A Mild and Efficient Protocol For the Synthesis of Novel Series of Chalcones and Homoisoflavonoids as Potential Antimicrobial Agents

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Abstract: A novel approach was adopted for the synthesis of series of new chalcones and dihydrochalcones under ultrasonication. The dihydrochalcones were then converted into homoisoflavonoids under Vilsmeier-Haak reaction condition with excellent yield. This method offers significant advantages such as efficiency and mild reaction conditions with shorter reaction time. Structures of these compounds were established on the basis of IR, 1H NMR, 13C NMR and mass spectral data. The title compounds were evaluated for their antimicrobial activity. Compounds 3c, 3e, 3f, 4c and 4e exhibited potent antimicrobial activity as that of standard drugs.

Keywords: Ultrasound, Chalcones, 2-Hydroxydihydrochalcones, Homoisoflavonoids, Antimicrobial activity.

1. Introduction

Homoisoflavonoids are an uncommon subclass of flavonoids, mainly found in the Fabaceae, Asparagaceae, Polygonaceae, Portulaceae, Orchidaceae, and Gentianaceae families. Although reports on homoisoflavonoids have dated back to the late 1960s [1], studies of this family of compounds were scant at the turn of the century, and the situation did not change until approximately 2002. Then, the interest in homoisoflavonoids began to grow gradually, presumably because more and more homoisoflavonoids were isolated. Presently, approximately 240 naturally occurring homoisoflavonoids have been identified despite their rare occurrence in nature [2,3], and a range of biological properties, including anti-inflammatory, antioxidant, antidiabetic, and antimicrobial activities, have been observed for various homoisoflavonoids [2,3]. In connection with structural elucidations and investigations of the bioactivity of homoisoflavonoids, many synthetic studies have also been performed [3]. They mainly include a chromanone, chromone, or chromane skeleton and are ubiquitous in
plants such as Ophiopogon [4], Polygonatum [5], Scilla [6], Eucomis [7], and Muscari [8]. Recently, several homoisoflavonoids have been successfully isolated from plants and evaluated for their bioactivities [9,10]. Also these compounds and their synthetic analogues have shown important biological properties such as anti-tuberculosis activity [11] and inhibition of protein tyrosine kinase (PTK) [12].

Chalcones have been the center of attention owing to their significant biological activities [13-17]. Besides they are the most important precursors for the formation of α,β-unsaturated carbonyl system in flavonoid classes. Homoisoflavonoids including chalcone system have shown selective biological activities [18]. The isolated natural homoisoflavonoids having 3-benzylidenechroman-4-one skeleton were found to be potent and selective MAO-B inhibitors. Compounds involving benzylidene chromanone have depicted significant medicinal properties such as antioxidant [19], anticancer [20], anti-inflammatory [21], anti-human-immune deficiency virus (HIV-I) activities [22].

Search for new methodologies for the synthesis of homoisoflavonoids continues to be of great interest for organic chemists due to the immense interest of this system from synthetic and biological standpoints. The two most popular pathways for the synthesis of homoisoflavonoids are the deoxybenzoin and the chalcone routes [23]. A few methods of synthesis have been reported in the literature for homoisoflavonoids and these were based on (i) the condensation of 4-chromanones with arylaldehydes in MeOH by passing HCl gas or by using piperidine as a base [24] or condensation using acetic anhydride [25] followed by isomerisation of the C=C bond using Pd/C at 250 ° and, (ii) hydrogenation of chalcones followed by one carbon extension using ethyl formate/Na [26] or methanesulfonyl chloride/DMF [27]. (iii) Condensation of 2-hydroxy-dihydrochalcones with dimethylaminodimethoxymethane, a one carbon unit molecule [28]. Recently V. M. Rao et.al. reported synthesis of homoisoflavones by one carbon extension using triethylorthoformate and 70% perchloric acid followed by aqueous hydrolysis of the intermediate perchlorates [29] etc. However these methods are associated with many drawbacks, such as, formation of undesired side reactions, hazardous, toxic, expensive reagents and have moderate yields. Therefore, developing a milder and more general procedure for the synthesis of homoisoflavonoids is still highly desirable.

On the synthesis of bioactive heterocycles containing oxygen specially chalcones and homoisoflavonoids; herein, we focused on to design a series of novel substituted homoisoflavonoids (Scheme 1) by Vilsmeier reagent (DMF-POCl₃) with dihydrochalcones in moderate to high yields. Then, we evaluated their antimicrobial activities.

