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Synthesis antimicrobial and antioxidant studies of new oximes of steroidal chalcones

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ABSTRACT

A convenient synthesis of oximes of steroidal chalcones (**4a–4j**) was performed and structural assignment of the products was confirmed on the basis of IR, ^{1}HNMR, ^{13}C NMR, MS and analytical data. The synthesized compounds were screened for *in vitro* antioxidant activity by using DPPH method and *in vitro* antimicrobial activity against different bacterial and fungal strains by agar diffusion method. The activity of the tested compounds against each microbe varied due to structural differences between them. Presence and position of different substituents on the benzene ring of the chalconyl pendent had a marked effect on the activity of the compounds. From the results it can be inferred that the compounds **4a–j** showed significant antioxidant activity and antimicrobial activity against all microbial strains used for testing.

1. Introduction

In the last few decades there has been an extensive focus of research towards the rational modification of steroid molecules. This is due to the fact that such type of compounds are less toxic, less vulnerable to multi-drug resistance (MDR) and highly bioavailable because of being capable of penetrating the cell wall. Oxime compounds are used as antidotes for nerve agents. A nerve agent inactivates acetylcholinesterase molecules by phosphorylation of the molecule. Oxime compounds can reactivate acetylcholinesterate by attaching to the phosphorus atom and forming an oxime-phosphonate which then splits away from the acetylcholinesterase molecule. The most effective oxime nerve-agent antidotes are pralidoxime (also known as 2-PAM), obidoxime, methoxime, HI-6, Hlo-7, and TMB-4 [1]. Methyl Ethyl Ketoxime is a skin-preventing additive in many oil-based paints. Steroidal oximes are different class of compounds and have well validated biological effects. Steroids and their synthetic congeners including their oxime dervatives have extensively been studied during the last decade [2,3]. These molecules have always attracted considerable attention because of being a fundamental class of biological signaling molecules and their profound biological, scientific and clinical importance [4]. They can regulate a variety of biological processes and thus have the potential to be developed as drugs for the treatment of a large number of diseases including cardiovascular [5], autoimmune diseases [6], brain tumors, breast cancer, prostate cancer, osteoarthritis, etc. [7]. Most of the steroid based pharmaceuticals are semi-synthetic compounds prepared by connecting a special functionality to the core structure of a steroid [8]. Structural modification of steroids would provide a platform to approach the synthesis of new drugs for tackling important biological problems. To meet these ends, attention has been devoted in the literature to the synthesis of steroidal compounds because of their potent receptor binding properties and valuable pharmacological activities [9–11]. Steroid molecules and their oxime derivatives have been tested against variety of microorganisms for antimicrobial activities, cholestanes, deoxycorticosterone, progesterone and androsterone are notable ones [12]. The advantage of employing hydrophobic steroid units with oxime group (=NOH) enhance their ability to interact with cell membranes and thus pave the way for biological activity of such hybrid molecules. This has been proved by different ring modification studies of steroidal molecules and their chalcone dervatives involving the A and D-ring whereby incorporation of heteroatom (N or O) have been reported to enhance the biological activities of these molecules. Such systems have been shown to bear a lot of different biological activities such as anti-microbial, anti-inflammatory, hypotensive, hypocholesterolemic and diuretic activities [13–17]. Very fewer efforts have been reported related to the synthesis of oximes of chalcones of steroidal moieties and their biological screening. Though there are reports for the synthesis of other such analogs, the same is not true for the oximes of chalconyl derivatives at the D-ring of pregnenolone. Taking inspiration from the number of reported biological activities associated with structurally related analogs, we, in continuation of our efforts towards the synthesis of oximes of chalcones of preglenenolone

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derivatives starting from readily available 20-keto pregnenanes [18]. We efficiently synthesized new oxime derivatives and studied their antimicrobial and antioxidant properties which we wish to report herein.

2. Experimental

2.1. General methods

Solvents and organic reagents were purchased from Sigma Aldrich, Merck (Germany) and Loba Chemie (India) and were used without further purification. Melting points were recorded on Bucci Melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. NMR spectra were recorded on Bruker DPX200 instrument in $CDCl_3$ with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants are given in Hz. Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. The progress of all reactions was monitored by TLC on 2×5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine.

