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ZnO in ionic liquid under microwave irradiation: A novel medium for synthesis of phloroglucide derivatives as antimicrobial agents

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Owing to the wide applications and significance of phloroglucide analogues in organic synthesis, pharmacology and industry, there is considerable interest in the synthesis of these compounds. Nevertheless, there are few methods for preparation of these compounds. Here we reported a simple, clean, and highly efficient procedure for the green synthesis of tricyclic polyhydroxyl aromatic compounds. Microwave-assisted alkylation reaction between 4-substituted-2, 6-bis(chloromethyl)phenols and various phenol derivatives, in the presence of zinc oxide (ZnO) in 1-butyl-3-methylimidazolium hexaflourophosphate ([Bmim]PF_6), affords the title compounds in short duration with high yields. We have synthesized 18 derivatives of the title compounds **3a**-**3r** by this procedure. Chemical structures of all synthesized compounds have been confirmed by spectrophotometric methods such as IR, NMR and mass spectroscopy. Some of the synthesized compounds have been screened for their antimicrobial activities. Antifungal and antibacterial activities have been evaluated against different species of microorganisms including gram positive and gram negative bacteria as well as fungi. Broth microdilution method as recommended by Clinical and Laboratory Standard Institute has been used for this purpose. The results show compounds **3i**, **3k** and **3m** have the best antibacterial and antifungal activity against most of the examined species. Compounds **3b, 3f, 3o, 3p** and **3q** also show good activity against some species.

Keywords: ZnO, ([Bmim]PF_6), microwave, phloroglucide, antimicrobial

Phloroglucides are a group of polyhydroxy aromatic compounds and they have very important applications in pharmacology and organic synthesis. The existence of key hydroxyl groups in a proper 3-D array makes these compounds as potent antimicrobial agents since it provides a perfect situation to accommodate metal ions of enzymes. Aspidin-BB, Trisaspidin-BBB, arzanol, zantrin Z1 and CBHBP are typical examples (Figure 1)[1-4]. They are also available from natural sources, *e.g.* ebracteolatain A and B which were isolated from *Euphorbia ebracteolata Hayata* [5]. In addition these compounds were used as veterinary medicines for treatment of trematods or nematod in warm blooded animals[6].

A traditional procedure for the synthesis of the title compounds involves Baeyer condensation using formalin and sulfuric acid. This procedure is restricted to preparation of the analogs containing three similar phenol rings in which they are obtained in low yields as side products[7]. Other conventional procedure includes acid (mineral and solid acids) catalyzed alkylation of two equivalents of a phenol with 2,6-bis(hydroxymethyl)phenol derivatives. Although using of solid acids reduces environmental pollutions and don't have other problems related with inorganic acids, but excess amounts of phenols which should be use in these reactions, is still a serious problem[6-10]. Another technique for preparing phloroglucide derivatives, is the condensation of phenols with 2, 6-bis(chloromethyl)phenols, in which large excess amounts of phenol derivative (20 folds) is needed and phenol itself work as catalyst. This autocatalytic method is not suitable for phenols which are deactivated by ring substituents, intermolecular hydrogen bonded or satirically hindered[11].

Recently, mineral oxides have verified to be valuable in laboratory research due to good activation of adsorbed compounds and reaction rate enhancement, simpler workup, recyclability of the supports, and eco-friendly reaction conditions. Zinc oxide (ZnO) is surely one of the most exciting of these oxides that has been applied in different organic transformations[12-14].

Owing to the fact that microwave irradiation (MW) has been used to increase organic reaction rates, some organic reactions have been explained using a mixture of microwave irradiation and ionic liquids (ILs)[15]. Some of the reactions in common organic solvents

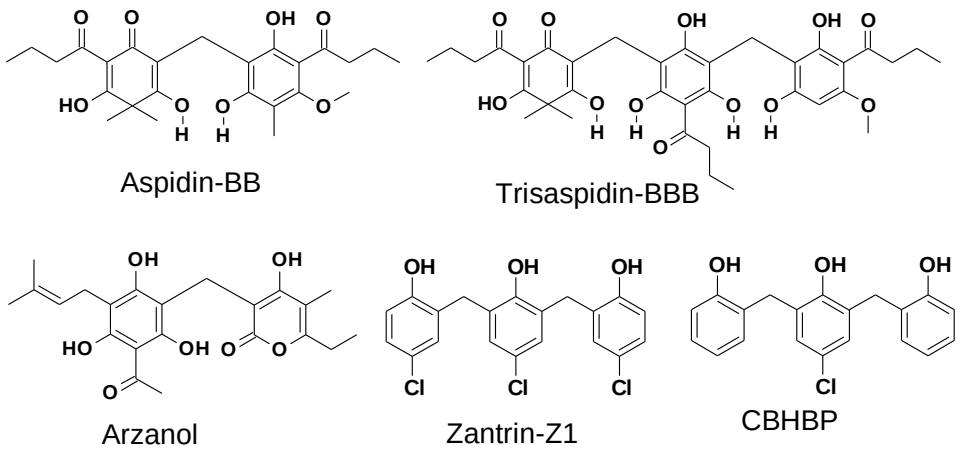
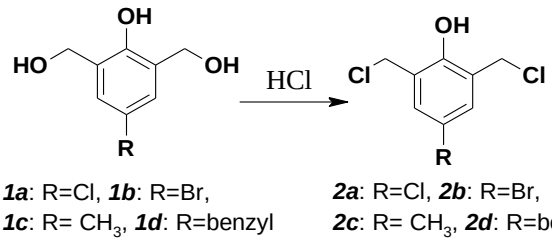


Figure 1 — Chemical structure of some biologically active phloroglucides

have limitations such as long reaction time, harsh reaction conditions and expensive catalysts. This justifies the growing interest in ionic liquids over the past decades[16]. Taking the above evidences in mind and also in extension of our previous study on the synthesis of poly-hydroxyl aromatic compounds[9] and adding of ionic liquids to the chemical reaction media[16], we reported herein a clean, facile and rapid solvent-free procedure for alkylation reaction of 2, 6-bis(chloromethyl)phenols and substituted phenols in the presence of ZnO-[Bmim]PF_6 under microwave condition. We also evaluated our new compounds in the point of antifungal and antibacterial activities by broth micro dilution method.

