Silica gel Supported Boric tri-Sulfuric Anhydride (SiO_2-BTSA): an Efficient Catalytic System for the Synthesis of Phloroglucide analogs as Antimicrobial Agents

Rahele Bargebid[a], Ali Khalafi-Nezhad[b], Kamiar Zomorodian[c], Mahkameh Moradi[a], Soghra Khabnadideh[a*]

[a]*Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, I.R. Iran*

[b]*Department of Chemistry, College of Sciences, Shiraz University, Shiraz 71454, Iran*

[c]*Department of Parasitology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

Abstract

An efficient procedure for the synthesis of polyhydroxyl aromatic compounds (phloroglucide analogs) is described. In this procedure a reaction was done between different 4-substituted phenols and 2,6-bis(hydroxymethy) phenols. The reactions proceed in the presence of catalytic amount of silica gel supported boric tri-sulfuric anhydride (SiO_2-BTSA) in excellent yields. 16 compounds were synthesized ***(I_1-I_{16})***. Chemical structures of all compounds were confirmed by spectroscopic methods. We optimized the chemical reactions in the presence of different acidic catalysts, different solvents and also different temperatures. Catalytic amounts of SiO_2-BTSA in dichloroethane (DCE) was the best conditions. Some of the synthesized compounds were screened for their antimicrobial activities. Antifungal and antibacterial activities of the synthesized compounds were evaluated by broth micro dilution method as recommended by CLSI. Some of the tested compounds show good in vitro biological properties.

Keywords: Phloroglucide, Polyhydroxyl aromatic compounds, SiO_2-BTSA, Antifungal, Antibacterial.

Introduction

In recent years modification of already existing synthetic methodologies in industrial and academic research has received greater attention. One of the major areas of research is the development of heterogeneous catalysts for

*Corresponding author: Tel.: +98-71-32424127-8; Fax: +98-71-32424126; E-mail: khabns@sums.ac.ir, Orcid No: 0000 0003 1176 9143

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excellent chemical synthesis of materials (1). For example, applications of solid acids have been extensively studied and have been revealed to be highly efficient catalysts in organic synthesis (2). These catalysts possess some advantages including, easy application potential, decreased reactor and plant corrosion problems, and are more environmentally safe and available (3-5). Polyhydroxyl aromatic compounds are one of the most significant categories of compounds in medicine, cosmetics, paints, pesticides and dying (6). A vast array of activities such as anti-inflammatory, anticancer, anti-microbial, anti-allergic, enzyme inhibitory, neuro-regenerative, antioxidant have been exhibited by these compounds (7-10). The presence of essential functional groups (hydroxyl groups) in a suitable three-dimensional arrangement for chelation with metal ions of enzymes is a significant feature of many antibiotics. Hydroxy substitutions makes these compounds as potent antibacterials, since it provides a perfect situation to accommodate metal ions of enzymes (11). In view of the great importance of poly-hydroxyl aromatic compounds (phloroglucides) and in continuation of our previous work (12), we report here, silica supported boric tri-sulfuric anhydride (SiO_2-BTSA) as a novel, reusable and efficient catalyst for the synthesis of structurally diverse poly-hydroxyl aromatic compounds under a mild reaction condition. To evaluate the catalytic performance of this catalyst we checked some reactions as acid catalyzed processes. We also are going to screen our compounds in the point of antimicrobial activity.

Materials and methods

All chemicals were obtained from *Merck* and *Fluka* companies. All yields refer to isolated products after chromatography or other indicated purification methods. Infrared (IR) spectra were run on a *Shimadzu FTIR-8300* spectrophotometer; ν_{max} in cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra were run on a *Bruker Avanced DPX-250, FT-NMR* spectrometer in pure deuterated solvent (DMSO-d_6 and D_2O). Chemical shifts are given in the δ scale in part per million (ppm) and *J* in Hz. Mass spectra were determined on a Shimadzu GCMS-QP 1000 EX instruments at 70 or 20 ev. Microanalyses were performed on a *thermofinnigan flash EA1112-1CHNS.* Melting points were recorded on a *Büchi B 545* apparatus in open capillary tubes and all are uncorrected. The progress of reactions was followed with TLC using silica gel *SILG/UV 254* plates. Column chromatography was carried out on silica gel 60 Merck (230-240 mesh) in glass columns (2 or 3 cm diameter) using 15-30 grams of silica gel per one gram of the crude product. The eluent solvents were petroleum ether, ethyl acetate or mixtures of these. Solvents for chromatography were purified by distillation before use.

Preparation of Silica gel Supported Boric tri-Sulfuric Anhydride (SiO_2-BTSA)

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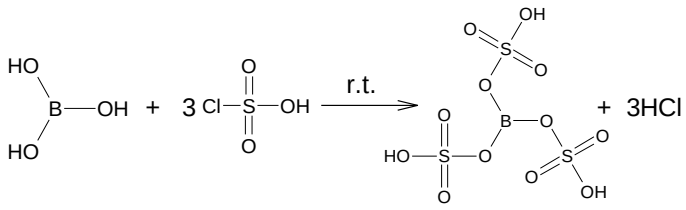
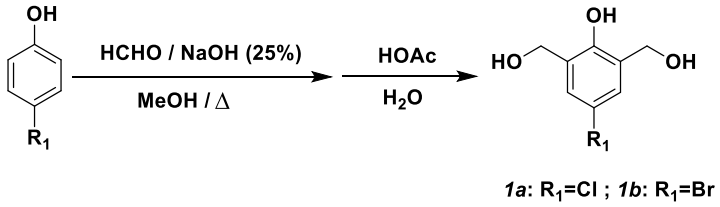