2.2. Chemical synthesis

2.2.1. General procedure for the synthesis of oxime derivatives

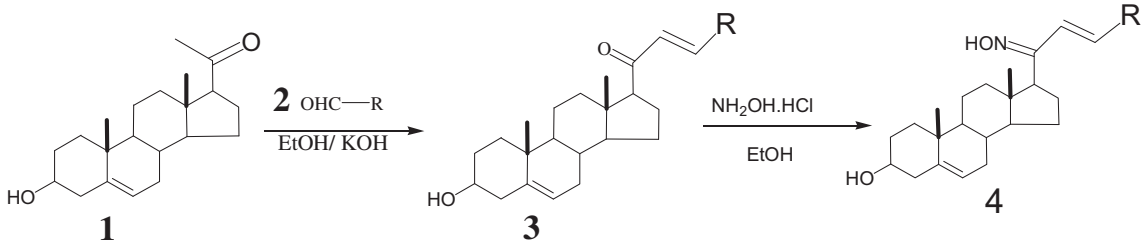
To a solution of pregnenolone 1 (0.316 g, 1 mmol, 1 eq.) in ethanol (10 ml) was added a conc. aq. solution of KOH (2 eq.). Then aldehyde 2 (1.2 eq.) was charged into the reaction mixture and the reaction mixture was stirred for 1–2 h at room temperature to get the corresponding benzylidine derivative 3. The benzylidene dervatives were isolated and recrystallized from ethylacetate and were further reacted (0.2 g, 1 mmol), with hydroxylamine hydrochloride (2 ml) in 10 ml of ethanol to get the corresponding oxime 4. After completion as revealed by thin layer chromatography (TLC run in ethylacetate:hexane) in an average span of around 3–4 h (Scheme 1). The precipitate obtained was filtered, dried and monitored through TLC for the purity. Thin layer chromatography revealed just a single spot which proved the presence of a single product. For further purification, the product was recrystallized from ethylacetate to give product as solid powder. In some cases the products were purified by column chromatography using ethylacetate: hexane (70:30 v/v) as eluent. It is to be mentioned that when non-aromatic aldehydes were used, the chalcones were formed in a very minor quantity and that too not stable enough at ambient conditions. Thus the study was restricted to the use of aromatic aldehydes only. The spectral data of various oxime dervatives are given as under (Most of the peaks due to steroidal skelton were merged and could not be differentiated in the ^{1}H NMR. Thus δ values of only those peaks that distinguish the product and could easily be differentiated are reported as under).

2.2.1.1. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-p-methylphenylprop-2-en-1-imine (4a). Coloured powder (78%). M.P: 235–240 °C; IR (KBr) cm^{-1}: 3525, 3385, 2948, 1814, 1617, 1512, 1423, 1051, 609; ^{1}H NMR ($CDCl_3$): δ 0.78 (s, 3H), 1.200 (s, 3H), 1.54–2.0 (m, 6H), 2.41–2.48 (m, 3H), 2.42 (t, J = 8.80, 1H); 3.21 (m, 1H); 5.98 (s, J = 16.00, 1H), 6.89 (m, 3H), 7.05 (m, 3H), 8.68 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 14.23, 21.06, 22.07, 24.02, 31.03, 31.48, 32.09, 36.52, 42.11, 45.21, 48.01, 48.75, 49.32, 49.79, 50.84, 57.44, 61.07, 73.12, 123.26, 127.69, 128.89, 131.41, 135.72, 142.15, 158.6, 201.12; ESI-MS: 420 (M+H); Anal. Calcd. for $C_{28}H_{37}NO_2$: C, 80.15; H, 8.89; N, 3.34; O, 7.63; Found C, 80.10; H, 8.71; N, 3.04; O, 7.52.