Experimental Section

All chemicals were purchased from Merck and Fluka companies. Infrared (IR) spectra were run on a Shimadzu FTIR-8300 spectrophotometer; ν_{max} in cm^{-1}. The ^1H- and ^{13}C NMR spectra were run on a Bruker Avanced DPX-250, FT-NMR spectrometer in pure deuterated solvent ($CDCl_3$, 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. Microwave assisted reactions were carried out using a household microwave oven (Panasonic NN-ST757W-1100W, Matsushita Electric Industrial Co., Ltd). 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. All yields refer to isolated products after chromatography or other indicated purification methods. 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.



Scheme I — Synthesis of 4-substituted-2,6-bis(chloromethyl)phenols

General procedure for synthesis of 4-substituted-2,6-bis(chloromethyl)phenols 2a-d

A sample of 4-substituted-2,6-bis (hydroxymethyl) phenol **1a-d** (30 mmol), (prepared according to literature reported procedures)[8], was suspended in concentrated hydrochloric acid (37%, 60 mL) and the mixture was stirred overnight. The resulting suspension was extracted with dichloromethane (3×50 mL). The combined dichloromethane phases were washed with brine, dried overnight with Na_2SO_4 and the liquid was decanted and vacuum evaporated, leaving the product which was recrystallized from petroleum ether in the case of solids (Scheme I)[17].

4-Chloro-2,6-bis(chloromethyl)phenol, 2a: Yellow crystals. m.p. 88-90ºC. ^1H NMR (DMSO-d_6, 250 MHz): 4.75 (s, 4H, $2CH_2$), 7.41 (s, 2H, aromatic), 9.76 (br., 1H, OH, exchangeable with D_2O); ^{13}C NMR

(DMSO-d_6, 62.9 MHz): 41.34, 122.87, 127.39, 130.74, 152.39.

4-Bromo-2,6-bis(chloromethyl)phenol, 2b: Yellow crystals. m.p. 93-95ºC. ^{1}H NMR (DMSO-d_6, 250 MHz): 4.75 (s, 4H, 2CH_2), 7.48 (s, 2H, aromatic), 9.35 (br., 1H, OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 41.28, 110.48, 127.81, 133.63, 152.88.

4-methyl-2,6-Bis(chloromethyl)phenol, 2c: Yellow crystals. m.p. 84-86ºC. ^{1}H NMR ($CDCl_3$, 250 MHz): 2.28 (s, 3H, CH_3), 4.66 (s, 4H, 2CH_2), 7.09 (s, 2H, aromatic), 9.12 (br, 1H, OH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 62.9 MHz): 20.23, 38.89, 124.98, 130.51, 131.82, 150.94.

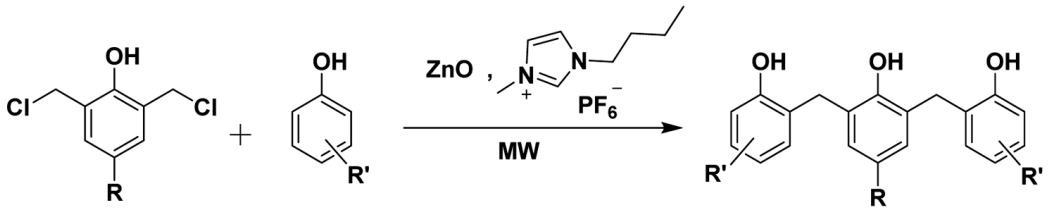
4-Benzyl-2,6-bis(chloromethyl)phenol, 2d: Colorless oil. ^{1}H NMR (DMSO-d_6, 250 MHz): 3.81 (s, 2H, CH_2), 4.67 (s, 4H, 2CH_2Cl), 6.85 (s, 2H, aromatic), 7.02-7.17 (m, 5H, aromatic), 9.58 (br., 1H, OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 41.29, 41.38, 125.33, 126.51, 128.41, 129.72, 131.09, 135.01, 141.78, 152.23.

General procedure for synthesis of tris-Phenol phloroglucide analogs 3a-r

To a mixture of 4-substituted-2,6-bis(chloromethyl) phenol (**2a-d**, 1 mmol), well-ground ZnO (0.08 g, 1 mmol) and different substituted phenols (2 mmol), 1-butyl-3-methylimidazolium hexaflourophosphate ([Bmim]PF_6) (0.05 g) was added and mixed carefully. The resulting mixture was then irradiated in a MW oven at 220 W for the desired time. Afterward, the reaction mixture was cooled to RT and was extracted with Et_2O (3×30 mL). The organic extracts were then combined. After removal of the solvent, the crude product was purified by column chromatography on silica gel using EtOAc-petroleum ether as eluent. After isolating the products and evaporating the reminder Et_2O from ionic liquid, the ionic liquid containing ZnO ([Bmim]PF_6-ZnO) was used for the next run under identical reaction conditions (Scheme II).

