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Bromophenols as *Candida albicans* isocitrate lyase inhibitors

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ABSTRACT

A new series of bromophenols was synthesized by reactions of corresponding phenol analogs with bromine. The synthesized compounds were tested for inhibitory activity against isocitrate lyase (ICL) of *Candida albicans* and antimicrobial activity against Gram-positive and, Gram-negative bacteria and fungi. Among the synthesized bromophenols, bis(3-bromo-4,5-dihydroxyphenyl)methanone (**11**) and (3-bromo-4,5-dihydroxyphenyl)(2,3-dibromo-4,5-dihydroxyphenyl)methanone (**12**) displayed potent inhibitory activities against ICL, showing a stronger inhibitory effects than were found with natural bromophenol **1**. The preliminary structure–activity relationships were investigated in order to determine the essential structural requirements for the inhibitory activities of these compounds against ICL of *C. albicans*.

The glyoxylate cycle serves to bypass the CO_2-generating steps of the tricarboxylic acid (TCA) cycle and facilitates the net assimilation of carbon from C_2 compounds, allowing microorganisms to replenish the pool of TCA cycle intermediates necessary for gluconeogenesis and other biosynthetic processes.[1] Enzymes unique to this route include isocitrate lyase (ICL) and malate synthase (MS). The glyoxylate cycle is widespread and well documented in archaea, bacteria, protists, plants, fungi, and nematodes. Genetic regulation of the glyoxylate cycle during bacterial growth on acetate has been reviewed and in the last several years it has become evident that this pathway is important in fungal and bacterial pathogenesis.[2,3]

The role of the glyoxylate cycle in microbial virulence has been reported in many pathogens including *Candida albicans*. The genes of the glyoxylate cycle are strongly induced when *C. albicans* is phagocytosed by macrophage.[3] The inside environment of phagolysosome, abundant in carbon sources such as fatty acids or their breakdown products allows *C. albicans* to utilize the enzymes of the glyoxylate cycle and permits the use of C_2 carbon sources. The *C. albicans* mutant strain lacking the glyoxylate cycle enzyme ICL is markedly less virulent in a mouse model of systemic candidiasis and less persistent in internal organs than the wild-type strain.[3,4] Since expression of glyoxylate cycle genes is detected during specific stages of the interaction between host and pathogen in a variety of human-pathogenic bacteria and fungi, the development of specific inhibitors against ICL and MS is an attractive prospect.

Several inhibitors of ICL have been identified, including 3-nitropropionate,[5] 3-bromopyruvate,[6] 3-phosphoglycerate,[7] mycenon,[8] oxalate,[9] and itaconate.[9] However, these inhibitor are not pharmacologically suitable for testing in vivo because of their toxicity and low activity. The development of selective ICL inhibitors with suitable pharmacological properties would open the door to testing in animal models to explore the role of the glyoxylate cycle in disease and serve as proof of concept experiments to evaluate the therapeutic potential of ICL inhibition. As a result of our efforts to discover ICL inhibitors, we recently reported on the isolation and evaluation of ICL inhibitory activities of sesterterpene sulfates[10] from marine sponges.

In the course of our search for novel natural products with potent antimicrobial activities from marine sources, we identified bromophenols **1** from red alga *Odonthalia corymbifera* that showed promising antibacterial activities.[11] Further experiments demonstrated that compound **1** exhibited potent isocitrate lyase (ICL) inhibitory activity and protected rice plants from rice blast fungus *Magnaporthe grisea* infection, whereas a synthetic bromophenol **2** showed antibacterial effects against Gram-negative and Gram-positive bacteria.[12] Furthermore we previously reported preliminary structure–activity relationships associated with halogenated derivatives of bis(3,4-dihydroxydiphenyl)methane as a novel class of antimicrobial agents (Fig. 1).[12,13]

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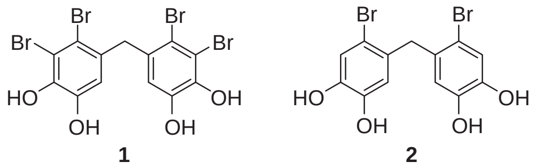


Figure 1. Bioactive bromophenols.

