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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antimicrobial screening of some novel chalcones and flavanones substituted with higher alkyl chains

Uppuluri Venkata Mallavadhani · Laxmidhar Sahoo · Koochana Pranay Kumar · Upadyayula Suryanarayana Murty

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Abstract As a part of our program to generate some novel flavonoid frameworks substituted with higher alkyl groups as possible antimicrobial agents, we have in total synthesized twelve novel chalcones (**11**–**16**) and their corresponding flavanones (**17**–**22**) substituted with either nonyl or dodecyl chains in ring B in very good to excellent yields. The synthesized compounds have been screened for their antimicrobial potential against six bacterial and four fungal strains. The tested compounds, in general, showed significant antibacterial and comparable antifungal activities. While the chalcone (**16**) with a dodecyl chain showed highly promising antibacterial activity against almost all the organisms tested, the chalcone (**13**) with nonyl chain showed promising antifungal activity against *Candida rugosa* and *Aspergillus niger* strains.

Keywords Chalcones · Flavanones · Nonyl chalcones · Nonyl flavanones · Dodecyl chalcones · Dodecyl flavanones · Antibacterial activity · Antifungal activity

Introduction

Chalcones and flavanones, the two important sub groups of flavonoids, are ubiquitous in the plant kingdom with many diverse functions including defense and UV protection (Buer *et al*., 2010). These compounds are reported to exhibit a wide spectrum of biological activities such as antimicrobial, antiviral, antihypertensive, antioxidant, anti-inflammatory, and cytotoxic (Nowakowska, 2007). Especially, their antimicrobial activity is gaining importance and increasingly documented. The antimicrobial effectiveness of chalcones and flavanones depends on their abilities to form complexes with both extracellular and soluble proteins as well as bacterial membranes, penetration into cell membranes, and the maintenance of intercellular concentrations in infecting species (Fowler *et al*., 2011). The antimicrobial activity of these compounds mainly attributed to the presence of phenolic hydroxyl groups, phenyl rings, and α,β-unsaturated ketones or benzopyrone rings, which have high affinity to proteins and thus may inhibit microbial enzymes (Cushnie and Lamb, 2005). It has been realized that certain degree of lipophilicity enhances the antimicrobial activity. The lipophilicity of chalcones and flavanones can be increased by substituting with higher alkyl chains (Cushnie and Lamb 2011). The two retrochalcones of liquorice viz. licochalcone A and licochalcone C, with 1,1-dimethallyl and 3,3-dimethally groups, respectively, were reported to exhibit potent antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, and Micrococcus luteus* (Haraguchi *et al*., 1998). Kromann *et al*. (2004) reported that the introduction of an hexyl group in licochalcone A significantly enhances its antibacterial activity against *S. aureus*. They further reported that a clear positive correlation is seen when the Clog P values are compared with antibacterial activity (Kromann *et al*., 2004). Stapleton *et al*. (2004) also reported that substitution with C8 and C10 alkyls enhances the activity of some flavonoids against *S. aureus*. Chalcones and flavanones substituted with higher alkyl chains especially in ring B are novel and expected to

U. V. Mallavadhani (✉) · L. Sahoo
Natural Products Chemistry Division, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 607, India
e-mail: uvmavadani@yahoo.com

Present Address:
L. Sahoo
Sai Life Sciences, Hyderabad, India

K. P. Kumar · U. S. Murty
Biology Division, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500607, India

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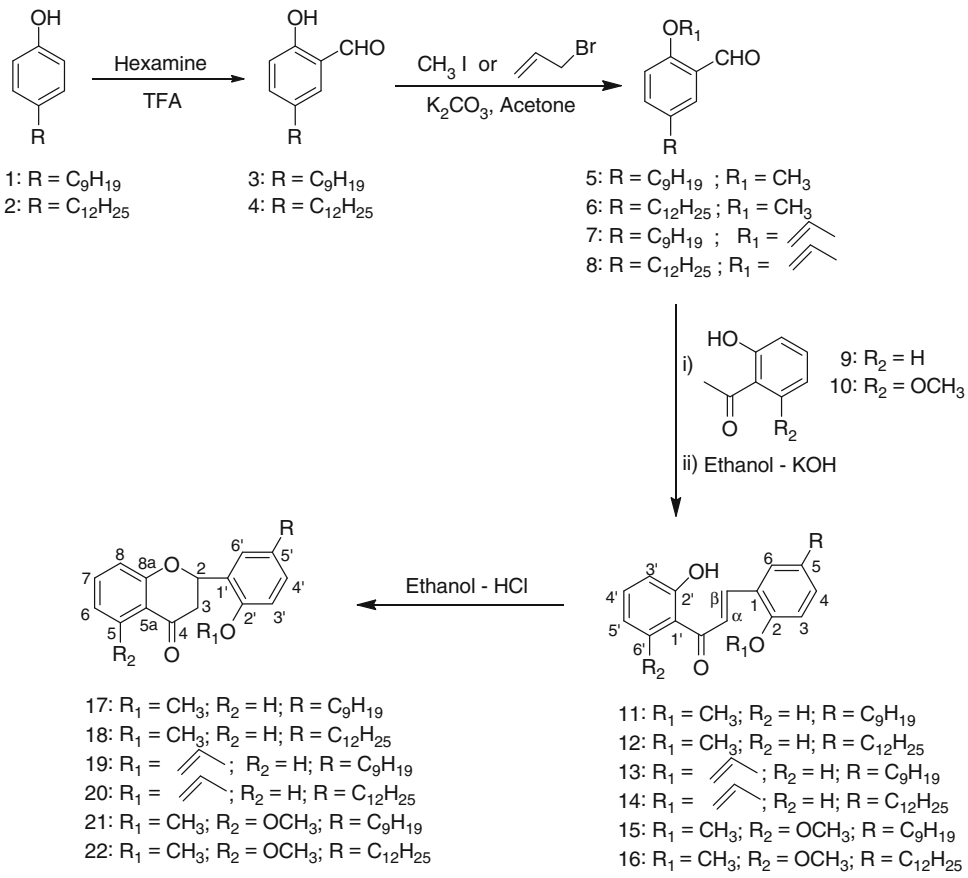
exhibit potent antimicrobial activities. To synthesize these frameworks, the alkyl substitution has to come from the benzaldehyde core. For this purpose, 2-hydroxy-5-nonyl-benzaldehyde and 5-dodecyl-2-hydroxy-benzaldehyde were found to be the highly attractive templates. In fact, these two benzaldehydes are the key precursors for the synthesis of liquid ion exchange reagents, which are highly useful in hydrometallurgy for extraction of precious metals like Cu and Ni (Sperline *et al*., 1998; Cheng *et al*., 2000; Gordon *et al*., 2008; Pouramini and Moradi, 2012). Further, these two benzaldehydes can be conveniently prepared from the commercially available 4-nonyl-phenol and 4-dodecyl-phenol respectively. With this background, we have now synthesized a series of twelve rings B substituted nonyl and dodecyl chalcones and flavanones from the two key 4-alkyl-phenols and screened for their antimicrobial potential.