Scheme 1: Preparation of Boric tri-sulfuric anhydride

A 50 mL suction flask was equipped with a constant pressure dropping funnel. The gas outlet was connected to a vacuum system through an adsorbing solution i.e. water. Boric acid (1.55 g, 25 mmol) was charged in to the flask and chlorosulfonic acid (8.74 g, 5 mL, 75 mmol) was added dropwise over a period of 1 hr at room temperature; HCl evolved immediately. Then the reaction mixture was shaken for 1 h, while the residual HCl was eliminated by suction. After that, the mixture was washed with CH_2Cl_2 to remove the unreacted chlorosulfonic acid. A grayish solid material (Boric tri-sulfuric anhydride) $[B(OSO_3H)_3]$ (7.0 g) was obtained in 92.5% yield (Scheme 1). In the next step boric tri-sulfuric anhydride (0.87 g) was supported on the surface of silicagel (0.13 g) to get the Silica gel supported boric tri-Sulfuric Anhydride (SiO_2-BTSA). The acidic capacity of the catalyst was determined 8.05 mmol/g related to the acidic functionalities ($-SO_3H$) (12).

Synthesis of 4-substituted-2,6-bis(hydroxymethyl)phenols ***(1a-1b)***



Scheme 2: Synthesis of 4-substituted-2,6-bis(hydroxymethyl)phenol

To an aqueous solution of NaOH (25%, 50 mL) containing *p*-substituted phenol (0.1 mol) and methanol (25 mL), formaldehyde (38%, 90 mL) was added. The reaction mixture was shaken at 60-80˚C for 1 h and then was allowed to stand at R.T. for 24 h. A mixture of water (50 mL) and glacial acetic acid (15 mL) was added and stirred for 4 h at 25˚C to give a precipitate. Filtration gave the corresponding 4-substituted-2,6-bis(hydroxymethyl)phenol (Scheme 2).

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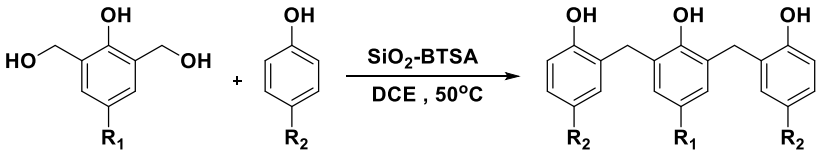
Synthesis of poly-hydroxyl aromatic compounds using Silica Gel Supported Boric Tri-Sulfuric Anhydride (SiO_2-BTSA)



	*I*1	*I*2	*I*3	*I*4	*I*5	*I*6	*I*7	*I*8	*I*9	*I*10	*I*11	*I*12	*I*13	*I*14	*I*15	*I*16
R1	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Cl	Br	Br	Br	Cl
R2	H	Cl	F	Br	I	OH	CH_3	OCH_3	NO_2	CHO	t-butyl	Ph	Br	Cl	benzyl	benzyl

Scheme 3: Synthesis of poly-hydroxyl aromatic compounds

To a required solution of 4-substituted -2,6-bis(hydroxymethyl)phenols ***(1a-1b)***, (1.0 mmol) in 1,2-dichloroethane (10 mL), substituted phenol (3.5 mmol) and catalytic amount of silica supported boric tri-sulfuric anhydride (SiO_2-BTSA, 0.1 g) were added, and the reaction mixture was heated at 50°C while TLC monitoring indicated no further progress in the reaction. Subsequently, the catalyst was removed by filtration and washed with hot dichloroethane (2×20 mL). The combined filtrates were evaporated under reduced pressure. Then, the residue was suspended in boiling water (3×10 mL) and decanted to remove the unreacted phenol. Finally, the crude product was purified by silica gel column chromatography using petroleum ether-EtOAc as eluent (Scheme 3).

Spectral data

4-Chloro-2,6-bis(hydroxymethyl)phenols ***(1a)***

White crystals, M.p. 167 °C; Lit. M.p. 166-168 °C (13) . ^{1}H-NMR (DMSO-d6, 250 MHz): 4.51 (s, 4H, $2CH_2$); 5.29 (s, 2H, 2OH, exchangeable with D_2O); 7.17 (s, 2H,aromatic); 8.71 (s, 1H, OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d6,62.9 MHz): 58.84; 122.94; 124.79; 130.89; 149.75. IR (KBr): 3412w; 3300w; 2967m; 2914m; 2888s; 1478m; 1456m; 1211s; 1068s; 1010m.

4-Bromo-2,6-bis(hydroxymethyl)phenol ***(1b)***

White crystals, M.p. 163 °C; Lit. M.p. 163-164 °C (13). ^{1}H-NMR (DMSO-d6, 250 MHz): 4.34 (s, 2H, $2CH_2$); 5.37 (s, 2H, 2OH, exchangeable with D_2O); 7.27 (s, 2H,aromatic); 8.42 (s, 1H, OH, exchangeable with D_2O).