2. Results and discussion

2.1 Chemistry.- As a part of our ongoing research in development of new biologically active chalcones and flavonoids [30,31] from readily available starting materials with our main strategy is to develop a efficient organic reaction enhancement method, which is relatively faster and cleaner than the reported methods. The strategies adopted for the synthesis of the intermediates and to target compounds are depicted in (Scheme 1). In order to obtain the key α,β-unsaturated intermediates as starting materials for the synthesis of the target products 5a-5g, 3-((1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one derivatives, with high purity, 3a-3g have been prepared by the Claisen–Schmidt condensation of commercially available 1-methyl-1H-pyrrole-2-carbaldehyde 2 was reacted with substituted α-hydroxy acetonaphenones 1a-1g in EtOH under basic condition at room temperature under ultrasonic irradiation. This process afforded...
the desired chalcones 3a-3g with excellent yields. Then 1-(2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-2-yl)propan-1-one 4a-4g were synthesized by the chemoselective reduction of 3a-3g using inexpensive and readily available Zn dust in AcOH under ultrasound irradiation at 60 °C within 15 min [32]. Under this reaction condition, C=O conjugated C=C bond in chalcones can be cleanly reduced C=C bond. The dihydrochalcones 4a-4g was added to the Vilsmeier reagent (DMF-POCl₃) prepared by drop-wise addition of POCl₃ in ice cooled DMF. The mixture was heated to reflux for 60 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into ice cold H₂O, neutralized with NaHCO₃, the solid separated was filtered, washed with H₂O and crystallized from EtOH to obtain target molecules 5a-5g in good yields (78–88%). Thus, our methodology added a new efficient procedure for the generation of 5a-5g in excellent yields, to the existing reported methods.

2.2 Spectral data

The novel series of compounds 3a-3g, 4a-4g and 5a-5g were fully characterized by spectroscopic techniques such as IR, ¹H NMR, ¹³C NMR, EIMS and elemental analysis, as summarized in the experimental section before being evaluated for in vitro antimicrobial activities. The [M + H]+ peak of all the compounds confirmed the respective molecular weights. The IR spectrum of 3a showed a sharp and strong absorption band in the region of 1627–1635 cm⁻¹ which is due to C=O stretching of the formed chalcone. Another sharp and strongly absorption band at 3211-3220 cm⁻¹ was assigned to OH bond. The ¹H-NMR spectrum showed trans olefinic protons Hₐ and Hₖ as a doublets at δ 7.92 (J = 14.56 Hz) and δ 7.40 (J = 14.96 Hz) ppm, respectively. The value of spin–spin coupling constant J₀₉ in the range J >15 Hz is indicative of the E-configuration of the chalcone. The MeN group of the N-methyl pyrrole moiety was apparent as a singlet at δ 3.79 ppm. The aromatic protons of the chalcones were present in the form of multiples. In the IR spectrum of 5a, the C=O absorption band of homoisoflavonoid observed at 1661 cm⁻¹. The absorption bands at 1635 and 1555 cm⁻¹ were assigned to C=C and C–N groups of the pyrrole moiety, respectively. In the ¹H-NMR spectra of 5a, showed one singlet in the region δ 6.91 ppm of chromone ring, these results supported the ring cyclization in the

![Scheme 1. Synthesis of novel chalcones, dihydrochalcones and homoisoflavonoids.](image-url)
products. The MeN group proton was present as sharp singlet at δ 3.65 ppm whereas protons of homoisoflavonoid CH₂ appeared at δ 3.46 ppm as another sharp singlet. Further evidence of the structure was given by mass spectrometry, which has given [M]+ ion peak at 230.09 as base peak. Furthermore, all compounds showed signals due to aromatic, substituent protons in the expected region. Further confirmations of the structures were given by mass spectra and elemental analysis. The spectroscopic data of other compounds followed similar pattern.

3. Materials and methods

3.1 General

The melting points of all synthesized compounds were determined in open capillary tubes and are uncorrected. IR spectra were recorded on Jasco FT-IR-4100, Japan, in KBr disc, ¹H NMR spectra were recorded on a Varian As 400 MHz spectrometer in CDCl₃/DMSO-d₆, chemical shift (δ) are in ppm relative to TMS and coupling constant (J) are expressed in hertz (Hz). Mass spectra were taken on a Macro mass spectrometer (Water) by electro-spray method (ES). Elemental analysis was performed on Perkin-Elmer EAL-240 elemental analyzer. Bandelin Sonorex (with a frequency of 35 kHz and a nominal power 200 W) ultrasonic bath was used for ultrasonic irradiation. Built-in heating, 30–80 ° there-mostatically adjustable. The reaction vessel placed inside the ultrasonic bath containing water. The reactions were monitored by TLC on F254 silica-gel precoated sheets (Merck, Darmstadt, Germany) and the purified compounds each showed a single spot.

3.2 General procedure for the Synthesis of (E)-1-(2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-2-yl)prop-2-en-1-ones 3a-3g

1-(5-Chloro-2-hydroxy-4-methylphenyl) Ethanone (1d, 0.0027 mmol) in EtOH (10 ml) was added drop wise to a cooled 1-methyl-1H-pyrole-2-carbaldehyde (2, 0.0027 mmol) in 10% KOH (0.0081 mmol). Then the reaction mixture was sonicated at 20 kHz for 2 h at 25–30 °C. The progress of the reaction was monitored by thin layer chromatography by using ethyl acetate: pet ether (2:8) as eluent. After completion of the reaction, the mixture was poured into crushed ice and acidified with HCl (2N) till pH = 4. The solid was filtered under vacuum and crystallized from ethanol to given the title chalcone 3d. The yield after crystallization of chalcones was 62–87%. In a similar fashion using general procedure we carried out the reactions of 1a-1c, 1e-1g with 2 to afford 3a-3c, 3e-3g. Their structures have been confirmed by ¹H-NMR, ¹³C-NMR, Mass, IR spectra and elemental analysis.

