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Rapid microwave assisted synthesis and antimicrobial bioevaluation of novel steroidal chalcones

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ABSTRACT

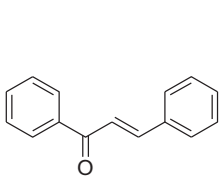
A novel class of chalconoyl pregnenolones has been prepared via Claisen–Schmidt condensation under microwave activation and solvent free reaction conditions. The compounds were screened for antimicrobial activity against two bacterial strains *Bacillus subtilis* and *Escherichia coli* and two fungal strains *Aspergillus niger* and *Candida albicans*. Some of the compounds exhibited significant inhibitory activity against the microbial strains. Presence of the α,β-unsaturated carbonyl moiety in the synthesized compounds was found to be essential for the activity as manipulation of the same through epoxidation of the double bond diminished the activity.

1. Introduction

The occurrences of life-threatening fungal infections have continued to rise over the last two decades, particularly within populations of immune compromised individuals [1]. Bacterial infections are caused by multidrug resistant Gram-positive and Gram-negative pathogens which affect millions of people in the subtropical regions of the world and 20,000 deaths every year are due to these parasitic bacterial infections [2]. Different antibiotics are extensively used for the treatment of these infectious diseases but with commonly increasing multidrug resistant microbial strains there is a constant need for the development of novel antimicrobial agents.

Steroids find widespread application as anti-inflammatory, diuretic, anabolic, contraceptive, antiandrogenic, progestational and anticancer agents [3]. Steroid based compounds turn out to be nontoxic, less vulnerable to multi-drug resistance (MDR) and highly bioavailable because of being capable of penetrating the cell wall [4].

Chalcones (1,3-diphenyl-2-propen-1-one) are well known for their diverse array of bioactivities. In particular, they have been reported to possess pharmacological activities like anticancer, antimalarial, anti-inflammatory [5], anti-tubercular, cytotoxic, gastroprotective [6] and antimicrobial activities [7–8]. Chalcone structure (**1**) consists of three important components, viz. two phenyl rings and an α,β-unsaturated carbonyl system joining them. Literature studies reveal that, the nature of substituents on the phenyl rings and their conformation in the core structure play a vital role in determining their activities. In some specific study it was found that variations leading to conformational changes like epoxidation or substitution on the double bond result in a decreased bioactivity [9].



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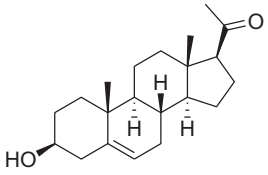

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Pregnenolone (**2**) is a naturally occurring neurosteroid that is synthesized from cholesterol in the adrenal gland and the central nervous system known as a precursor to other hormones, including cortisone, oestrogen, testosterone and progesterone [10–11]. It is used in the synthesis of anti-depressive drugs [12] and its derivatives and analogues were prepared and evaluated for different biological activities [13].

The coupling of two or more natural products to make hybrids leads to an almost inexhaustible reservoir of new types of compounds with diverse structures [14]. The underlying expectation being that a combination of structural features of two or more functionally active substances into one molecule or their covalent

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coupling may either enhance or modulate the desired characteristics of individual components or lead to new types of properties [15].

Working close to the realm of hybrid molecules, we prepared a novel class of modified steroids or chalcone analogues or chalconoyl pregnenolones using pregnenolone acetate as the steroid precursor. The 17-acetyl group of pregnenolone acetate was used to form the enol using our own method of Claisen–Schmidt condensation using I_2-Al_2O_3 [6] to condense with different aldehydes in order to synthesize a novel class of chalconoyl pregnenolones. During the preparation of this manuscript, Banday et al. [4] reported the synthesis of 17-chalconoyl derivatives of pregnenolone with their antimicrobial bioevaluation. We report here the synthesis of another novel class of steroidal chalcones using pregnenolone acetate with potent antimicrobial activities.

2. Experimental

2.1. General remarks

All commercially available chemicals and reagents were purchased from Aldrich and used without further purification. Melting points were determined with a Buchi B 540 apparatus in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1640 FT-IR instrument. The ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker DPX-300 NMR machine using $CDCl_3$ as solvent with TMS as the internal standard. Mass spectra were recorded with a Trace DSQ GCMS system. Elemental analyses were carried out using a Perkin-Elmer series II CSNS/O Model 2400 analyser.

2.2. Chemistry

2.2.1. Preparation of the catalyst

The catalyst was prepared as reported earlier [6].

