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Efficient Synthesis of 'Redox-Switched' Naphthoquinone Thiol-Crown Ethers and Their Biological Activity Evaluation

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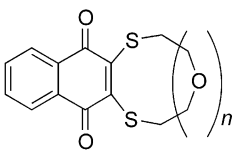
Abstract—Series of naphthoquinone thiol-crown ethers had been prepared. The antibacterial, antifungal, and cytotoxic activities of these synthetic naphthoquinone thiol-crown ethers were investigated. All of the compounds tested displayed antibacterial, cytotoxic and antifungal activities. The bis-naphthoquinone thiol-crown ether **7a** was the most potent inhibitor among tested analogues against *Staphylococcus aureus* methicillin resistance with MIC value of 2.68 μM.

Introduction

The quinone structure is common in numerous natural products that are associated with antitumor, antibacterial, antimalarial, and antifungal activities.[1] In many cases, the biological activity of quinone is attributed to the ability to accept electrons to form the corresponding radical anion or dianion species. The variable capacity of quinones to accept electrons is due to the electron-attraction or -donation substituents at the quinone moiety which modulate the redox properties responsible for the resulting oxidative stress.[1] Quinone moiety substituted with the cation bind crown ether could affect its redox properties, and this type of molecule is classified as redox-switched crown ethers.[2] The redox switched crown ethers were first synthesized by Misumi et al.[3] The detail investigation on the redox properties of these redox switched crown ethers provided strong evidence that the presence of alkali metal salts made the quinone easier to reduce.[2] The concept of the redox-switched crown ether had been applied in studies for electrochemical switch because the redox-active groups are mostly responsive to the electrochemical signals.[2] Although research in this area remains active, an extensive survey of the literature did not reveal any biological data of these redox-switched crown ethers on their antitumor, antibacterial, and antifungal activities.

As part of an ongoing studies directed toward redox-switched naphthoquinone thiol-crown ethers[4] disclosed herein is the synthesis of a series of naphthoquinone thiol-crown ethers **3a**–**e** and **7a**–**e**, from commercially available naphthoquinone. Disclosed herein is the preliminary result on the biological properties, antibacterial, antifungal, and cytotoxic activities, of these analogues. All of these synthetic naphthoquinone thiol-crown ethers exhibited weak antifungal activities, considerable cytotoxicities, and potent antibacterial activities against *Staphylococcus aureus* methicillin resistant. We believe the synthetic route disclosed herein provide an efficient preparation of naphthoquinone thiol-crown ethers and holds promise for providing new generation of potent antibacterial molecules.



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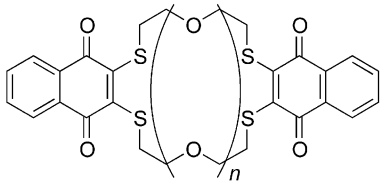
3b $n = 2$
3c $n = 3$
3d $n = 4$
3e $n = 5$