(E)-1-(2-Hydroxyphenyl)-3-(1-methyl-1H-pyrrol-2-yl)prop-2-en-1-one (3a): Yield: 88%, M.p. 88-90 °C. ¹H-NMR (400 MHz, CDCl₃): 3.79 (s, 1H, N-CH₃), 6.25 (dd, J = 3.28 and J = 7.9 Hz, 1H, Ar-H), 6.86 (d, 1H, Ar-H), 6.90 (m, 2H, Ar-H), 7.02 (m, 1H, Ar-H), 7.40 (d, J = 14.96 Hz, 1H, Hb), 7.45 (dd, J = 7.31 and J = 1.56 Hz, 1H, Ar-H), 7.87 (dd, J = 6.31 and J = 1.89 Hz, 1H, Ar-H), 7.92 (d, J = 14.56 Hz, 1H, Ha), 13.13 (s, 1H, OH). ¹³C-NMR: 34.1, 109.2, 111.4, 123.4, 128.4, 130.4, 130.9, 131.2, 135.5, 137.4, 138.7, 190.3. MS: 228.12 [M]+; IR (KBr) cm⁻¹: 3215 (OH), 1629 (C=O), 1530 (C-N). Elemental Analysis Calcd. for C₁₄H₁₃NO₂: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.02; H, 5.70; N, 6.22.

(E)-1-(2-Hydroxy-3,5-dimethylphenyl)-3-(1-methyl-1H-pyrrol-2-yl)prop-2-en-1-one (3b): Yield: 83%, M.p. 115-117 °C. ¹H-NMR (400 MHz, CDCl₃): 2.17 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 3.76 (s, 3H, N-CH₃), 6.24 (dd, J = 3 Hz, 1H, Ar-H), 6.84 (d, J = 1.88 Hz, 1H, Ar-H), 6.91 (d, J = 2.72 Hz, 1H, Ar-H), 7.17 (d, J = 7.81 Hz, 1H, Ar-H), 7.37 (d, J = 8.2 Hz, 1H, Ar-H), 7.41 (d, J = 15.00 Hz, Hb), 7.86 (d, J = 14.96 Hz, Ha), 13.24 (s, 1H, -OH). ¹³C-NMR: 25.3, 25.9, 34.3,
109.5, 111.7, 123.7, 128.9, 130.1, 130.4, 136.5, 137.1, 138.3, 139.4, 190.3. MS: 256.05 [M]+1; IR (KBr) cm⁻¹: 3211 (OH), 1631 (C=O), 1527 (C-N). Elemental Analysis Calcd. for C75.27; H, 6.71; N, 5.49; Found: C, 75.33; H, 6.65; N, 5.56.

(E)-1-(3,5-Dichloro-2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-2-yl)prop-2-en-1-one (3c): Yield: 86%, M.p. 76-80 °C. ¹H-NMR (400 MHz, CDCl₃): 3.78 (s, 3H, N-CH₃), 6.25 (dd, J = 3.83 Hz, 1H, Ar-H), 6.88 (dd, J = 8.8 Hz, 1H, Ar-H), 6.90 (dd, J = 1.14 Hz, 1H, Ar-H), 7.32 (d, 1H, Ar-H), 7.53 (d, J = 1.8 Hz, 1H, Ar-H), 7.64 (d, J = 14.92 Hz, H₂), 7.89 (d, J = 14.90 Hz, H₁), 13.11 (s, 1H, -OH). ¹³C-NMR: 34.4, 109.6, 111.8, 123.7, 128.6, 129.4, 129.9, 137.2, 137.5, 137.9, 141.7, 190.2. MS: 297.10 [M]+1 and 299.15 [M]+3; IR (KBr) cm⁻¹: 3215 (OH), 1629 (C=O), 1522 (C-N). Elemental Analysis Calcd. for C₁₄H₁₂ClNO₂; C, 71.98; H, 4.32; N, 4.73; Found: C, 72.01; H, 4.42; N, 4.79.

(E)-1-(5-Chloro-2-hydroxy-4-methylphenyl)-3-(1-methyl-1H-pyrrol-2-yl)prop-2-en-1-one (3d): Yield: 85%, M.p. 58-60 °C. ¹H-NMR (400 MHz, CDCl₃): 2.17 (s, 3H, CH₃), 3.79 (s, 3H, N-CH₃), 6.26 (dd, J = 3 Hz, 1H, Ar-H), 6.86 (dd, J = 8.45 Hz, 1H, Ar-H), 6.93 (dd, J = 1.12 Hz and J = 7.5 Hz, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.67 (d, J = 14.98 Hz, H₂), 7.80 (s, 1H, Ar-H), 7.91 (d, J = 14.96 Hz, H₁), 13.02 (s, 1H, -OH). ¹³C-NMR: 16.3, 34.4, 109.6, 111.7, 123.8, 128.8, 130.1, 130.5, 131.6, 135.8, 137.3, 142.7, 190.9. MS: 276. 13 [M]+1 and 277.12 [M]+3; IR (KBr) cm⁻¹: 3215 (OH), 1627 (C=O), 1529 (C-N). Elemental Analysis Calcd. for C₁₄H₁₂ClNO₂; C, 71.98; H, 4.32; N, 5.81; Found: C, 72.01; H, 4.42; N, 5.88.