2.2.2. Pregnenolone acetate (**3**)

Pregnenolone acetate (**3**) was prepared by chemoselective catalytic hydrogenation of 16-DPA over Pd-charcoal. 16-DPA (2.0 g, 5.61 mmol) was dissolved with brief warming in a minimum volume of absolute ethanol (70 mL). 10% Pd-C (0.2 g) was added to it and subjected to hydrogenation at 45 psi. The reaction was monitored by taking ^{1}H NMR of the reaction mixture at regular intervals. Disappearance of the signal for 16-C*H* proton at δ 6.72 indicated the complete reduction of the 16–17 carbon–carbon double bond after 3.5 h. After separating the Pd-C by filtration, ethanol was distilled off at reduced pressure to obtain **3** in the form of pink crystals which was used in the synthesis of compounds **5**–**20** without further purification. Yield: 1.96 g, 98%; mp 132–133 °C. IR (KBr) 1732, 1698, 1631, 1366, 1248, 1036 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.38 (d, J = 4.95 Hz, 1H), 4.64–4.55 (m, 1H), 2.57–2.51 (m, 1H), 2.33 (d, J = 7.08 Hz, 2H), 2.12 (s, 3H), 2.03 (s, 3H), 1.89 (d, J = 9.89 Hz, 2H), 1.68–1.53 (m, 11H), 1.2–1.15 (m, 4H), 1.02 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.6, 170.5, 139.6, 122.3, 73.8, 63.6, 56.8, 49.8, 43.9, 38.7, 38.0, 36.9, 36.5, 31.7, 31.5, 27.7, 24.4, 22.8, 21.4, 21.0, 19.2, 13.2; MS (ESI): *m/z* 359 $(M + H)^+$; Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56; Found C, 77.01; H, 9.53.

2.2.3. General experimental procedure for the synthesis of compounds **5**–**20**

Pregnenolone acetate (**3**, 0.2 g, 0.557 mmol), benzaldehyde (0.557 mmol) and I_2-Al_2O_3 (20 mol% I_2 as 10% I_2 in Al_2O_3) were taken and homogenized in a mortar. Then the reaction mixture was transferred to a reaction vessel of Anton Paar Synthos 3000 microwave reactor and irradiated for the stipulated time at 250 W power. Maximum internal temperature observed was 88–95 °C and pressure 5.5–5.7 bar. After cooling to room temperature, ethyl acetate was (15 mL) added to the RM and washed with $Na_2S_2O_3$ solution (15 mL × 1) followed by H_2O (2 × 15 mL). The separated organic layer was dried over anhydrous sodium sulphate and concentrated in reduced pressure. Products were purified by column chromatography over silica gel (100–200 mesh) with ethyl acetate and hexane as the mobile phase.

*2.2.3.1. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-phenyl-2-propen-1-one (**5**).* Reaction time: 6 min. Brown solid (86%); mp 171–173 °C; IR (KBr) 1732, 1681, 1631, 1605, 1366, 1248, 1037 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61–7.52 (m, 3H), 7.40–7.38 (m, 3H), 6.81 (d, J = 15.9 Hz, 1H), 5.39 (d, J = 4.56 Hz, 1H), 4.65–4.55 (m, 1H), 2.90–2.84 (m, 1H), 2.34 (d, J = 6.9 Hz, 2H), 2.04 (s, 3H), 1.88 (d, J = 11.2 Hz, 2H), 1.62–1.50 (m, 5H), 1.28–1.25 (m, 6H), 1.01 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.36, 170.58, 141.5, 139.6, 134.8, 130.3, 128.9, 128.3, 126.8, 122.3, 73.8, 62.0, 57.1, 49.9, 44.9, 39.0, 38.0, 36.9, 36.6, 31.9, 27.7, 26.7, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) *m/z* 447 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{38}O_3$: C, 80.68; H, 8.58. Found: C, 80.64; H, 8.55.

*2.2.3.2. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-methoxyphenyl)-2-propen-1-one (**6**).* Reaction time: 7 min. Brown solid (82%); mp 179 °C. IR (KBr) 2941, 1731, 1677, 1598, 1511, 1251, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55–7.52 (m, 2H), 7.50 (d, J = 1.38 Hz, 1H), 6.93–6.89 (m, 2H), 6.69 (d, J = 15.9 Hz, 1H), 5.39 (d, J = 4.62 Hz, 1H), 4.66–4.55 (m, 1H), 3.84 (s, 3H), 2.88–2.82 (m, 1H), 2.37–2.31 (m, 3H), 2.05–1.98 (m, 5H), 1.88 (d, J = 9.96 Hz, 2H), 1.75–1.46 (m, 12H), 1.28–1.25 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.3, 170.6, 161.4, 141.3, 139.6, 129.9, 127.4, 124.7, 122.4, 114.3, 73.8, 61.9, 57.1, 55.4, 49.9, 44.9, 39.0, 38.0, 36.9, 36.6, 31.9, 31.8, 27.7, 24.6, 22.7, 21.4, 21.0, 19.3, 13.4. MS (ESI) *m/z* 477 $(M + H)^+$. Anal. Calcd. for $C_{31}H_{40}O_4$: C, 78.11; H, 8.46. Found: C, 78.09; H, 8.43.