2.2.1.2. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-p-nitrophenylprop-2-en-1-imine (4b). Yellow powder (81%). M.P: 255–260 °C; IR (KBr) cm^{-1}: 3410, 3315, 2941, 1764, 1651, 1323, 1061, 797; ^{1}H NMR ($CDCl_3$): δ 0.71 (s, 3H), 1.09 (s, 3H), 1.93–2.0 (m, 6H), 2.26–2.39 (m, 3H), 2.31 (s, 3H), 3.12 (t, J = 8.43, 1H); 3.82 (m, 1H), 6.12 (s, 1H), 7.01 (d, J = 15.78, 1H), 7.72 (m, 3H), 7.89 (d, J = 6.83, 1H), 8.12 (d, J = 15.78, 1H), 9.31 (s, 1H),; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.94, 19.96, 20.15, 21.11, 22.14, 24.87, 31.07, 32.19, 36.71, 37.37, 37.82, 39.82,42.12, 45.18, 51.23, 57.75, 63.27, 71.30, 71.86, 121.82, 126.57, 128.11, 129.18, 130.58, 131.93, 134.81, 138.87, 139.76, 140.53, 160.3, 200.51; ESI-MS: 465 (M+H); Anal. Calcd. for $C_{28}H_{36}N_2O_4$: C, 72.39; H, 7.81; N, 6.03; O, 13.77; Found: C, 72.09; H, 7.68; N, 5.83; O, 13.57.

2.2.1.3. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(3,3,4-trimethoxyphenyl)prop-2-en-1-imine (4c). Solid yellowish powder (74%). M.P: 230–235 °C; IR (KBr) cm^{-1}: 3407, 3323, 2981, 1627, 1433, 1216, 1029, 778; ^{1}H NMR ($CDCl_3$): δ 0.73 (s, 3 H), 1.00 (s, 3H), 1.81–2.10 (m, 6H), 2.30 (m, 3H), 2.55 (s, 3H), 2.88 (t, J = 8.43, 1H); 3.55 (m, 1H), 5.76 (s, 1H), 6.94 (d, J = 15.98, 1H), 7.32 (d, J = 7.42, 2H), 7.58 (d, J = 7.42, 2H), 7.64 (d, J = 15.98, 1H), 10.41 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 12.82, 18.48, 20.43, 20.88, 22.18, 23.76, 30.72, 30.85, 31.13, 35.64, 36.67, 38.34, 41.57, 44.96, 50.11, 57.21, 58.89, 71.73, 120.24, 125.14, 128.29, 128.95, 131.28, 139.63, 159.9, 199.42; ESI-MS: 510 (M+H); Anal. Calcd. for $C_{31}H_{43}NO_5$: C, 73.05; H, 8.5; N, 2.75; O, 15.70; Found C, 72.85; H, 8.05; N, 2.25; O, 15.12.

2.2.1.4. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(4-dimethylamine)prop-2-en-1-imine (4d). Solid powder (75%). M.P: 231–236 °C; IR (KBr) cm^{-1}: 3415, 3335, 2944, 1723, 1621, 1512, 1413, 1112, 789; ^{1}H NMR ($CDCl_3$): δ 0.58 (s, 3H), 1.00 (s, 3H), 1.71–1.80 (m, 6H), 2.10–2.44 (m, 3H), 2.60 (s, 3H), 2.90 (t, J = 8.73 ,1H); 3.65 (m, 1H), 6.36 (s, 1H), 6.95 (d, J = 15.93, 1H), 7.37 (m, 4H), 7.61 (d, J = 15.93, 1H) 10.10 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.36, 20.18, 20.48, 21.78, 24.19, 30.78, 30.99, 31.63,



Scheme 1. Synthesis of D-ring substituted oximes of steroidal chalcones.

35.64, 36.17, 38.64, 44.26, 45.16, 49.51, 56.61, 61.99, 72.73, 121.54, 124.04, 127.19, 128.05, 132.18, 139.73, 159.54, 198.42; ESI-MS: 462 (M+H); Anal. Calcd. for $C_{30}H_{42}N_2O_2$: C, 77.88; H, 9.15; N, 6.05; O, 6.92; Found: C, 77.68; H, 9.03; N, 5.95; O, 6.81.