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^{13}C-NMR (DMSO-d6,62.9 MHz): 58.47; 110.72; 127.76; 131.27; 150.32. IR (KBr): 3420w; 3354w; 3100m; 1590s; 1480s; 1070m; 667s.

2,2'-(5-chloro-2-hydroxy-1,3-phenylene)bis(methylene)diphenol ***(I$_1$)***

White crystals, ^{1}H-NMR (DMSO-d$_6$, 250 MHz): 3.81 (s, 4H, 2CH$_2$); 6.69-6.82 (m, 6H, aromatic); 6.99-7.05 (m, 4H, aromatic); 9.48 (br., 3H, 3OH, exchangeable with D$_2$O). ^{13}C-NMR (DMSO-d$_6$, 62.9 MHz): 29.64; 114.9; 119.09; 122.58; 125.87; 126.57; 127.29; 129.99; 130.39; 151.24; 154.76. Anal. calc. for $C_{20}H_{17}ClO_3$: C 70.49; H 5.03, found: C 70.52; H 4.78. Mass m/z (%): 340 (M^+, 57.6). IR (KBr): 3300w; 3010m; 2900m; 1600s; 1200m.

2,2'-(5-Chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-chlorophenol) ***(I$_2$)***

Yellow crystals, ^{1}H-NMR (DMSO-d$_6$, 250 MHz): 3.81 (s, 4H, 2CH$_2$); 6.77-6.83 (m, 4H, aromatic); 6.99-7.09 (m, 4H, aromatic); 8.88 (br., 1H, OH, exchangeable with D$_2$O); 9.81 (br., 2H, 2OH, exchangeable with D$_2$O). ^{13}C-NMR (DMSO-d$_6$, 62.9 MHz): 29.58; 116.38; 122.38; 122.84; 126.87; 127.18; 128.35; 129.43; 129.52; 151.41; 153.86. Anal. calc. for $C_{20}H_{15}Cl_3O_3$: C 58.63; H 3.69, found: C 58.61; H 3.73. Mass m/z (%): 409 (M^+, 13.2). IR (KBr): 3150w; 3010m; 2980m; 1610s; 1220m.

2,2'-(5-chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-fluorophenol) ***(I$_3$)***

White crystals, ^{1}H-NMR (DMSO-d$_6$, 250 MHz): 3.81 (s, 4H, 2CH$_2$); 6.77-6.89 (m, 8H, aromatic); 8.75 (s, 1H, OH, exchangeable with D$_2$O); 9.57 (s, 2H, 2OH, exchangeable with D$_2$O). ^{13}C-NMR (DMSO-d$_6$, 62.9 MHz): 29.75; 113.06; 113.41; 115.46; 115.59; 116.12; 116.48; 122.85; 127.06; 127.65; 127.76; 129.52; 151.06; 151.35; 153.53; 157.25. Anal. calc. for $C_{20}H_{15}ClF_2O_3$: C 63.75; H 4.01, found: C 63.69; H 3.95. Mass m/z (%): 376 (M^+, 4.8). IR (KBr): 3192w; 1606m; 1496s; 1446s; 1236.3m; 1190s.

2,2'-(5-Chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-bromophenol) ***(I$_4$)***

White crystals, ^{1}H-NMR (DMSO-d$_6$, 250 MHz): 3.84 (s, 4H, 2CH$_2$); 6.78-6.85 (m, 4H, aromatic); 7.15-7.21 (m, 4H, aromatic); 9.67 (br., 3H, 3OH, exchangeable with D$_2$O). ^{13}C-NMR (DMSO-d$_6$, 62.9 MHz): 29.57; 110.12; 116.97; 122.87; 127.21; 128.93; 129.43; 129.81; 132.39; 151.37; 154.30. Anal. calc. for $C_{20}H_{15}Br_2ClO_3$: C 48.18; H 3.03, found: C 48.21; H 3.06. Mass m/z (%): 498 (M^+, 26.3). IR (KBr): 3150w; 3020m; 2990m; 1600s; 1220m.

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2,2'-(5-Chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-iodophenol) ***(I5)***

White crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.77 (s, 4H, $2CH_2$); 6.63-7.51 (m, 8H, aromatic); 8.75 (s, 1H, OH, exchangeable with D_2O); 9.88 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.29; 114.79; 117.61; 122.73; 127.01; 129.53; 132.89; 135.79; 138.23; 151.26; 154.91. Anal. calc. for $C_{20}H_{15}ClI_2O_3$: C 40.54; H 2.55, found: C 40.45; H 2.51. Mass m/z (%): 592 (M^+, 37.9). IR (KBr): 3380w; 3060w; 2980m; 1576s; 1464m; 1224s; 1032s; 728s.

2,2'-(5-chloro-2-hydroxy-1,3-phenylene)bis(methylene)dibenzene-1,4-diol ***(I6)***

Light-brown crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.8 (s, 4H, $2CH_2$); 6.49-6.83 (m, 8H, aromatic); 8.65 (s, 2H, 2OH, exchangeable with D_2O); 8.77 (s, 1H, OH, exchangeable with D_2O); 9.00 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.73; 113.58; 115.53; 116.85; 122.66; 126.67; 126.82; 130.02; 147.01; 149.67; 151.18. Anal. calc. for $C_{20}H_{17}ClO_5$: C 64.44; H 4.60, found: C 64.42; H 4.67. Mass m/z (%): 372 (M^+, 48.1). IR (KBr): 3200w; 3020m; 2990m; 1610s; 1600s; 1500s; 1450m; 1250m; 850s.

