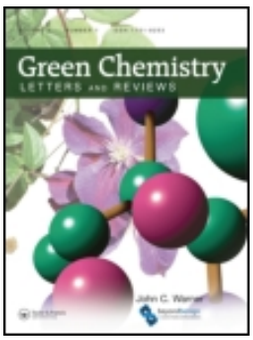

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Solvent-free microwave-assisted synthesis of *E*-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones and their antibacterial activity

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A series of *E*-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones were synthesized by the Claisen–Schimdt reaction of 2-acetyl-3,5-dimethyl-6-benzoyl benzodifuran with aromatic/heteroaromatic aldehydes under solvent-free microwave irradiation. All these compounds were characterized by means of their IR, ^{1}H NMR, and ^{13}C NMR spectra and elemental analysis. All the compounds were screened for their antibacterial activity.

Keywords: chalcones; benzofuran; benzodifuran; antibacterial activity; microwave irradiation

Introduction

Benzofuran derivatives have been reported to possess a wide variety of biological activities. They have been reported to possess antimicrobial (*1–3*), antitumor (*4*), and anti-inflammatory (*5*) activity. Chalcones are α,β-unsaturated ketones and have great existence in plant kingdom. They have been reported to possess antioxidant (*6*), antimalarial (*7*), anti-inflammatory (*8*), anticancer (*9*), antiviral (*10*), and antimicrobial activity (*11*). Benzodifurans exhibit interesting biological activities such as antibacterial (*12*), antifungal (*12*), anti-implantation (*12*), and antiviral activity (*13*). In view of the biological activities exhibited by benzofurans, benzodifurans, and α,β-unasaturated ketones, we have synthesized some new *E*-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones (**3a**–**g**).

Results and discussion

Recently microwave-induced organic reaction enhancement (*14*) chemistry has gained popularity as a nonconventional technique for rapid organic synthesis; it is eco-friendly and economical and is believed to be a step toward green chemistry. The solvent-less reactions offer a number of advantages: solvents are often expensive, toxic, and difficult to remove in the case of aprotic solvents with high boiling points. The most popular way of synthesis of chalcones is the Claisen–Schmidt condensation of an appropriate acetophenone with benzaldehyde in the presence of aqueous bases such as NaOH (*15*), KOH (*16*), $Ba(OH)_2$ (*17*), magnesium *t*-butoxide (*18*), potassium carbonate (*19*), alumina (*20*), and piperidine (*21*). We have developed a simple and solvent-free method for the synthesis of *E*-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones (**3a**–**g**). The mild reaction conditions, clean reaction profiles, minimal side products, and cost efficiency render this approach as a useful alternative to the existing methods.

The required starting material 2-acetyl-3,5-dimethyl-6-benzoyl benzodifuran was synthesized by reacting 5-acetyl-2-benzoyl-6-hydroxy-3-methyl benzofuran (*22, 23*) with 2-chloroacetone in the presence of anhydrous K_2CO_3 under conventional and microwave irradiation methods.

The targeted molecules *E*-(*1*)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones (**3a**–**g**) were synthesized in excellent yields by condensing 2-acetyl-3,5-dimethyl-6-benzoyl benzodifuran with aromatic/heteroaromatic aldehydes in the presence of sodium hydroxide in ethanol under conventional heating and microwave irradiation. The reaction time has been brought down from hours to minutes using microwave-assisted synthesis, with improved yields. The antibacterial activity of synthesized *E*-(1)-(6-benzo

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yl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones (**3a**–**g**) was carried out by using cup plate method (Scheme 1).

Experimental

Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked routinely by the silica gel F_{254} (Merck). Microwave reactions were carried out in the Milestone MultiSYNTH microwave system. IR spectra were recorded on Shimadzu FTIR-8400s spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Avance 300 spectrometer, and mass spectra were recorded on Shimadzu mass spectrometer. Elemental analysis was determined by using ThermoFinnigan CHNS analyzer.

Experimental procedure

*General procedure for the synthesis of 2-acetyl-3,5-dimethyl-6-benzoyl benzodifuran (**2**)*

Microwave irradiation method. A thoroughly blended mixture of 5-acetyl-2-benzoyl-6-hydroxy-3-methyl benzofuran (**1**) (5 mmol), chloroacetone (5 mmol), and anhydrous potassium carbonate (**3g**) was taken in a quartz tube and inserted into a Teflon vial with screw capped and then subjected to microwave irradiation at the constant temperature 120°C for 4 min. Progress of the reaction was monitored by thin-layer chromatography (TLC). After completion of the reaction it was diluted with cold water, and the precipitate formed was filtered, washed with water, and recrystallized from dichlormethane (DCM)/pet ether (40–60°C) in 1:3 ratio.