*2.2.3.3. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-chlorophenyl)-2-propen-1-one (**7**).* Reaction time: 6 min. Brown solid (83%); mp 126–127 °C; IR (KBr)1732, 1680, 1591, 1511, 1058 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52–7.46 (m, 3H), 7.37–7.34 (m, 2H), 6.77 (d, J = 15.96 Hz, 1H), 5.38 (d, J = 4.14 Hz, 1H), 4.65–4.55 (m, 1H), 2.57–2.51 (m, 1H), 2.33 (d, J = 6.94 Hz, 2H), 2.12 (s, 3H), 1.89 (d, J = 10.1 Hz, 2H), 1.69–1.50 (m, 9H), 1.47 (d, J = 9.06 Hz, 2H), 1.25–1.15 (m, 4H), 1.02 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.0, 170.5, 140.0, 139.6, 136.1, 133.2, 129.4, 127.1, 122.3, 73.8, 63.6, 56.7, 49.8, 43.9, 38.7, 38.0, 36.9, 36.5, 31.9, 31.78, 31.73, 31.5, 27.7, 24.4, 22.7, 21.0, 19.2, 13.2. MS (ESI) *m/z* 482 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{37}ClO_3$: C, 74.90; H, 7.75. Found: C, 74.87; H, 7.73.

*2.2.3.4. 1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(2-bromophenyl)-2-propen-1-one (**8**).* Reaction time: 5 min. White solid (86%); mp 64.5 °C; IR (KBr) 1732, 1686, 1631, 1606, 1246, 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.91 (d, J = 16.02 Hz, 1H), 7.63 (d, J = 7.9 Hz, 2H), 7.35–7.30 (m, 1H), 7.23–7.20 (m, 1H), 6.72 (d, J = 15.96 Hz, 1H), 5.39 (d, J = 4.35 Hz, 1H), 4.65–4.59 (m, 1H), 2.98–2.93 (m, 1H), 2.34 (d, J = 7.23 Hz, 2H), 2.03 (s, 3H), 1.88 (d, J = 10.29 Hz, 2H), 1.74–1.58 (m, 9H), 1.51 (d, J = 9.96 Hz, 2H), 1.29–1.25 (m, 4H), 1.01 (s, 3H), 0.65 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.3, 170.6, 140.1, 139.6, 134.8, 133.5, 131.1, 129.8, 127.7, 125.8, 122.3, 73.8, 61.2, 57.0, 49.9, 44.9, 39.2, 38.07, 36.9, 36.6, 35.5, 31.9, 31.8, 27.7, 24.7, 22.9, 21.0, 19.3, 13.3. MS (ESI) *m/z* 526 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{37}BrO_3$: C, 68.57; H, 7.10. Found: C, 68.55; H, 7.08.

*2.2.3.5. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-fluorophenyl)-2-propen-1-one (**9**).* Reaction time: 6 min. Brown solid (84%); mp 151–152 °C, IR (KBr) 1729, 1682, 1599, 1509, 1234, 1159 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.01 (d, J = 15.6 Hz, 1H), 7.78–7.75 (m, 2H), 7.72–7.62 (m, 3H), 5.38 (d, J = 4.57 Hz, 1H), 4.65–4.54 (m, 1H), 2.90–2.84 (m, 1H), 2.33 (d, J = 6.89 Hz, 2H), 2.04 (s, 3H), 1.88 (d, J = 11.1 Hz, 2H), 1.73–1.54 (m, 9H), 1.52 (d, J = 8.68 Hz, 2H), 1.28–1.25 (m, 4H), 1.01 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.3, 170.5, 141.2, 139.1, 136.7, 132.1, 129.1, 126.6, 122.7, 122.4, 73.8, 62.5, 57.08, 49.9, 45.3, 39.1, 38.0, 36.7, 36.4, 31.8, 31.7, 27.7, 24.6, 22.6, 21.0, 19.3, 13.5. MS (ESI) m/z 465 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{37}FO_3$: C, 77.55; H, 8.03. Found: C, 77.53; H, 8.01.

*2.2.3.6. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-nitrophenyl)-2-propen-1-one (**10**).* Reaction time: 7 min. Yellow solid (79%); mp 184–185 °C, IR ($CHCl_3$) 1732, 1704, 1510, 1436, 1326, 1242, 1036 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.26 (d, J = 8.64 Hz, 2H), 7.71 (d, J = 8.55 Hz, 2H), 7.58 (d, J = 15.96 Hz, 1H), 6.89 (d, J = 15.9 Hz, 1H), 5.40 (d, J = 5.13 Hz, 1H), 4.62–4.58 (m, 1H), 2.66–2.60 (m, 1H), 2.34 (d, J = 4.11 Hz, 2H), 2.03 (s, 3H), 1.88 (d, J = 9.96 Hz, 2H), 1.69–1.52 (m, 9H), 1.43–1.38 (m, 2H), 1.28–1.13 (m, 4H), 1.01 (s, 3H), 0.65 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 199.87, 170.6, 141.0, 139.6, 138.3, 130.2, 130.1, 128.8, 124.1, 123.3, 122.3, 73.8, 62.5, 57.0, 49.9, 45.2, 39.1, 38.0, 36.9, 31.9, 31.7, 27.7, 24.6, 22.6, 21.0, 19.3, 13.5. MS (ESI) m/z 492 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{37}NO_5$: C, 73.29; H, 7.59; N, 2.85. Found: C, 73.26; H, 7.57; N, 2.84.