2,2'-(5-chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-methylphenol) ***(I7)***

White crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 2.13 (s, 6H, $2CH_3$); 3.77 (s, 4H, $2CH_2$); 6.68-6.84 (m, 8H, aromatic); 8.69 (s, 1H, OH, exchangeable with D_2O); 9.34 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 20.14; 29.62; 114.79; 122.59; 125.62; 126.56; 127.46; 127.65; 130.09; 130.88; 151.12; 152.39. Anal. calc. for $C_{22}H_{21}ClO_3$: C 71.64; H 5.74, found: C 71.65; H 5.77. Mass m/z (%): 368 (M^+, 23.7). IR (KBr): 3200w; 3020m; 2920m; 2880m; 1600m; 1450m; 1210s; 810s.

2,2'-((5-chloro-2-hydroxy-1,3-phenylene)bis(methylene))bis(4-methoxyphenol) ***(I8)***

White crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.60 (s, 6H, $2OCH_3$); 3.81 (s, 4H, $2CH_2$); 6.61-6.79 (m, 8H, aromatic); 9.15 (br., 3H, 3OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.92; 55.11; 111.89; 115.36; 116.16; 122.71; 126.77; 126.91; 129.93; 148.47; 151.18; 152.18. Anal. calc. for $C_{22}H_{21}ClO_5$: C 65.92; H 5.28, found: C 65.90; H 5.25. Mass m/z (%): 400 (M^+, 0.8). IR (KBr): 3170w; 3020m; 2930m; 1604m; 1498s; 1434m; 1377w; 1215s; 1099m; 1043s.

2,2'-(5-chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-nitrophenol) ***(I9)***

Pale yellow crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.93 (s, 4H, $2CH_2$); 6.84-7.06 (m, 4H, aromatic); 7.89-8.03 (m, 4H, aromatic); 10.49 (br., 3H, 3OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.64;

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114.98; 122.92; 123.99; 125.72; 127.56; 128.93; 129.43; 139.45; 151.61; 161.71. Anal. calc. for $C_{20}H_{15}ClN_2O_7$: C 55.76; H 3.51; N 6.50, found: C 55.80; H 3.47; N 6.53. Mass m/z (%): 430 (M^+, 47.7). IR (KBr): 3400w; 3090m; 2920m; 1630s; 1590s; 1500s; 1300s; 1250s; 1080m.

3,3'-(5-Chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-hydroxybenzaldehyde) ***(I10)***

Yellow crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.9 (s, 4H, $2CH_2$); 6.81 (s, 2H, aromatic); 6.93-7.05 (m, 2H, aromatic); 7.53 (s, 2H, aromatic); 7.62-7.66 (m, 2H, aromatic); 9.71 (s, 2H, 2CHO); 10.15 (br., 3H, 3OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.59; 115.2; 122.75; 127.12; 127.26; 128.34; 129.32; 130.59; 131.56; 151.7; 161.14; 191.00. Anal. calc. for $C_{22}H_{17}ClO_5$: C 66.59; H 4.32, found: C 66.54; H 4.35. Mass m/z (%): 396 (M^+, 100.0). IR (KBr): 3200w; 3020w; 2950w; 2850m; 2750m; 1690s; 1600s; 1210m.

2,2'-(5-Chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-tert-butylphenol) ***(I11)***

White crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 1.17 (s, 18H, $6CH_3$); 3.79 (s, 4H, $2CH_2$); 6.70-6.73 (m, 4H, aromatic); 7.01-7.10 (m, 4H, aromatic); 8.74 (s, 1H, OH, exchangeable with D_2O); 9.40 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.99; 31.37; 33.54; 114.4; 122.49; 123.86; 125.01; 126.46; 127.41; 130.15; 141.2; 151.08; 152.30. Anal. calc. for $C_{28}H_{33}ClO_3$: C 74.24; H 7.34, found: C 74.19; H 7.23. Mass m/z (%): 453 (M^+, 29.3). IR (KBr): 3300w; 3020s; 2980s; 1590m; 1510s; 1450s; 1260m.

3,3'-(5-Chloro-2-hydroxy-1,3-phenylene)bis(methylene)dibiphenyl-4-ol ***(I12)***

White crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.77 (s, 4H, $2CH_2$); 6.71 (d, J =8.2 Hz, 2H, aromatic); 7.08 (s, 2H, aromatic); 7.23-7.61 (m, 14H, aromatic); 8.82 (br., 3H, 3OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 33.38; 115.69; 117.28; 125.19; 125.77; 125.86; 126.69; 128.31; 128.6; 129.73; 129.76; 130.95; 140.57; 157.21; 159.74. Anal. calc. for $C_{32}H_{25}ClO_3$: C 77.96; H 5.11, found: C 78.04; H 5.10. Mass m/z (%): 492 (M^+, 94.0). IR (KBr): 3200w; 3030m; 2910m; 1610s; 1600s; 1520s; 1490m; 1220s; 820s.