(E)-1-(5-Fluoro-2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-2-yl)prop-2-en-1-one (3g): Yield: 73%, M.p. 130-132 °C. ¹H-NMR (400 MHz, CDCl₃): 3.79 (s, 3H, N-CH₃), 6.22 (dd, J = 3.76 Hz, 1H, Ar-H), 6.83 (dd, J = 8.43 Hz, 1H, Ar-H), 6.84 (dd, J = 1.16 Hz, 1H, Ar-H), 7.08 (d, J = 1.8 Hz, 1H, Ar-H), 7.45 (dd, 1H, Ar-H), 7.61 (d, J = 7.4 Hz, 1H, Ar-H), 7.65 (d, J = 14.86 Hz, 1H, H₂), 7.89 (d, J = 14.91 Hz, 1H, H₁), 13.09 (s, 1H, -OH). ¹³C-NMR: 34.6, 109.7, 111.1, 115.4, 122.4, 123.8, 126.3, 128.4, 131.4, 131.8, 135.5, 140.4, 164.7, 190.3. MS: 246.18 [M]+1; IR (KBr) cm⁻¹: 3214 (OH), 1633 (C=O), 1529 (C-N). Elemental Analysis Calcd. for C₁₅H₁₃FO₂S: C, 71.98; H, 4.32; N, 5.71; Found: C, 72.01, H, 4.42, N: 5.78.
3.3 General procedure for the preparation of 1-(2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-2-yl)propan-1-ones 4a-4g

Chalcone (3a, 0.0022 mmol) and Zn dust (0.0044 mmol) were well mixed in a round bottom flask and then AcOH (10 ml) was added. The reaction mixture was irradiated in ultrasonic at 65ο for 28 min. After the completion of reaction monitor by TLC, the mixture was filtrated through filter paper and the filter cake was washed by EtOAc (10 ml) for three times. The filtrate was poured into H2O (30 ml) and was adjusted to pH 5-6 with diluted NaOH aqueous solution, then extracted with EtOAc for three times. The combined organic layers were dried over anhydrous MgSO4 and purified by column chromatography on silica gel eluted with mixture of petroleum ether/EtOAc (5:1) to afford 4a. In a similar fashion using general procedure we carried out the reactions of 3b-3g with Zn dust to afford 4b-4g. The product obtained 4a-4g was used for further reaction without any purification. Their structures have been confirmed by 1H-NMR, Mass, IR spectra and elemental analysis.

1-(2-Hydroxyphenyl)-3-(1-methyl-1H-pyrrol-3-yl)propan-1-one (4a): Yield: 86%, M.p. 62-64 οC. 1H-NMR (400 MHz, CDCl3): 3.02 (t, J = 8.2 Hz, 2H, CH2), 3.29 (t, J = 8.2 Hz, 2H, CH2), 3.56 (s, 3H, N-CH3), 6.20 (dd, 1H, Ar-H), 6.26 (dd, 1H, Ar-H), 6.48 (dd, 1H, Ar-H), 6.80 (dd, 1H, Ar-H), 7.28-7.87 (m, 3H, Ar-H), 12.11 (s, 1H, -OH). 13C-NMR: 23.3, 34.3, 46.4, 108.6, 109.7, 116.8, 122.3, 122.8, 123.5, 125.6, 131.1, 135.5, 161.6, 201.8. MS: 230.20 [M]+; IR (KBr) cm⁻¹: 3015 (OH), 2920, 2722 (CH2), 1655 (C=O). Elemental Analysis Calcd. for C21H15FO2S: C: 73.34, H: 6.59, N: 6.11; Found: C: 73.30, H: 6.63, N: 6.15.

1-(2-Hydroxy-3,5-dimethylphenyl)-3-(1-methyl-1H-pyrrol-3-yl)propan-1-one (4b): Yield: 82%, M.p. 74-76 οC. 1H-NMR (400 MHz, CDCl3): 2.46 (s, 3H, CH3), 2.59 (s, 3H, CH3), 3.03 (t, J = 8.4 Hz, 2H, CH2), 3.26 (t, J = 8.4Hz, 2H, CH2), 3.54 (s, 3H, N-CH3), 6.23 (dd, 1H, Ar-H), 6.44 (dd, 2H, Ar-H), 6.89 (d, 1H, Ar-H), 7.67 (d, 1H, Ar-H), 12.13 (s, 1H, -OH). 13C-NMR: 15.3, 23.6, 34.7, 46.1, 108.2, 109.3, 122.6, 122.9, 123.6, 125.3, 128.3, 131.3, 137.5, 158.6, 201.3. MS: 258.16 [M]+; IR (KBr) cm⁻¹: 3010 (OH), 2918, 2726 (CH2), 1658 (C=O), 1589 (C=C). Elemental Analysis Calcd. for C21H15FO2S: C: 74.68, H: 7.44, N: 5.44; Found: C: 74.72, H: 7.49, N: 5.48.