Conventional heating method. A solution of 5-acetyl-2-benzoyl-6-hydroxy-3-methyl benzofuran (**1**) (0.03 mol), chloroacetone (0.03 mol), K_2CO_3 (30 g), and acetone (150 mL) was refluxed for 8 h. Progress of the reaction was monitored by TLC. After completion of the reaction, acetone was distilled under vacuum and chilled water was added to the residue. The precipitate formed was filtered, washed with water, and recrystallized from DCM/pet ether (40–60°C) in 1:3 ratio.

*General procedure for the synthesis of E-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[b]furan-2-yl)-3-(aryl)-2-propen-1-ones (**3a**–**g**)*

Microwave irradiation method. A thoroughly blended mixture of 2-acetyl-3,5-dimethyl-6-benzoyl benzodifuran (**2**) (0.001 mol), appropriate aromatic/heteroaromatic aldehydes (0.001 mol), and powdered NaOH (0.001 mol) was taken in a quartz tube and inserted into a Teflon vial with screw capped and then subjected to microwave irradiation at the constant temperature 90°C for 4–5 min. Progress of the reaction was monitored by TLC. After completion of the reaction it was diluted with cold water and acidified with dil. HCl. The precipitate formed was filtered, washed with water, and recrystallized from methanol as a yellow solid.

Conventional heating method. A mixture of 2-acetyl-3,5-dimethyl-6-benzoyl benzodifuran (**2**) (0.001 mol), appropriate aromatic/heteroaromatic aldehydes (0.001 mol), NaOH (0.001 mol), and EtOH (10 mL) was taken in a 50 mL round-bottom flask and then it was refluxed for 6–8 h. Progress of the reaction was monitored by TLC. After completion of the reaction it was diluted with cold water and acidified with dil. HCl. The precipitate formed was filtered, washed with water, and recrystallized from methanol as a yellow solid.

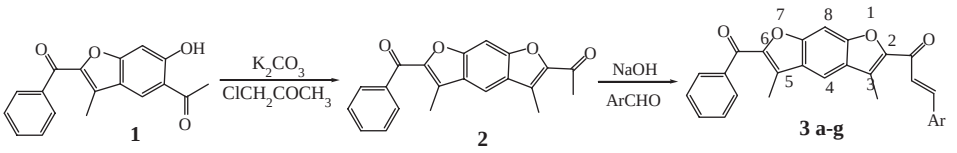
IR, NMR, and mass spectral data of 2 and (3a-g)

2: IR (KBr): 3076, 2923, 1672 (>C=O), 1643 (>C=O), 1622, 1600, 1568, 1502, 1494, 1448, 1423, 1361, 1315, 1263, 1218, 1182, 1166, 1141, 1128, 1110, 1076, 1010, 958, 941, 923 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 2.63 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 7.50–7.65 (m, 4H, C_8–H, C_3, C_4, C_5, –H), 7.86 (s, 1H, C_4–H), 8.05 (d, 2H, *ortho* protons of benzoyl ring). MS: $[M]^+$ m/z = 332 (100%).

3a: IR (KBr): 3058, 2962, 2923, 2852, 1654 (>C=O), 1623, 1604, 1566, 1496, 1448, 1425, 1371, 1357, 1325, 1259, 1201, 1163, 1105, 1016, 937 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 2.66 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.20 (s, 1H, Ar–H), 7.43 (d, 1H, α-H), 7.79 (s, 1H, Ar–H), 7.60–7.91 (m, 8H, Ar–H), 7.87 (d, 1H, β-H). MS: $[M+H]^+$ m/z = 421 (100%). Analysis calculated for $C_{28}H_{20}O_4$ (%): C 80.0, H 4.76. Found (%): C 81.4, H 4.85.