Results and discussion

Chemistry

The two key precursor benzaldehydes viz. 2-hydroxy-5-nonyl-benzaldehyde and 5-dodecyl-2-hydroxy-benzaldehyde were prepared from the commercially available 4-nonyl and 4-dodecyl phenols by modified Duff method using hexamine and trifluoroacetic acid (Blazevic *et al*., 1979). In this connection, it is to mention here that the commercial 4-nonyl-phenol and 4-dodecyl-phenol contain both the ring and side-chain isomers. Repetitive chromatographic purification of these two phenols removes the ring impurities completely and afforded pure nonyl and dodecyl phenols with the alkyl substituents as inseparable linear and branched isomeric mixtures. Hence, the purified 4-nonyl-phenol and 4-dodecyl- phenol with mixed isomeric chains have been taken up for the synthesis. Thus the two synthesized o-hydroxybenzaldehydes or their corresponding O-alkyl ethers on condensation with an appropriate 2-hydroxyacetophenone in the presence of 60 % ethanolic KOH yielded the chalcones substituted with nonyl or dodecyl groups in ring B. The resulting chalcones on acid catalyzed cyclization afforded the corresponding flavanones (Marais *et al*., 2006). In total, 6 flavanones with three different frameworks, such as i) 2′-methoxy, ii) 2′-allyloxy, and iii) 2′,5-dimethoxy, were synthesized from their corresponding chalcones (Scheme 1).

All the synthesized compounds have been characterized by their spectroscopic data (IR, ^{1}H, and ^{13}C NMR and Mass) (Mandge *et al*., 2007; Hwang *et al*., 2011; Yoon *et al*., 2011). While chalcones **11**–**16** showed characteristic



Scheme 1 Synthesis of novel alkyl substituted chalcones and flavanones

α,β-unsaturated carbonyl absorptions around 1640–1630 cm^{-1} in IR and α and β protons as doublets around 7.75–7.90 and δ 8.2–8.1, respectively, with coupling constants of 16 Hz each in ^{1}H NMR, the flavanones **17–22** showed the carbonyl absorption around 1690 cm^{-1} in IR and H-2 and H-3 protons around δ 5.9–5.80 and 3.0–2.9, respectively, in ^{1}H NMR and C_2 and C_3 around δ 75.0–74.0 and 45.0–44.0, respectively, in ^{13}C NMR. The nonyl and dodecyl chains (mixed isomers) showed the characteristic peaks in NMR between 0.6 and 1.8 (^{1}H) and 8.40–52.70 (^{13}C).

Antibacterial screening

The twelve synthesized chalcones and flavanones (**11–22**) have been subjected to antibacterial screening against six bacterial strains such as *B. subtilis, S. aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* by employing broth dilution method (Clinical and Laboratory standards Institute, 2008) with penicillin G and streptomycin as standards. The microorganisms were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures of test organism were maintained on nutrient agar (Himedia labs, Mumbai) slants and were sub cultured in Petri dishes prior to testing the antimicrobial activity was expressed in terms of MIC values, which are the lowest concentrations able to inhibit the bacterial growth. The results are presented in Table 1 (Fig. 1). All the tested compounds, in general, showed significant antibacterial activity. The highly active compounds for each organism are: (i) *B. subtilis*—**11, 12, 16, 17, 18, 22** (ii) *S. aureus*—**15, 16** (iii) *S. epidermidis*—**16, 21, 22** (iv) *E. coli*—**16** (v) *P. aeruginosa*—**16** (vi) *K. pneumoniae*—**16**. From this analysis, it is evident that the nonyl and dodecyl chalcones with 2,6′-dimethoxy-2′-hydroxy substitution patterns such as compounds **15** and **16** are highly active than the other synthesized compounds. Further, it is interesting to note that the dodecyl chalcone (**16**) is the most active one and showed promising antibacterial activity against all the six organisms tested.

Antifungal screening

The twelve synthesized novel chalcones and flavanones have been subjected to antifungal screening against four fungal strains such as *Candida albicans, Candida rugosa, Saccharomyces cerevisiae*, and *Aspergillus niger* by employing Agar cup method (Linday, 1962) with amphotericin-B as standard. The organisms were obtained from Institute of Microbial Technology, Chandigarh, India. Cultures of test organisms were maintained on Potato Dextrose Agar (PDA) slants and were sub cultured in Petri dishes prior to testing. The antifungal activity was expressed in terms of inhibition of zones, expressed in mm diameters, and the results are presented in Table 2; (Fig. 2).