1-(3,5-Dichloro-2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-3-yl)propan-1-one (4c): Yield: 85%, M.p. 108-110 οC. 1H-NMR (400 MHz, CDCl3): 0.6 (t, J = 8.0 Hz, 2H, CH2), 3.26 (t, J = 8.0 Hz, 2H, CH2), 3.57 (s, 3H, N-CH3), 6.20 (dd, 1H, Ar-H), 6.41 (dd, 2H, Ar-H), 6.86 (d, 1H, Ar-H), 7.57 (d, 1H, Ar-H), 12.10 (s, 1H, -OH). 13C-NMR: 15.3, 23.6, 34.3, 46.1, 108.2, 109.4, 122.5, 122.7, 125.5, 125.8, 126.4, 129.1, 137.3, 158.6, 201.9. MS: 298.15 [M]+ and 300.13 (M+2) [M]+2; IR (KBr) cm⁻¹: 3018 (OH), 2925, 2727 (CH2), 1653 (C=O), 1586 (C=C), 765 (C-Cl). Elemental Analysis Calcd. for C21H15FO2S: C: 56.39, H: 4.39, H: 4.70; Found: C: 56.34, H: 4.43, N: 4.73.

1-(5-Chloro-2-hydroxy-4-methylphenyl)-3-(1-methyl-1H-pyrrol-3-yl)propan-1-one (4d): Yield: 86%, M.p. 46-48 οC. 1H-NMR (400 MHz, CDCl3): 2.59 (s, 3H, CH3), 3.05 (s, 3H, CH3), 3.29 (t, J = 8.1 Hz, 2H, CH2), 3.52 (s, 3H, N-CH3), 6.21 (dd, 1H, Ar-H), 6.48 (d, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.63 (s, 1H, Ar-H), 12.10 (s, 1H, -OH). 13C-NMR: 16.4, 23.8, 34.9, 46.2, 108.7, 109.3, 117.3, 121.8, 123.3, 125.8, 127.5, 131.2, 142.5, 159.6, 201.4. MS: 278.15 [M]+ and 280.12 [M]+3; IR (KBr) cm⁻¹: 3016 (OH), 2921, 2725 (CH2), 1657 (C=O), 1590 (C=C), 762 (C-Cl). Elemental Analysis Calcd. for C21H15FO2S: C: 64.87, H: 5.81, N: 5.04; Found: C: 64.91, H: 5.84, N: 5.00.
1-(5-Chloro-2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-3-yl)propan-1-one (4e): Yield: 82%, M.p. 40-42 °C. 1H-NMR (400 MHz, CDCl3): 3.06 (t, J = 7.6 Hz, 2H, CH2), 3.27 (t, J = 7.6 Hz, 2H, CH2), 3.55 (s, 3H, N-CH3), 6.26 (dd, 1H, Ar-H), 6.38 (d, 1H, Ar-H), 6.81 (d, 1H, Ar-H), 7.25 (d, 1H, Ar-H), 7.53 (dd, 1H, Ar-H), 7.67 (d, 1H, Ar-H), 12.14 (s, 1H, -OH). 13C-NMR: 23.9, 34.3, 46.2, 108.4, 109.3, 118.8, 123.3, 124.8, 125.1, 127.6, 131.4, 135.3, 159.6, 201.3.

MS: 263.5 [M]+ and 265.02 [M]+2; IR (KBr) cm−1: 3015 (OH), 2916, 2721 (CH2), 1652 (C=O), 1592 (C=C). Elemental Analysis Calcd. for C21H15FO2S: C: 63.76, H: 5.35, N: 5.31; Found: C: 63.72; H: 5.39; N: 5.36.

1-(2-Hydroxy-5-methylphenyl)-3-(1-methyl-1H-pyrrol-3-yl)propan-1-one (4f): Yield: 80%, M.p. 48-50 °C. 1H-NMR (400 MHz, CDCl3): 2.32 (s, 3H, CH3), 3.05 (t, J = 7.6 Hz, 2H, CH2), 3.26 (t, J = 7.6 Hz, 2H, CH2), 3.54 (s, 3H, N-CH3), 6.23 (dd, 1H, Ar-H), 6.40 (d, 1H, Ar-H), 6.80 (d, 1H, Ar-H), 7.31 (d, 1H, Ar-H), 7.49 (dd, 1H, Ar-H), 7.65 (d, 1H, Ar-H), 12.11 (s, 1H, -OH). 13C-NMR: 23.5, 25.3, 34.4, 46.6, 108.1, 109.2, 116.2, 122.5, 123.8, 125.2, 131.3, 131.5, 135.2, 158.6, 201.2. MS: 244.12 [M]+1; IR (KBr) cm−1: 3013 (OH), 2928, 2728 (CH2), 1654 (C=O), 1591 (C=C). Elemental Analysis Calcd. for C21H15FO2S: C: 74.05, H: 7.04, N: 5.76; Found: C: 74.11; H: 7.09; N: 5.71.

3.4 General procedure for the synthesis of various substituted 3-(1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one 5a-5g

In a similar fashion using general procedure we carried out the reactions of 4b-4g with DMF/POCl3 to afford 5b-5g. Their structures have been confirmed by 1H-NMR, 13C-NMR, Mass, IR spectra and elemental analysis.