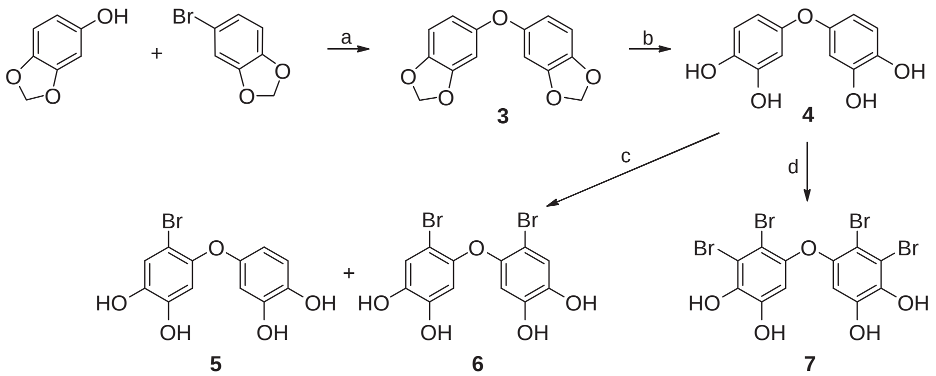
To understand more about the activities of bromophenols, derivatives of compound **1** were prepared and their antimicrobial activities were examined. The promising results of experiments with certain derivatives prompted us to further investigate these compounds. A variety of bis-bromophenols with different linkers such as oxygen, a carbonyl group, ethylene, or oxybis(methylene) were prepared. Most derivatives were synthesized in the lab by a simple halogenation process as a mixture of multiple products with different degrees of substitutions, which were then separated using HPLC techniques.

The synthesis of bis(2,3-dibromo-4,5-dihydroxyphenyl) ether (**7**) via compound **4**, which has an electron-donating ether linkage between two phenols, is illustrated in Scheme 1. Compound **3** was obtained by the sequential treatment of sesamol and 4-bromo-1,2-(methylenedioxy)benzene in the presence of cesium carbonate, copper bromide, and 2-oxocyclohexane carboxylate for a 62% yield.[14] Subsequently, deprotection of the two methylene groups in **3** with BBr_3 yielded 90% 4,4′-oxydibenzene-1,2-diol (**4**).[15] The treatment of phenolic compound **4** with excess bromine in acetic acid resulted in a 67% conversion to bromophenol **7**.[15] Meanwhile, (2-bromo-4,5-dihydroxyphenyl)(3,4-dihydroxyphenyl) ether (**5**) and bis(2-bromo-4,5-dihydroxyphenyl) ether (**6**) were also prepared from the reaction[15] of compound **4** with 2 equiv of bromine in acetic acid: CH_2Cl_2 (1:1) yielding 20% and 56% yields, respectively.

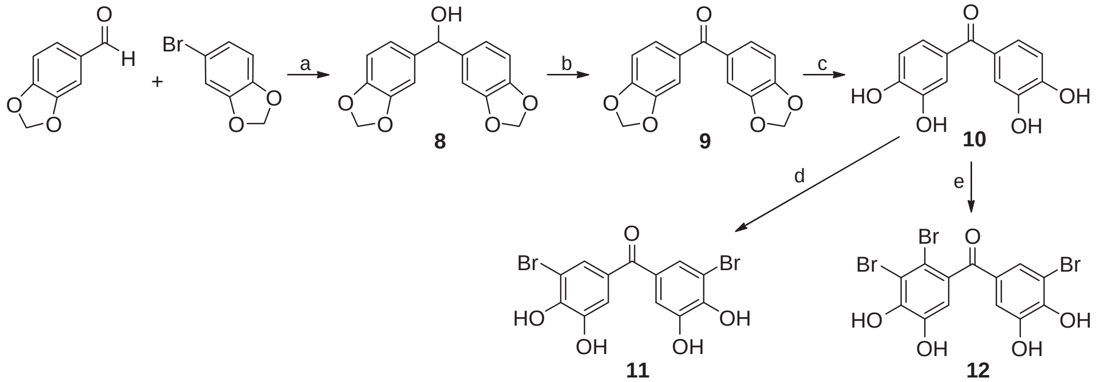
The second set of bromophenols, **11** and **12**, bearing a carbonyl group between two phenyl rings, was prepared following a sequence of reactions similar to the first one described. The treatment of 4-bromo-1,2-(methylenedioxy)benzene with the mixture of *n*-butyllithium and piperonal afforded a 64% yield of compound **8**, which was reacted with PDC to form dibenzo[*d*][1,3]dioxol-5-ylmethanone (**9**) with a 95% yield. Bis(3,4-dihydroxyphenyl)methanone (**10**)[16] was prepared from compound **9** by deprotection and then treated with 2 equiv bromine furnished in a 50% of bis(3-bromo-4,5-dihydroxyphenyl)methanone (**11**). And the reaction of compound **10** with excess bromine yielded 47% of (3-bromo-4,5-dihydroxyphenyl)(2,3-dibromo-4,5-dihydroxyphenyl)methanone (**12**)[16] (Scheme 2).