Surprisingly, compounds **12, 13,** and **19** only showed antifungal activity. While compounds **13** and **19** showed significant activity against 2 organisms, compound **12** showed moderate activity against one organism. Compounds **13** and **19** are the allyloxy-nonyl substituted chalcone and its corresponding flavanone, respectively.

Table 1 Antibacterial activity of alkyl substituted chalcones and flavanones

	MIC(μg/mL)					
Compound	*Bacillus subtilis*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
11	75	37.5	150	150	150	150
12	75	150	150	150	150	150
13	150	150	150	150	150	150
14	150	150	150	150	150	150
15	150	18.75	150	150	150	150
16	75	18.75	75	37.5	37.5	75
17	75	37.5	150	150	150	150
18	75	75	150	150	150	150
19	150	150	150	150	150	150
20	150	150	150	150	150	150
21	150	75	75	150	150	150
22	75	37.5	75	150	75	150
Penicillin	1.562	1.562	3.125	2.5	12.5	6.25
Streptomycin	6.25	6.25	3.125	6.25	1.562	3.125

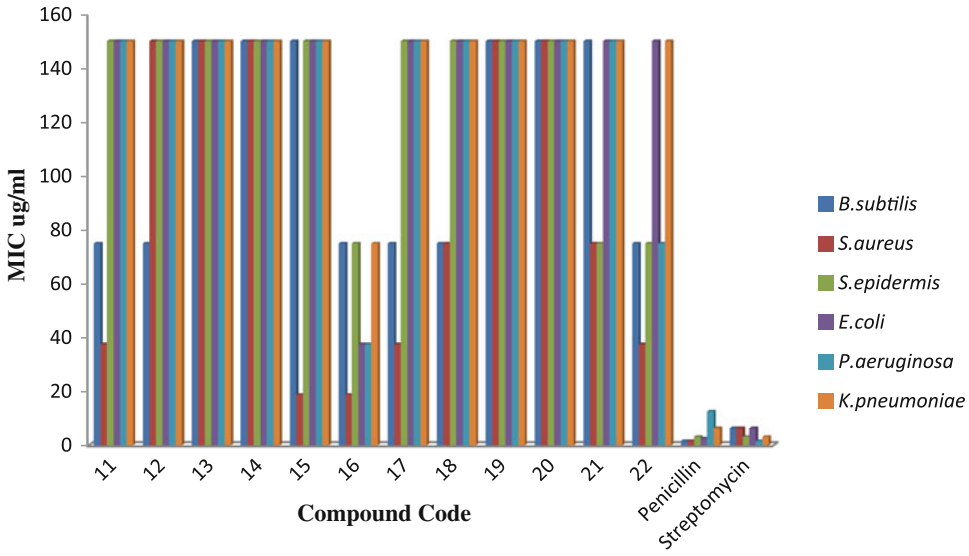


Fig. 1 Antibacterial activity of alkyl substituted chalcones and flavanones

Table 2 Antifungal activity of alkyl substituted chalcones and flavanones

	Zone of Inhibition (mm)							
Compound	*Candida albicans*		*Candida rugosa*		*Saccharomyces cerevisiae*		*Aspergillus niger*	
	100 µg	150 µg	100 µg	150 µg	100 µg	150 µg	100 µg	150 µg
12	0	0	9	0	0	0	0	0
13	0	0	11	16	0	0	8	12
19	0	0	9	13	0	0	8	12
Amphotericin-B (50 µg)	23.5		21		22		25	

Compounds 11, 14–18 and 20–22 did not show any antifungal activity

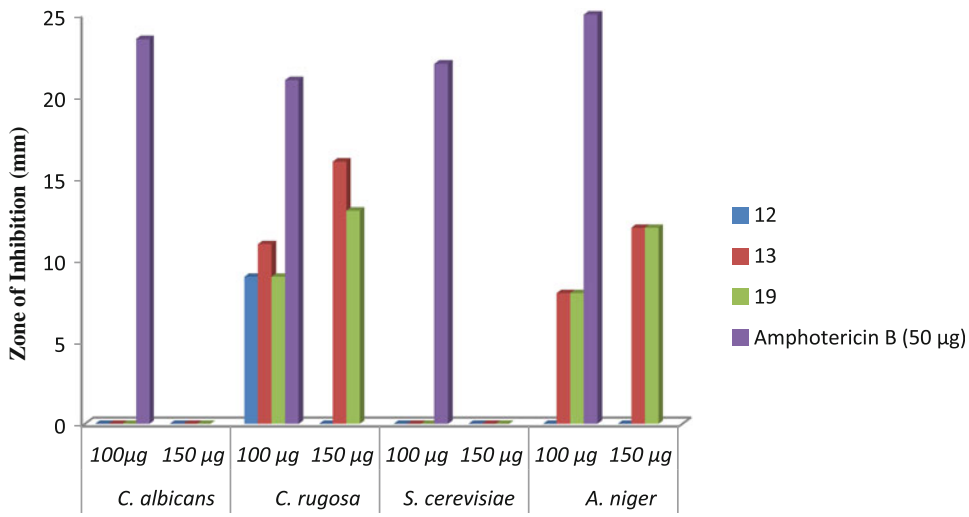


Fig. 2 Antifungal activity of alkyl substituted chalcones and flavanones

Interestingly, these two compounds also found selective in exhibiting antifungal activity against *C. rugosa* and *A. niger*. The highest antifungal activity (16 mm zone inhibition) was found with Compound **13**, when tested against the organism *C. rugosa* at 150 µg concentration.