2,2'-(5-Chloro-2-hydroxy-1,3-phenylene)bis (methylene)bis(4-chlorophenol), 3a: 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_2O), 9.81 (br., 2H, 2OH, exchangeable with D_2O); ^{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 $C_{20}H_{15}Cl_3O_3$ (409.69): 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.

6,6'-(5-Chloro-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-chloro-3-methylphenol), 3b: White crystals. ^{1}H NMR (DMSO-d_6, 250 MHz): 2.19 (s, 6H, 2CH_3), 3.77 (s, 4H, 2CH_2), 6.75 (s, 2H, aromatic), 6.99 (s, 2H, aromatic), 7.15 (d, J = 8.65 Hz, 2H, aromatic), 9.67 (br., 3H, 3OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 19.3, 29.17, 114.01, 117.33, 122.59, 125.72, 126.92, 129.92, 133.81, 135.16, 151.26, 153.66. Anal. calc. for $C_{22}H_{19}Cl_3O_3$: C 60.36; H 4.37, found: C 60.37; H



2a-2d **3a-3r**

Compd	R	R′
3a	Cl	4-Cl
3b	Cl	3-Methyl,4-Cl
3c	Cl	2-Methoxy,4-allyl
3d	Br	4-F
3e	Br	4-OH
3f	Br	3-Methyl,4-Cl
3g	Br	2-Methoxy,4-allyl
3h	CH_3	4-Cl
3i	CH_3	4-Br
3j	CH_3	4-CH_3
3k	CH_3	3-Methyl,4-Cl
3l	CH_3	4-Octyl
3m	CH_3	3-OH
3n	Benzyl	4-Cl
3o	Benzyl	4-Br
3p	Benzyl	4-F
3q	Benzyl	4-OCH_3
3r	Benzyl	3-Methyl,4-Cl

Scheme II — Synthesis of tris-Phenol phloroglucide analogs

4.44. Mass *m/z* (%): 437 (M^+, 10.0). IR (KBr): 3350w, 3100m, 2950m, 1650m, 1620m, 1460s, 1225m, 1170m.

6,6'-(5-Chloro-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-allyl-2-methoxyphenol), 3c: Light-yellow crystals. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.11 (d, *J* = 6.6 Hz, 4H, $2CH_2$), 3.61 (s, 4H, $2CH_2$), 3.67 (s, 6H, $2OCH_3$), 4.81 (dd, *J* = 16.5, 10.3 Hz, 4H, $2H_2C$=C), 5.16 (br., 3H, 3OH, exchangeable with D_2O), 5.70 (m, 2H,=C(H)-C), 6.54-7.49 (m, 6H, aromatic); ^{13}C NMR ($CDCl_3$, 62.9 MHz): 30.02, 40.01, 56.34, 111.36, 115.36, 117.52, 122.67, 124.61, 125.81, 128.65, 134.01, 138.81, 141.69, 143.02, 156.37. Anal. calc. for $C_{28}H_{29}ClO_5$: C 69.92; H 6.08, found: C 69.98; H 6.10. Mass *m/z* (%): 480 (M^+, 17.4). IR (KBr): 3450w, 3090m, 2950m, 2985m, 1600m, 1510m, 1450m, 1410m, 1210w, 1200s.

2,2'-(5-Bromo-2-hydroxy-1,3-phenylene)bis-(methylene)bis(4-fluorophenol), 3d: White crystals. ^{1}H NMR (DMSO-d_6, 250 MHz): 3.80 (s, 4H, $2CH_2$), 6.74-6.86 (m, 6H, aromatic), 6.91 (s, 2H, aromatic), 9.35 (br., 3H, 3OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 29.69, 110.65, 113.02, 113.38, 115.44, 115.57, 116.10, 116.46, 127.64, 127.75, 129.91, 129.99, 151.01, 151.86, 153.50, 157.22. Anal. calc. for $C_{20}H_{15}BrF_2O_3$: C 57.03; H 3.59, found: C 56.97; H 3.60. Mass *m/z* (%): 421 (M^+, 7.9). IR (KBr): 3188w, 2940w, 1600w, 1496s, 1446s, 1236m, 1025w.

2,2'-(5-Bromo-2-hydroxy-1,3-phenylene)bis-(methylene) dibenzene-1,4-diol, 3e: Light-brown crystals. ^{1}H NMR (DMSO-d_6, 250 MHz): 3.73 (s, 4H, $2CH_2$), 6.43-6.64 (m, 6H, aromatic), 6.88 (m, 2H, aromatic), 8.61 (br., 2H, 2OH, exchangeable with D_2O), 8.76 (br., 1H, OH, exchangeable with D_2O), 8.97 (br., 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 29.64, 110.56, 113.56, 115.49, 116.83, 126.62, 129.64, 130.52, 146.99, 149.84, 151.65. Anal. calc. for $C_{20}H_{17}BrO_5$: C 57.57; H 4.11, found: C 57.39; H 3.98. Mass *m/z* (%): 417 (M^+, 37.5). IR (KBr): 3209w, 2353m, 1704m, 1604s, 1458m, 1373m, 1211s, 1049m, 956s.

6,6'-(5-Bromo-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-chloro-3-methylphenol), 3f: White crystals. ^{1}H NMR (DMSO-d_6, 250 MHz): 2.17 (s, 6H, $2CH_3$), 3.78 (s, 4H, $2CH_2$), 6.39-7.17 (m, 6H, aromatic), 8.88 (br., 1H, OH, exchangeable with D_2O), 9.72 (br., 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 19.30, 29.11, 114.02, 117.34, 122.61, 125.73, 127.66, 129.93, 133.83, 135.17, 151.74, 153.65. Anal. calc. for $C_{22}H_{19}BrCl_2O_3$: C 54.80; H 3.97, found: C 54.69; H 3.58. Mass *m/z* (%): 482 (M^+, 7.8). IR (KBr): 3350w, 3120m, 2952m, 1645m, 1445s, 1245m, 1180m.