2.2.1.5. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(3-fluorophenyl)-prop-2-en-1-imine (4e). Solid powder (74%). M.P: 240–245 °C; IR (KBr) cm^{-1}: 3407, 3312, 2940, 1773, 1618, 1518, 1232, 1040, 781; ^{1}H NMR ($CDCl_3$): δ 0.66 (s, 3H), 1.00 (s, 3H), 1.81–2.00 (m, 6H), 2.40–2.62 (m, 3H), 2.94 (t, J = 8.92, 1H); 4.12 (m, 1H), 5.36 (s, 1H), 6.90 (d, J = 15.93, 1H), 7.18 (m, 2H), 7.73 (m, 3H) 9.36 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 12.16, 19.18, 20.43, 22.78, 25.90, 30.82, 30.96, 31.13, 35.74, 36.87, 38.24, 43.56, 44.96, 49.71, 56.81, 60.99, 72.73, 122.14, 124.14, 126.79, 129.12, 132.98, 159.73, 196.92; ESI-MS: 438 (M+H); Anal. Calcd. for $C_{28}H_{36}FNO_2$ C, 76.85; H, 8.29; N, 3.02; O, 7.31 F, 4.34; Found: C, 76.76; H, 8.21; N, 2.92; O, 7.11 F, 4.14.

2.2.1.6. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(4-fluorophenyl)-prop-2-en-1-imine (4f). Solid powder (71%). M.P:243–250 °C; IR (KBr) cm^{-1}: 3417, 3315, 2946, 1774, 1641, 1518, 1232, 1045, 759; ^{1}H NMR ($CDCl_3$): δ 0.67 (s, 3H), 1.09 (s, 3H), 1.71–1.99 (m, 6H), 2.23–2.53 (m, 3H), 2.83 (t, J = 8.61, 1H), 3.73 (m, 1H), 5.57 (s, 1H), 6.65 (d, J = 15.84, 1H), 7.18 (m, 2H), 7.14–7.22 (m, 2H), 7.52 (d, J = 15.84, 1H) 8.86 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 12.13, 19.36, 20.23, 20.58, 21.68, 23.45, 30.42, 31.86, 32.03, 35.34, 36.17, 38.24, 43.16, 44.96, 49.21, 56.01, 61.99, 70.73, 121.52, 125.04, 127.19, 128.35, 132.08, 139.53, 158.14, 196.82; ESI-MS: 438 (M+H); Anal. Calcd. for $C_{28}H_{36}FNO_2$ C, 76.85; H, 8.29; N, 3.02; O, 7.31 F, 4.34; Found: C, 76.74; H, 8.22; N, 2.91; O, 7.12 F, 4.14.

2.2.1.7. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(4-methoxyphenyl)prop-2-en-1-imine (4g). Solid powder (72%). M.P: 250–255 °C; IR (KBr) cm−1: 3405, 3322, 2916, 2853, 1803, 1637, 1521, 1275, 1036, 769; ^{1}H NMR (CDCl3): δ 0.68 (s, 3H), 1.2(s, 3H), 1.7–1.90 (m, 6H), 2.3–2.56 (m, 3H), 2.93 (t, J = 8.89, 1H), 3.13 (m, 1H), 4.17 (s, 3H), 5.46 (s, 1H), 6.55 (d, J = 15.92, 1H), 6.81 (d, J = 8.72, 2H), 7.61 (d, J = 8.72, 2H), 7.83 (d, J = 15.84, 1H), 9.36 (s, 1H); ^{13}C NMR (500 MHz, CDCl3): δ 12.23, 19.31, 21.21, 21.78, 24.23, 31.51, 32.13, 34.42, 36.17, 38.24, 43.56, 44.96, 49.12, 56.31, 61.89, 75.83, 121.54, 124.74, 127.19, 128.45, 132.46, 139.63, 160.14, 199.94; ESI-MS: 450 (M+H); Anal. Calcd. for C29H39NO3: C, 77.47; H, 8.74; N, 3.12; O, 10.68 Found:: C, 77.38; H, 8.64; N, 3.02; O, 10.56.