Conclusion

In conclusion, a series of 12 novel nonyl and dodecyl ring B substituted chalcones and flavanones were synthesized from the two precursor aldehydes viz. 2-hydroxy-5-nonyl-

benzaldehyde and 5-dodecyl-2-hydroxy-benzaldehyde in very good to excellent yields. The synthesized compounds have been screened against six bacterial and four fungal strains. In general, the synthesized compounds showed significant antibacterial activity and comparable antifungal activity. Significantly, the chalcone (**16**) with a dodecyl chain showed highly promising antibacterial activity against almost all the organisms tested. Whereas, the chalcone (**13**) with nonyl chain showed promising antifungal activity against the *C. rugosa* and *A. niger* strains. Thus the two compounds **13** and **16** can be considered as lead compounds and can be fine tuned further to develop some highly potent antimicrobial agents.

Experimental

General

4-Nonylphenol and 4-dodecylphenol were procured from Herdillia Chemicals Ltd., Mumbai, India and purified by column chromatography before use. All the reagents and solvents were purchased from commercial suppliers and used after purification (distillation/crystallization). Purity of the compounds was routinely checked by thin layer chromatography (TLC) and ^{1}H NMR. TLC was performed on silica gel-coated plates, and the components were visualized under UV light or exposing to Iodine vapors or by spraying with methanolic sulfuric acid (5 % v/v) followed by heating the plates at $\sim$105 °C in an oven/hot plate. IR spectra were recorded on JASCO FT IR- 5300 or Perkin Elmer Spectrum 1000 spectrophotometers. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 360 and DPX-300 Spectrometers using TMS as internal standard. Positive ion electronspray mass spectra and high resolution mass spectra were obtained with Micromass Quattro II triple quadrupole spectrometer and Micromass Q-Tof 2 Mass spectrometer.

*2-Hydroxy-5-nonyl-benzaldehyde (**3**)*

To a mixture of 4-nonylphenol (10 g, 0.045 mol) and hexamine (12.7 g, 0.090 mol), trifluoroacetic acid (34.98 mL, 0.454 mol) was added drop wise. The resulting reaction mixture was heated on a water bath at 75 °C for 10 h. After work up followed by silica gel column chromatography gave 2-hydroxy-5-nonyl-benzaldehyde as pale reddish oil (4.5 g, 40 %), IR (Neat, cm^{-1}): 2961, 1658, 1485, 1284 and 756; ^{1}H NMR ($CDCl_3$): δ 0.4–1.8 (m, 19 H), 6.9 (d, J = 8.9 Hz, 1H, Ar–H), 7.25–7.60 (m, 2H, Ar–H), 9.9 (s, 1H, –CHO) and 10. 90 (s, 1H, –OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 196.73 (–CHO), 159.24 (C_2), 139.46 (C_5), 135.75 (C_1), 135.24 (C_4), 130.68 (C_6), 116.96 (C_3), 51.41–8.47 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 249 (M^++H).

*5-Dodecyl-2-hydroxy-benzaldehyde (**4**)*

To a mixture of 4-dodecylphenol (10 g, 0.038 mol) and hexamine (10.68 g, 0.067 mol), trifluoroacetic acid (29 mL, 0.381 mol) was added drop wise. The resulting reaction mixture was heated on a water bath at 75 °C for 10 h. After work up followed by silica gel column chromatography gave 5-dodecyl-2-hydroxybenzaldehyde as pale reddish oil (4.42 g, 40 %). IR (Neat, cm^{-1}): 2961, 1658, 1485, 1284 and 756; ^{1}H NMR ($CDCl_3$): δ 0.4–1.8 (m, 25 H), 6.9 (d, J = 8.9 Hz, 1H, Ar–H), 7.25–7.60 (m, 2H, Ar–H), 9.9 (s, 1H, –CHO) and 10. 90 (s, 1H, –OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 196.68 (–CHO), 159.20 (C_2), 139.51 (C_5), 135.78 (C_1), 135.19 (C_4), 130.65 (C_6), 116.90 (C_3), 51.40-8.45 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 291 (M^++H).

*2-Methoxy-5-nonyl-benzaldehyde (**5**)*

A mixture of 2-hydroxy-5-nonyl-benzldehyde (**3**, 700 mg, 2.82 mmol), methyl iodide (0.27 mL, 4.23 mmol), and anhydrous K_2CO_3 (778 mg, 5.64 mmol) in dry acetone (10 mL) was refluxed over night. The solvent acetone was removed under reduced pressure, and crushed ice was added to the residue. The resulting product was extracted with ethyl acetate (3 × 25 ml), the organic layer washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with hexane–ethyl acetate (98:2) as eluent to yield compound **5** as colorless oil, 591 mg (yield: 80 %). R_f: 0.23 (n-hexane–ethyl acetate 95:5); IR (Neat, cm^{-1}): 1640 (C=O). ^{1}HNMR (200 MHz) ($CDCl_3$) :δ 0.6–1.7 (m, 19H, alkyl chain), 3.95 (s, –OCH_3, 3H), 6.90 (d, J = 12.0 Hz, 1H0, 7.54 (m, 1H), 7.78 (m, 1H), 10.45 (s, –CHO, 1H). ^{13}C NMR (300 Hz) ($CDCl_3$): 190.15 (–CHO), 159.67 (C_2), 134.21 (C_5), 133.85 (C_4), 125.64 (C_1), 124.06 (C_6), 111.04 (C_3), 55.45 (–OCH_3), 51.44–8.50 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 263 (M^++H).

*5-Dodecyl-2-methoxy-benzaldehyde (**6**)*

This compound was prepared from the aldehyde **4** as per the above procedure. Obtained as colorless oil, yield: 83 %, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.8 (m, 25H, alkyl chain), 3.95 (s, –OCH_3, 3H), 6.98 (d, J = 8.4 Hz, 1H), 7.54 (m, 1H), 7.82(m, 1H), 10.48 (s, –CHO, 1H). Mass (*m/z*): 304 $[M+H]^+$. ^{13}C NMR (300 Hz) ($CDCl_3$): 190.20 (–CHO), 159.79 (C_2), 134.36 (C_5), 133.67 (C_4), 126.72 (C_1), 123.94 (C_6), 111.04 (C_3), 55.59 (–OCH_3), 50.40–8.50

($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 305 (M^++H).