*2.2.3.7. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-bromophenyl)-2-propen-1-one (**11**).* Reaction time: 6 min. White solid (85%); mp 99–100 °C; IR ($CHCl_3$) 1736, 1682, 1630, 1601, 1243, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.57–7.49 (m, 3H), 7.11–7.02 (m, 2H), 6.73 (d, J = 15.9 Hz, 1H), 5.39 (d, J = 4.44 Hz, 1H), 4.62–4.59 (m, 1H), 2.87–2.83 (m, 1H), 2.34 (d, J = 6.45 Hz, 2H), 2.04 (s, 3H), 1.87 (d, J = 9.9 Hz, 2H), 1.73–1.49 (m, 9H), 1.47 (d, J = 11.4 Hz, 2H), 1.39–1.25 (m, 4H), 1.04 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 170.5, 164.4, 141.1, 139.5, 134.7, 127.7, 127.6, 122.3, 115.3, 115.1, 73.6, 61.2, 57.1, 49.9, 44.9, 39.1, 38.0, 37.1, 36.8, 35.6, 31.8, 27.8, 24.6, 22.9, 21.0, 19.3, 13.5. MS (ESI) m/z 526 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{37}BrO_3$: C, 68.57; H, 7.10. Found: C, 68.54; H, 7.07.

*2.2.3.8. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(2,4-difluorophenyl)-2-propen-1-one (**12**).* Reaction time: 6 min. Brown solid (84%); mp 128–129 °C; IR ($CHCl_3$) 1729, 1686, 1611, 1489 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.63–7.59 (m, 2H), 7.09 (s, 1H), 6.85 (d, J = 16.1 Hz, 1H), 5.39 (d, J = 5.01 Hz, 1H), 4.63–4.59 (m, 1H), 2.87–2.84 (m, 1H), 2.34 (d, J = 6.42 Hz, 2H), 2.04 (s, 3H), 1.88 (d, J = 11.2 Hz, 2H), 1.71–1.54 (m, 9H), 1.53 (d, J = 8.5 Hz, 2H), 1.29–1.26 (m, 4H), 1.01 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 170.1, 164.4, 162.3, 141.4, 139.1, 128.1, 126.4, 122.1, 118.1, 109.9, 73.8, 62.6, 57.08, 49.9, 45.3, 39.1, 38.2, 36.7, 31.9, 31.8, 27.7, 24.6, 22.7, 21.1, 19.3, 13.3. MS (ESI) m/z 483 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{36}F_2O_3$: C, 74.66; H, 7.52. Found: C, 74.63; H, 7.49.

*2.2.3.9. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(2,3,5-trifluorophenyl)-2-propen -1-one (**13**).* Reaction time: 6 min. Brown liquid (84%); IR ($CHCl_3$) 1736, 1688, 1615, 1491, 1145 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.79 (d, J = 16.0 Hz, 1H), 7.57 (s, 1H), 7.02 (s, 1H), 6.78 (d, J = 16.0 Hz, 1H), 5.39 (d, J = 4.24 Hz, 1H), 4.62–4.59 (m, 1H), 2.85–2.83 (m, 1H), 2.34 (d, J = 6.37 Hz, 2H), 1.74–1.70 (m, 9H), 1.64 (d, J = 4.9 Hz, 2H), 1.57–1.50 (m, 4H), 1.01 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.8, 170.6, 158.1, 151.5, 145.2, 141.5, 139.5, 126.3, 122.3, 121.2, 114.3, 103.1, 73.8, 62.0, 57.1, 49.9, 44.8, 39.0, 38.8, 36.7, 31.9, 31.8, 27.7, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) m/z 501 $(M + H)^+$.

*2.2.3.10. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-hydroxyphenyl)-2-propen-1-one (**14**).* Reaction time: 7 min. Thick brown oil (82%); IR ($CHCl_3$) 3390, 1735, 1686, 1631, 1556, 1366, 1248, 1037 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, J = 7.75 Hz, 2H), 7.79 (d, J = 15.5 Hz, 1H), 7.65–7.55 (m, 2H), 6.88 (d, J = 15.9 Hz, 1H), 5.39 (d, J = 4.35 Hz, 1H), 4.65–4.57 (m, 1H), 2.98–2.91 (m, 1H), 2.33 (d, J = 10.1 Hz, 2H), 2.03 (s, 3H), 1.89 (d, J = 10.1 Hz, 2H), 1.73–1.57 (m, 9H), 1.54 (d, J = 9.3 Hz, 2H), 1.28–1.23 (m, 4H), 1.02 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 170.5, 156.6, 141.5, 139.6, 132.7, 127.8, 126.3, 122.2, 117.2, 73.7, 62.0, 57.1, 49.9, 44.8, 39.2, 38.0, 36.9, 36.7, 32.0, 31.9, 27.7, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) m/z 463 $(M + H)^+$.