7a $n = 1$
7b $n = 2$
7c $n = 3$
7d $n = 4$
7e $n = 5$

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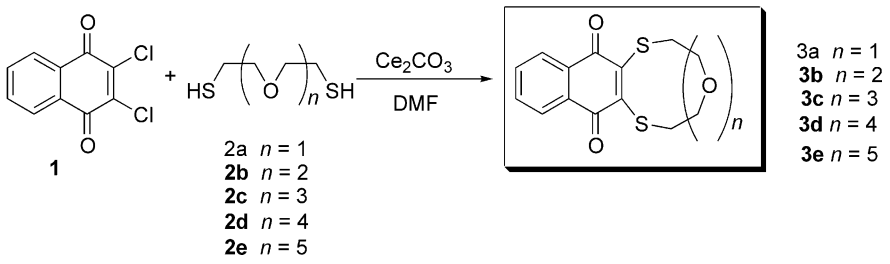
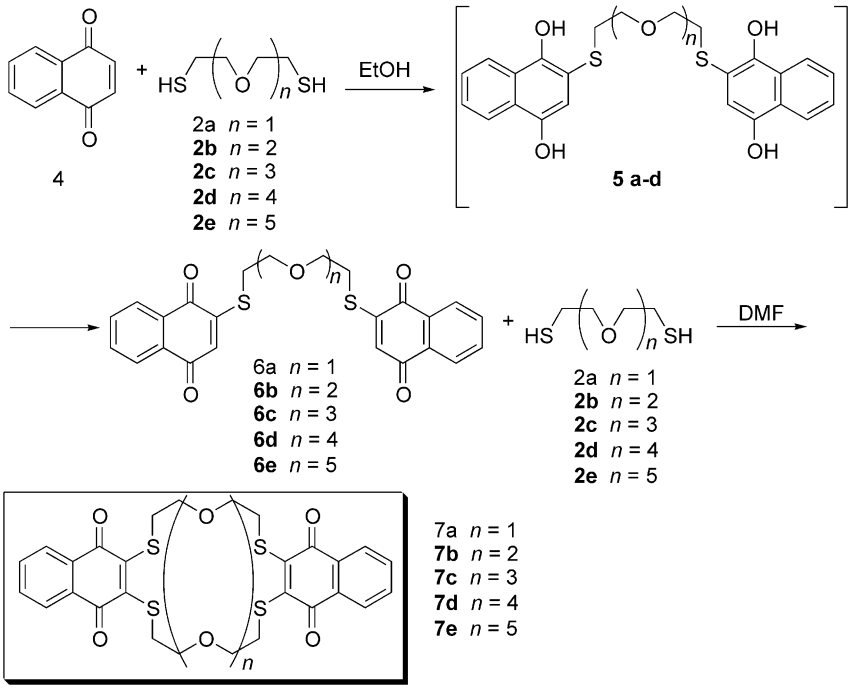


Scheme 1.



Scheme 2.

Results

Synthesis

The naphthoquinone thiol-crown ethers **3a**–**e** were prepared from 2,3-dichloronaphthoquinone (**1**) as outlined in Scheme 1. Double nucleophilic 1,4-addition-elimination of the quinone moiety of 2,3-dichloronaphthoquinone (**1**) to β,β′/dimercaptodiethyl ether[5] **2a**–**e** under basic condition gave crowned naphthoquinone **3a**–**e** in moderate yield (35–57%). The DMF and Ce_2CO_3 were found to be the best solvent and the base of choice for the reaction.

A series of thiol-crowned bis-naphthoquinone **7a**–**e** were prepared from naphthoquinone (**4**) by the route as describe in Scheme 2. Bis-naphthoquinone thiol ethers **6a**–**e** were synthesized by direct 1,4-type addition followed by in situ oxidation; 4 equivalent of 1,4-napthoquonine (**4**) were employed. Direct 1,4-type addition of the quinone moiety of 1,4-napthoquonine (**4**) to ether **2a**–**e** yielded nonisolated bis-hydroxylnaphthoquinone **5a**–**d**. The bis-hydroxylnaphthoquinone **5a**–**d** were spontaneously oxidized, to allow regeneration of the quinone moiety in the final product **6a**–**d**, and 1,4-dihydroxylnaphthoquinone (not shown) was isolated as the by-product of this reaction. The regeneration of the quinone moiety from the initial intermediates **5a**–**e** by excess naphthoquinone, as the oxidation reagent was unprecedented; the molecular oxygen was the common reagent for regeneration of quinone moiety.[6] Treatment of equal molar of 1,4-napthoquonine (**4**) with ether **2a**–**e** under positive pressure of oxygen lead to complex mixtures with minor amount of desired products; finally the yield was optimized with 4 equivalent of 1,4-napthoquonine (**4**). This result suggested that excess amount of 1,4-naphthoquonine (**4**) could served as the oxidation reagent.

The final transformation to complete the synthesis is outlined in the latter half of Scheme 2. Treatment of equal molar of **6a**–**e** and ether **2a**–**e** gave the crowned bis-naphthoquinone **7a**–**e**. Another direct 1,4-type addition of the quinone moiety of bis-naphthoquinone thiol ethers **6a**–**e** to ether **2a**–**e** yielded nonisolated thiol-substituted bis-hydroxylnaphthoquinone intermediates (not shown) which were spontaneously oxidized by excess molecular oxygen, to allow regeneration of the quinone moiety in the final product. The two-step synthesis of **7a**–**e** gave the overall yield of 35–45%.