*2-Allyloxy-5-nonyl-benzaldehyde (**7**)*

A mixture of 2-hydroxy-5-nonyl-benzldehyde (**3,** 1.0 g, 4.0 mmol), allyl bromide (0.34 mL, 4.0 mmol), and anhydrous K_2CO_3 (1.1 g, 8.0 mmol) in dry acetone (10 mL) was refluxed for 8 h on a water bath. The completion of the reaction was monitored by TLC. The solvent acetone was removed under reduced pressure, and crushed ice was added to the residue. The resulting product was extracted with ethyl acetate (3 × 25 ml); the organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography with hexane–ethyl acetate (98:2) as eluent to yield compound **7** as colorless oil, 1.0 gm (yield: 86 %). R_f: 0.43 (n-hexane–ethyl acetate 95:5); IR (Neat, cm^{-1}): 1680, 1640. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.5–1.8 (s, 19H, alkyl chain), 4.70 (d, J = 6 Hz, 2H), 5.40 (m, 2H), 6.10 (m, 1H), 6.90 (d, J = 10 Hz, 1H), 7.45 (m, 1H), 7.8 (m, 1H), 10.50 (s, 1H). ^{13}C NMR (300 Hz) ($CDCl_3$): 190.10 (–CHO), 158.75 (C_2), 134.35 (C_5), 134.05 (C_4), 133.85 (C_6), 133.49 (C_1), 132.52 (=CH_2), 117.75 (–CH=), 112.30 (C_3), 69.10 (–OCH_2–), 50.65–8.50 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 289.1 (M^++H).

*2-Allyloxy-5-dodecyl-benzaldehyde (**8**)*

This compound was prepared from the aldehyde **4** as per the above procedure. It was obtained as colorless oil, (Yield: 88 %), IR: (Neat, cm^{-1}): 1680, 1640. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.4–1.7 (s, 25H, alkyl chain), 4.62 (d, J = 6 Hz, 2H), 5.38 (dd, J = 16, 8.7 Hz, 1H), 5.41 (m, 1H), 6.15 (m, 1H), 6.91 (d, J = 8.6 Hz, 1H), 7.47 (m, 1H), 7.8 (d, J = 9.4 Hz, 1H), 10.50 (s, 1H). ^{13}C NMR (300 Hz) ($CDCl_3$): 190.02 (–CHO), 158.79 (C_2), 134.40 (C_5), 134.09 (C_4), 133.82 (C_1), 133.50 (C_6), 132.52 (=CH_2), 117.78 (–CH=), 112.34 (C_3), 69.09 (–OCH_2–), 50.64–8.53 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 331 (M^++H).

*1-(2′-Hydroxy-phenyl)-3-(2-methoxy-5-nonyl-phenyl)-propen-1-one (**11**)*

A mixture of 2-hydroxyacetophenone (**9**,186 mg, 1.37 mmol) and 2-methoxy-5-nonyl- benzaldehyde (**5**, 400 mg, 1.52 mmol) was stirred in 10 mL of 60 % ethanolic KOH for 48 h. Ethanol was removed under reduced pressure. The reaction mixture was neutralized with dilute HCl and extracted with EtOAc. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography with hexane–ethyl acetate (98:2) as eluent to yield compound **11** as a colorless oil, 360 mg (Yield: 60 %), R_f: 0.21 (n-hexane–ethyl acetate 95:5). IR (Neat, cm^{-1}): 1640 (C=O). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.8 (m, 19H, alkyl chain), 3.96 (s, 3H, –OMe), 6.80 (t, 2H), 7.06 (d, J = 8.0 Hz, 1H), 7.38 (m, 1H), 7.54 (t, 2H), 7.85 (d, J = 16.0, 1H), 7.85 (d, J = 8.0, 1H), 8.10 (d, J = 16, 1H), 13.0 (s, 1H, OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 190.33 (–C=O), 110.67 (C_1), 156.73 (C_2), 118.46 (C_3), 129.72 (C_4), 136.03 (C_5), 129.72 (C_6), 142.25 (α-C), 120.57 (β-C), 120.57 ($C_{1'}$), 163.48 ($C_{2'}$), 118.68 ($C_{3'}$), 136.03 ($C_{4'}$), 120.57 ($C_{5'}$), 129.72 ($C_{6'}$), 55.52 (2–OCH_3), 52.20–8.50 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 381.1 (M^++H). Anal. calcd for $C_{25}H_{32}O_3$: C, 78.89; H, 8.48. Found: C, 78.92; H, 8.52.

*1-(2′-Hydroxy-phenyl)- 3-(5-dodecyl-2-methoxy-phenyl)-propen-1-one (**12**)*

This compound was prepared from the aldehyde **6** as per the above procedure. Yield: 54 %, IR (Neat, cm^{-1}): 1640. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.6 (m, 25H, alkyl chain), 3.94 (s, 3H, –OCH3), 6.90 (m, 3H), 6.96 (d, J = 8.4 Hz, 1H), 7.42–7.56 (m, 1H), 7.78 (d, J = 10.0 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 16.0 Hz, 1H), 13.00 (s, 1H, OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 194.49 (–C=O), 110.68 (C_1), 156.92 (C_2), 118.48 (C_3), 129.72 (C_4), 136.01 (C_5), 129.72 (C_6) 142.39 (α-C), 120.58 (β-C), 120.21 ($C_{1'}$), 163.52 ($C_{2'}$), 118.68 ($C_{3'}$), 136.01 ($C_{4'}$), 120.58 ($C_{5'}$), 129.72 ($C_{6'}$), 55.53 (–OCH_3), 52.49–8.64 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 423 (M^++H). Anal. calcd for $C_{28}H_{38}O_3$: C, 79.07; H, 9.28. Found: C, 79.11; H, 9.32.