1-(5-Fluoro-2-hydroxyphenyl)-3-(1-methyl-1H-pyrrol-3-yl)propan-1-one (4g): Yield: 83%, M.p. 72-74 °C. 1H-NMR (400 MHz, CDCl3): 3.07 (t, J = 7.2 Hz, 2H, CH2), 3.26 (t, J = 7.2 Hz, 2H, CH2), 3.50 (s, 3H, N-CH3), 6.27 (dd, 1H, Ar-H), 6.42 (d, 1H, Ar-H), 6.84 (d, 1H, Ar-H), 7.36 (d, 1H, Ar-H), 7.56 (dd, 1H, Ar-H), 7.72 (d, 1H, Ar-H), 12.15 (s, 1H, -OH). 13C-NMR: 23.5, 34.8, 46.2, 108.8, 109.4, 116.2, 118.4, 122.0, 123.7, 125.2, 156.1, 157.5, 201.0. MS: 248.11 [M]+1; IR (KBr) cm−1: 3011 (OH), 2927, 2730 (CH2), 1654 (C=O), 1588 (C=C). Elemental Analysis Calcd. for C21H15FO2S: C: 68.00, H: 5.71, N: 5.66; Found: C: 68.05; H: 5.76; N: 5.70.

1-(2-Hydroxy-5-methylphenyl)-3-(1-methyl-1H-pyrrol-2-yl)propan-1-one (4h): Yield: 80%, M.p. 208-210 °C. 1H-NMR (400 MHz, CDCl3): 2.34 (s, 3H, CH3), 2.52 (s, 3H, Ar-CH3), 3.46 (s, 3H, N-CH3), 6.23 (d, 1H, Ar-H), 6.27 (d, 1H, Ar-H), 6.40 (d, 1H, Ar-H), 6.91 (s, 1H, Ar-H), 7.15-7.86 (m, 4H, Ar-H). 13C-NMR: 28.5, 35.1, 108.2, 109.7, 116.5, 118.0, 124.0, 124.6, 125.7, 125.9, 131.1, 136.5, 152.2, 158.3, 184.4. MS: 230.09 [M]+1; IR (KBr) cm−1: 1661 (C=O), 1632 & 1552 (C=C). Elemental Analysis Calcd. for C15H13NO2: C, 75.30; H, 5.48; N, 5.85; Found: C, 75.27; H, 5.52; N, 5.80.

6,8-Dimethyl-3-((1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one (5b): Yield: 78%, M.p. 215-218 °C. 1H-NMR (400 MHz, CDCl3): 2.35 (s, 3H, Ar-CH3), 2.52 (s, 3H, Ar-CH3), 3.47 (s, 2H, CH2), 3.65 (s, 3H, N-CH3), 6.25 (d, 1H, Ar-H), 6.27 (d, 1H, Ar-H), 6.31 (d, 1H, Ar-H), 6.93 (s, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.56 (d,
1H, Ar-H). $^1$C-NMR: 15.4, 25.6, 28.2, 35.3, 108.5, 109.2, 116.4, 123.4, 124.5, 125.6, 128.7, 128.9, 134.1, 138.5, 152.6, 155.3, 184.6. MS: 268.10 [M]$^+$; IR (KBr) cm$^{-1}$: 1667 (C=O), 1635 and 1555 (C=C). Elemental Analysis Calcd. for C$_{17}$H$_{17}$NO$_2$ C, 76.38; H, 6.41; N, 5.24; Found: C, 76.34; H, 6.44; N, 5.28.

6,8-Dichloro-3-((1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one (5c): Yield: 80%, M.p. 110-113 °C. $^1$H-NMR (400 MHz, CDCl$_3$): 3.51 (s, 2H, CH$_2$), 3.67 (s, 3H, N-CH$_3$), 6.24 (dd, 1H, Ar-H), 6.29 (d, 1H, Ar-H), 6.35 (d, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 7.26 (d, 1H, Ar-H), 7.60 (d, 1H, Ar-H). $^1$C-NMR: 28.6, 35.5, 108.5, 109.9, 116.7, 123.8, 125.2, 127.9, 128.1, 129.1, 131.5, 151.2, 153.6, 184.8. MS: 307.02 [M]$^+$; IR (KBr) cm$^{-1}$: 1660 (C=O), 1631 and 1552 (C=C), 671 (C-Cl). Elemental Analysis Calcd. for C$_{15}$H$_{11}$Cl$_2$NO$_2$ C, 58.46; H, 3.60; N, 4.55; Found: C, 58.50; H, 3.65; N, 4.60.

6-Chloro-7-methyl-3-((1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one (5d): Yield: 79%, M.p. 155-157 °C. $^1$H-NMR (400 MHz, CDCl$_3$): 2.55 (s, 3H, Ar-CH$_3$), 3.43 (s, 2H, CH$_2$), 3.69 (s, 3H, N-CH$_3$), 6.24 (dd, 1H, Ar-H), 6.25 (d, 1H, Ar-H), 6.26 (d, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 7.86 (s, 1H, Ar-H). $^1$C-NMR: 16.4, 28.7, 35.4, 108.6, 109.4, 116.1, 118.6, 123.3, 123.8, 125.0, 130.6, 131.7, 143.9, 152.1, 156.5, 184.7. MS: 288.07 [M]$^+$ and 290.12 [M]$^+$; IR (KBr) cm$^{-1}$: 1665 (C=O), 1636 & 1550 (C=C). Elemental Analysis Calcd. for C$_{16}$H$_{14}$ClNO$_2$ C, 66.79; H, 3.60; N, 4.81; Found: C, 66.75; H, 4.88; N, 4.86.