2.2.1.8. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(2-methoxyphenyl)prop-2-en-1-imine (4h). Solid powder (73%). M.P: 255–260 °C; IR (KBr) cm^{-1}: 3485, 3330, 2950, 1718, 1623, 1528, 1246, 1014, 763; ^{1}H NMR ($CDCl_3$): δ 0.70 (s, 3H), 1.70 (s, 3H), 1.9–2.10 (m, 6H), 2.60–2.72 (m, 3H), 2.99 (t, J = 8.85, 1H), 3.52 (m, 1H), 3.98 (s, 3H), 5.57 (s, 1H), 6.84 (d, J = 16.16, 1H), 7.01 (m, 2H), 7.46 (m, 1H), 7.59 (d, J = 6.37, 1H), 7.98 (d, J = 16.16, 1H), 10.06 (s, 1H),; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.34, 19.14, 20.38, 21.78, 25.11, 30.67, 30.88, 31.03, 35.44, 36.27, 38.44, 43.56, 44.76, 49.01, 55.29, 61.89, 71.13, 118.75, 124.44, 127.09, 128.15, 130.38, 140.93, 160.34, 199.93; ESI-MS: 450 (M+H); Anal. Calcd. for $C_{29}H_{39}NO_3$: C, 77.47; H, 8.74; N, 3.12; O, 10.68 Found:: C, 77.38; H, 8.64; N, 3.02; O, 10.56.

2.2.1.9. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(4-chlorophenyl)-prop-2-en-1-imine (4i). Brown powder (70%). M.P: 240–250 °C; IR (KBr) cm^{-1}: 3425, 3300, 2917, 1718, 1611, 1513, 1296, 1024, 763; ^{1}H NMR ($CDCl_3$): δ 0.61 (s, 3H), 1.03 (s, 3H), 1.65–1.92 (m, 6H), 2.22–2.54 (m, 3H), 2.76 (t, J = 9.12, 1H), 3.62 (m, 1H), 5.45 (s, 1H), 6.74 (d, J = 15.31, 1H), 6.96–7.21 (m, 4H), 7.54 (d, J = 15.31, 1H), 9.96 (s, 1H),; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.13, 19.58, 20.23, 24.04, 25.21, 30.92, 31.66, 35.04, 36.17, 38.44, 43.16, 45.76, 49.21, 57.28, 61.29, 75.03, 121.64, 124.04, 127.19, 129.45, 130.98, 142.13, 160.04, 199.83; ESI-MS: 455 (M+H); Anal. Calcd. for $C_{28}H_{36}ClNO_2$: C, 74.07; H, 7.99; N, 3.08; O, 7.05; Cl, 7.81; Found C, 74.01; H, 7.89; N, 3.01; O, 6.99; Cl, 7.78.

2.2.1.10. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3-(4-bromophenyl)-prop-2-en-1-imine (4j). Solid brown powder (69%). M.P:238–244 °C; IR (KBr) cm^{-1}: 3454, 3305, 2939, 2872, 1605, 1511, 1355, 1200, 754; ^{1}H NMR ($CDCl_3$): δ 0.61 (s, 3H), 1.03 (s, 3H), 1.71–1.81 (m, 6H), 2.21–2.35 (m, 3H), 2.79 (t, J = 8.68, 1H), 3.62 (m, 1H), 5.54 (s, 1H), 6.38 (s, 1H), 6.67–6.89 (dd, J = 3.68, 2H), 7.37 (m,1H), 7.58 (s, 1H), 10.16 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.26, 20.18, 21.34, 22.48, 22.78, 24.11, 30.52, 30.96, 32.13, 35.59, 37.85, 38.74, 43.96, 49.12, 56.23, 61.39, 72.53, 121.04, 124.91, 127.09, 121.05, 130.19, 138.95, 160.14, 197.44; ESI-MS: 499 (M+H); Anal. Calcd. for $C_{28}H_{36}BrNO_2$: C, 67.46; H, 7.28; N, 2.81; O, 6.42; Br, 16.03; Found: C, 67.34; H, 7.21; N, 2.76; O, 6.36; Br, 15.96.

