMS m/z 377 [MH^+]; EIMS m/z 204, 172, 104, 76.

2-Hydroxymethyl-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione (13). To a solution of **12** (1.0 g, 2.7 mmol) in pyridine (40 mL) was added propargyl alcohol (1.6 mL, 27 mmol) and cuprous oxide (1.0 g) at 80 °C. The mixture solution was stirred at this temperature for 2 h. The solution was filtered and diluted with 10% aqueous HCl. The acidic solution was extracted three times with EtOAc. The combined organic phase was washed with H_2O and dried over Na_2SO_4. Evaporation and repeated column chromatography on Si gel with $CHCl_3$−Me_2CO gave compound **13** as an orange solid (150 mg, 25%): mp 202−203 °C; ^{1}H NMR δ 4.69 (2H, s), 6.87 (1H, s), 7.78 (2H, m), 8.17 (2H, m); ^{13}C NMR δ 56.1, 104.7, 126.3, 126.4, 131.0, 132.0, 132.6, 133.5, 133.6, 151.6, 162.4, 173.2, 180.3; EIMS m/z 228 [M^+], 212, 183, 172, 113, 104, 76.

2-Formyl-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione (14). To a solution of **13** (90 mg, 0.39 mmol) in CH_2Cl_2 (5 mL) was added pyridinium chlorochromate (0.3 g). The mixture was stirred for 20 h at room temperature. Then the solution was diluted with H_2O and extracted with EtOAc three times. The combined extract was washed with H_2O and dried over Na_2SO_4. Evaporation and column chromatography on Si gel with $CHCl_3$−Me_2CO afforded **14** as a yellow solid (63 mg, 70%): mp 183−186 °C; ^{1}H NMR δ 7.67 (1H, s), 7.81 (2H, m), 8.24 (2H, m), 9.96 (1H, s); ^{13}C NMR δ 114.4, 127.3, 127.4, 130.4, 132.5, 133.1, 134.4, 134.6, 153.8, 154.8, 173.9, 179.1, 179.5; HREIMS m/z 226.0266 [$M^{\bullet+}$] (calcd for $C_{13}H_6O_4$, 226.0265); EIMS m/z 226 [$M^{\bullet+}$], 197, 169, 141, 114.

General Procedure for the Preparation of 15 and Its Analogues. To a solution of **14** (10 mg, 0.044 mmol) in CH_2Cl_2 (3 mL) was added 2-amino-2-methyl-1,3-propanediol; 2-amino-2-methylpropanol; 2-amino-2-ethyl-1,3-propanediol; 2-amino-2-hydroxymethyl-1,3-propanediol; or 3-amino-2,3-propanediol (0.88 mmol) and anhydrous $MgSO_4$ (100 mg). The mixture was stirred for 24 h. The solution was filtered and evaporated to dryness. The residue was dissolved in MeOH (2 mL). The resulting solution was adjusted to pH 6−7 by HOAc, and sodium cyanoborohydride was added (14 mg, 0.22 mmol). The mixture was stirred for 30 min. The solution was diluted with H_2O and extracted with EtOAc three times. The EtOAc extracts were collected and dried over Na_2SO_4. Evaporation

and purification by preparative TLC eluting with $CHCl_3$−MeOH afforded pure compounds.

2-Methyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1,3-propanediol (15): yellow solid (8.6 mg, 61%), mp 193−195 °C; ^{1}H NMR (DMSO) δ 0.92 (3H, s), 2.08 (1H, br s), 3.27 (4H, d, J = 5.5), 3.88 (2H, s), 4.47 (2H, dd, J = 5.5), 6.91 (1H, s), 7.85 (1H, m), 8.07 (2H, m); ^{13}C NMR (DMSO) δ 18.8, 39.4, 57.5, 64.9, 79.6, 105.1, 126.7, 126.8, 131.5, 132.6, 133.1, 134.4, 134.7, 151.6, 164.8, 172.9, 180.8; HRFABMS *m/z* 316.1182 [MH$^+$] (calcd for $C_{17}H_{18}NO_5$, 316.1185); EIMS *m/z* 226 [M$^+$ − $C_4H_9O_2$], 197, 169, 141, 114, 113, 76.