2,2'-(5-bromo-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-bromophenol) ***(I13)***

Yellow crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.81 (s, 4H, $2CH_2$); 6.76-6.83 (m, 2H, aromatic); 6.98 (s, 2H, aromatic); 7.13-7.19 (m, 4H, aromatic); 8.80 (s, 1H, OH, exchangeable with D_2O); 9.89 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.43; 110.05; 110.7; 116.97; 128.93; 129.82;

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129.95; 130.04; 132.35; 151.85; 154.31. Anal. calc. for $C_{20}H_{15}Br_3O_3$: C 44.23; H 2.78, found: C 44.19; H 2.73. Mass m/z (%): 543 (M^+, 29.6). IR (KBr): 3200w; 3090m; 2950m; 1600s; 1250m.

2,2'-(5-bromo-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-chlorophenol) ***(I$_{14}$)***

Pale-yellow crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.82 (s, 4H, $2CH_2$);6.78-7.07 (m, 8H, aromatic); 8.8 (br., 1H, OH, exchangeable with D_2O); 9.86 (br., 2H, 2OH, exchangeable with D_2O).^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.49; 110.71; 116.37; 122.36; 126.88; 128.35; 129.51; 129.94; 130.05; 151.88; 153.85. Anal. calc. for $C_{20}H_{15}BrCl_2O_3$: C 52.89; H 3.33, found: C 52.85; H 3.30. Mass m/z (%): 454 (M^+, 43.1). IR (KBr): 3150w; 3050m; 2950m; 1600s; 1220m.

2,2'-(5-bromo-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-benzylphenol) ***(I$_{15}$)***

White crystals, ^{1}H-NMR (DMSO-d6, 250 MHz): 3.77 (s, 4H, 2CH2); 3.82 (s, 4H, 2CH2); 6.78-7.23 (m, 18H, aromatic); 9.36 (br., 3H, 3OH, exchangeable with D2O). 13C-NMR (DMSO-d6, 62.9 MHz): 29.64; 48.20; 115.03; 125.68; 127.93; 128.24; 128.43; 128.89; 129.92; 131.04; 131.66; 141.86; 144.39; 151.63; 153.01; 155.40. Anal. calc. for C34H29BrO3: C 72.21; H 5.17, found: C 72.13; H 5.10. Mass m/z (%): 565 (M+, 6.8). IR (KBr): 3335w; 3045m; 2940m; 1640m; 1555m; 1455w; 1225m.

2,2'-(5-chloro-2-hydroxy-1,3-phenylene)bis(methylene)bis(4-benzylphenol) ***(I$_{16}$)***

White crystals, ^{1}H-NMR (DMSO-d_6, 250 MHz): 3.78 (s, 4H, $2CH_2$); 3.80 (s, 4H, $2CH_2$); 6.69-7.27 (m, 18H, aromatic); 8.73 (s, 1H, OH, exchangeable with D_2O); 9.47 (s, 2H, 2OH, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6, 62.9 MHz): 29.72; 48.21; 115.02; 122.69; 125.69; 125.74; 126.46; 127.61; 128.24; 128.45; 130.05; 131.03; 141.86; 151.13; 153.02. Anal. calc. for $C_{34}H_{29}ClO_3$: C 78.37; H 5.61, found: C 78.23; H 5.55. Mass m/z (%): 521 (M^+, 8.9). IR (KBr): 3330w; 3040m; 2980m; 1610m; 1515m; 1435w; 1220m.

Biological study:

Microorganisms

Antifungal activities of the synthetic compounds against some American Type Culture Collection (ATCC) strains of fungi, including *Candida albicans* (ATCC 10261), *Candida glabarata* (ATCC 90030), *Candida dubliniensis* (CBS 8500), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 4344), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformane* (ATCC 9011), *Aspergillus fumigatus* (ATCC 14110),

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Aspergillus flavus (ATCC 64025), *Aspergillus clavatus* (CBS 51465), *Exophiala dermatitidis* (ATCC 109136), and *Pseudallescheria boydii* were determined. The susceptibility of all clinical isolates of fungi against selected antibiotics was examined by micro dilution method. The antibacterial activities of the above compounds against standard species of *Escherichia coli* (*ATCC 25922*), *Staphylococcus aureus* (*ATCC 25923*), *Enterococcus faecalis (ATCC 11700*), and *Pseudomonas aeruginosa* (*ATCC 27853*) were also determined in this study.

Determination of minimum inhibitory concentration

MICs were determined using the broth microdilution method recommended by the CLSI with some modifications (14, 15). Briefly, for determination of antimicrobial activities against fungi, serial dilutions of the synthetic compounds (1–256 µg/ml) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St. Louis, MO, USA) buffered with MOPS (Sigma). Stock inoculums were prepared by suspending three colonies of the examined yeast in 5 ml sterile 0.85% NaCl, and adjusting the turbidity of the inoculums to 0.5 McFarland standards at 530 nm wavelengths (this yields stock suspension of 1-5 × 106 cells/ml). For moulds (Aspergillus spp. and dermatophytes), conidia were recovered from the 7-day old cultures grown on potato dextrose agar by a wetting loop with tween-20. The collected conidia were transferred in sterile saline and their turbidity was adjusted to OD=0.09-0.11 that yields 0.4-5 × 10^6 conidia/ml. Working suspension was prepared by making a 1/50 and 1/1000 dilution with RPMI of the stock suspension for moulds and yeasts, respectively. Working inoculums (0.1 ml) were added to the microtiter plates, which were incubated in a humid atmosphere at 30ºC for 24–48 h. Uninoculated medium (200 µL) was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without antibiotics or the synthetic compounds) were also included. The growth in each well was compared with that of the growth in the control well.