*1-(2′-Hydroxy-phenyl)-3-(2-allyloxy-5-nonyl-phenyl)-propen-1-one (**13**)*

This compound was prepared from the aldehyde **7** as per the above procedure. Yield: 60 %, ^{1}H NMR ($CDCl_3$, 200 MHz) : δ 0.6–1.8 (m, 19H, alkyl chain), 2.34 (d, J = 8.0 Hz, 2H), 4.60 (d, J = 5.0 Hz, 2H), 5.40 (m, 2H), 6.20 (m, 1H), 6.90 (m, 3H), 7.02 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 5.0 Hz, 1H), 7.54 (s,1H), 7.90 (d, J = 16 Hz, 2H), 8.20 (d, J = 16.0 Hz, 1H), 13.0 (s, 1H, OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 194.60 (–C=O), 111.86 (C_1), 155.25 (C_2), 118.07 (C_3), 129.65 (C_4), 136.54 (C_5), 129.65 (C_6), 142.15 (α-C), 120.50 (β-C), 121.15 ($C_{1'}$), 163.55 ($C_{2'}$), 118.35 ($C_{3'}$), 136.54 ($C_{4'}$), 121.15 ($C_{5'}$), 129.65 ($C_{6'}$), 132.95 (=CH_2), 118.64 (–CH=), 69.28 (–OCH_2–), 52.70–8.40 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 407.3 (M^++H). Anal. calcd for $C_{27}H_{34}O_3$: C, 79.75; H, 8.43. Found: C, 79.78; H, 8.47.

*1-(2′-Hydroxy-phenyl)-3-(2-allyloxy-5-dodecyl-phenyl)-propen-1-one (**14**)*

This compound was prepared from the aldehyde **8** as per the above procedure. Yield : 55 %, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.6 (m, 25H, alkyl chain), 4.62 (d, $J = 8.0$ Hz, 2H), 5.48 (m, 2H), 6.20 (m, 1H), 6.82 (d, $J = 8.6$ Hz, 2H), 7.04 (m, 1H), 7.55 (m, 2H), 7.94 (m, 3H), 13.0 (s, 1H, -OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 194.42 (–C=O), 111.96 (C_1), 155.93 (C_2), 118.17 (C_3), 129.70 (C_4), 136.04 (C_5), 129.70 (C_6), 142.45 (α-C), 120.23 (β-C), 120.95 ($C_{1'}$), 163.46 ($C_{2'}$), 118.47 ($C_{3'}$), 136.04 ($C_{4'}$), 120.95 ($C_{5'}$), 129.70 ($C_{6'}$), 132.95 (=CH_2), 118.64 (–CH=), 69.28 (–OCH_2–), 52.70–8.44 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 449.2 (M^++H). Anal. calcd for $C_{30}H_{40}O_3$: C, 80.30; H, 8.99. Found: C, 80.32; H, 8.92.

*1-(2′-Hydroxy-6′-methoxy-phenyl)-3-(2-methoxy-5-nonyl-phenyl)-propen-1-one (**15**)*

This compound was prepared from the aldehyde **5** and the acetophenone **10** as per the above procedure. Yield: 75 %, IR (Neat, cm^{-1}): 1684, 1640. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.8 (m, 19H), 3.90 (s. 3H), 3.95 (s, 3H), 6.40 (d, $J = 8.4$ Hz, 1H), 6.62 (d, $J = 8.4$ Hz 1H), 6.86 (d, $J = 8.4$ Hz, 1H), 7.40 (t, 2H), 7.54 (m, 1H), 7.95 (d, $J = 16.0$ Hz, 1H), 8.15 (d, $J = 16.0$ Hz), 13.29 (s, 1H, OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 194.82 (–C=O), 111.17 (C_1), 156.59 (C_2), 111.17 (C_3), 129.94 (C_4), 135.72 (C_5), 127.84 (C_6), 139.65 (α-C), 123.27 (β-C), 111.17 ($C_{1'}$), 164.84 ($C_{2'}$), 112.40 ($C_{3'}$), 135.72 ($C_{4'}$), 101.53 ($C_{5'}$), 160.81 ($C_{6'}$), 55.65 (2′-OCH_3), 55.42 (2-OCH_3), 51.67-8.61 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 411 (M^++H). Anal. calcd for $C_{26}H_{34}O_4$: C, 76.35; H, 8.35. Found: C, 76.37; H, 8.37.

*1-(2′-Hydroxy-6′-methoxy-phenyl)-3-(5-dodecyl-2-methoxy-phenyl)-propen-1-one (**16**)*

This compound was prepared from the aldehyde **6** and the acetophenone **10** as per the above procedure. Yield: 54 %. IR (Neat, cm^{-1}): 1635. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.6 (m, 25H), 3.90 (s, 3H), 3.94 (s. 3H), 6.40 (d, $J = 16$ Hz, 1H), 6.60 (d, $J = 16$ Hz 1H), 6.84 (d, $J = 8.4$ Hz, 1H), 7.26 (m, 2H), 7.60 (m, 1H), 7.95 (d, $J = 16$ Hz, 1H), 8.10 (d, $J = 16$ Hz), 13.29 (s, 1H, OH). ^{13}C NMR (300 Hz) ($CDCl_3$): 194.84 (–C=O), 111.19 (C_1), 156.78 (C_2), 111.19 (C_3), 129.60 (C_4), 135.56 (C_5), 127.68 (C_6), 139.78 (α-C), 123.29 (β-C), 111.19 ($C_{1'}$), 164.85 ($C_{2'}$), 112.41 ($C_{3'}$), 135.56 ($C_{4'}$), 101.54 ($C_{5'}$), 161.00 ($C_{6'}$), 55.68 (2′-OCH_3), 55.46 (2–OCH_3), 51.77–8.63 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 453 (M^++H). Anal. calcd for $C_{29}H_{40}O_4$: C, 76.94; H, 8.91. Found: C, 76.96; H, 8.93.