The synthesis of a third set, bromophenol **16** and **17**, with an ethylene between two phenyl rings, involved subjecting piperonal to a mixture of zinc and $TiCl_4$ in tetrahydrofuran to obtain coupling compound **13** with a 53% yield.[17] Compound **13** was reduced under hydrogen in the presence of Pd/C to give compound **14** with an 81% yield. When compound **14** was subjected to the same reaction sequence (deprotection and bromination) used for the synthesis of compound **12**, the corresponding compounds of 2,2′-dibromo-4,4′,5,5′-tetrahydroxybibenzyl (**16**) and 2,2′,3-tribromo-4,4′,5,5′-tetrahydroxybibenzyl (**17**) were prepared[18] via compound **15**[19] (Scheme 3).

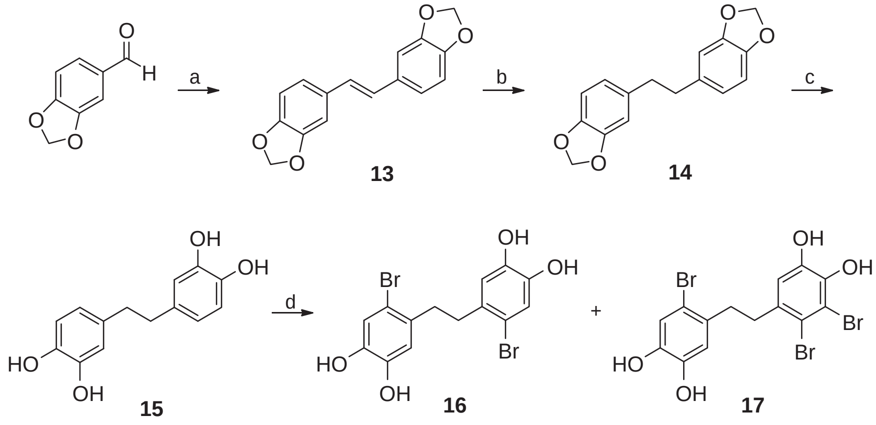
The final set of bromophenols, **20** and **21**, was prepared following a reaction sequence similar to the first one. Compound **18** was prepared by the treatment of 5-(bromomethyl)benzo[*d*][1,3]diox-



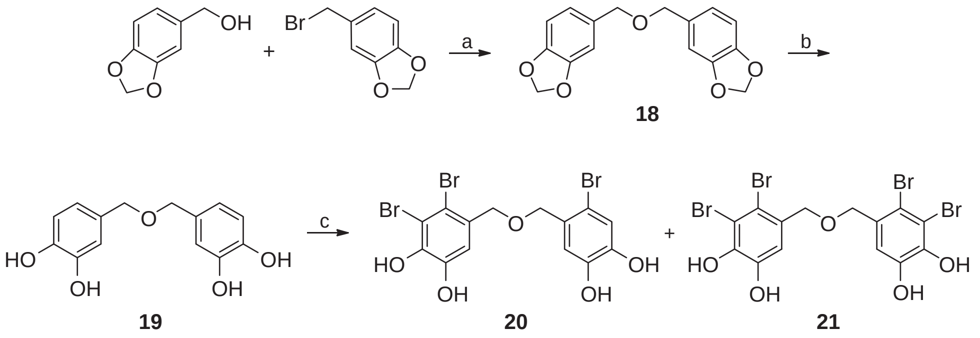
Scheme 1. Reagents and conditions: (a) CuBr, Cs_2CO_3, oxocyclohexane carboxylate, DMSO; (b) BBr_3, CH_2Cl_2, rt; (c) Br_2 (2 equiv), CH_2Cl_2 + AcOH, rt; (d) excess Br_2, AcOH, 50 °C.