6,6'-(5-Bromo-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-allyl-2-methoxyphenol), 3g: Light-brown crystals. ^{1}H NMR ($CDCl_3$, 250 MHz): 3.34 (d, *J* = 6.3 Hz, 4H, $2CH_2$), 3.84 (s, 4H, $2CH_2$), 4.38 (s, 6H, $2OCH_3$), 4.97 (dd, *J* = 16.8, 10.3 Hz, 4H, 2 H_2C=C), 5.1 (br., 3H, 3OH, exchangeable with D_2O), 5.9 (m, 2H, 2=C(H)-C), 6.46-7.28 (m, 6H, aromatic); ^{13}C NMR ($CDCl_3$, 62.9 MHz): 32.48, 36.97, 56.03, 112.82, 115.82, 115.91, 123.88, 129.17, 129.32, 129.86, 131.15, 137.20, 144.07, 145.52, 151.24. Anal. calc. for $C_{28}H_{29}BrO_5$: C 64.00; H 5.56, found: C 64.10; H 5.58. Mass *m/z* (%): 525 (M^+, 43.6). IR (KBr): 3450w, 3090m, 2950m, 2985m, 1600m, 1520m, 1430s, 1420m, 1200w.

2,2'-(2-Hydroxy-5-methyl-1,3-phenylene)bis-(methylene)bis (4-chlorophenol), 3h: White crystals.. m.p^{1}H NMR (DMSO-d_6, 250 MHz): 2.06 (s, 3H, CH_3), 3.78 (s, 4H, $2CH_2$), 6.63 (s, 2H, aromatic), 6.72-6.88 (m, 4H, aromatic), 7.00-7.04 (m, 2H, aromatic), 8.31 (s, 1H, OH, exchangeable with D_2O), 9.79 (s, 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 20.30, 29.56, 116.18, 122.32, 126.38, 126.70, 127.73, 127.98, 128.59, 129.18, 144.92, 153.71. Anal. calc. for $C_{21}H_{18}Cl_2O_3$: C 64.79; H 4.66, found: C 64.77; H 4.63. Mass *m/z* (%): 389 (M^+, 15.9). IR (KBr): 3136w, 3027m, 2931w, 1604w, 1485s, 1421w, 1388w, 1240s.

2,2'-(2-Hydroxy-5-methyl-1,3-phenylene)bis-(methylene)bis (4-bromophenol), 3i: White crystals. ^{1}H NMR (DMSO-d_6, 250 MHz): 2.07 (s, 3H, CH_3), 3.81 (s, 4H, $2CH_2$), 6.66-6.79 (m, 4H, aromatic), 7.04-7.17 (m, 4H, aromatic), 8.35 (br, 1H, OH, exchangeable with D_2O), 9.77 (br, 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 20.27, 29.48, 110.05, 116.78, 126.88, 127.93, 128.80, 129.34, 130.02, 132.06, 149.89, 154.16. Anal. calc. for $C_{21}H_{18}Br_2O_3$: C 52.75; H 3.79, found: C 52.67; H 3.53. Mass *m/z* (%): 478 (M^+, 7.9). IR (KBr): 3126w, 3017m, 2915w, 1614m, 1485s, 1420w, 1385w, 1245s.

2,2'-(2-Hydroxy-5-methyl-1,3-phenylene)bis-(methylene)bis (4-methylphenol), 3j: White crystals. ^{1}H NMR (DMSO-d_6, 250 MHz): 2.06 (s, 3H, CH_3), 2.14 (s, 6H, $2CH_3$), 3.82 (s, 4H, $2CH_2$), 6.67-

6.84 (m, 8H, aromatic), 8.26 (s, 1H, OH, exchangeable with D_2O), 9.41 (s, 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 20.19, 20.32, 29.73, 114.70, 126.76, 127.24, 127.43, 127.52, 127.61, 128.31, 130.70, 149.89, 152.16. Anal. calc. for $C_{23}H_{24}O_3$: C 79.28; H 6.94, found: C 79.32; H 7.01. Mass *m/z* (%): 348 (M^+, 45.3). IR (KBr): 3436w, 3290w, 2999w, 2921m, 1575s, 1423s, 1340w, 1230m.

6,6'-(2-Hydroxy-5-methyl-1,3-phenylene)bis-(methylene)bis (4-chloro-3-methylphenol), 3k: White crystals. ^1H NMR (DMSO-d_6, 250 MHz): 2.06 (s, 6H, $2CH_3$), 2.16 (s, 3H, CH_3), 3.76 (s, 4H, $2CH_2$), 6.19-7.14 (m, 6H, aromatic), 8.32 (br., 1H, OH exchangeable with D_2O), 9.71 (br., 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 19.25, 20.25, 29.15, 117.17, 122.57, 126.83, 126.88, 127.09, 128.57, 129.64, 133.29, 150.02, 153.48. Anal. calc. for $C_{23}H_{22}Cl_2O_3$: C 66.19; H 5.31, found: C 66.05; H 5.24. Mass *m/z* (%): 417 (M^+, 6.7). IR (KBr): 3345w, 3020m, 2905m, 1615m, 1445s, 1245m, 1180m.