3. Biology

3.1. Antimicrobial activity

The bacterial strains used for the analysis were *Bacillus subtilis* (MTCC 619), *Staphylococcus epidermidis* (MTCC 435), *Proteus vulgaris* (MTCC 426) and *Pseudomonas aeruginosa* (MTCC 424). The fungal strains used were *Aspergillus niger* (MTCC 1344) and *Penicillium chrysogenum* (MTCC 947). All the bacterial and fungal strains were obtained from The Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. Kanamycin and Fluconazole were used as standard antibacterial and antifungal substances respectively, under similar conditions for comparison. Dimethyl sulphoxide (DMSO) was used as negative control.

The test organisms were cultured on agar slants, incubated 24 h at 37 ± 0.5 °C and 24–48 h at 27 ± 0.2 °C for bacteria and fungi respectively to get the freshly prepared cultures. The steroidal oxime derivatives were evaluated for antimicrobial activity against these freshly prepared strains of test organisms by agar diffusion method [19–21]. Muller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) were used as nutrient media for bacterial and fungal strains respectively. The media (MHA &PDA) was prepared using distilled water and 20 ml of it was transferred into 50 ml test tubes, the test tubes were tightly plugged with cotton and sterilized in autoclave at 15 lb/in^2 for 15 min as directed by the manufacturer. After sterilization the medium was inoculated with freshly cultured bacterial strains under sterile condition i.e. under Laminar Flow. The inoculation was done when the temperature of the medium reached to 50–40 °C, so that test organism may not die at higher temperature. The medium inoculated with test microorganisms was transferred into the plates of 90 mm size under sterile conditions. The medium was allowed to solidify and the wells (4/plate) of 6 mm diameter and 50 μl volume were bored on it using sterile cork borer. The solution of test compound 1000 μg/ml was prepared in DMSO and the wells bored on the medium were each filled (50 μg) with test compound using micropipette (20–200 μl). Four wells were bored on the plates and each filled with same

Table 1
Antibacterial and antifungal screening data of compounds **4a–j**.

Compound	(Zone of inhibition in "mm")					
	Antibacterial activities				Antifungal activities	
	B. subtilis (MTCC 619)	*S. epidermidis* (MTCC 435)	*P. Vulgaris* (MTCC 426)	*P. aeruginosa* (MTCC 424)	*A. niger* (MTCC1344)	*P. chrysogenum* (MTCC 947)
4a	20	18	12	13	11	11
4b	18	17	10	14	14	13
4c	13	-	12	15	11	–
4d	17	15	14	14	14	15
4e	14	11	–	12	11	17
4f	13	15	15	14	15	20
4g	15	13	–	12	11	14
4h	16	–	11	12	14	12
4i	11	14	12	15	14	19
4j	21	10	11	12	12	14
Control	–	–	–	–	–	–
Kenamycin	24	24	22	17	–	–
Flucanazole	–	–	–		18	14

P. vulgaris-*Proteus vulgaris*, B. subtilis-*Bacillus subtilis*, *S.* epidermidis-*Staphylococcus epidermidis*, P. aeruginosa *Pseudomonas aeruginosa.* DMSO-Negative control, well diameter/vol.- 6 mm/50 μl, Kanamycin- Standard for antibacterial activity. Fluconazole-Standard for anti fungal activity.

compound and two plates for each test compound were taken and the experiment was repeated thrice. The discs of Kanamycin and Fluconazole were also incorporated into the medium for comparison (10–30 μg). The plates containing test organism and test material in contact were incubated at 37 ± 0.5 °C for 24 h. Same procedure was employed for antifungal activity however, the culture strains of fungi were maintained on potato dextrose agar and spores were transferred in the PDA medium and the plates were incubated at 27 ± 0.2 °C for 24–48 h. Inhibition of growth of test organisms (bacterial & fungal) in presence of test material and standard was measured with the help of standard scale and the mean values of inhibition zones are reported in Table 1.