Scheme 2. Reagents and conditions: (a) *n*-BuLi, THF, rt; (b) PDC, CH_2Cl_2, rt; (c) BBr_3, CH_2Cl_2, rt; (d) excess Br_2, AcOH, rt; (e) excess Br_2, AcOH, 50 °C.



Scheme 3. Reagents and conditions: (a) $TiCl_4$, Zn, THF, reflux; (b) H_2, Pd/C, EtOAc/EtOH; (c) BBr_3, CH_2Cl_2, rt; (d) excess Br_2, CH_2Cl_2 + AcOH, rt.



Scheme 4. Reagents and conditions: (a) NaH, THF, rt; (b) BBr_3, CH_2Cl_2, rt; (c) excess Br_2, AcOH, rt.

ole in the presence of piperonyl alcohol and NaH in THF. The next deprotection and bromination reactions, as used previously in Scheme 1, gave the corresponding bromophenols, (2-bromo-4,5-dihydroxybenzyl)(2,3-dibromo-4,5-dihydroxybenzyl) ether (**20**) and bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (**21**) via compound **19**[20] (Scheme 4). Compound **21**, which had been reported as a natural product from red alga *O. corymbifera*,[21] was synthesized for the first time.

The cloning, expression, and purification of ICL from the genomic DNA of *C. albicans* (ATCC 10231) were carried out as described previously.[9] The eleven bromophenols **1**, **2**, **5**–**7**, **11**, **12**, **16**, **17**, **20**, **21** and four bisphenols **4**, **10**, **15**, **19** were evaluated for their inhibitory activities against ICL of *C. albicans* according to a previously documented procedure.[9,22,23] The inhibitory potencies of the tested compounds, expressed as IC_{50} values, are shown in Table 1 and are compared to that of a known ICL inhibitor, 3-nitropropinate.[24] As expected, the synthesized bromophenols did significantly affect *C. albicans* ICL. The most active compound was compound **12** (IC_{50}: 2.65 μM) possessing an activity nearly 20 times stronger than 3-nitropropinate (IC_{50}: 50.7 μM). Interestingly, the introduction of a carbonyl group between two phenols resulted in compounds **11** and **12** which displayed nearly 2–7 times higher activity than the natural compound **1** (IC_{50}: 18.44 μM). The reason for this increased activity is not clear at this moment. It might be due to the changes in electron density of the phenyl ring caused by the electron-withdrawing properties of carbonyl groups, or it could be due to formation of an additional binding site between the carbonyl group of the compounds and enzyme. In contrast, compounds **5**–**7**, bearing an ether linkage between two phenols, were found to be similar or less active (IC_{50}: 11.63–68.19 μM) as compared to **1**. The effects of the substitution of an ethylene or an oxybis(methylene) group instead of methylene in bromophenols were also investigated. However, the lengths of linkers had little effect on *C. albicans* ICL inhibition activities.

Table 1
Inhibitory effect of synthetic compounds on the activity of ICL enzyme

Compound	ICL IC_{50} (μM) (μg/ml)
1	18.44 (10.1)
2	46.15 (18.0)
4	>100 (61.23)
5	68.19 (21.35)
6	30.88 (12.10)
7	11.63 (6.39)
10	22.58 (5.56)
11	8.89 (3.57)
12	2.65 (1.27)
15	58.47 (14.40)
16	21.21 (8.57)
17	14.77 (7.13)
19	(>100)
20	15.61 (7.74)
21	28.06 (16.1)
3-Nitropropionate[a]	50.70 (6.0)
Amphotericin B[b]	ND[b]

[a] 3-Nitropropinate and amphotericin B inhibitors of ICL and fungus, respectively, were used as positive controls.
[b] ND, not determined.