Results

Chemistry

Optimization of the reaction conditions

To optimize the reaction conditions, the reaction of 4-chloro-2,6-bis(hydroxymethyl)phenol (1.0 mmol) and *p*-chlorophenol (5.0 mmol) in the presence of different acidic catalysts in 1,4-dioxane (10 mL) was studied as a model reaction (Scheme 4).

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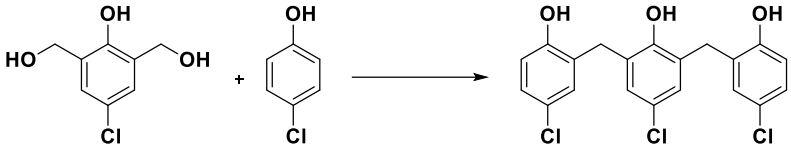

Scheme 4: Reaction 4-chloro-2,6-bis(hydroxymethyl)phenol and *p*-chlorophenol as a model to optimize the reaction conditions

In the initial step, the effect of various acidic catalysts such as $B(OH)_3$, $ZnCl_2$, LiCl, $MgSO_4$, …. were examined (Table 1, entries 1-15). As shown in this Table, the best results were obtained when silica gel supported boric tri-sulfuric anhydride was used as catalyst (Table 1, entry 10-12). The effect of heating on the rate and yield of the reactions was also studied. For this purpose, the model reaction was done under different thermal conditions (Reflux, 50°C and r.t.). The best result was obtained in 50°C (Table 1, entry 11).

Table 1: Effects of different catalysts (2 mmol) and conditions on the reaction of 4-chloro-2,6-bis(hydroxylmethyl)phenol and *p*-chlorophenol in 1,4-dioxane

Entry	Catalyst	Time (h)	Condition	Yield (%)
1	$B(OH)_3$	24	Reflux	15
2	$ZnCl_2$	24	Reflux	10
3	LiCl	18	Reflux	10
4	$MgSO_4$	24	Reflux	Trace
5	$FeCl_3$	24	Reflux	Trace
6	TiO_2	24	Reflux	Trace
7	I_2	24	Reflux	Trace
8	SiO_2	24	Reflux	Trace
9	$AlCl_3$	18	Reflux	10
10	SiO_2-BTSA	5	Reflux	41
11	**SiO_2-BTSA**	**5**	**50°C**	**58**
12	SiO_2-BTSA	10	r.t.	35
13	BTSA	2	50°C	39
14	SPA	3	50°C	32

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15	SC	2.5	50ºC	27

SPA: Silica phosphoric acid, SC: Sulfonated charcoal

In continuance of our study, the model reaction was tested in several solvents to compare the efficiency and capacity of the different solvents. Thus, a mixture of 4-chloro-2,6-bis(hydroxymethyl)phenol (1.0 mmol) and *p*-chlorophenol (5.0 mmol) were reacted in different solvents (10 ml) (Table 2). As the results indicate, desirable yield was obtained in dichloroethane (Table 2, entry 6). It should also be considered that three electron withdrawing groups (-OSO_3H), in the SiO_2-BTSA catalyst lead to increase the Lewis acidity of boron. In a non-coordinate solvent such as 1,2-dichloroethane (DCE) the amount of product has increased to some extent (Table 2, entry 6). This could be due to the availability of the Lewis acidic sites in such solvents and this solvent was selected for all other reactions.

Table 2. Effect of different solvents on the reaction of 4-chloro-2,6-bis(hydroxylmethyl)phenol and *p*-chlorophenol in the presence of SiO_2-BTSA (0.25 g) at 50°C.

Entry	Solvent	Time (h)	Yield (%)
1	CH_3CN	5	24
2	CH_3OH	5	38
3	EtOAc	5	15
4	1,4-Dioxane	5	58
5	DMF	5	Trace
6	**DCE**	**5**	**71**
7	THF	5	30
8	DMSO	5	Trace
9	EtOH	5	41

In the next experiment, to determine the optimum amount of the catalyst, we used different amounts of SiO_2-BTSA on the model reaction (Table 3). Increasing the amounts of the catalyst up to 0.1g improved the yield of reactions. But using of more than 0.1g of catalyst had no any significant effects on the yield of products (Table 3, entry 3).

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Table 3. Effect of different amounts of SiO_2-BTSA on the reaction of 4-chloro-2,6-bis(hydroxylmethyl)phenol and *p*-chlorophenol at 50°C in DCE

Entry	catalyst (g)	Time (h)	Yield (%)
1	0.01	3	35
2	0.05	3	60
3	**0.1**	**1**	**85**
4	0.2	1	77
5	0.25	1	71
6	0.5	1	76
7	1.0	1	70

Finally to establish the best stoichiometric ratio of phenol to bis(hydroxymethyl)phenol, we performed the model reaction in the presence of different amounts of *p*-chlorophenol (Table 4). The best results were obtained when 3.5 eq of phenol was used per 1 eq of bis(hydroxymethyl)phenol (Table 4, entry 4).