6-Chloro-3-((1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one (5e): Yield: 72%, M.p. 220-222 °C. $^1$H-NMR (400 MHz, CDCl$_3$): 3.49 (s, 2H, CH$_2$), 3.66 (s, 3H, N-CH$_3$), 6.21 (dd, 1H, Ar-H), 6.25 (d, 1H, Ar-H), 6.27 (d, 1H, Ar-H), 6.97 (s, 1H, Ar-H), 7.14 (d, 1H, Ar-H), 7.45 (dd, 1H, Ar-H), 7.68 (d, 1H, Ar-H). $^1$C-NMR: 28.7, 35.5, 108.7, 109.2, 116.8, 120.0, 123.0, 125.3, 125.8, 130.9, 131.8, 136.5, 152.6, 154.3, 184.1. MS: 274.00 [M]$^+$; IR (KBr) cm$^{-1}$: 1667 (C=O), 1630 & 1558 (C=C), 670 (C-Cl). Elemental Analysis Calcd. for C$_{15}$H$_{13}$ClNO$_2$ C, 58.42; H, 3.64; N, 4.04; Found: C, 58.57; H, 3.64; N, 3.96.

6-Methyl-3-((1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one (5f): Yield: 70%, M.p. 228-230 °C. $^1$H-NMR (400 MHz, CDCl$_3$): 3.51 (s, 2H, CH$_2$), 3.63 (s, 3H, N-CH$_3$), 6.25 (dd, 1H, Ar-H), 6.29 (d, 1H, Ar-H), 6.34 (d, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.42 (dd, 1H, Ar-H), 7.71 (d, 1H, Ar-H). $^1$C-NMR: 25.4, 28.1, 35.4, 108.9, 109.5, 116.2, 118.4, 123.2, 124.4, 124.8, 131.7, 134.9, 136.1, 136.5, 152.8, 155.3, 184.7. MS: 254.10 [M]$^+$; IR (KBr) cm$^{-1}$: 1660 (C=O), 1630 & 1557 (C=C). Analysis Calcd. for C$_{16}$H$_{15}$NO$_2$ C, 75.87; H, 5.97; N, 5.53; Found: C, 75.83; H, 6.01; N, 5.50.

6-Fluoro-3-((1-methyl-1H-pyrrol-3-yl)methyl)-4H-chromen-4-one (5g): Yield: 68%, M.p. 104-106 °C. $^1$H-NMR (400 MHz, CDCl$_3$): 3.54 (s, 2H, CH$_2$), 3.65 (s, 3H, N-CH$_3$), 6.21 (dd, 1H, Ar-H), 6.25 (d, 1H, Ar-H), 6.30 (d, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 7.18 (d, 1H, Ar-H), 7.47 (dd, 1H, Ar-H), 7.68 (d, 1H, Ar-H). $^1$C-NMR: 16.4, 28.7, 35.4, 108.5, 109.4, 116.2, 123.4, 124.3, 125.2, 126.9, 152.8, 154.3, 158.3, 184.8. MS: 258.09 [M]$^+$; IR (KBr) cm$^{-1}$: 1659 (C=O), 1632 & 1551 (C=C). Elemental Analysis Calcd. for C$_{16}$H$_{14}$FNO$_2$ C, 70.03; H, 4.70; N, 5.44; Found: C, 70.08; H, 4.74; N, 5.39.

3.5 Biological evaluation

The synthesized novel N-methyl pyrrole incorporated chalcones 3a-3g and homoisoflavonoids 5a-5g were then assayed for their in vitro antibacterial activity against Gram positive bacterial strain and Gram negative bacterial strain, and antifungal activity against all pathogenic fungal strains tested
using standardized agar well diffusion method for antibacterial [33] and antifungal activity [34]. The results were determined using the minimum inhibitory concentration (MIC) and the minimum inhibitory concentration (MIC) values that inhibited the growth of the tested microorganisms (MIC) and minimal bactericidal/fungicidal concentration were determined. The results have been reported in (Table 1, 2 and 3), along with those of standard drugs Gentamicin and Nystatin.

**Antibacterial activity**

The newly prepared compounds chalcones and homoisoflavonoids were tested for their in vitro antibacterial activity against two gram positive bacterial strain *Staphylococcus aureus* (ATCC No. 25923), *Bacillus subtilis* (ATCC 6633), and two gram negative bacterial strain, *Echerichia coil* (ATCC No, 25922) and *Pseudomonas aeruginosa* (ATCC No. 27853) (Table 1 and 2). The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Standard antibiotics Gentamicin namely was used for comparison with antibacterial activity data shown by compounds 3a-3g and 5a-5g. All the compounds of the tested series possessed good antimicrobial activity. Four of these compounds 3d, 3f, 5d and 5f exhibited good antibacterial activity against Gram positive and Gram negative bacteria. In case of Gram positive bacteria compounds 3c, 3f and 5c were found to be most effective against *Staphylococcus aureus* in the range of concentration 23 and 24 and compounds 3e and 4e were most effective against *Bacillus subtilis*, with the range of concentration between 23 and 24. However, in case of Gram-negative bacteria, compounds 3d, 3f, 5d and 5f were found to be most effective against *E. coil* and *P. aeruginosa* with zone of inhibition ranging between 18-20 (Table 1). Of the compound tested, compounds 3a and 5f expressed moderate to good activity against *S.