In the current series, an increase of the number of Br atom in bromphenols resulted in an increase in activity. Concerning the number of bromines, compounds **1**, **7**, **11**, **12**, **17**, **20**, and **21**, bearing three or four bromines, were more active than the others. The reason for this increase in activity remains unclear. To our surprise,

Table 2
Antibacterial activity

Compound	MIC (μg/ml)				
	S. aureus[a]	*B. subtilis*[a]	*M. luteus*[a]	*P. vulgaris*[a]	*S. typhimurium*[a]
1[b]	25	25	25	50	50
2[c]	25	25	12.5	25	25
4	50	>100	100	>100	100
5	100	>100	100	>100	100
6	100	100	50	100	100
7	50	25	50	25	50
10	>100	>100	>100	>100	>100
11	>100	100	100	>100	>100
12	100	50	50	100	100
15	50	100	100	100	100
16	50	50	50	50	50
17	100	50	100	100	100
19	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100
Ampicillin	1.56	1.56	1.56	3.12	3.12

[a] Microorganisms: *Staphylococcus aureus* ATCC 6538p; *Bacillus subtilis* ATCC 6633; *Micrococcus luteus* IFO 12708; *Proteus vulgaris* ATCC 3851; *Salmonella typhimurium* ATCC 14028.

[b] Published results.[13]

[c] Published results.[12]

compounds with no halogen substituents (**10**, and **15**) also showed potent ICL inhibition activities. In particular, compound **10,** bearing carbonyl groups, was more effective than 3-nitropropionate, with an IC_{50} value of 5.56 μg/mL. Activities obtained with compound **10** can be regarded as more interesting results considering that the brominated derivative **12** might cause possible concerns regarding toxicities.

The in vitro antimicrobial activities of the nine bromophenols **5**–**7**, **11**, **12**, **16**, **17**, **20**, **21** and four bisphenols **4**, **10**, **15**, **19** were assessed against three representative Gram-positive bacteria including *Staphylococcus aureus* (ATCC 6538p), *Bacillus subtilis* (ATCC 6633), and *Micrococcus luteus* (IFO 12708), two Gram-negative bacteria, *Proteus vulgaris* (ATCC 3851) and *Salmonella typhimurium* (ATCC 14028), and four fungi, *C. albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC6094), *Trichophyton rubrum* (IFO 9185), and *T. mentagrophytes* (IFO 40996).[25,26]

The minimum inhibitory concentrations (MIC) of the compounds are displayed in Tables 2 and 3. Among the bromophenols tested, compounds **7** and **16** exhibited only weak inhibitory activities against Gram-positive and Gram-negative bacteria with minimum inhibitory concentration (MIC) values in the range of 25–50 μg/ml, as shown in comparison to ampicillin. In an antifungal activity assay using medically important pathogenic fungi, most of the synthesized bromophenols, except compound **7** and **16**, were inactive at 100 μg/ml. In our previous Letters,[12,13] natural bromophenol **1** exhibited inhibitory activity against *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes* with MIC values in the range of 1.56–12.5 μg/ml. These results revealed that the methylene group in bromophenol was important for antimicrobial activity.

Table 3
Antifungal activity

Compound	MIC (μg/ml)			
	C. albicans[a]	*A. fumigatus*[a]	*T. rubrum*[a]	*T. mentagrophytes*[a]
1[b]	1.56	0.78	1.56	1.56
2[c]	>100	>100	>100	50
4	>100	>100	>100	>100
5	>100	>100	>100	>100
6	>100	>100	>100	>100
7	25	25	12.5	25
10	>100	>100	>100	>100
11	>100	>100	>100	>100
12	100	>100	>100	>100
15	>100	>100	>100	100
16	50	50	50	100
17	100	>100	>100	>100
19	>100	>100	>100	>100
20	>100	>100	>100	>100
21	>100	>100	>100	>100
Amphotericin B	6.25	3.12	3.12	3.12

[a] Microorganisms: *Candida albicans* ATCC 10231; *Aspergillus fumigatus* HIC6094; *Trichophyton rubrum* IFO 9185; *Trichophyton mentagrophytes* IFO 40996.