3.2. Minimum inhibitory concentration

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an anti-microbial that will inhibit the visible growth of a microorganism after overnight incubation and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture onto antibiotic-free media. Minimum inhibitory concentrations (MICs) are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing [22]. MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing, or when disc diffusion methods are not appropriate. The Minimum Inhibitory Concentration (MIC) was determined *in vitro* by macrodilution method for four bacterial isolates. Antibiotic dilution ranges were prepared from stock solution having concentration of 1000 μg/mL as 0, 4, 8, 16, 32, 64, 128, 256, 512 (μg/mL) using DMSO as solvent with the help of micropipette. The Muller Hinton Agar medium was prepared using distilled water and 20 mL of it was transferred into 50 mL test tubes, the test tubes were tightly plugged with cotton and sterilized in autoclave at 15 lb/in^2 for 15 min as directed by the manufacturer. The inoculums was prepared by transferring the bacterial colonies with the help of sterile loop into the freshly prepared and autoclaved 1 mL of Muller Hinton Broth under laminar flow and were taken for incubation for 24 h at 37 ± 0.5 °C. The visible turbity of these organism suspensions were matched with 0.5 Mc Farland standard (0.5 mL of 0.048 M $BaCl_2$ in 99.5 mL of 0.18 M H_2S0_4) by measuring the absorbance of organism suspension and Mc Farland so that suspension should contain 10^7–10^8 CFU/mL. From the bacterial suspensions with the help of sterile loop, a loop of organisms were transferred into the different antibiotic dilutions (4 mg/mL, 8 mg/mL etc.) under laminar flow and incubated for 24 h at 37 ± 0.5 °C. The inoculums after incubation were tested for bacterial growth by transferring them with the help of sterile loop on the nutrient agar plates. The nutrient agar plates were incubated for 24 h at 37 ± 0.5 °C to determine the visible growth of microorganism. 0 mg/mL is antibiotic free inoculums, was taken as growth or positive control for microorganism.

3.3. Antioxidant activity

3.3.1. General free radical scavenging-DPPH assay

The antioxidant potential of any compound can be determined on the basis of its capacity to trap the stable 1, 1-diphenyl-2-pieryl hydrazyl (DPPH) free radical [23,24]. The antioxidant activity by this method is measured as the decrease in the absorbance of DPPH at 517 nm resulting from the colour change from purple to yellow. The decrease in absorbance is because of formation of stable molecule of DPPH on reaction with an antioxidant through donation of hydrogen or electron by an antioxidant. The free electron on DPPH radical is responsible for giving absorbance peak at 517 nm and appears purple in colour. The antioxidant agent pair up through donation of electron or release of hydrogen with the free electron on DPPH radical and form stable molecule of DPPH–H. The change of colour from purple to yellow is attributed to decrease of molar absorptivity of DPPH radical when odd electron of DPPH pair up with the antioxidant agent. The resulting decrease in colour is also stiochiometric with number of electrons captured. Antiradical activity of Compounds was performed by DPPH model. Stock solution of DPPH (1.3 mg/ml) in methanol was prepared. Stock solution of DPPH 100 μl was added in 3 ml of methanol and absorbance was recorded at 517 nm. The various concentrations of Compounds (25, 50, 75, 100, 125 mg/ml) were prepared. All sample solutions 1 ml each is diluted to 3 ml and 100 μl of stock solution of DPPH was added then absorbance were recorded at 517 nm.

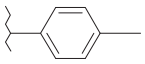
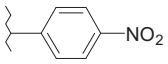
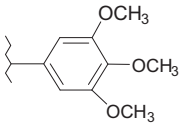
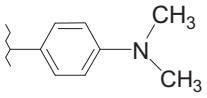
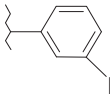
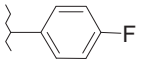
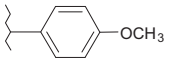
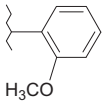
$$\% \text{ Inhibition} = [\text{Blank} - \text{Test}]/\text{Blank} \times 100$$

4. Results and discussion

4.1. Chemistry

Present study was undertaken to synthesize some novel D-ring oxime derivatives of chalcones of 20-keto pregnenolone to

investigate their probable antimicrobial and antioxidant effects. Target compounds were obtained in two step reaction, in first step pregnenolone was converted into corresponding chalcone with different aldehydes. In the second step the chalcones on reaction with hydroxylamine hydrochloride got converted into corresponding oximes which were isolated and recrystallized from ethylacetate. The preparation of the latter involves the following synthetic approach (Scheme 1). Most products were found to be homogeneous by TLC (ethylacetae:hexane) and ^{1}H NMR analyses, but when needed, heterogeneous products were readily purified by silica gel column chromatography using ethylacetate/hexane eluent. The structure of all the compounds was confirmed using NMR, IR, mass spectrometry, and elemental analysis.