Table 4. Effect of different ratio of *p*-chlorophenol to the 2,6-bis(hydroxymethyl)phenol in the presence of SiO_2-BTSA (0.1 g) at 50°C in DCE

Entry	eq of phenol	Time (h)	Yield (%)
1	2.0	1	35
2	2.5	1	40
3	3.0	1	50
4	**3.5**	**1**	**90**
5	4.0	1	81
6	5.0	1	80
7	6.0	1	65

The catalyst can also be recovered, activated and reused for three consecutive runs with only slight variation in the yields of the products (Table 5).

Table 5: Recovery of the catalyst

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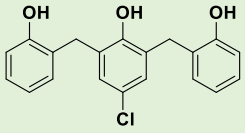
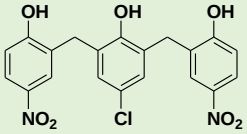
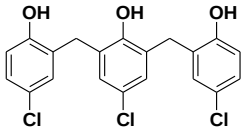
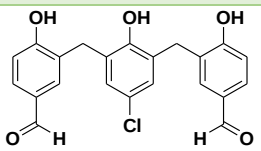
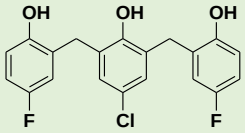
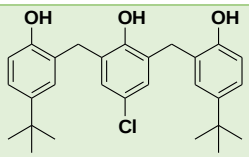
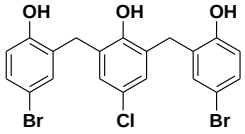
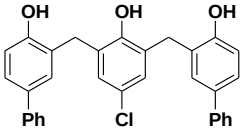
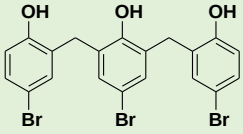
Entry	Cycle	Time (h)	Yield (%)
1	1	1	90
2	2	1	85
3	3	1	82

To evaluate the generality and scope of the above procedure, a variety of 2,6-bis(hydroxymethyl)phenols and different 4-substituted phenols with electron donating and electron withdrawing groups were introduced to the optimized reaction conditions. The results are summarized in Table 6. In all these cases, the reaction proceeded quickly and the desired products were obtained in desirable yields.

Table 6: Chemical structure and characterization of the synthesized compounds using SiO_2-BTSA

Entry	Chemical structure of the Products	Time (h:min)	Yield (%)	m.p. (°C)	Entry	Chemical structure of the Products	Time (h:min)	Yield (%)	m.p. (°C)
I1		1:00	82	190	*I9*		1:30	84	225
I2		1:00	90	233	*I10*		1:30	82	202
I3		1:00	83	223	*I11*		2:30	80	190
I4		1:00	91	251	*I12*		1:30	83	144
I5		1:30	85	189	*I13*		1:20	87	236

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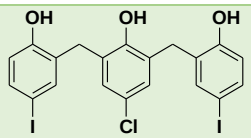
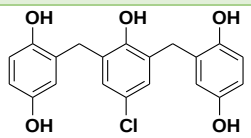
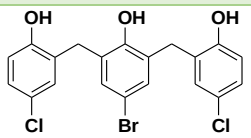
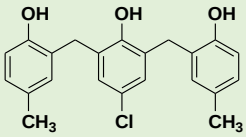
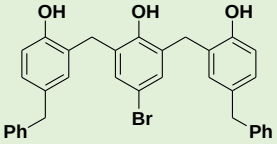
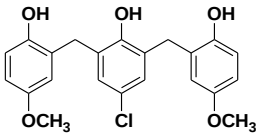
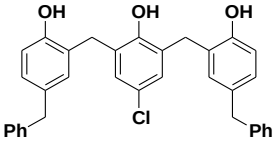
									
I6		1:30	79	179	***I14***		1:20	89	235
I7		1:00	81	136	***I15***		1:30	85	180
I8		1:30	80	193	***I16***		1:45	86	190

As table 6 indicates, alkyl substituted phenols such as *p*-cresol, *p-tert*-butyl phenol, *p*-methoxy phenol and *p*-benzyl phenol show good activity in the reaction and desired products were formed with good yields (Table 6, entries ***I7***, ***I8***, ***I11***, ***I15*** and ***I16***). On the other hand, strong electron-withdrawing groups such as nitro and formyl can be used in this modified procedure (Table 6, entries ***I9*** and ***I10***).

Biological activities

Antimicrobial activities of the synthetic derivatives

Antimicrobial activities of the compounds were evaluated against different yeasts, filaments fungi, gram positive and gram negative bacteria. Fluconazole, grisofulvin and ciprofloxacin were used as positive control respectively. MIC values of the compounds are shown in table 7, 8 and 9.

Table 7: MIC values (µg/mL) for tested compounds against different yeasts

		I1	***I3***	***I6***	***I7***	***I8***	***I9***	***I13***	***I14***	***I15***	***I16***	***Flu***
C.albicans	MFC	32	1	***1***	***2***	G	32	16	2	32	16	G
	MIC_{90}	32	1	1	2	G	32	4	2	32	16	8
	MIC_{50}	16	0.5	0.5	1	G	16	2	1	16	8	2
C.glabrata	MFC	128	G	0.5	G	G	32	4	0.5	G	G	G
	MIC_{90}	8	G	0.5	G	G	32	4	0.5	G	G	4
	MIC_{50}	4	G	0.25	G	G	16	2	0.25	G	G	2