2,2'-(2-Hydroxy-5-methyl-1,3-phenylene)bis-(methylene)bis (4-octylphenol), 3l: White crystals. ^1H NMR (DMSO-d_6, 250 MHz): 0.83 (t, J = 6.2 Hz, 6H, $2CH_3$), 1.23 (m, 24H, $12CH_2$), 2.04 (s, 3H, CH_3), 2.3 (t, J = 7.5 Hz, 4H, $2CH_2$), 3.8 (s, 4H, $2CH_2$), 6.64-6.81 (m, 8H, aromatic), 9.2 (br., 3H, 3OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 14.11, 20.32, 22.73, 29.32, 29.47, 29.55, 29.65, 31.76, 31.97, 35.11, 114.71, 126.79, 127.18, 127.36, 127.51, 127.55, 128.26, 130.68, 149.87, 152.15. Anal. calc. for $C_{37}H_{52}O_3$: C 81.57; H 9.62, found: C 81.62; H 9.70. Mass *m/z* (%): 544 (M^+, 72.3). IR (KBr): 3240w, 3140s, 2930m, 1620m, 1500s, 1430m, 1220s, 725w.

4,4'-(2-Hydroxy-5-methyl-1,3-phenylene)bis-(methylene) dibenzene-1,3-diol, 3m: White crystals. ^1H NMR (DMSO-d_6, 250 MHz): 2.06 (s, 3H, CH_3), 3.65 (s, 4H, $2CH_2$), 6.17 (d, J = 8.0 Hz, 2H, aromatic), 6.28 (s, 2H, aromatic), 6.49 (s, 2H, aromatic), 6.75 (d, J = 8.0 Hz, 2H, aromatic), 9.02 (s, 2H, 2OH, exchangeable with D_2O), 9.16 (s, 1H, OH, exchangeable with D_2O), 9.36 (br., 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 20.36, 28.99, 102.21, 106.25, 117.35, 127.24, 127.82, 127.95, 130.64, 149.68, 155.11, 156.33. Anal. calc. for $C_{21}H_{20}O_5$: C 71.58; H 5.72, found: C 71.67; H 5.79. Mass *m/z* (%): 352 (M^+, 69.4). IR (KBr): 3255w, 2931w, 1604s, 1396w, 1288m, 1226m, 1095m, 833s.

2,2'-(5-Benzyl-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-chlorophenol), 3n: White crystals. ^1H NMR (DMSO-d_6, 250 MHz): 3.74 (s, 2H, CH_2), 3.85 (s, 4H, $2CH_2$), 6.81-7.25 (m, 13H, aromatic), 8.34 (br., 1H, OH, exchangeable with D_2O), 9.87 (br., 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 29.68, 40.41, 116.18, 122.43, 125.66, 126.41, 126.96, 128.19, 128.36, 129.04, 129.16, 129.41, 131.95, 141.74, 150.86, 153.71. Anal. calc. for $C_{27}H_{22}Cl_2O_3$: C 69.68; H 4.76, found: C 69.47; H 4.33. Mass *m/z* (%): 465 (M^+, 11.4). IR (KBr): 3236w, 3127m, 2910w, 1614w, 1455s, 1420w, 1366w, 1240s.

2,2'-(5-Benzyl-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-bromophenol), 3o: White crystals. ^1H NMR (DMSO-d_6, 250 MHz): 3.74 (s, 2H, CH_2), 3.85 (s, 4H, $2CH_2$), 6.78-7.26 (m, 13H, aromatic), 9.72 (br, 3H, 3OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 29.58, 40.39, 110.12, 116.77, 125.66, 126.95, 128.21, 128.34, 129.05, 129.34, 129.99, 131.89, 131.99, 141.74, 150.84, 154.18. Anal. calc. for $C_{27}H_{22}Br_2O_3$: C 58.51; H 4.00, found: C 58.37; H 3.73. Mass *m/z* (%): 554 (M^+, 27.1). IR (KBr): 3430w, 3125m, 2945w, 1620w, 1475m, 1421w, 1380m, 1242s.

2,2'-(5-Benzyl-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-fluorophenol), 3p: Brown oil. ^1H NMR ($CDCl_3$, 250 MHz): 3.89-4.06 (m, 6H, $3CH_2$), 6.86-7.46 (m, 13H, aromatic), 8.96 (br., 3H, 3OH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 62.9 MHz): 29.12, 41.15, 114.24, 114.61, 116.86, 116.91, 126.31, 126.36, 127.11, 127.36, 128.03, 128.69, 128.73, 129.02, 129.79, 130.06, 134.82, 141.43, 147.95, 155.63, 159.43. Anal. calc. for $C_{27}H_{22}F_2O_3$: C 74.99; H 5.13, found: C 75.23; H 5.03. Mass *m/z* (%): 432 (M^+, 9.5). IR (KBr): 3350m, 3055m, 2915m, 1614m, 1485m, 1385, 1210s.

2,2'-(5-Benzyl-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-methoxyphenol), 3q: White crystals. ^1H NMR ($CDCl_3$, 250 MHz): 3.58 (s, 6H, $2OCH_3$), 3.74 (s, 4H, $2CH_2$), 3.77 (s, 2H, CH_2), 6.41-7.17 (m, 13H, aromatic), 8.55 (br., 2H, 2OH, exchangeable with D_2O), 9.28 (br., 1H, OH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 62.9 MHz): 31.82, 41.02, 55.73, 113.00, 116.09, 116.79, 126.08, 127.62, 128.33, 128.48, 128.93, 129.74, 134.34, 141.40, 145.83, 148.22, 154.12. Anal. calc. for $C_{29}H_{28}O_5$: C 76.30; H 6.18, found: C 76.25; H 6.08. Mass *m/z* (%): 456 (M^+, 100.0). IR (KBr):

3420w, 3110m, 2970w, 1625s, 1415s, 1395w, 1315w, 1205s.