*2.2.3.11. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-hydroxy 3-methoxyphenyl)-2-propen-1-one (**15**).* Reaction time: 7 min. Yellow oil (84%); IR (KBr) 3340, 1731, 1680, 1599, 1511, 1251, 1042 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.81 (d, J = 15.7 Hz, 1H), 7.32–7.26 (m, 4H), 5.38 (d, J = 4.41 Hz, 1H), 4.62–4.58 (m, 1H), 3.8 (s, 3H), 2.87–2.82 (m, 1H), 2.34 (d, J = 6.46 Hz, 2H), 2.04 (s, 3H), 1.88 (d, J = 9.8 Hz, 2H), 1.75–1.54 (m, 9H), 1.48 (d, J = 11.0 Hz, 2H), 1.41–1.29 (m, 4H), 1.04 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 170.1, 147.5, 141.2, 140.2, 139.0, 131.4, 127.1, 126.6, 120.9, 117.7, 113.4, 73.8, 62.0, 57.1, 56.0, 49.9, 44.9, 39.0, 38.0, 36.9, 36.6, 31.9, 27.7, 26.7, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) m/z 493 $(M + H)^+$.

*2.2.3.12. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(3,4-dihydroxyphenyl)-2-propen-1-one (**16**).* Reaction time: 6 min. Brown oil (81%); IR (KBr) 3410, 1737, 1683, 1636, 1556, 1361, 1248, 1037 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.01–7.95 (m, 2H), 7.81 (d, J = 15.9 Hz, 1H), 7.19–7.14 (m, 2H), 5.39 (d, J = 4.4 Hz, 1H), 4.61–4.58 (m, 1H), 2.88–2.83 (m, 1H), 2.34 (d, J = 6.46 Hz, 2H), 2.04 (s, 3H), 1.87 (d, J = 9.8 Hz, 2H), 1.75–1.53 (m, 9H), 1.49 (d, J = 10.9 Hz, 2H), 1.40–1.28 (m, 4H), 1.04 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 170.5, 146.5, 145.2, 141.5, 139.1, 138.1, 123.3, 122.3, 119.4, 114.1, 73.8, 61.9, 57.0, 49.9, 44.9, 39.1, 38.0, 36.9, 36.6, 31.9, 31.8, 27.7, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) m/z 479 $(M + H)^+$.

*2.2.3.13. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(2,4-dichlorophenyl)-2-propen-1-one (**17**).* Reaction time: 6 min. Brown gum (83%); IR ($CHCl_3$) 1732, 1681, 1590, 1513, 1062 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.61 (d, J = 15.9 Hz, 1H), 7.54 (d, J = 6.8 Hz, 1H), 6.86–6.81 (m, 2H), 5.39 (d, J = 4.21 Hz, 1H), 4.61–4.57 (m, 1H), 2.84–2.81 (m, 1H), 2.34 (d, J = 6.29 Hz, 2H), 2.03 (s, 3H), 1.89 (d, J = 10.9 Hz, 2H), 1.75–1.70 (m, 9H), 1.64 (d, J = 4.81 Hz, 2H), 1.56–1.51 (m, 4H), 1.01 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.7, 170.6, 141.1, 139.0, 136.1, 136.3, 131.4, 129.7, 127.1, 126.3, 122.1, 73.8, 62.0, 57.2, 49.9, 44.9, 39.0, 38.0, 36.9, 31.9, 31.8, 27.8, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) m/z 516 $(M + H)^+$.

*2.2.3.14. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(4-methylphenyl)-2-propen-1-one (**18**).* Reaction time: 6 min. Brown solid (84%); mp 171–173 °C; IR 3050, 2916, 1655, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.02–7.96 (m, 2H), 7.81 (d, J = 15.5 Hz, 1H), 7.60–7.46 (m, 4H), 5.38 (d, J = 4.58 Hz, 1H), 4.64–4.55 (m, 1H), 2.90–2.84 (m, 1H), 2.40 (s, 3H), 2.34 (d, J = 6.9 Hz, 2H), 2.03 (s, 3H), 1.88 (d, J = 11.2 Hz, 2H), 1.71–1.55 (m, 9H), 1.51 (d, J = 8.67 Hz, 2H), 1.29–1.25 (m, 4H), 1.01 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 170.3, 141.1, 139.4, 135.7, 136.2, 131.1, 130.5, 127.1, 126.9, 122.1, 73.8, 61.9, 57.1, 49.9, 44.9, 39.1, 38.3, 36.9, 36.6, 31.9, 31.7, 27.7, 24.6, 22.7, 21.2, 21.0, 19.3, 13.4.

MS (ESI) *m/z* 461 $(M + H)^+$. Anal. Calcd. for $C_{31}H_{40}O_3$: C, 80.83; H, 8.75. Found: C, 80.80; H, 8.71.