Biological evaluation

Antibacterial, antifungal and cytotoxic activities of naphthoquinone thiol-crown ether **3a–e** and bis-naphthoquinone thiol-crown ether **7a–e** were evaluated, and the results were reported in Tables 1–3. Table 1 shows MIC values of **3a–e** and **7a–e** against six strains of bacteria, *S. aureus* methicillin resistance, *Escherichia coli*, *Mycobacterium ranae*, *Pseudomanas aeruginosa*, *Klebsiella pneumoniae*, and *Prateus vulgaris*. Gentamycin was used as a positive control in all tests, and its MIC value was express in μg/mL. Both **3a–e** and **7a–e** showed significant activities against *S. aureus* methicillin resistance, and had no antibacterial activities against other strains of bacteria tested. The bis-naphthoquinone thiol crown ether **7a** was the most potent inhibitor among tested analogues against *S. aureus* methicillin resistance with MIC value of 2.68 μM (1.56 μg/mL), which was comparable to the MIC value (0.78 μg/mL) of gentamycin. The series of bis-naphthoquinone thiol-crown ethers **7a–e** analogues were the more potent inhibitors than the corresponding series of naphthoquinone thiol-crown ethers **3a–e** analogues.

The antifungal activities results were listed in Table 2. In this assay, two strains of fungi, *Candida albicans* and *Trichophyton mentagrophytes*, were used, and amphotericin B was used as a positive control in all tests. Both **3a–e** and **7a–e** exhibited weak antifungal activities compared to the control fungicide, amphotericin B. The most active inhibitors among tested were **3a** and **7a** with MIC value of 5.33 and 10.7 μM against *C. albicans* and 21.4 and 5.34 μM against *T. mentagrophytes*, respectively.

The cytotoxic activities of **3a–d** and **7a–e** against three human carcinoma cell lines, Hep2, CT-5′, and BC-M1 were investigated. MTT assay were employed to determine the IC_{50} values of the tested compounds, and the results are shown in Table 3. All compounds tested shown significant cytotoxicities with IC_{50} values at between 6 and 21 μM concentration ranges. Analogues, **3a–d** and **7a–e**, had the average IC_{50} value of 6.11 and 8.07 μM against Hep2 and CT-5′ carcinoma cell lines, respectively, and exhibited weaker cytotoxic activities against BC-M1 as compared to the other two cell lines.

Discussion

Although **3a–e** and **7a–e** exhibited weak antifungal activities, and moderated cytotoxicities, there were

Table 1. Antibacterial activity of naphthoquinone thiol-crown ethers obtained from the standard dilution techniques of various compounds tested against numerous strains of bacteria

Compd	*S. aureus* Methicillin resistance (ATCC 33591)	*E. coli* (ATCC 10536)	*M. ranae* (ATCC 110)	*Ps. aeruginosa* (ATCC 9027)	*K. pneumoniae* (ATCC 9977)	*P. vulgaris* (A 9539)
3a	5.31	>200	>200	>200	>200	>200
3b	37.2	>200	>200	>200	>200	>200
3c	32.9	>200	>200	>200	>200	>200
3d	29.4	>200	>200	>200	>200	>200
3e	26.6	>200	>200	>200	>200	>200
7a	2.68	21.1	150	>200	>200	>200
7b	9.28	>200	>200	>200	>200	>200
7c	8.21	>200	>200	>200	>200	>200
7d	14.0	>200	>200	>200	>200	>200
7e	6.73	>200	>200	>200	>200	>200
Gentamycin[a]	0.78	0.39	0.20	0.78	0.39	0.39

[a]Minimum inhibitory concentration (MICs) were reported in μM.
The minimum inhibitory concentration of gentamycin reported in μg/mL.

Table 2. Antifungal activity of naphthoquinone thiol-crown ethers obtained from the standard dilution techniques of various compounds tested against *C. albicans*, and *T. mentagrophytes*[a]

Compd	*C. albicans* (ATCC 10231)	*T. mentagrophytes* (CDC Atlanta)
3a	5.33	21.4
3b	>200	>200
3c	131	>200
3d	150	>200
3e	>200	>200
7a	10.7	5.34
7b	>200	150
7c	>200	65.8
7d	>200	58.9
7e	>200	150
Amphotericin B	0.027	0.422

[a]Minimum inhibitory concentration (MICs) were reported in μM.