2-Methyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1-propanol (16): yellow solid (12.6 mg, 96%), mp 187−189 °C; ^{1}H NMR (CD_3OD) δ 1.13 (6H, s), 3.42 (2H, s), 3.93 (2H, s), 6.91 (1H, s), 7.81 (2H, m), 8.16 (2H, m); ^{13}C NMR (DMSO) δ 24.0, 39.6, 54.2, 68.6, 105.1, 126.7, 126.9, 131.4, 132.6, 133.1, 134.4, 134.7, 151.6, 164.9, 173.0, 180.9; HRFABMS *m/z* 300.1244 [MH$^+$] (calcd for $C_{17}H_{17}NO_4$, 300.1234).

2-Hydroxymethyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]-furan-4′,9′-dionylmethyl)amino]-1,3-propanediol (17): yellow solid (13.7 mg, 94%), mp > 320 °C; ^{1}H NMR (DMSO) δ 3.38 (6H, d, J = 5.0), 3.99 (2H, s), 4.38 (3H, t, J = 5.0), 6.93 (1H, s), 7.84 (2H, m), 8.07 (2H, m); ^{13}C NMR (DMSO) δ 60.0, 61.3, 104.7, 126.3, 126.4, 131.1, 132.2, 132.6, 134.0, 134.3, 151.2, 164.3, 172.5, 180.5; HRFABMS *m/z* 332.1143 [MH$^+$] (calcd for $C_{17}H_{17}NO_6$, 332.1134).

2-Ethyl-2-[2′-(4′,9′-dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1,3-propanediol (18): yellow solid (12.9 mg, 89%), mp > 320 °C; ^{1}H NMR (CD_3OD) δ 0.93 (3H, t, J = 7.5), 1.50 (2H, q, J = 7.5), 3.53 (4H, d, J = 4.1), 3.95 (2H, s), 6.91 (1H, s), 7.81 (2H, m), 8.17 (2H, m); ^{13}C NMR (DMSO) δ 7.1, 22.3, 48.6, 58.9, 62.1, 104.7, 126.3, 126.4, 131.0, 132.2, 132.7, 134.0, 134.3, 151.2, 164.2, 172.5, 180.5; HRFABMS *m/z* 330.1354 [MH$^+$] (calcd for $C_{18}H_{19}NO_5$, 330.1341).

3-[2′-(4′,9′-Dihydronaphtho[2′,3′-*b*]furan-4′,9′-dionylmethyl)amino]-1,2-propanediol (19): yellow solid (12.6 mg, 95%), mp 148−150 °C; ^{1}H NMR (CD_3OD) δ 2.65 (1H, dd, J = 8.0, 12.0), 2.80 (1H, dd, J = 4.0, 12.0), 3.51 (2H, d, J = 5.0), 3.75 (1H, m), 3.99 (2H, s), 6.92 (1H, m), 7.82 (2H, m), 8.15 (2H, m); ^{13}C NMR (DMSO) δ 45.8, 52.0, 64.3, 70.6, 105.2, 126.3, 126.5, 131.0, 132.2, 132.7, 134.0, 134.3, 151.4, 163.3, 172.6, 180.5; HRFABMS *m/z* 302.1034 [MH$^+$] (calcd for $C_{16}H_{15}NO_5$, 302.1028).

8-Formyl-4-hydroxy-5-methoxynaphtho[2,3-*b*]furan (23). To a solution of **22**[19] (1.4 g, 4.5 mmol) in CH_2Cl_2 (50 mL) was added α,α-dichloromethyl methyl ether (4.15 mL, 45 mmol) and aluminum chloride (0.65 g, 6.7 mmol) at 0 °C. The mixture was stirred for 30 min. Then the solution was diluted with H_2O and extracted with EtOAc three times. The EtOAc was collected and extracted with 5% aqueous NaOH solution three times. The combined basic solution was acidified with HCl and extracted with EtOAc three times. The combined extracts were washed with H_2O and dried over Na_2SO_4. Evaporation and column chromatography on Si gel with $CHCl_3$ followed by preparative TLC eluting with hexane−EtOAc gave pure compound **23** as a yellow solid (275 mg, 25.3%): mp 158−161 °C; ^{1}H NMR δ 4.17 (3H, s), 6.74 (1H, d, J = 8.0), 7.01 (1H, d, J = 2.4), 7.63 (1H, d, J = 2.4), 7.75 (1H, d, J = 8.0), 9.11 (1H, s), 9.69 (1H, s), 10.11 (1H, s); ^{13}C NMR ($CDCl_3$) δ 56.5, 97.8, 100.4, 104.0, 109.9, 115.7, 125.8, 130.7, 140.0, 145.4, 148.5, 157.7, 162.4, 192.0; HREIMS *m/z* 242.0579 [M$^{\bullet+}$] (calcd for $C_{14}H_{10}O_4$, 242.0579); EIMS *m/z* 242 [M$^{\bullet+}$], 214, 199, 171, 115, 87.