*2.2.3.15. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(2,4-dimethoxyphenyl)-2-propen-1-one (**19**).* Reaction time: 7 min. Brown solid (83%); mp 188–189 °C; IR (KBr) 3062, 1732, 1699, 1643, 1249, 1044 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.01 (d, *J* = 15.7 Hz, 1H), 7.79–7.73 (m, 1H), 6.51–6.34 (m, 3H), 5.38 (d, *J* = 4.56 Hz, 1H), 4.65–4.55 (m, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 2.91–2.85 (m, 1H), 2.34 (d, *J* = 6.9 Hz, 2H), 2.03 (s, 3H), 1.88 (d, *J* = 11.1 Hz, 2H), 1.72–1.54 (m, 9H), 1.52 (d, *J* = 8.66 Hz, 2H), 1.29–1.26 (m, 4H), 1.01 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.4, 170.5, 161.2, 160.8, 141.7, 139.4, 129.3, 126.3, 116.1, 109.4, 101.0, 73.8, 62.0, 57.1, 49.9, 44.8, 39.1, 38.1, 36.9, 36.6, 31.9, 31.8, 27.7, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) *m/z* 507 $(M + H)^+$. Anal. Calcd. for $C_{32}H_{42}O_5$: C, 75.86; H, 8.36. Found: C, 75.84; H, 8.31.

*2.2.3.16. (E)-1-[(3β)-3-(Acetyloxy)-androst-5-en-17-yl]-3-(2-nitrophenyl)-2-propen-1-one (**20**).* Reaction time: 6 min. Brown solid (81%); mp 143–144 °C, IR ($CHCl_3$) 2990, 1732, 1704, 1510, 1436, 1329, 1247, 1039 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.14 (d, *J* = 15.7 Hz, 1H), 8.08 (d, *J* = 7.3 Hz, 1H), 8.03–8.01 (m, 3H), 7.51 (d, *J* = 15.9 Hz, 1H), 4.65–4.59 (m, 1H), 2.98–2.93 (m, 1H), 2.33 (d, *J* = 7.77 Hz, 2H), 2.04 (s, 3H), 1.88 (d, *J* = 10.27 Hz, 2H), 1.74–1.58 (m, 9H), 1.52 (d, *J* = 9.97 Hz, 2H), 1.28–1.25 (m, 4H), 1.01 (s, 3H), 0.65 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.4, 170.5, 161.2, 160.8, 141.7, 139.4, 129.3, 126.3, 116.1, 109.4, 101.0, 73.8, 62.0, 57.1, 56.1, 49.9, 44.8, 39.1, 38.1, 36.9, 36.6, 31.9, 31.8, 27.7, 24.6, 22.7, 21.0, 19.3, 13.4. MS (ESI) *m/z* 492 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{37}NO_5$: C, 73.29; H, 7.59; N, 2.85. Found: C, 73.27; H, 7.56; N, 2.82.

2.2.4. Epoxidation of compound **9**

Compound **9** was converted to its epoxide by reacting with H_2O_2 in the presence of K_2CO_3 (Scheme 2) [16]. Compound **9** (0.4 g, 0.86 mmol) and K_2CO_3 (0.356 g, 2.58 mmol) were added to MeOH (30 mL) to get a suspension. Excess aqueous hydrogen peroxide (35%, 1 mL, 10 mol equiv.) was then added to the suspension over 10 min. Then the mixture was stirred at room temperature and reaction progress was monitored by TLC. Upon completion (4 h), the MeOH was removed under reduced pressure and the resulting residue was dissolved in CH_2Cl_2 (50 mL) and washed with H_2O (2 × 30 mL). The organic phase was separated, dried (Na_2SO_4) and was concentrated under reduced pressure. The resulting crude oil was purified by column chromatography over silica gel (100–200 mesh) using EtOAc/hexane as the eluent to yield the corresponding epoxide (0.284 g, 0.591 mmol, 69%) as a brown oil.

Acetic acid 17-[3-(4-fluoro-phenyl)-oxiranecarbonyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester (**21**).

IR ($CHCl_3$) 1729, 1628, 1601, 1511, 1251, 1230, 1159, 1038 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.57–7.49 (m, 2H), 7.11–7.02 (m, 2H), 5.39 (d, *J* = 4.44 Hz, 1H), 4.62–4.59 (m, 1H), 4.23 (d, *J* = 1.75 Hz, 1H), 4.19 (d, *J* = 1.75 Hz, 1H), 2.87–2.83 (m, 1H), 2.34 (d, *J* = 6.45 Hz, 2H), 2.04 (s, 3H), 1.87 (d, *J* = 9.9 Hz, 2H), 1.73–1.49 (m, 9H), 1.47 (d, *J* = 11.4 Hz, 2H), 1.39–1.25 (m, 4H), 1.04 (s, 3H), 0.63 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.1, 170.5, 164.4, 139.5, 134.7, 127.7, 122.3, 115.3, 115.1, 73.6, 62.1, 61.2, 59.4, 57.1, 49.9, 44.9, 39.1, 38.0, 37.1, 36.8, 35.6, 31.8, 27.8, 24.6, 22.9, 21.0, 19.3, 13.5. MS (ESI) *m/z* 481 $(M + H)^+$.