Table 3. Cytotoxicity of naphthoquinone thiol-crown ethers obtained from MTT assay of various compounds, **3a–d** and **7a–e**, tested against human larynx (Hep2), oral (CT-5′) and breast (BC-M1) carcinoma cell lines[a]

Compd	Hep 2	CT-5′	BC-M1
3a	6.17	9.98	17.0
3b	5.80	7.05	20.5
3c	5.48	8.35	17.2
3d	6.02	7.75	16.2
7a	6.58	9.08	9.05
7b	5.64	8.25	10.6
7c	6.21	8.05	9.22
7d	6.17	6.42	8.57
7e	6.53	7.75	8.93

[a]Inhibitory concentration at 50% (IC_{50}) reported in μM.

encouraging results revealed on the antibacterial activities. The *S. aureus* methicillin resistant has become a major nosocomial pathogen in community hospitals, long term-care facilities, and tertiary care hospitals.[7] Development of new antibiotics to combat this epidemic is becoming a new challenge for many scientists. Both **3a–e** and **7a–e** exhibited selective antibacterial activities toward *S. aureus* methicillin resistance, and had no significant activity against other tested strains of bacteria. Taken together the preliminary data disclosed herein revealed circumstantial suggestion that the bacterial membranes of *S. aureus* methicillin resistance was more vulnerable toward to these naphthoquinone thiol-crown ethers penetration as compared to the other strains of bacteria. The more detailed investigation on the antibacterial mechanisms of these naphthoquinone thiol-crown ethers was under way.

Conclusion

The aim of this study was to develop an efficient synthetic approach to construct various sizes of naphthoquinone thiol-crown ethers and to screen for possible antibacterial, antifungal or antitumor activities. The efficient synthetic approach disclosed herein had lead to quick output of a series of naphthoquinone thiol-crown ethers for the evaluation of antibacterial, antifungal, and cytotoxic activities, and identified **7a** as the potent inhibitor for *S. aureus* methicillin resistance.

Experimental

Proton and carbon NMR were obtained on a Bruker AMX-500 spectrometer. Chemical shifts were reported in ppm relative to tetramethylsilane (δ units). Fast atom bombardment (FAB) mass spectra were recorded on a Micromass ZAB spectrometer at the Analytical Facility of The National Taiwan University. All of chemicals were purchased from Acros, Aldrich, or TCI and used without future purification.

General procedure for preparing naphthoquinone thiol-crown ethers 3a–d

A mixture of β,β′-dimercaptodiethyl ether (1.1 mmol) and Ce_2CO_3 (1.2 mmol) in DMF (15 mL) was cooled to 5–10 °C under nitrogen, and 1,4-naphthoquinone (1.0 mmol) in DMF (15 mL) was added to the mixture dropwise. The mixture was allowed to warm to room temperature. After 24 h, the mixture was poured into cold water (100 mL), and acidified with 5% HCl (30 mL). The aqueous solution was extracted with CH_2Cl_2 (50 mL×4). The combined extracts were dried with $MgSO_4$ and concentrated under reduced pressure to provide a crude product. The crude product was purified by silica gel chromatography.

2,3,5,6 - tetrahydronaphtho[2,3-*e*] [1,4,7] oxadithionine-8,13-dione (3a). As red solid (35% yield) Mp 208–210 °C; IR (KBr) ν_{max}: 1662, 1650 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.49(4H, t, $J=4.6$ Hz), 3.95 (4H, 4H, t, $J=4.6$ Hz), 7.69 (2H, dd, $J=5.6$ Hz, $J=3.2$ Hz), 8.02(2H, dd, $J=5.6$ Hz, 3.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.2, 74.2, 127.1, 132.3, 133.7, 149.0, 180.7; MS(EI) *m*/*z*: 292 (M+), 104 (100%). Anal. calcd for $C_{14}H_{12}O_3S_2$: C, 57.51; H, 4.14. Found: C, 57.40; H, 4.33.

2,3,5,6,8,9,-Hexahydronaphtho [2,3][1,4,7,10]dioxadithiacyclododecine (3b). As red solid (72% yield) Mp: 175–176 °C; IR (KBr) ν_{max}: 1668, 1652 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.31 (4H, s), 3.33 (4H, t, $J=4.5$ Hz), 3.63 (4H, t, $J=4.5$ Hz), 7.65 (2H, dd, $J=5.7$ Hz, 3.2 Hz), 8.03 (2H, dd, $J=5.7$ Hz, 3.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ35.0, 67.8, 70.5, 126.8, 133.1, 133.4, 149.3, 179.2; MS(EI) *m*/*z*: 336 (M+), 76 (100). Anal. calcd for $C_{16}H_{16}O_4S_2$: C, 57.12; H, 4.79. Found C, 57.32; H,4.56.