[b] Published results.[13]

[c] Published results.[12]

In conclusion, a new series of bromophenols was synthesized and their inhibitory activities against isocitrate lyase of *C. albicans* were investigated. Among the synthesized bromophenols, (3-bromo-4,5-dihydroxyphenyl)(2,3-dibromo-4,5-dihydroxyphenyl)methanone (**12**) displayed a potent inhibitory activity against isocitrate lyase (ICL) of *C. albicans*, which showed a much stronger inhibitory effect than did natural bromophenol **1**. During the investigation of bromophenols, bis(3,4-dihydroxyphenyl)methanone (**10**), which showed very high activity despite its lack of halide substituents, was discovered as a new and promising lead compound for the development of potent ICL inhibitors. Investigations into this compound are already underway. Since the enzymes of the glyoxylate cycle are not found in mammals, the synthesized bromophenol compounds tested in this study are good starting candidates for ICL inhibitor design.

Acknowledgments

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References and notes

1. Dunn, M. F.; Ramirez-Trujillo, J. A.; Hernandez-Lucas, I. *Microbiology* **2009**, *155*, 3166.
2. Vereecke, D.; Cornelis, K.; Temmerman, W.; Jaziri, M.; Van Montagu, M.; Holsters, M.; Goethals, K. *J. Bacteriol.* **2002**, *184*, 1112.
3. Lorenz, M. C.; Fink, G. R. *Nature* **2001**, *412*, 83.
4. Goldstein, A. L.; McCusker, J. H. *Genetics* **2001**, *159*, 499.
5. McFadden, B. A.; Purohit, S. *J. Bacteriol.* **1977**, *131*, 136.
6. Ko, Y. H.; McFadden, B. A. *Arch. Biochem. Biophys.* **1990**, *278*, 373.

7. Ko, Y. H.; Vanni, P.; McFadden, B. A. *Arch. Biochem. Biophys.* **1989**, *274*, 155.
8. Hautzel, R.; Anke, H.; Sheldrik, W. S. *J. Antibiot.* **1990**, *43*, 1240.
9. Shin, D. S.; Kim, S.; Yang, H. C.; Oh, K. B. *J. Microbiol. Biotechnol.* **2005**, *15*, 652.
10. Lee, D.; Shin, J.; Yoon, K. M.; Kim, T. I.; Lee, S. H.; Lee, H. S.; Oh, K. B. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5377.
11. Lee, H.-S.; Lee, T.-H.; Lee, J. H.; Chae, C.-S.; Chung, S.-C.; Shin, D.-S.; Shin, J.; Oh, K.-B. *J. Argic. Food Chem.* **2007**, *55*, 6923.
12. Oh, K.-B.; Lee, J. H.; Lee, J. W.; Yoon, K.-M.; Chung, S.-C.; Jeon, H. B.; Shin, J.; Lee, H.-S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 945.
13. Oh, K.-B.; Lee, J. H.; Chung, S.-C.; Shin, J.; Shin, H. J.; Kim, H.-K.; Lee, H.-S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 104.
14. Lv, X.; Bao, W. *J. Org. Chem.* **2007**, *72*, 3863.
15. *Experimental*: The 1D and 2D NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a Varian UNITY 500 spectrometer in chloroform-*d*, methanol-d_4 and acetone-d_6 with solvent peaks as references. Mass spectra were recorded on a ThermoFinnigan Surveyor MSQ spectrometer. Column chromatography was performed with RP-18 reversed-phase silica gel (43–60 μm).

    General procedure: A mixture of 4,4′-oxydibenzene-1,2-diol (**4**) (26 mg, 0.11 mmol) of and bromine (12 μl, 0.23 mmol) in a mixture of acetic acid and dichloromethane (2 ml, 1:1) was stirred at room temperature for 1 h. The excess of bromine was removed by blowing with N_2, and the solvent was evaporated under reduced pressure. The crude products were purified by reversed-phase HPLC (YMC ODS-A column, 1 cm × 25 cm, 45% aqueous CH_3OH) to give 6.9 mg (20%) and 24 mg (56%) of compounds **5** and **6**, respectively.

    4,4′-Oxydibenzene-1,2-diol (**4**): mp 124–126 °C; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.60 (2H, d, *J* = 8.4 Hz), 6.33 (2H, d, *J* = 2.4 Hz), 6.19 (2H, dd, *J* = 8.4, 2.4 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 152.4, 146.9, 141.8, 116.5, 110.4, 107.5; ESI (−) *m*/*z* 233.1 $(M-H)^-$.