2.3. Antimicrobial bioevaluation

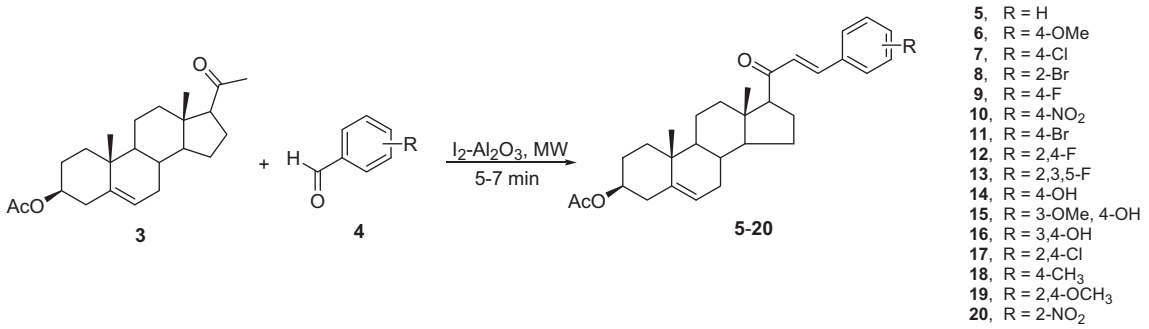
2.3.1. Microorganisms and media

The antimicrobial activity of the new chalconoids of pregnenolone acetate was studied against fungal and bacterial pathogens. All the pathogens, *Aspergillus niger* (MTCC 281), *Candida albicans* (MTCC 183), *Bacillus subtilis* (MTCC 736) and *Escherichia coli* (MTCC 1692) were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

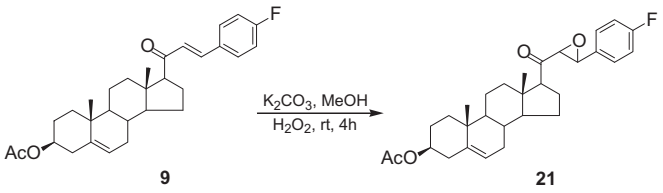
The bacterial strains were grown in nutrient broth (NB) at 30 ± 2 °C with continuous agitation at 200 rpm for 24 h, whereas the fungal strains were grown in potato dextrose broth (PDB) at 25 ± 2 °C. The compounds were solubilized in 10% dimethyl sulfoxide (DMSO). The bactericidal as well as fungicidal effects were studied with 1000 μg/mL solution of the compounds using nutrient agar (NA) and potato dextrose agar (PDA) medium, respectively through agar well diffusion assay [17]. 100 μL of compound suspension and 60 μL of bacterial/fungal cultures (approx. 10^8 colony forming units [CFU]) were applied for each study. The zone of inhibition was recorded after 24 h of incubation at 30 ± 2 °C for bacterial strains and after 72 h at 25 ± 2 °C for fungal strains. Kanamycin for bacteria and fluconazole for fungus were used as positive control with same dilution as of the compounds.

2.3.2. Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of the applied material that can inhibit the growth of an organism. MIC was determined through batch cultures containing different volumes of the compounds (1000 μg/mL, 500 μg/mL, 300 μg/mL, 250 μg/mL, 200 μg/mL, 150 μg/mL, 100 μg/mL and 50 μg/mL). 100 μL of compound suspensions of different concentrations was added to 100 mL of sterile NB and PDB taken in Erlenmeyer flask (250 mL).The flasks were then inoculated with 1 mL of 24 h old bacterial and 72 h old fungal suspension (approx. 10^8 CFU) and incubated at 30 ± 2 °C and 25 ± 2 °C with continuous shaking at 200 rpm for 18–24 h for bacteria and 72 h for fungus. After the incubation period, microbial growth was observed by taking optical density (OD) at 600 nm. Microbial culture with only DMSO was treated as control for the experiments. Kanamycin for bacteria and



Scheme 1. Synthesis of compounds **5**–**20**.



Scheme 2. Conversion of **9** to its epoxide **21**.

fluconazole for fungus were used as standard drugs. The value of OD for each concentration of compound and standard was subtracted from the value of control for the calculation of the killing percentage.

3. Results and discussions

3.1. Chemistry

The condensation between pregnenolone acetate (**3**) and benzaldehyde was tried using I_2-Al_2O_3 as catalyst under microwave activation and solvent free conditions. The desired product was isolated in 86% pure yield after 6 min of MW exposure at 250 W of microwave power in the presence of 20 mol% catalyst. Using **3** as the ketone component sixteen novel steroidal chalcones were prepared via its condensation with different substituted benzaldehyde (Scheme 1). In general, the reactions were clean and no side products were detected. Products were isolated in good yields (79–86%); further enhancement of the same was not possible as increment of microwave power or reaction time or catalyst loading resulted in the decomposition of the product formed. No significant variation in product yield was observed with variations in the nature of substituents in the benzaldehyde aromatic ring. In activity screening, compound **9** was found to be the most potent antimicrobial agent. In order to have a gist of the structural requirements for the activity, it was converted to its epoxide using H_2O_2 in the presence of K_2CO_3.