6,6'-(5-Benzyl-2-hydroxy-1,3-phenylene)bis-(methylene)bis (4-chloro-3-methylphenol), 3r: White crystals. ^{1}H NMR (DMSO-d_6, 250 MHz): 2.18 (s, 6H, $2CH_3$), 3.46 (s, 2H, CH_2), 3.78 (s, 4H, $2CH_2$), 6.25-7.24 (m, 11H, aromatic), 8.40 (br., 1H, OH, exchangeable with D_2O), 9.71 (br., 2H, 2OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 62.9 MHz): 19.27, 29.24, 40.37, 113.87, 117.15, 122.59, 122.62, 125.58, 125.62, 127.15, 128.08, 128.17, 128.21, 129.53, 133.28, 150.60, 153.49. Anal. calc. for $C_{29}H_{26}Cl_2O_3$: C 70.59; H 5.31, found: C 70.43; H 5.28. Mass *m/z* (%): 493 (M^+, 14.4). IR (KBr): 3315m, 3127w, 2931w, 1615s, 1421w, 1376w, 1245s.

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 8501), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 4344), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformane* (ATCC 9011), *Aspergillus flavus* (ATCC 64025) and *Aspergillus fumigatus* (ATCC 14110) were determined. The susceptibility of all clinical isolates of fungi against selected compounds 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. The susceptibility of all clinical isolates of bacteria against selected compounds was examined by microdilution method[18,19]. Fluconazole and ciprofloxacin were used as positive control for antifungal and antibacterial activities respectively.

Determination of minimum inhibitory concentration

MICs were determined using the broth microdilution method recommended by the CLSI with some modifications. 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 × 10^6 cells/mL). For moulds, 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.

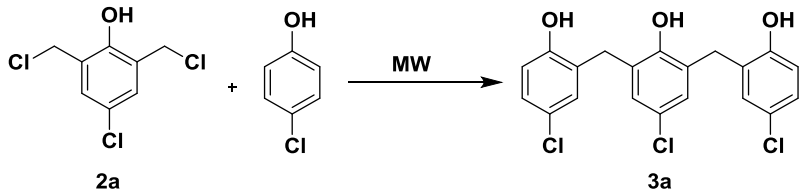
Results

Chemistry

Optimization of the reaction conditions

To optimize the reaction conditions, condensation of 4-chlorophenol (5 mmol) with 4-chloro-2,6-bis (chloromethyl)phenol **2a** was studied as a model reaction to provide compound **3a** (Scheme III).

The efficiency of several mediums was first examined in the presence of ZnO (1 mmol) under microwave irradiation (200 Watt) and 0.2 g of different ionic liquids or tetraalkylammonium salts. The results are collected in Table I. As shown in Table I, the best result was observed when ([Bmim]PF_6) was used as medium (entry 4) and compound **3a** was obtained in 91% yield in 2 minutes. The catalytic power of the ILs is markedly influenced by the counter ion, a decrease in



2a **3a**

Scheme III — Synthesis of **3a** in order to optimize the reaction conditions

Table I — Effect of different mediums for synthesis of compound **3a**

Entry	Medium	Time (min:sec)	Yield (%)
1	[Bmim]Br	1:00	85
2	[Bmim]Cl	2:10	81
3	[Bmim]BF_4	1:40	85
4	[Bmim]PF_6	2:00	91
5	[Bmim]OAc	1:30	57
6	[Bmim]HSO_4	1:30	41
7	TBAB	1:00	76
8	TBAC	1:00	77
9	TBAI	1:20	61
10	TEAB	2:15	53

the catalytic efficiency was observed in the case of tetraalkylammonium-based salts.

In the next experiment, to determine the optimum amount of ionic liquid, we examined the effect of different amounts of ([Bmim]PF_6) on the model reaction (Table II). Our results showed that 0.05 g of ([Bmim]PF_6) (entry 4) had the best activity for production of **3a**.

In the next step to examine the efficiency of ZnO comparative to other mineral oxides, we performed the model reaction using various catalysts (1 mmol) instead of ZnO (Table III). As shown in Table III, ZnO was the best catalyst for completion of the reaction (entry 2). Other catalysts afforded low yields of the product. Thus, ZnO was selected as the catalyst of choice in all further reactions.

Then to obtain the optimized amount of the ZnO, we examined different amounts of catalyst in the reaction. The results are summarized in Table IV. These data shows, that the best isolated yield of the product was obtained using 1 mmol of ZnO (entry 2).

Finally, to establish the best ratio of substituted phenols toward 1 mmol of bis(chloromethyl)phenol **2a**, we performed the model reaction in the presence of different ratio of this starting material (Table V). The best results were obtained when 2 mmol of substituted phenols was used per 1 mmol of **2a** (entry 5). Therefore, we decided to extend the scope of the reaction using 2 mmol of phenols, matching benign with atom economic processes.

These conditions were then applied to run all the subsequent reactions. To evaluate the generality and scope of the above procedure, a variety of 2,6-bis(chloromethyl)phenols **2a-d** and different substituted phenols with electron donating and electron withdrawing groups were introduced to the optimized reaction conditions. All the reactions proceeded in mild conditions under microwave irradiation with good to excellent yields. The results are summarized in Table VI. In all these cases, the reaction proceeded quickly and the desired products were obtained in desirable yields.