*2,3-Dihydro-2-(2′-methoxy-5′-nonyl-phenyl)-4H-1-benzopyran-4-one (**17**)*

A mixture of chalcone **11** (125 mg, 0.328 mmol) and conc. HCl (1 mL) in ethanol (10 mL) was refluxed for 48 h on a water bath. Ethanol was distilled off under reduced pressure, and the resulting residue was extracted with ethyl acetate (3 × 25 mL). The combined ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography with hexane–ethyl acetate (98:2) as eluent to yield compound **17** as colorless oil, 60 mg (yield: 48 %), R_f: 0.23 (n-hexane–ethyl acetate 95:5). IR (Neat, cm^{-1}): 1695. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.8 (m, 19H, alkyl chain), 2.96 (d, $J = 8.2$ Hz, 2H), 3.82 (s. 3H), 5.84 (t, 1H), 6.82 (d, $J = 8.8$ Hz 1H), 7.04 (m, 2H), 7.52 (m, 3H), 7.98 (d, $J = 7.8$ Hz, 1H). ^{13}C NMR (200 Hz) ($CDCl_3$): 193.00 (–C=O), 75.00 (C_2), 44.00 (C_3), 127.13 (C_5), 121.33 (C_6), 135.97 (C_7), 118.13 (C_8), 121.33 (C_{5a}), 162.20 (C_{8a}), 127.13 ($C_{1'}$), 152.60 ($C_{2'}$), 109.94 ($C_{3'}$), 129.75 ($C_{4'}$), 135.97 ($C_{5'}$), 129.75 ($C_{6'}$), 55.50 (2′-OCH_3), 52.50–8.55 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 381 (M^++H). Anal. calcd for $C_{25}H_{32}O_3$: C, 78.89; H, 8.48. Found: C, 78.92; H, 8.52.

*2,3-Dihydro-2-(5′-dodecyl-2′-methoxy-phenyl)-4H-1-benzopyran-4-one (**18**)*

This compound was prepared from the chalcone **12** as per the above procedure. Yield: 83 %, IR (Neat, cm^{-1}): 1695. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.7 (m, 25H, alkyl chain), 2.95 (d, $J = 8.0$ Hz, 2H), 3.80 (s, 3H, –OCH3), 5.90 (t, $J = 8.0$ Hz, 1H), 6.80 (d, $J = 8.0$ Hz, 1H), 7.04 (m, 2H), 7.50 (m, 3H), 7.90 (d, $J = 8.0$ Hz, 1H). ^{13}C: 192.47 (–C=O), 74.90 (C_2), 43.80 (C_3), 126.42 (C_5), 121.15 (C_6), 135.70 (C_7), 118.06 (C_8), 121.15 (C_{5a}), 161.98 (C_{8a}), 126.42 ($C_{1'}$), 153.35 ($C_{2'}$), 109.75 ($C_{3'}$), 126.90 ($C_{4'}$), 135.70 ($C_{5'}$), 126.90 ($C_{6'}$), 55.24 (2′–OCH_3), 52.00-8.67 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 423 (M^++H). Anal. calcd for $C_{28}H_{38}O_3$: C, 79.07; H, 9.28. Found: C, 79.11; H, 9.32.

*2,3-Dihydro-2-(2′-allyloxy-5′-nonyl-phenyl)-4H-1-benzopyran-4-one (**19**)*

This compound was prepared from the chalcone **13** as per the above procedure. Yield: 45 %,^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.54–1.8 (m, 19H, alkyl chain), 3.0 (d, $J = 8.0$ Hz, 2H), 4.60 (d, $J = 5.0$ Hz, 2H), 5.30 (m, 2H), 5.90 (t, 1H), 6.0 (m, 1H), 6.80 (d, $J = 4.6$ Hz, 1H), 7.16 (m, 3H), 7.52 (m, 2H), 8.00 (d, $J = 8.2$ Hz, 1H). ^{13}C NMR (300 Hz) ($CDCl_3$): 192.50 (–C=O), 74.92 (C_2), 43.62 (C_3), 126.60 (C_5), 121.00 (C_6), 133.06 (C_7), 117.55 (C_8), 121.40

(C_{5a}), 162.10 (C_{8a}), 126.39 ($C_{1'}$), 152.50 ($C_{2'}$), 111.11 ($C_{3'}$), 126.92 ($C_{4'}$), 135.85 ($C_{5'}$), 126.92 ($C_{6'}$), 133.06 (=CH_2), 118.18 (–CH=), 68.82 (–OCH_2–), 50.95-8.70 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*): 407.3 (M^++H). Anal. calcd for $C_{27}H_{34}O_3$: C, 79.75; H, 8.43. Found: C, 79.78; H, 8.47.

*2,3-Dihydro-2-(2′-allyloxy-5′-dodecyl-phenyl)-4H-1-benzopyran-4-one (**20**)*

This compound was prepared from the chalcone **14** as per the above procedure. Yield: 50 %,^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.7 (m, 25H, alkyl chain), 3.0 (d, $J = 8.0$ Hz, 2H), 4.60 (d, $J = 8.5$ Hz, 2H), 5.3 (t, 1H), 5.4 (m, 21H), 5.88 (t, 1H), 6.0 (m, 1H), 6.82 (d, $J = 8.6$ Hz, 1H), 7.10 (m, 3H), 7.52 (m, 2H), 7.96 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (300 Hz) ($CDCl_3$): 192.64 (–C=O), 74.94 (C_2), 43.82 (C_3), 126.65 (C_5), 121.03 (C_6), 133.06 (C_7), 117.34 (C_8), 121.29 (C_{5a}), 162.09 (C_{8a}), 126.49 ($C_{1'}$), 152.42 ($C_{2'}$), 111.01 ($C_{3'}$), 126.97 ($C_{4'}$), 135.89 ($C_{5'}$), 126.97 ($C_{6'}$), 133.06 (=CH_2), 118.18 (–CH=), 68.82 (–OCH_2), 50.92–8.76 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 449.2 (M^++H). Anal. calcd for $C_{30}H_{40}O_3$: C, 80.30; H, 8.99. Found: C, 80.32; H, 8.92.