    (2-Bromo-4,5-dihydroxyphenyl)(3,4-dihydroxyphenyl) ether (**5**): mp 93–95 °C (dec.); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.21 (1H, dd, *J* = 8.0, 2.6 Hz, H-6), 6.35 (1H, d, *J* = 2.6 Hz, H-6′), 6.43 (1H, s, H-3′), 6.67 (1H, d, *J* = 8.0 Hz, H-2′), 6.95 (1H, s, H-3); ^{13}C NMR (CD_3OD, 125 MHz) δ 103.5, 106.6, 109.2, 116.4, 119.9, 120.1, 141.9, 143.6, 146.9, 147.1, 148.2, 152.4; ESI (−) *m*/*z* 311.1 $(M-H)^-$.

    Bis(2-bromo-4,5-dihydroxyphenyl) ether (**6**): mp 146–149 °C (dec.); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.31 (1H, s, H-6, 6′), 6.96 (1H, s, H-3, 3′); ^{13}C NMR (CD_3OD, 125 MHz) δ 102.5, 107.9, 120.1, 143.5, 146.9, 148.1; ESI (−) *m*/*z* 390.9 $(M-H)^-$. Reacting 14 mg of **4** with 40 μl of bromine at 50 °C for 6 h yielded 22 mg (67%) of compound **7**.

    Bis(2,3-dibromo-4,5-dihydroxyphenyl) ether (**7**): mp 144–146 °C (dec.); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.37 (1H, s, H-6, 6′); ^{13}C NMR (CD_3OD, 125 MHz) δ 106.5, 106.8, 114.6, 142.3, 147.0, 148.5; ESI (−) *m*/*z* 548.9 $(M-H)^-$.
16. The reaction of compound **10** (25 mg, 0.10 mmol) with bromine (12 μl, 0.23 mmol) yielded compound **11** (20 mg, 50%).

    Bis(3,4-dihydroxyphenyl)methanone (**10**): mp 220–222 °C; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.28 (2H, d, *J* = 1.6), 7.20 (2H, dd, *J* = 8.4, 1.6 Hz) 6.88 (2H, d, *J* = 8.4); ^{13}C NMR (CD_3OD, 125 MHz) δ 197.4, 151.3, 146.1, 131.2, 124.8, 118.1, 115.6; ESI (−) *m*/*z* 245.1 $(M-H)^-$.

    Bis(3-bromo-4,5-dihydroxyphenyl)methanone (**11**): mp 183–185 °C (dec.); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.18 (2H, s, H-6, 6′), 7.38 (2H, s, H-2, 2′); ^{13}C NMR (CD_3OD, 125 MHz) δ 110.1, 116.6, 127.4, 130.9, 146.9, 149.1, 194.4; ESI (−) *m*/*z* 401.8 $(M-H)^-$.

    Reacting **10** (12 mg) with bromine (40 μl) yielded compound **12** (11 mg, 47%).

    (3-Bromo-4,5-dihydroxyphenyl)(2,3-dibromo-4,5-dihydroxyphenyl)methanone (**12**): ^{1}H NMR (CD_3OD, 500 MHz) δ 6.99 (1H, s, H-6′), 7.17 (1H, s, H-6), 7.36 (1H, s, H-2); ^{13}C NMR (CD_3OD, 125 MHz) δ 109.7, 110.4, 114.7, 116.5, 124.1, 128.1, 130.0, 134.2, 145.8, 146.9, 147.1, 150.4, 194.3; ESI (−) *m*/*z* 480.8 $(M-H)^-$.
17. Bringmann, G.; Pabst, T.; Henschel, P.; Michel, M. *Tetrahedron* **2001**, *57*, 1269.
18. The reaction of compound **15**[19] (21 mg, 85 μmol) with bromine (40 μl) yielded 5 mg (15%) and 9 mg (22%) of bromophenols **16** and **17**, respectively.