2,3,5,6,8,9,11,12 - Octahydronaphtho[2,3 - *k*][1,4,7,10,13] trioxadithiacyclopentadecine - 14,19 - dine (3c). As red solid (52% yield). Mp: 95–97 °C; IR (KBr) ν_{max}: 1683, 1655 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.08 (4H, t, $J=4.2$ Hz), 3.38 (4H, t, $J=4.2$ Hz), 3.46 (4H, t, $J=4.8$ Hz), 3.71 (4H, t, $J=4.8$ Hz), 7.63 (2H, dd, $J=5.7$ Hz, 3.3 Hz), 7.95 (2H, dd, $J=5.7$ Hz, 3.3 Hz): ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.2, 70.1, 70.2, 71.7, 126.4, 132.8, 133.9, 146.9, 179.5; MS(EI) *m*/*z*: 380 (M+), 104 (100%). Anal. calcd for $C_{18}H_{20}O_5S_2$: C, 56.82; H, 5.30. Found: C, 57.02; H, 5.42.

2,3,5,6,8,9,11,12,14,15-Decahydronaphtho[2,3-*n*] [1,4,7,10,13,16] tetraoxadithiacyclooctadecine-17,22-dione (3d). As red solid (38% yield). Mp: 80–82 °C; IR (KBr) ν_{max} 1655 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.38 (4H, s), 3.53 (12H, m), 3.71 (4H, t, $J=6.0$ Hz), 7.65 (2H, dd, $J=5.8$ Hz, 3.2 Hz), 8.0 (2H, dd, $J=5.8$ Hz, 3.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.1, 70.3, 70.4, 70.7, 71.0, 126.7, 133.2, 133.3, 147.6, 179.0; MS(EI) *m*/*z*: 424 (M+), 104 (100%). Anal. calcd for $C_{20}H_{24}O_6S_2$: C, 56.58; H, 5.70. Found: C, 56.70; H, 5.48.

2,3,5,6,8,9,11,12,14,15,17,18-Dodecahydronaphtho[2,3-*q*] [1,4,7,10,13,16,19] penta-oxadithiac-yclohenicosine-20,25-dione (3e). As red solid (39% yield). Mp: 59–61 °C; IR(KBr) ν_{max}: 1659 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.49 (20H, m), 3.72 (4H, t, $J=5.8$ Hz), 7.65 (2H, dd, $J=5.7$ Hz, 3.3 Hz), 8.01 (2H, dd, $J=5.7$ Hz, 3.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.4, 69.6, 70.3, 70.5, 70.6, 71.0, 126.8, 133.1, 133.3, 147.8, 178.9; MS(EI) *m*/*z*: 468 (M+), 104 (100%). Anal. calcd for $C_{22}H_{28}O_7S_2$: C, 56.39; H, 6.02. Found: C, 56.17; H, 6.34

General procedure for preparing bis-naphthoquinone thiol ethers 6a–d

A mixture of 1,4-naphthoquinone (**4**) (12 mmol) and ββ′-dimercaptodiethyl ether (3 mmol) in EtOH (100 mL) was stirred at room temperature for 48 h. The resulting brown precipitates were filtered off and washed well with EtOH. The crude brown precipitates were purified by re-crystallization.

2-[(2 - {2 - [(1,4 - Dioxo - 1,4 - dihydronaphthalen-2-yl)thio]-ethoxy}ethyl)thio]naphthoquin one (6a). As brown red solid (92% yield). Mp 202–203 °C (DMF); ^1H NMR

(500 MHz, $CDCl_3$) δ 3.06 (4H, t, $J=6.2$ Hz) 3.81 (4H, t, $J=6.2$ Hz), 6.66 (2H, s), 7.71 (4H, m), 8.07 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.4, 68.3, 126.5, 126.9, 127.3, 131.8, 132.1, 134.3, 135.2, 154.4, 181.5, 182.7; IR(KBr) ν_{max}: 1666 cm^{-1}; MS(EI) *m/z*: 450 (M+), 217, 55 (100%). Anal. calcd for $C_{24}H_{18}O_5S_2$: C, 63.98; H, 4.03. Found: C, 63.78; H, 4.30.