Table II — Effect of different amounts of ([Bmim]PF_6) for synthesis of compound **3a**

Entry	Weight (g)	Time (min:sec)	Yield (%)
1	0.2	1:30	91
2	0.15	1:30	92
3	0.1	1:30	94
4	0.05	2:00	95
5	0.02	2:00	90

Table III — Effect of different catalysts for synthesis of compound **3a**

Entry	Catalyst	Time (min:sec)	Yield (%)
1	CaO	15:00	25
2	ZnO	2:00	91
3	TiO_2	10:00	56
4	Al_2O_3	20:00	32

Table IV — Effect of different amounts of ZnO for synthesis of compound **3a**

Entry	mmol	Time (min:sec)	Yield (%)
1	1.5	1:40	87
2	1.0	2:00	91
3	0.75	1:00	85
4	0.5	1:30	81

Table V — Effect of different ratio of substituted phenols toward 1 mmol of **2a**

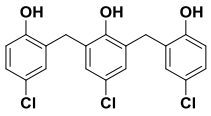
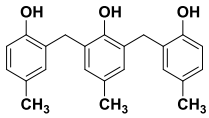
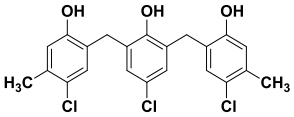
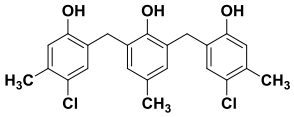
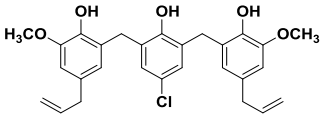
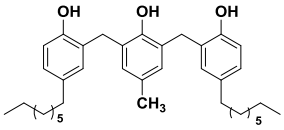
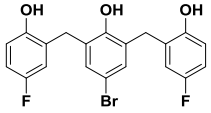
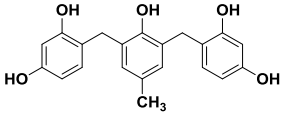
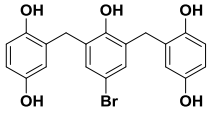
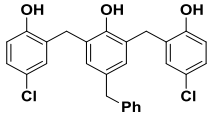
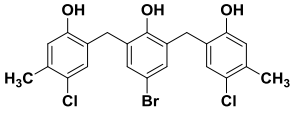
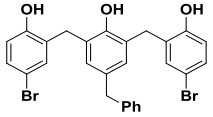
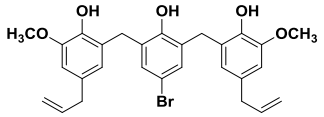
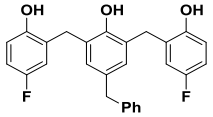
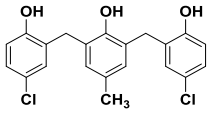
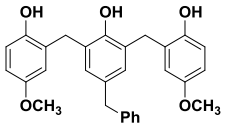
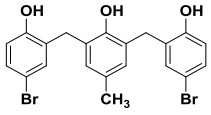
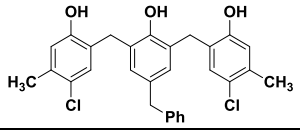
Entry	mmol	Time (min:sec)	Yield (%)
1	5	2:00	91
2	4	1:40	92
3	3	1:40	94
4	2.5	1:30	95
5	2	2:00	95

Biological activities

Antimicrobial activities of the synthetic derivatives

Some of the synthesized compounds were screened for antimicrobial activities and the results are summarized in Table VII. According to our results, compounds **3k** and **3m** exhibited considerable antifungal activities against most of the tested yeasts at concentration of 1-128 μg/mL. Compound **3b** showed desirable antifungal activities against *C. albicans*, *C. glabrata* and *C. tropicalis* at concentration 16-64 μg/mL. Compound **3i** showed desirable antifungal activities against *C. parapsilosis* at concentration 8-16 μg/mL. Compound **3o** showed desirable antifungal activities against *C. albicans*, *C. krusei* and *C. tropicalis* at concentration 1-64 μg/mL.

Table VI — Chemical structures of the synthesized compounds **3a-r** using optimized reaction conditions

Entry	Chemical Structure	Time (min:sec)	Yield (%)	m.p. (˚C)	Entry	Chemical Structure	Time (min:sec)	Yield (%)	m.p. (˚C)
3a		2:00	95	233-235	**3j**		1:00	90	214-216
3b		2:00	85	178-179	**3k**		3:00	89	198-200
3c		2:00	89	56-57	**3l**		4:30	81	116-117
3d		1:00	90	230-232	**3m**		1:00	90	134-136
3e		4:00	84	219-221	**3n**		2:30	80	187-188
3f		2:30	88	202-203	**3o**		2:00	87	192-193
3g		3:00	85	60-61	**3p**		1:30	90	oil
3h		1:00	89	245-247	**3q**		1:30	80	201-202
3i		1:00	88	187-188	**3r**		2:00	90	189-190

Compound **3r** showed desirable antifungal activities against *C. albicans* at concentration 4 μg/mL.

The antibacterial activities of the synthesized compounds were also evaluated. The results are summarized in Table VIII. Compounds **3f, 3i, 3p** and **3q** showed desirable antibacterial activities against *E. fecalis* and *P. aeruginosa* at concentration 0.5-32 μg/mL.

Based on the results displayed in Table VI, it is clear that both electron donating and electron withdrawing substituents on the phenol rings are suitable for this reaction. In the reaction of different substituted phenols *versus p*-cresol (Table VI, entries **3h-m**) the lower yield is related to **3l** which is suggested to be because of the bulkiness of *n*-octyl group. This method was also examined for an asymmetric phenol (4-chloro-3-methyl) which has two different *ortho* sites adjacent to its hydroxyl (Table VI, entries **3b**, **3f**, **3k** and **3r**). As the results indicate, these derivatives also alkylated selectively in less hindered *ortho* site with good to excellent yields.