3b: IR (KBr): 3141, 3089, 3033, 2941, 1643 (>C=O), 1618, 1581, 1560, 1496, 1467, 1440, 1380, 1353, 1319, 1290, 1180, 1151, 1058, 1022, 983 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): 2.70 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 7.22 (s, 1H, Ar–H), 7.35 (d, 1H, $=CH_\alpha$),7.59–7.90 (m, 7H, Ar–H), 7.93 (d, 2H, Ar–H), 8.10 (d, 2H, Ar–H), 8.13 (d, 1H, β-H). MS: $[M+H]^+$ m/z = 455 (90%). Analysis calculated for $C_{28}H_{19}O_4Cl$ (%): C 73.92, H 4.18. Found (%): C 74.04, H 4.25.

3c: IR (KBr): 3058, 3004, 2929, 2853, 1654 (>C=O), 1643, 1623, 1596, 1566, 1512, 1446, 1423, 1373, 1357, 1325, 1255, 1197, 1172, 1105, 1027, 1012,



Ar = a) Phenyl
b) 2-Chlorophenyl
c) 4-Methoxy phenyl
d) 2- Furyl
e) α- Naphthyl
f) 1, 3-Diphenyl-1*H*-pyrazol-4-yl
g) 1-Phenyl-3-(p-bromophenyl)-1-*H*-pyrazol-4-yl

Scheme 1. Synthesis of *E*-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones (**3a**–**g**).

979 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 2.71 (s, 3H, CH_3), 2.77 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 6.95 (d, 1H, $=CH_\alpha$), 7.25 (s, 1H, Ar–H), 7.52–7.78 (m, 7H, Ar–H), 7.83 (s, 1H, Ar–H), 7.9 (d, 1H, β-H), 8.11 (d, 2H, Ar–H). ^{13}C NMR (300 MHz, DMSO-d_6): △ 9.35, 9.55, 9.85, 45.93, 54.96, 55.41, 94.93, 95.25, 113.81, 114.30, 114.58, 119.50, 122.96, 123.82, 124.47, 126.49, 126.93, 128.55, 129.22, 130.29, 132.83, 137.39, 142.16, 143.59, 148.18, 149.05, 153.93, 154.22, 157.72, 161.64, 180.50, 184.93. MS: $[M+H]^+$ $m/z=451$ (100%). Analysis calculated for $C_{29}H_{22}O_5$ (%): C 77.33, H 4.88. Found (%): C 77.48, H 4.85.

3d: IR (KBr): 3093, 3058, 2958, 2916, 1654 ($>C=O$), 1637, 1623, 1604, 1560, 1477, 1467, 1425, 1373, 1325, 1259, 1211, 1184, 1107, 1008, 970, 927 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 2.60 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.70 (d, 1H, β-H, furyl), 7.43 (d, 1H, β′-H, furyl), 7.11 (d, 1H, $=CH_\alpha$), 7.21 (s, 1H, Ar–H), 7.56–7.71 (m, 4H, 3, Ar–H and 1H, $=CH_\beta$), 7.93–7.96 (d, 2H–Ar–H), 8.05 (s, 1H, Ar–H), 8.29 (s, 1H, Ar–H). MS: $[M+H]^+$ $m/z=410$ (20%). Analysis calculated for $C_{26}H_{18}O_5$ (%): C 76.09, H 4.39. Found (%): C 76.18, H 4.39.

3e: IR (KBr): 3056, 2954, 2920, 1649 ($>C=O$), 1623, 1604, 1596, 1566, 1510, 1492, 1446, 1425, 1396, 1373, 1323, 1259, 1211, 1184, 1163, 1103, 1010, 937 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 2.65 (s, 3H, CH_3), 2.78 (s, 3H, CH_3), 7.61 (s, 1H, Ar–H), 7.63 (d, 1H, $=CH_\alpha$), 7.68–7.83 (m, 3H, Ar–H), 7.88 (d, 2H, Ar–H), 7.94 (d, 1H, $=CH_\beta$), 8.02–8.33 (m, 5H, Ar–H), 8.66 (d, 2H, Ar–H). MS: $[M+H]^+$ $m/z=471$ (100%). Analysis calculated for $C_{32}H_{22}O_4$ (%): C 81.70, H 4.68. Found (%): C 81.63, H 4.76.