*2,3-Dihydro-2-(2′-methoxy-5′-nonyl-phenyl)-5-methoxy-4H-1-benzopyran-4-one (**21**)*

This compound was prepared from the chalcone **15** as per the above procedure. Yield: 53 %, IR (Neat, cm^{-1}): 1680, 1690. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.8 (m, 19H, alkyl chain), 2.95 (d, $J = 8.8$ Hz), 3.85 (s. 3H), 3.95 (s, 3H), 5.80 (t, 1H), 6.54 (d, $J = 8.4$ Hz 1H), 6.72 (d, $J = 8.4$ Hz, 1H), 6.82 (d, $J = 8.8$ Hz, !H), 7.2 (m, 1H), 7.42 (m, 2H). ^{13}C NMR (200 MHz) ($CDCl_3$) : 191.00 (–C=O), 74.00 (C_2), 45.00 (C_3), 161.45 (C_5), 103.45 (C_6), 136.27 (C_7), 103.45 (C_8), 111.23 (C_{5a}), 162.20 (C_{8a}), 126.50 ($C_{1'}$), 154.20 ($C_{2'}$), 111.00 ($C_{3'}$), 127.50 ($C_{4'}$), 136.27 ($C_{5'}$), 127.50 ($C_{6'}$), 56.67 (2′–OCH_3), 55.89 (5–OCH_3), 52.00-8.50 (C_9H_{19}: mixed side-chain isomers). ESI-Mass (*m/z*):0.411.2 (M^++H). Anal. calcd for $C_{26}H_{34}O_4$: C, 76.35; H, 8.35. Found: C, 76.37; H, 8.37.

*2,3-dihydro-2-(5′-dodecyl-2′-methoxy-phenyl) -5-methoxy-4H-1-benzopyran-4-one (**22**)*

This compound was prepared from the chalcone **16** as per the above procedure. Yield: 40 %, IR (Neat, cm^{-1}): 1692. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.6–1.64 (m, 25H, alkyl chain), 2.95 (d, $J = 6.8$ Hz, 2H), 3.80 (s, 3H, –OCH3), 3.98 (s, 3H, –OCH3), 5.80 (dd, 8.4 Hz, 1H), 6.58 (d, $J = 8.4$ Hz, 1H),6.74 (d, $J = 8.0$ Hz, 1H), 6.82 (d, $J = 8.0$ Hz, 1H), 7.38 (t, 2H), 7.56 (m, 1H). ^{13}C NMR (200 MHz) ($CDCl_3$) : 191.66 (–C=O), 74.38 (C_2), 45.18 (C_3), 160.78 (C_5), 103.77 (C_6), 135.54 (C_7), 103.77 (C_8), 111.52 (C_{5a}), 163.83 (C_{8a}), 126.46 ($C_{1'}$), 153.54 ($C_{2'}$), 110.92 ($C_{3'}$), 127.69 (C_4'), 135.54 (C_5'), 127.69 (C_6'), 56.19 (2′–OCH_3), 55.34 (5–OCH_3), 52.00–8.69 ($C_{12}H_{25}$: mixed side-chain isomers). ESI-Mass (*m/z*): 453 (M^++H). Anal. calcd for $C_{29}H_{40}O_4$: C, 76.94; H, 8.91. Found: C, 76.96; H, 8.93.

Antibacterial activity

The minimum inhibitory concentrations (MIC) of the synthesized compounds were determined against three representative gram-positive organisms viz. *B. subtilis* (MTCC 441), *S. aureus* (MTCC 96), *S. epidermidis* and three Gram-negative organisms viz *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 741), *K. pneumoniae* (MTCC 618) by micro dilution method recommended by CLSI Standard Protocol in liquid medium (Nutrient agar) distributed in 96-well plates. Serial dilutions of the tested compounds were performed (concentrations from 150 to 0.97 μg/mL) in a 200 μL culture medium; afterward each well was seeded with a 50 μL microbial suspension of 0.5 Mac-Farland density. In each test, a microbial culture control and a sterility control (negative) were performed. The plates were incubated for 24 h at 37 °C. The lowest concentration which inhibited the visible microbial growth was considered as the MIC (μg/mL) value for the tested compound. Spectrophotometer has been used for quantitative measurement of microbial growths. Penicillin and Streptomycin were used as standard drugs. The experiments were carried out in triplicate. The MIC values are presented in the Table 1.

Antifungal activity

In vitro, antifungal activity of the newly synthesized compounds was studied against the fungal strains, *C. albicans* (MTCC 227), *C. rugosa* (NCIM 3467), *Saccharomyces cervisiae* (MTCC 36), and *A. niger* (MTCC 282) by Agar Well Diffusion method. The PDA medium was suspended in distilled water (39 g in 1000 mL) and heated to boiling until it dissolved completely; the medium and Petri dishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. Agar well bioassay was employed for testing antifungal activity. The medium was poured into sterile petri dishes under aseptic conditions in a laminar air flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound in DMSO and Chloroform, and different concentrations were made. After inoculation, wells were

scooped out with 6 mm sterile cork borer, and the lids of the dishes were replaced. To each well, different concentrations of test solutions were added. Controls were maintained. The treated and the controls were kept at 27 °C for 48 h. Inhibition zones were measured, the diameter calculated in millimeter. The experiments were carried out in triplicate. The results were presented in Table 2.

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