Moreover, this method was suitable for the condensation of acid sensitive phenols such as eugenol which is prone to double bond migration and conversion to its more stable isomer (isoeugenol) in acidic media (Table VI, entries **3c** and **3g**). It is suggested that, the higher condensation rates encouraged by ionic liquid compared to the

Table VII — MIC values (μg/mL) for tested compounds against different fungi

Entry	*C. albicans*		*C. glabrata*		*C. dubliniesis*		*C. krusei*		*C. parapilopsis*		*C. tropicalis*		*C. neoformans*		*A. flavus*		*A. fumigatus*	
	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC	MIC_{90}	MFC
3b	64	12	16	16	G	G	G	G	G	G	16	G	G	G	G	G	G	G
3f	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
3i	G	G	G	G	G	G	G	G	8	16	G	G	G	G	G	G	G	G
3k	16	16	16	16	G	G	16	256	4	16	1	8	G	G	G	G	G	G
3m	32	32	8	64	G	G	64	128	64	64	32	64	64	64	256	256	256	256
3o	16	G	G	G	G	G	64	G	G	G	1	1	G	G	G	G	G	G
3p	G	G	128	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
3q	G	G	G	G	G	G	G	G	G	G	32	G	G	G	G	G	G	G
3r	4	4	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
Flu[a]	8	G	4	G	0.5	64	128	G	2	16	32	G	32	G	32	G	32	G

[a] Fluconazole

Table VIII — MIC values (μg/mL) for tested compounds against different bacteria

Entry	*E. coli*		*S. aureus*		*E. faecalis*		*P. aeruginosa*	
	MIC_{90}	MBC	MIC_{90}	MBC	MIC_{90}	MBC	MIC_{90}	MBC
3b	G	G	G	G	G	G	G	G
3f	G	G	G	G	0.5	G	0.5	0.5
3i	G	G	G	G	1	2	1	2
3k	G	G	G	G	G	G	G	G
3m	G	G	G	G	G	G	G	G
3o	G	G	G	G	G	G	G	G
3p	G	G	G	G	8	G	8	16
3q	G	G	G	G	0.5	G	8	32
3r	G	G	G	G	G	G	G	G
cip[a]**.**	0.25	G	0.5	G	0.5	G	0.5	G

[a] ciprofloxacin

competing isomerization reaction which is much slower in the neutral medium is the cause for progress of the desired reaction.

Alkyl and alkoxy substituted phenols such as *p*-cresol **(3j)** and *p*-*n*-octylphenol **(3l)** and *p*-methoxy phenol **(3q)** were also used and the products were obtained in good to excellent yields.

Conclusion

In conclusion, we have introduced a highly efficient procedure for ionic-liquid-accelerated alkylation reaction of 4-substituted-2,6-bis (chloromethyl) phenols with different substituted phenols under microwave irradiation. The promising points for the presented methodology are high conversion, ease of handling and low cost of ZnO, cleaner reaction profile, and short reaction times. These properties make our procedure a useful and attractive process for the rapid synthesis of tricyclic polyhydroxy aromatic compounds as biologically interesting compounds. Some of the synthesized compounds were screened for possibly antimicrobial activity. In compare to standard drugs our compounds showed moderate to good antifungal and antibacterial activities.

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References

1 Oryan A, Khalafi-Nezhad A, Toloo N & Soltani Rad M N, *Journal of Veterinary Medicine Series A*, 54(10) (2007) 585.
2 Widén C-J, Vida G, Von Euw J & Reichstein T V, *Helv Chim Acta*, 54(8) (1971) 2824.
3 Urgaonkar S, La Pierre H S, Meir I, Lund H, Ray-Chaudhuri D & Shaw J T, *Org Lett*, 7(25) (2005) 5609.
4 Appendino G, Ottino M, Marquez N, Bianchi F, Giana A & Ballero M, *J Natural Products*, 70(4) (2007) 608.
5 Fu G-M, Yu B-Y & Zhu D-N, *Journal of Asian Natural Products Research*, 8(1-2) (2006) 149.
6 McGarry E J & Forsyth B A, *Google Patents* (1981).
7 Moshfegh A A, Mazandarani B, Nahid A & Hakimelahi G H, *Helv Chim Acta*, 65(4) (1982) 1229.
8 Khalafi-Nezhad A, Rad M N S & Hakimelahi G H, *Helv Chim Acta*, 86(7) (2003) 2396.
9 Khalafi-Nezhad A, Parhami A, Bargebid R, Molazade S, Zare A & Foroughi H, *Molecular Diversity*, 15(2) (2011) 373.
10 Fareghi-Alamdari R, Khalafi-Nezhad A & Zekri N, *Synthesis*, 46(07) (2014) 887.
11 Rodia J S, *J Org Chem*, 26(8) (1961) 2966.
12 Zare A, Hasaninejad A, Khalafi-Nezhad A, Zare A M & Parhami A, *Arkivoc*, 13 (2007) 105.
13 Zare A, Hasaninejad A, Zare A R M, Parhami A, Sharghi H & Khalafi-Nezhad A, *Can J Chem*, 85(6) (2007) 438.
14 Tamaddon F, Amrollahi M A & Sharafat L, *Tetrahedron Lett*, 46(45) (2005) 7841.

15 Tierney J & Lidström P, *Microwave Assisted Organic Synthesis* (John Wiley and Sons) (2009).
16 Khabnadideh S & Harper J B, *Science Research*, 2(5) (2014) 125.
17 Paine R T, Tan Y-C & Gan X-M, *Inorg Chem*, 40(27) (2001) 7009.
18 Clinical, Institute L S: Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard-Third Edition M27-A3 (2008) 28.
19 Wayne P, Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard. CLSI document M27-A2 (2002).