Results of antimicrobial activity screening indicate that the presence of the fluoro group in the para position of the benzaldehyde aromatic ring enhances the activity. A comparison of the zone of inhibition produced by the unsubstituted compound **5** and the most active compound **9** is shown in Fig. 1. The presence of Cl group on that position also increased the activity compared to unsubstituted **5**. However, the presence of the other halogen Br or any other groups whether electron withdrawing or electron donating, reduces the activity compared to **5**. In addition to the one in para position, another F in the ortho position of benzaldehyde or in both of the meta and one ortho position could not enhance the activity more than the para substituted compound. It is also found that compounds **3** and **4** ($R = 4–F$), which are the starting compounds in the synthesis of **9**, possess no activity against the select microbes (entry 1 and 2, Table 1). Again manipulation of the α,β-unsaturation of the most active compound **9** via its epoxidation (**21**), resulted in the total loss of activity. This signifies the necessity of the condensation between **3** and **4** and the presence of the specific conformation for the bioactivity.

Table 1
Antimicrobial activity of steroidal chalcones.

Entry	Compound	Zone of inhibition (mm)[a]			
		E. coli	*B. subtilis*	*C. albicans*	*A. niger*
1	**3**	–	–	–	–
2	**4** ($R = 4–F$)	–	–	–	–
3	**5**	20	22	–	–
4	**6**	–	–	–	–
5	**7**	18	–	16	–
6	**8**	–	–	–	–
7	**9**	26	28	22	19
8	**10**	–	–	–	–
9	**11**	–	–	–	–
10	**12**	22	24	20	17
11	**13**	24	25	20	18
12	**14**	–	–	–	–
13	**15**	–	–	–	–
14	**16**	–	–	–	–
15	**17**	21	–	18	16
16	**18**	–	–	–	–
17	**19**	–	–	–	–
18	**20**	–	–	–	–
19	Kanamycin	28	31	–	–
20	Fluconazole	–	–	24	20

[a] Zone of inhibitions less than 10 mm are not shown.

Table 2
MIC of the active compounds.

Entry	Compound	MIC (μg/mL)			
		E. coli	*B. subtilis*	*C. albicans*	*A. niger*
1	**5**	300	300	–	–
2	**7**	350	–	500	–
3	**9**	150	150	300	300
4	**12**	300	200	350	500
5	**13**	200	200	350	500
6	**17**	300	–	500	350
7	Kanamycin	100	100	–	–
8	Fluconazole	–	–	150	150

'–' No activity.

3.2. Biology

The synthesized compound **5**, **7**, **9**, **12**, **13** and **17** showed good antimicrobial activity in general (Table 1). Bacterial strain *E. coli* was sensitive to all of these compounds with inhibition zones varying from 18 to 26 mm. *B. subtilis* was found to be resistant to **7** and **17**, while sensitive to samples **5**, **9**, **12** and **13** with the zone of inhibitions from 22 to 28 mm. In the case of fungal pathogens *C. albicans* was found to be resistant to **5** and *A. niger* to **5** and **7**. Compound **9** was found to be much effective against *C. albicans* with the inhibition zone at 22 mm. In the case of *A. niger* zones of inhibition were 19, 18, 17 and 16 mm for samples **9**, **13**, **12**

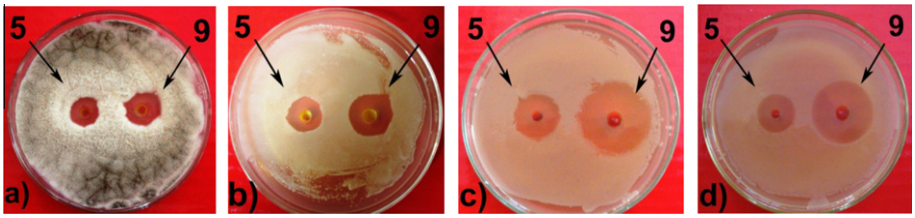


Fig. 1. Zone of inhibition produced by compounds **5** & **9** in (a) *A. niger*, (b) *C. albicans*, (c) *B. subtilis* and (d) *E. coli*.

and **17**, respectively. MIC was determined for the active compounds **5**, **7**, **9**, **12**, **13**, **17** and the results are plotted in Table 2. Except compounds **7** and **17**, all have the property to inhibit the growth of both of the bacterial strains-Gram positive *B. subtilis* as well as Gram negative *E. coli*. Among all the compounds analysed, compound **9** was found to be the most promising having both bactericidal and fungicidal activities with MIC values 150 and 300 μg/mL, respectively.

4. Conclusions

In conclusion, we have synthesized a novel series of chalconoyl pregnenolones for the first time under convenient reaction conditions in a very short reaction time. These new steroidal compounds possess potent antimicrobial activities against some common pathogens. Necessity of the presence of the α,β-unsaturated carbonyl system in the compounds was also verified by manipulation of the conformation via epoxidation. Further studies on structure activity relationships and on the scope of application of the compounds is going on.

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