8-Formyl-5-methoxy-4,9-dihydronaphtho[2,3-*b*]furan-4,9-dione (24). Salcomine (20 mg) was added to a solution of **23** (90 mg, 0.37 mmol) in MeCN (10 mL), and the mixture was stirred for 40 min while oxygen was bubbled through. The solution was then filtered and evaporated. Column chromatography of the residue on Si gel with $CHCl_3$ afforded pure compound **24** as a yellow solid (55 mg, 58%): mp 199 °C; ^{1}H NMR δ 4.09 (3H, s), 6.98 (1H, d, J = 2.0), 7.40 (1H, d, J = 8.8), 7.78 (1H, d, J = 2.0), 8.04 (1H, d, J = 8.8), 10.64 (1H, s); ^{13}C NMR δ 56.9, 109.0, 118.1, 120.6, 131.8, 132.1, 135.1, 135.2, 149.4, 150.6, 163.3, 174.5, 179.1, 191.6; HREIMS *m/z* 256.0372 [M$^{\bullet+}$] (calcd for $C_{14}H_8O_5$, 256.0372); EIMS *m/z* 256 [M$^{\bullet+}$], 241, 149, 115, 91, 83, 69, 57.

The Formation of 25. To a solution of **24** (14 mg, 0.055 mmol) in CH_2Cl_2 (3 mL) was added 2-amino-2-methyl-1,3-propanediol (115 mg, 1.1 mmol) and anhydrous $MgSO_4$ (100 mg), and the mixture was stirred for 24 h. The solution was then filtered and evaporated to dryness, and the residue dissolved in MeOH (2 mL). The resulting solution was adjusted to pH 6−7 by HOAc, sodium cyanoborohydride (17 mg, 0.28 mmol) was added, and the mixture was stirred for 30 min. The solution was diluted with H_2O and extracted with EtOAc three times, and the combined EtOAc extracts were dried over Na_2SO_4. Evaporation and purification by preparative TLC, eluting with $CHCl_3$−MeOH afforded pure compound **25** as a yellow solid (9.4 mg, 50%): ^{1}H NMR (CD_3OD) δ 1.43 (3H, s), 3.57 (1H, d, J = 10.8), 3.71 (1H, d, J = 10.8), 4.02 (3H, s), 4.17 (1H, d, J = 8.4), 4.55 (1H, d, J = 8.4), 6.98 (1H, d, J = 2.0), 7.57 (1H, d, J = 8.8), 7.78 (1H, d, J = 8.8), 7.99 (1H, d, J = 2.0); ^{13}C NMR δ 23.3, 57.2, 68.7, 72.9, 77.4, 109.6, 119.7, 122.0, 123.3, 132.7, 134.9, 138.0, 151.3, 152.2, 163.3, 167.9, 173.5, 180.9; HRFABMS *m/z* 342.0975 [M + 1$^+$] (calcd for $C_{18}H_{17}NO_6$, 342.0978); CIMS *m/z* 342 [MH$^+$], 309, 254, 212, 140.

Protection of 2-Amino-2-methyl-1,3-propanediol. To a solution of 2-amino-2-methyl-1,3-propanediol (4.0 g, 38 mmol) in *N*,*N*-dimethylformanide (25 mL) was added benzyl chloroformate (5.72 mL, 38 mmol). After the mixture was stirred for 4 h, 2,2-dimethoxypropane (10 mL) and pyridinium *p*-toluenesulfonate (300 mg) were added to this solution, which was allowed to stir for another 20 h. Then the solution was diluted with H_2O and extracted with EtOAc three times. The combined extracts were washed with H_2O and dried over Na_2SO_4. Evaporation gave crude product, which was crystallized from hexane−EtOAc to afford pure **27** as white crystals (8.0 g, 75%): mp 109 °C; ^{1}H NMR δ 1.28 (3H, s), 1.42 (3H, s), 1.43 (3H, s), 3.66 (2H, d, J = 12.0), 3.90 (2H, d, J = 12.0), 5.08 (2H, s), 7.31−7.37 (5H, m); ^{13}C NMR δ 18.8, 19.2, 27.8, 49.3, 66.3, 67.1, 98.2, 128.0, 128.1, 128.5, 136.5, 155.3; EIMS *m/z* 279 [M$^{\bullet+}$], 264, 248, 221.