3f: IR (KBr): 3056, 2960, 2916, 1647 ($>C=O$), 1623, 1596, 1560, 1541, 1506, 1446, 1411, 1373, 1323, 1257, 1010, 937 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 2.61 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 7.97 (s, 1H, Ar–H), 7.40 (d, 1H, $=CH_\alpha$), 7.52–7.73 (m, 14H, 13Ar–H and 1H, $=CH_\beta$), 8.0 (d, 2H, Ar–H), 8.33 (s, 1H, Ar–H), 9.45 (s, 1H, triazolyl-H). MS: $[M+H]^+$ $m/z=563$ (100%). Analysis calculated for $C_{37}H_{26}O_4N_2$ (%): C 79.0, H 4.62, N 4.98. Found (%): C 78.89, H 4.62, N 5.05.

3g: IR (KBr): 3056, 2950, 2916, 1649 ($>C=O$), 1623, 1596, 1566, 1533, 1500, 1446, 1413, 1323, 1257, 1105, 1008, 958, 937 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): 2.64 (s, 3H, CH_3), 2.72 (s, 3H, CH_3), 7.71 (s, 1H, Ar–H), 7.42 (d, 1H, $=CH_\alpha$), 7.55–7.68 (m, 12H, 11Ar–H and 1H, $=CH_\beta$), 8.01 (d, 2H, Ar–H), 8.39 (s, 1H, Ar–H), 9.5 (s, 1H, triazolyl-H). MS: $[M+H]^+$ $m/z=642$ (40%). Analysis calculated for $C_{37}H_{25}O_4N_2Br$ (%): C 69.24, H 3.90, N 4.36. Found (%): C 69.10, H 3.95, N 4.41.

Table 1. Physical and analytical data for *E*-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones.

		Conventional method		Microwave method	
Compound no.	M.P. (°C)	Time (h)	Yield (%)	Time (min)	Yield (%)
3a	242—244	6	68	5	92
3b	220—223	7	64	5	90
3c	270—271	7	65	4	94
3d	272—274	6	64	4	89
3e	246—248	8	66	5	92
3f	275—276	8	56	5	87
3g	255—257	8	53	5	93

Table 2. Antibacterial activity of compounds **3a**–**g** and inhibition zones.

	Gram–positive bacteria		Gram-negative bacteria	
Compound no.	*Bacillus subtilis* (ATCC-6633) (mm)	*Staphylococcus aureus* (ATCC-29737) (mm)	*Escherichia coli* (ATCC-10536) (mm)	*Pseudomonas aeruginosa* (ATCC-27853) (mm)
3a	15	10	9	10
3b	16	9	10	9
3c	10	8	7	7
3d	10	8	8	6
3e	9	7	7	8
3f	16	10	10	9
3g	17	10	11	10
Standard	22 (streptomycin)	15 (tetracycline)	13 (chloramphenicol)	13 (carbenicillin)

Antibacterial activity

All the compounds were screened for their antibacterial activity (*24*) against bacterial strains such as *Bacillus subtilis* (ATCC-6633), *Staphylococcus aureus* (ATCC-29737), *Escherichia coli* (ATCC-10536), and *Pseudomonas aeruginosa* (ATCC-27853) using streptomycin, tetracycline, chloramphenicol, and carbenicillin as standard drugs. The activity was determined using the cup-plate agar diffusion method by measuring the inhibition zone in millimeters. Nutrient agar was used as a culture medium. A 1 mg/mL solution in dimethylformamide was used. The agar medium was inoculated with bacterial cultures tested. After 24 h of incubation at 37°C, the diameter of inhibition zone (in millimeters) was measured. The results of the antibacterial activity are given in Table 2. Among the compounds screened, **3a**, **3b**, **3f**, and **3g** showed good activity against all bacteria. The remaining compounds (**3c**, **3d**, and **3e**) were found to be moderately active against all bacteria.

Conclusion

In conclusion, we have successfully synthesized *E*-(1)-(6-benzoyl-3,5-dimethyl furo[3′,2′:4,5]benzo[*b*]-*b*]furan-2-yl)-3-(aryl)-2-propen-1-ones (**3a**–**g**) under solvent-free microwave irradiation conditions. In this method there is no need to recover, purify, and reutilize the solvent, which reduces the pollution arising from such operations. This methodology provides an easily facile, economical, and environmentally benign synthesis in which the reaction time is reduced with better yields. The *E*-(1)-(6-benzoyl-3,5-dimethylfuro[3′,2′:4,5]benzo[*b*]furan-2-yl)-3-(aryl) -2-propen-1-ones exhibit moderate antibacterial activity.