2-{[2-(2-{2-[(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)thio]-ethoxy}ethoxy)ethyl]thio}naphthoqui-none (6b). As brown red solid (94% yield). Mp: 178–179 °C (DMF); IR(KBr) ν_{max}: 1670, 1640 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.06 (4H, t, $J=6.2$ Hz), 3.66 (4H, s), 3.81 (4H, t, $J=6.2$ Hz), 6.67 (2H, s), 7.68 (4H, m), 8.04 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.6, 68.4, 70.7, 126.5, 126.8, 127.2, 131.9, 132.2, 133.2, 134.3, 154.7, 181.6, 182.0; MS(EI) *m/z*: 494 (M+), 129, 55 (100%). Anal. calcd for $C_{26}H_{22}O_6S_2$: C, 63.14; H, 4.48. Found: C, 63.02; H,4.41.

2-({2-[2-(2-{2-[(1,4-Dioxo-1,4-dihydronaphthalen - 2 - yl)-thio]ethoxy}ethoxy)ethoxy]ethyl}thio) - naphthoquinone (6c). As brown red soild (82% yield). Mp: 114–6 °C (DMF); IR(KBr) ν_{max}: 1664, 1647 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.04 (4H, t, $J=6.2$ Hz), 3.65 (8H, s), 3.79 (4H, t, $J=6.2$ Hz), 6.64 (2H, s), 7.67 (4H, m), 8.03 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.6, 68.2, 70.7, 70.8, 126.5, 126.8, 127.2, 131.8, 132.1, 133.2, 134.3, 154.7, 181.5, 182.0; MS(FAB) *m/z*: 539.1 [M+1]. Anal. calcd for $C_{30}H_{30}O_8S_2$: C, 61.84; H, 5.19. Found: C, 61.66; H, 4.88.

2-({14-[(1,4-Dioxo - 1,4 - dihydronaphthalen - 2 - yl)thio]-3,6,9,12 - tetraoxatetradec - 1 - yl}thio)naphthoquinone (6d). As brown red solid (62% yield). Mp: 60–61 °C (DMF); IR(KBr) ν_{max}: 1664, 1647 cm^{-1}; ^1H NMR: δ 3.04 (4H, t, $J=6.4$ Hz), 3.63 (12H, s), 3.78 (4H, t, $J=6.4$ Hz), 6.64 (2H, s), 7.67 (4H, m), 8.04 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.5, 68.1, 76.7, 77.0, 77.3, 126.5, 126.8, 127.1, 131.8, 132.1, 133.2, 134.3, 154.7, 181.5, 182.0; MS(FAB) *m/z*: 583 [M+1]. Anal. calcd for $C_{30}H_{30}O_8S_2$: C, 61.84; H, 5.19. Found: C, 61.60; H, 5.40.

2-({17-[(1,4-Dioxo - 1,4 - dihydronaphthalen - 2 - yl)thio]-3,6,9,12,15 - pentaoxaheptadec - 1 - yl}thio)- naphthoquinone (6e). As brown red soild (66% yield). Mp: 56–57 °C (DMF); IR(KBr) ν_{max}: 1670, 1641 cm^{-1}; ^1H NMR: δ 3.04 (4H, t, $J=6.4$ Hz), 3.63 (16H, s), 3.79 (4H, t, $J=6.4$ Hz), 6.65 (2H, s), 7.68 (4H, m), 8.05 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.5, 68.2, 70.7, 76.5, 77.0, 77.3, 126.5, 126.8, 127.1, 131.9, 132.2, 133.3, 134.3, 154.7, 181.5, 182.0; MS(FAB) *m/z*: 627.1 [M+1]. Anal. calcd for $C_{32}H_{34}O_9S_2$: C, 61.32; H, 5.47. Found: C, 61.14; H, 5.28.

General procedure for preparing bis-naphthoquinone thiol-crown ethers 7a–d

A mixture of bis-naphthoquinone thiol ethers (1 mmol) and β,β′-dimercaptodiethyl ether (1 mmol) in DMF (25 mL) was stirred under positive pressure of oxygen. After 72 h, the reaction mixture was pour into cold water (150 mL) and stirred for additional 2 h. The resulting precipitate was filtered off and washed well with water. The crude product was purified by re-crystallization or silica gel chromatography.