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C.dubliniensis	MFC	16	G	16	G	G	G	G	256	G	G	64
	MIC_{90}	16	G	16	G	G	G	G	2	G	G	0.5
	MIC_{50}	8	G	8	G	G	G	G	1	G	G	0.25
C.krusei	MFC	4	1	4	G	G	G	128	0.5	G	G	G
	MIC_{90}	1	1	1	64	G	64	1	0.5	G	G	128
	MIC_{50}	0.5	0.5	0.5	32	G	32	0.5	0.25	G	G	32
C.parapsilosis	MFC	8	8	8	G	G	G	256	G	G	G	16
	MIC_{90}	8	4	8	G	G	32	4	G	G	G	2
	MIC_{50}	4	2	4	G	G	16	2	G	G	G	1
C.tropicalis	MFC	2	2	2	1	G	4	1	1	64	256	G
	MIC_{90}	1	1	1	0.5	G	1	1	0.5	2	1	32
	MIC_{50}	0.5	0.5	0.5	0.25	G	0.5	0.5	0.25	1	0.5	16
C.neoformance	MFC	G	G	G	G	G	G	2	4	G	G	G
	MIC_{90}	G	G	G	G	G	G	1	4	G	G	1
	MIC_{50}	G	G	G	G	G	G	0.5	2	G	G	0.5

Flu: Fluconazole, G: >256 µg/mL

According to our results, compounds ***I_1, I_6 and I_{14}*** exhibited considerable antifungal activities against most of the tested *Candida* species at concentration of 0.5–16 µg/mL. Compound ***I_3*** showed desirable antifungal activities against the tested *Candida* species except *C. glabrata* and *C. dubliniensis* at concentration 0.5-8 µg/mL. Compound ***I_7*** showed desirable antifungal activities against *C. albicans, C. krusei and C. tropicalis* at concentration 0.25-64 µg/mL. Compound ***I_9*** showed desirable antifungal activities against the tested *Candida* species except *C. dubliniensis* at concentration 0.5-64 µg/mL.

Table 8: MIC values (µg/mL) for tested compounds against different filaments fungi

		I_1	***I_3***	***I_6***	***I_7***	***I_8***	***I_9***	***I_{13}***	***I_{14}***	***I_{15}***	***I_{16}***	***Gris***
A.fumigatus	MFC	G	G	G	G	G	G	G	G	G	G	G
	MIC_{90}	G	G	G	G	G	G	G	G	G	G	4
	MIC_{50}	G	G	G	G	G	G	G	G	G	G	2
	MFC	G	G	G	G	G	G	G	G	G	G	G

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A.flavus	MIC_{90}	G	G	G	G	G	G	G	G	G	G	1
	MIC_{50}	G	G	G	G	G	G	G	G	G	G	0.5
A.clavatus	MFC	G	G	G	G	G	G	G	G	G	G	G
	MIC_{90}	G	G	G	G	G	G	G	G	G	G	4
	MIC_{50}	G	G	G	G	G	G	G	G	G	G	2
E.dermatitidis	MFC	G	G	32	G	G	G	G	2	G	G	32
	MIC_{90}	G	G	32	G	G	G	G	2	G	G	16
	MIC_{50}	G	G	16	G	G	G	G	1	G	G	8
P.boydii	MFC	128	G	32	G	G	16	G	G	G	G	G
	MIC_{90}	128	G	32	G	G	16	G	G	G	G	G
	MIC_{50}	64	G	16	G	G	8	G	G	G	G	G

Gris: Grisogulvin, G: >256 µg/mL

As the results show, compounds ***I1, I6, I9*** and ***I14*** exhibited considerable antifungal activities against *E.dermatitidis* and *P. boydii* at concentration of 1-128 µg/mL.

Table 9: MIC values (µg/mL) for tested compounds against different bacteria

		I1	***I3***	***I6***	***I7***	***I8***	***I9***	***I13***	***I14***	***I15***	***I16***	***Cip***
E.coli	MBC	G	G	G	G	G	G	4	2	G	G	G
	MIC_{90}	G	G	G	G	G	G	4	2	G	G	0.25
S.aureus	MBC	G	G	G	G	G	G	2	1	G	G	G
	MIC_{90}	G	G	G	G	G	G	2	1	G	G	0.5
E.fecalis	MBC	G	G	G	G	G	G	G	G	G	G	G
	MIC_{90}	G	G	G	G	G	G	G	G	G	G	G
P.aeruginosa	MBC	G	G	G	G	G	G	G	G	G	G	G
	MIC_{90}	G	G	G	G	G	G	G	G	G	G	G

Cip: Ciprofuxacin, G: >256 µg/mL

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Compounds ***I*$_{14}$** and ***I*$_{15}$** showed desirable antibacterial activities against *E. coli* and *S. aureus* at concentration 1-4 µg/mL. Our other compounds did not exhibit significant bactericidal activities at concentration up to 256 µg/mL.

Discussion

In summary, this new strategy offers several advantages including high yields of condensation products, short reaction times with respect to classical methods, minimum amount of polymerization of the starting bis(hydroxymethyl)phenols as well as simple experimental procedure which makes it useful for the synthesis of poly-hydroxyl aromatic compounds (phloroglucide analogs). The use of inexpensive and recyclable silica gel supported boric tri-sulfuric anhydride (SiO_2-BTSA) as catalyst makes this procedure quite simple and more convenient. Further studies still needed to investigate other biological activities of these compounds.

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