    2,2′-Dibromo-4,4′,5,5′-tetrahydroxybibenzyl (**16**): mp 205–206 °C (dec.); ^{1}H NMR (CD_3OD, 500 MHz) δ 2.86 (4H, m, H-7, H-7′), 6.61 (2H, s, H-6, H-6′), 6.91 (2H, s, H-3, H-3′); ^{13}C NMR (CD_3OD, 125 MHz) δ 146.0, 145.8, 132.8, 120.0, 118.1, 113.3, 37.2; ESI (−) *m*/*z* 402.9 $(M-H)^-$.

    2,2′,3-Tribromo-4,4′,5,5′-tetrahydroxybibenzyl (**17**): mp 220–225 °C (dec.); ^{1}H NMR (CD_3OD, 500 MHz) δ 2.86 (4H, m, H-7-7′), 6.73 (1H, s, H-6), 6.91 (1H, s, H-6′), 6.98 (1H, s, H-3′); ^{13}C NMR (CD_3OD, 125 MHz) δ 35.3, 38.2, 113.4, 113.8, 117.9, 118.9, 120.0, 131.7, 132.8, 145.8, 146.1; ESI (−) *m*/*z* 480.9 $(M-H)^-$.
19. Nishimura, N.; Kobayashi, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 6255.
20. The reaction of 26 mg (0.10 mmol) of **19** with bromine (40 μl) yielded 8 mg (16%) and 26 mg (45%) of bromophenols **20** and **21**,[21] respectively.

    Bis(3,4-dihydroxybenzyl) ether (**19**): ^{1}H NMR (CD_3OD, 500 MHz) δ 6.77–6.53 (m, 6H), 4.28 (s, 4H); ^{13}C NMR (CD_3OD, 125 MHz) δ 146.2, 146.1, 130.7, 121.1, 116.5, 116.1, 75.7; ESI (−) *m*/*z* 261.1 $(M-H)^-$.

    (2-Bromo-4,5-dihydroxybenzyl)(2,3-dibromo-4,5-dihydroxybenzyl) ether (**20**): mp 155–157 °C (dec.); ^{1}H NMR (CD_3OD, 500 MHz) δ 4.40 (4H, s, H-7, 7′), 6.95 (1H, s, H-6), 7.00 (1H, s, H-6′), 7.15 (1H, s, H-3′); ESI (−) *m*/*z* 495.8 $(M-H)^-$.
21. Kurihara, H.; Mitani, T.; Kawabata, J.; Takahashi, K. *J. Nat. Prod.* **1999**, *62*, 882.
22. Dixon, G. H.; Kornberg, H. L. *J. Biochem.* **1959**, *72*, 3P.
23. Hautzel, R.; Anke, H.; Sheldrick, W. S. *J. Antibiot.* **1990**, *43*, 1240.
24. Sharma, V.; Sharma, S.; Hoener zu Bentrup, K.; McKinney, J. D.; Russell, D. G.; Jacobs, W. R., Jr.; Sacchettini, J. C. *Nat. Struct. Biol.* **2000**, *7*, 663.
25. Three Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* IFO 12708) and two Gram-negative bacteria (*Proteus vulgaris* ATCC 3851 and *Salmonella typhimurium* ATCC) were used for antimicrobial activity tests. Bacteria were grown overnight in Luria Bertani (LB) broth at 37 °C, harvested by centrifugation, and then washed twice with sterile distilled water. Stock solutions of the series compound were prepared in DMSO. Each stock solution was diluted with Standard method broth (Difco) to prepare serial twofold dilutions in the range of 100–0.78 μg/ml. Ten microliters of the broth containing approximately 10^5 colony-forming units (cfu)/ml of test bacteria were added to each well of a 96-well microtiter plate. Culture plates were incubated for 24 h at 37 °C.
26. *Candida albicans* ATCC 10231, *Aspergillus fumigatus* HIC 6094, *Trichophyton rubrum* IFO 9185 and *Trichophyton mentagrophytes* IFO 40996 were used for antifungal activity tests. *C. albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2% peptone and 2% dextrose), harvested by centrifugation, and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco), incubated at 28 °C for 2 weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculum size of 10^5 spores/ml. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial twofold dilutions in the range of 100–0.8 μg/ml. Ten microliters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/ml of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for 48–72 h at 28 °C.