7,8,10,11,20,21,23,24 - Octahydrodinaphtho[2,3-*e*:2′,3′-*n*][1,10,4,7,13,16] dioxatetrathiacyclooctadecine-5,13,18,26-tetrone (7a). As yellow solid (82% yield). Mp 228–229 °C (DMF) IR(KBr) ν_{max}: 2928, 2850, 1679, 1658 cm^{-1}; ^1H NMR: δ 3.24 (8H, t, $J=6.0$ Hz), 3.61 (8H, t, $J=6.0$ Hz), 7.62 (4H, dd, $J=5.8$, 3.3 Hz), 7.98 (4H, dd, $J=5.8$, 3.3 Hz m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.1, 71.1, 126.8, 133.0, 133.4, 148.0, 178.8; MS(FAB) *m/z*: Calc. 584.4. Found 585.0. Anal. calcd for $C_{28}H_{24}O_6S_4$: C, 57.12; H, 4.14. Found: C, 57.41; H, 4.16.

7,8,10,11,13,14,23,24,26,27,29,30 - Dodecahydrodinaphtho - [2,3-*h*:2′,3′-*t*] [1,4,13,16,7,10,19,22]- tetraoxatetrathiacyclo-tetracosine-5,16,21,32-tetrone (7b). As yellow solid (80% yield). Mp 176–177 °C (EtOH); IR(KBr) ν_{max}: 2926, 2849, 1670, 1653, 1590 cm^{-1}; ^1H NMR: δ 3.30 (16H, m), 3.64 (8H, t, $J=5.0$ Hz), 7.65 (4H, dd, $J=5.8$, 3.3 Hz), 8.04 (4H, dd, $J=5.8$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.9, 70.5, 76.8, 126.8, 133.1, 133.3, 149.0,179.2; MS(FAB) *m/z*: calcd 672.9. Found 673.1. Anal. calcd for $C_{32}H_{32}O_8S_4$: C, 57.12; H, 4.79. Found: C, 57.03; H, 4.70.

7,8,10,11,13,14,16,17,26,27,29,30,32,33,35,36 - Hexadeca-hydrodinaphtho[2,3-*k*:2′,3′-*z*] [1,4,7,16,19,22,10,13, 25,28] hexaoxatetrathiacyclotriacontine-5,19,24,38-tetrone (7c). As yellow solid (78% yield). Mp 113–114 °C (EtOH–water); IR(KBr) ν_{max}: 2927, 2847,1670, 1653, 1590 cm^{-1}; ^1H NMR: δ 3.09 (8H, t, $J=4.8$ Hz), 3.43 (16H, m), 3.71 (8H, t, $J=4.8$ Hz) 7.64 (4H, dd, $J=5.8$, 3.3 Hz), 7.95 (4H, dd, $J=5.8$, 3.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 33.7, 70.2, 70.7, 71.7, 126.4, 132.8, 133.9, 146.9, 179.5; MS(FAB) *m/z*: calcd 761.0 found 761.1. Anal. calcd for $C_{36}H_{40}O_{10}S_4$: C, 56.82; H, 5.30. Found: C, 56.87; H, 5.29.

7,8,10,11,13,14,16,17,19,20,29,30,32,33,35,36,38,39,41,42-Icosahydrodinaphtho[2,3-*n*:2′,3′-f_1] [1,4,7,10,19,22,25,28, 13,16,31,34] octaoxatetrathiacyclohexatriacontine-5,22, 27,44-tetrone (7d). As yellow solid (25% yield). Mp 79–82 °C (column purification); IR(KBr) ν_{max}: 2937, 2851, 1656, 1630, 1591 cm^{-1}; ^1H NMR: δ 3.47 (32H, m), 3.71 (8H, t, $J=4.8$ Hz) 7.64 (4H, dd, $J=5.8$, 3.3 Hz), 8.01 (4H, dd, $J=5.8$, 3.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ35.0, 71.3, 71.4, 71.6, 77.9, 127.6, 134.1, 134.2, 148.5, 180.0; MS(FAB) *m/z*: cald 849.1. Found 849.0.

7,8,10,11,13,14,16,17,19,20,22,23,32,33,35,36,38,39,41,-42,45,47,48 - Tricosahydrodinaphtho[2,3 - *q*:2′,3′ - l_1] [1,4,7, 10,13,22,25,28,31,34,16,19,37,40] decaoxatetrathiacyclo-dotetracontine-5,25,30,50-tetrone (7d). As yellow solid (31% yield). Mp 57–55 °C (EtOH–water); IR(KBr) ν_{max}: 2942, 2870, 1659, 1592 cm^{-1}; ^1H NMR: δ 3.51 (40H, m), 3.73 (8H, t, $J=5.7$ Hz) 7.67 (4H, dd, $J=5.8$, 3.3 Hz), 8.01 (4H, dd, $J=5.8$, 3.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.5, 70.5, 70.7, 70.8, 70.9, 80.0, 126.8, 133.2, 133.3, 147.8, 178.9; MS(FAB) *m/z*: cald 937.2. Found 937.1.