**Table 1. Antibacterial activity of compounds 3a-3g and 5a-5g**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of growth of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>3a</td>
<td>10</td>
</tr>
<tr>
<td>3b</td>
<td>20</td>
</tr>
<tr>
<td>3c</td>
<td>23</td>
</tr>
<tr>
<td>3d</td>
<td>19</td>
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<tr>
<td>3e</td>
<td>23</td>
</tr>
<tr>
<td>3f</td>
<td>19</td>
</tr>
<tr>
<td>3g</td>
<td>20</td>
</tr>
<tr>
<td>5a</td>
<td>11</td>
</tr>
<tr>
<td>5b</td>
<td>19</td>
</tr>
<tr>
<td>5c</td>
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<td>5e</td>
<td>22</td>
</tr>
<tr>
<td>5f</td>
<td>14</td>
</tr>
<tr>
<td>5g</td>
<td>19</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>24</td>
</tr>
</tbody>
</table>

**Table 2. Minimum inhibitory concentration (MIC), (in µg/mL) of compounds 3a-3g and 5a-5g**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>3a</td>
<td>62</td>
</tr>
<tr>
<td>3b</td>
<td>30</td>
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<tr>
<td>3c</td>
<td>125</td>
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<tr>
<td>3f</td>
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</tr>
<tr>
<td>3g</td>
<td>6</td>
</tr>
<tr>
<td>5a</td>
<td>62</td>
</tr>
</tbody>
</table>
Antifungal activity

Antifungal activity was also done by all synthesized compounds. For assaying the antifungal activity two fungi *Candida albicans* and *Aspergillus niger* were tested *in vitro* for their antifungal activity. Nystatin was as standard drug used for comparison with antifungal activity shown by compounds 3a-3g and 5a-5g. A careful analysis revealed that almost all of the newly synthesized compounds 3a-3g and 5a-5g showed maximum potent inhibition against all the fungal strains as shown in (Table 3). Compounds 3e, 3g, 5e and 5g showed maximum inhibition against both of the fungi, *Candida albicans* and *Aspergillus niger*. Compounds 3c and 5c was found more effective against both fungi. Compounds 3b, 3d, 5d and 5g displayed moderate inhibition against both of the fungi.

**Table 3.** *In vitro* antifungal activity of compounds 3a-3g and 5a-5g by using agar well diffusion method.

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>Candida albicans</em></th>
<th><em>Aspergillus niger</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>3b</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>3c</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>3d</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>3e</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>3f</td>
<td>08</td>
<td>22</td>
</tr>
<tr>
<td>3g</td>
<td>18</td>
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</tr>
<tr>
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<td>08</td>
<td>16</td>
</tr>
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<td>12</td>
<td>16</td>
</tr>
<tr>
<td>5c</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>5d</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>5e</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>5f</td>
<td>12</td>
<td>09</td>
</tr>
<tr>
<td>5g</td>
<td>20</td>
<td>24</td>
</tr>
</tbody>
</table>

*Nystatin* 30 30

*aZone of inhibition*

It is interesting to note that different substituent’s
moreover affect antibacterial and antifungal activity of compounds 3a-3g and 5a-5g. The presence of different substituents’s on phenyl ring i.e. chloro substitution in compounds influenced these molecules to exhibit inhibition to greater extent against the organisms tested. Methyl and Fluoro substitution present in compounds also increase the inhibitory effect of these compounds against the organism tested, which may be due to the electron withdrawing nature of this functional group. The presence of electron donating groups at ortho position or no substitution resulted with moderate activity. A possible mechanism was suggested in (Scheme 2): firstly, chalcone accepted electron from the zinc to form the anion free radical (1). Secondly, the anion free radical abstracted a proton from the solution of the reaction, then (2) was formed. Thirdly, (2) accepted an electron from the zinc to form the enol anion (3). Finally, the enol anion became the saturated alkone by passing an proton from acetic acid ion.

Conclusions

In summary, these studies should not only enable an extension of applicability of the ultrasound wave, but also introduce environmentally friendlier method for novel chalcones 3a-3g, dihydrochalcones 4a-4g and efficient protocol developed for homoisoflavonoid 5a-5g and screened for their in vitro antimicrobial activity against four bacterial and two fungi. As a result, derivatives having electron withdrawing substituent’s showed broad antimicrobial spectrum. Among the tested compounds, compounds 3c, 3e, 3f, 4c, 4e and 5c displayed good antimicrobial activity against all the tested strains and comp. 3c and 5c exhibited excellent antifungal activity against both fungi. We believed the insights gained in this study would be useful for the development of potential drug candidates derived from homoisoflavonoids.

Acknowledgments

The authors are grateful to the Head, Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for

Scheme 2. Proposed mechanism for the formation of dihydrochalcone
providing the laboratory facility and Director, SIAF, Chandigarh for providing spectral analysis of newly synthesized compounds and Y. B. Chavhan College of pharmacy, Aurangabad for biological screening of novel compounds.

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