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References

(1) Aruna Kumar, D.B.; Prakash, G.K.; Kumaraswamy, M.N.; Nandeshawarappa, B.P.; Sheringara, B.S.; Mahadevan, K.M. *Ind. J. Chem.* **2007,** *46B,* 336–343.
(2) Manna, K.; Agarwal, Y.K. *Bioorg. Med. Chem. Lett.* **2009,** *19* (10), 2688–2692.
(3) Urzúa, A.; Rezende, M.C.; Mascayano, C.; Vásquez, L. *Molecules*, **2008,** *13* (10), 882–891.
(4) Hayakawa, I.; Shioya, R.; Agatsuma, T.; Furukawa, H.; Naruto, S.; Sugano, Y. *Bioorg. Med. Chem. Lett.* **2004,** *14,* 455–458.
(5) Santana, L.; Teijeira, M.; Uriate, E.; Teran, C.; Linares, B.; Villar, R.; Laguna, R.; Cano, E. *Eur. J. Pharm. Sci.* **1999,** *7,* 161–166.
(6) Anto, J.R.; Sukumaran, K.; Kuttan, G.; Rao, M.N.A.; Subbaraju, V.; Kuttan, R. *Cancer Lett.* **1995,** *97,* 33–37.
(7) Soon, S.L.; Hye-Sook, K.; Dong-Ung, L. *Bull. Korean Chem. Soc.* **2007,** *28* (12), 2495–2497.
(8) Avila, H.P.; Smania, E.F.A.; Monache, F.D.; Smania, Jr., A. *Bioorg. Med. Chem.* **2008,** *16,* 9790–9794.
(9) Shen, J.W.; Chang, T.L.; Lo-ti, T.S.; Jing-Ru, W.; Horng-Heuy, K., Chun-Nan, L. *Eur. J. Med. Chem.* **2005,** *40,* 103–112.
(10) Yu, D.C.; Panfilova, L.V.; Boreko, E.I. *Pharm. Chem.* **1982,** *16,* 103–105.
(11) Batovska, D.; Parushev, S.; Stamboliysa, B.; Tsvetkova, I.; Ninova, M.; Najdenski, H. *Eur. J. Med. Chem.* **2009,** *44,* 2211–2218.

(12) Krishna Murthy, K.S.; Rajitha, B.; Kanakalingeswara Rao M. *Ind. J. Chem.* **2003,** *42B,* 425—428.
(13) Viano, I.; Dianzani, C.; Gia, O.; Gastaldi, S.; Genazzani, E. *Drugs, Exp. Clin. Res.* **1985,** *11* (12), 865–867.
(14) Ahluwalia, V.K.; Kidwai, M. *New Trends in Green Chemistry*; Anamaya: New Delhi, India, 2004; p 59 (and references cited therein).
(15) Satyanarayana, M.; Tiwari, P.; Tripathi, B.K.; Srivastava, A.K.; Pratap, R. *Bioorg. Med. Chem.* **2004,** *12,* 883–889.
(16) Bu, X.; Zhao, L.; Li, Y. *Synthesis* **1997,** *11*, 1246–1248.
(17) Sinisterra, J.V.; Garcia-Raso, A.; Cabello, J.A.; Marians, J.M. *Synthesis*, **1984,** *6*, 502—503.
(18) Rochus, W.; Kickuth, R. Potassium-carbon compounds as catalysts for base catalyzed organic reactions. German Patent 1 095 832, 1957.
(19) Guthrie, J.L.; Rabjhon, N. *J. Org. Chem.* **1957,** *22,* 176–179.
(20) Varma, R.S.; Kabalka, G.W.; Evans, L.T.; Pagani, R.M. *Synth. Commun.* **1985,** *15,* 279–284.
(21) Trivedi, J.C.; Bariwal, J.B.; Upadhyay, K.D.; Naliapara, Y.T.; Joshi, S.K.; Pannecouque, C.C.; Clercq, E.D.; Shah, A.K. *Tetrahedron Lett.* **2007,** *48,* 8472–8474.
(22) Sharada, J.; Ratna Kumari, Y.; Kanakalingeswara, R.M. *Ind. J. Chem.* **1986,** *25B,* 334–337.
(23) Vishnu Vardhan Reddy, K.; Sampath Rao, P.; Ashok, D. *Synth. Commun.* **2000,** *30* (10), 1825–1836.
(24) The United States Pharmacopeia, *Biological tests and assay*, 25th ed, Rockwille, MD, 2001.