General procedure for minimum inhibitory concentration (MICs)

The MICs of **3a**–**e** and **7a**–**e** were determined in microtiter plates and capped test tube for bacteria and fungi, respectively. A 2-fold serial dilution of tested compound in Mueller–Hinton broth or yeast nitrogen base with glucose (YNBG) broth was added to the test organism. The microtiter plates were incubated for 16–20 h[8] and the capped test tube the at 37 °C for 48 h.[9] The tested bacteria were grown in Muller–Hinton broth for 4 h and diluted to an OD_{600} of 0.1. Microtiter plates were covered with a sterile lid and incubated at 37 °C for 18–20 h. The MICs were determined by examine the wells were for bacterial growth at OD_{600}. The MICs for fungi were determined after 48 h incubation. The capped tubes were examined for fungal growth at OD_{600}. Growth was presented in the medium control and absent from the inoculum control.[8] The MIC was defined as the lowest drug concentration of the agent that prevents growth of test organism.

General procedure for the human cell lines cultured

The cell lines CT-5′ (human oral epidermoid carcinoma), Hep-2 (human larynx epidermal carcinoma) and BC-M1 (human breast adenocarcinoma) were cultured in the RPMI 1640 medium with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 25 mM Hepes, pencillin-streptomycine and fungizone. All the medium and supplements were purchased from Gibico Laboratories (Grand Island, NY, USA). All cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Cell cultures were subcultured once or twice weekly using trypsin-EDTA to detach the cell from their culture flask. Numbers of cells were counted after trypsinization by a Neubauer hemocytometer (VWR, Scientific Corp. Philadelphia, PA, USA)

General procedure for cytotoxicity assay (MTT assay)

The MTT assay was according the method of Brusselbach et al.[10] One day before drug application cells were seeded in 96-well flat-bottomed microtiter plates (3000–5000 cells/well). Cells were incubated for 24 h with drugs, applied as serial 1:2 dilutions (100 μL/well) ranging from 20 μM down to 2 μM. Twenty microliters of MTT (5 mg/mL) were added to each well and incubated for 4 h at 37 °C. The formazan product was dissolved by adding 100 μL DMSO to each well, and the plates were read at 550 nm. All measurements were performed in triplicate and each experiment was repeated at least three times. The LD_{50} was calculated from the 50% formazan formation compared with control without drugs addition.

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References and Notes

1. O'Brien, P. J. *Chem. Biol. Interact*. **1991**, *80*, 1.
2. Shinkai, S. In *Bioorganic Chemistry Frontiers*; Dugas, H., Ed.; Springer: Berlin, 1990; p 162.
3. Sugihara, K.; Kamiya, H.; Yamaguchi, M.; Kaneda, T.; Misumi, S. *Tetrahedron Lett*. **1981**, *22*, 1619.
4. Chang, E. E.; Cheng, H. H.; Kai, J. E.; Kuo, H. S. *Chinese Pharm. J*. **1995**, *47*, 531.
5. Martin, D. J.; Greco, C. C. *J. Org. Chem*. **1968**, *33*, 1275.
6. Kallmayer, H. J. *Arch. Pharmaz*. **1974**, *37*, 806.
7. Mulligan, M. E.; Murray-Leisure, K. A.; Ribner, B. S.; Standiford, H. C.; John, J. F.; Korvicj, J. A.; Kauffman, C. A.; Yu, V. L. *Am. J. Med*. **1993**, *94*, 313.
8. Phillips, I.; Willians, J. D.; Wise, R. In *Laboratory Methods in Antimicrobial Chemotherapy*; Garrod, L., Ed.; Churchill Livingston: Edinburgh, 1978; p 3.
9. Warnock, D. W. In *Medical Mycology: A Practical Approach*; Evans, E. G. V., Richardson, M. D., Eds.; Oxford University: New York, 1989; p 235.
10. Brusselbach, S.; Nettelbeck, D. M.; Sedlacek, H. H.; Muller, R. *Int. J. Cancer* **1988**, *77*, 146.