Hydrogenolysis of 27. To a solution of **27** (1.92 g, 6.9 mmol) in absolute MeOH (30 mL) was added palladium on activated carbon (10%, 200 mg). The mixture was stirred for 2 h under hydrogen, and the solution filtered, and evaporated under vacuum to give pure **28** as a colorless liquid (0.98 g, 98%): ^{1}H NMR (CD_3COCD_3) δ 0.96 (3H. s), 1.32 (3H, s), 1.34 (3H, s), 1.97 (2H, br s), 3.40 (2H, d, J = 12.0), 3.60 (2H, d, J = 12.0); ^{13}C NMR (CD_3COCD_3) δ 20.8, 20.9, 25.6, 46.6, 70.1, 97.4; CIMS *m/z* 146 [MH$^+$], 130, 88, 73, 57.

The Formation of 29. To a solution of **24** (10 mg, 0.039 mmol) in CH_2Cl_2 (3 mL) was added **28** (180 mg, 1.2 mmol) and $MgSO_4$ (100 mg). The mixture was stirred for 20 h. The solution was filtered, diluted with H_2O, and extracted with EtOAc. The combined extracts were washed with H_2O and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was dissolved in MeOH (2 mL). The resulting solution was adjusted to pH 6−7 by HOAc and sodium cyanoborohydride (12 mg, 0.19 mmol) added, and the mixture was stirred for 30 min. The solution was diluted with H_2O and extracted with EtOAc three times, and the EtOAc extracts were combined and dried over Na_2SO_4. Evaporation and purification by preparative TLC, eluting with $CHCl_3$−MeOH, afforded pure compound **29** as a pink solid (6.8 mg, 46%): ^{1}H NMR (CD_3OD) δ 1.25 (3H, s), 1.58 (3H, s), 1.76 (3H, s), 4.12 (3H, s), 4.45 (1H, d, J = 13.1), 4.69 (1H, d, J = 13.1), 7.06 (1H, d, J = 2.0), 7.28 (1H, d, J = 8.8), 7.66 (1H, d, J = 2.0), 8.25 (1H, d, J = 8.8), 8.30 (1H, s); ^{13}C NMR (CD_3OD) δ 19.4, 21.4, 28.6, 57.1, 58.3, 67.7, 99.1, 109.1, 109.7, 112.5, 119.4, 120.9, 123.3, 125.2, 126.1, 132.5, 141.6, 149.4, 164.0, 176.6; HREIMS *m/z* 367.1420 [M$^{\bullet+}$] (calcd for $C_{21}H_{21}NO_5$, 367.1420); EIMS *m/z* 367 [M$^{\bullet+}$], 255, 239, 210, 152, 71, 59.

Hydrolysis of 29. To a solution of **29** (4.5 mg, 0.012 mmol) in a mixture of MeOH and H_2O (95:5, 2 mL) was added *p*-toluenesulfonic acid (10 mg). The mixture was stirred for 20 h at room temperature. The solution was then diluted with H_2O and extracted with EtOAc three times. The combined extracts were washed with H_2O and dried over Na_2SO_4.

Evaporation and purification by preparative TLC, eluting with $CHCl_3$−MeOH, afforded pure compound **33** as a pink solid (3.2 mg, 80%): ^{1}H NMR (CD_3OD) δ 1.91 (3H, s), 4.17 (3H, s), 4.18 (2H, d, J = 12.0), 4.40 (2H, d, J = 12.0), 7.16 (1H, d, J = 2.0), 7.32 (1H, d, J = 8.8), 7.65 (1H, d, J = 2.0), 8.29 (1H, s), 8.34 (1H, d, J = 8.8); ^{13}C NMR (CD_3OD) δ 22.6, 55.7, 64.7, 67.0, 108.0, 108.8, 110.0, 110.8, 118.3, 119.7, 125.2, 127.9, 133.5, 140.6, 149.0, 165.2, 179.2; HRFABMS *m/z* 328.1200 [MH^+] (calcd for $C_{18}H_{18}NO_5$, 328.1185).

Bioassays. The yeast bioassays were carried out as previously described.[21] The cytotoxicity assays were carried out by standard methods with H4IIE rat hepatoma cells, using the XTT protocol for visualization.[22]

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