Entry	Nature of R	Entry	Nature of R
4a		4f	
4b		4g	
4c		4h	
4d		4i	
4e		4j	

4.2. Biology

4.2.1. Antimicrobial activity

The antimicrobial activity of the synthesized compounds was determined against some bacterial and fungal strains. Table 1 summarizes the *in vitro* activity of both the types of microbial strains against all the synthesized test chalconyl oximes. Evaluation of antimicrobial activity showed that all the test compounds were active *in vitro* against all the tested microorganisms with varying degrees of inhibition. The oxime derivatives showed enhanced antimicrobial activity than the reported chalcones of pregnenolone. The substituents on aromatic ring in **4a**, **4e**, **4f**, **4g** and **4h** were same as chalcones of the reported paper [25], However, the antimicrobial activity of afore mentioned oxime derivatives were enhanced against some or all bacterial and fungal strains used for testing, this can be attributed to the incorporation of oxime group (=NOH) in the core molecule of steroidal chalcone, thus the aim of incorporating oxime group in the steroidal chalcone to enhance its biological properties proved to be successful. Among the oxime derivatives, the compounds **4a** and **4j** were found more potent against *Bacillus subtilis,* similar behavior were observed in other oxime derivatives as well, **4a** and **4b** against *Staphylococcus epidermidis,***4f** against *Proteus vulgaris,***4c** and **4i** against *Pseudomonas aeruginosa*, **4b, 4d, 4h** and **4i** against *Aspergillus niger*. The compounds **4d**, **4e, 4f,** and **4i** showed the highest inhibition against *Penicillium chrysogenum*.

SAR studies on these oxime derivatives revealed that the oxime derivatives of steroids were more potent than its chalcones. Both electron donating and electron withdrawing groups on the aromatic nucleus had no remarkable effect on the antimicrobial properties of oximes. Since the compound exist in two isomeric forms i,e *syn*- and *anti*- isomers, However, only one isomer formed predominantly may be due to the bulky nature of steroid nucleus and the methyl group present at D-ring of the steroid molecule.

4.2.2. Minimum inhibitory concentration

The MIC of the synthesized compounds was determined against two gram positive and two gram negative bacteria and two fungal strains using macrodilution method. Table 2 summarizes the *in vitro* susceptibilities of all the types of isolates against all the synthesized test steroidal oximes. Evaluation of MIC showed that all the test compounds were active *in vitro* against all the tested microorganisms with varying degrees of inhibition except *P. vulgaris*, within the reference range. Our study revealed that all the compounds had stronger antibacterial activity against gram positive bacteria when compared to gram negative bacteria, which may be due to the presence of the lipopolysaccharides in the outer membrane of the gram-negative bacteria, which may hinder the penetration of these oximes into the cells. As already demonstrated, our results also showed that the activity of the tested agents against each microbe varies due to structural differences between the microorganisms. It was interesting to observe that the gram negative *P. vulgaris* bacteria did not show susceptibility against any of the steroidal oxime at the concentration $\leqslant$512 μg/mL.

4.2.3. Antioxidant activity

The *in vitro* antioxidant activity and scavenging effects of the steroidal oximes were evaluated by using different reactive species assay containing DPPH radical scavenging activity. The free radical scavenging activity of all the steroidal oximes (**4a–j**) were

Table 2
MIC values (μg/mL) of steroidal oximes against bacterial and fungal strains.

Bacteria	Compound									
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**
B. subtilis	⩽64	⩽128	⩽64	⩽128	⩽64	⩽128	⩽64	⩽128	⩽128	⩽512
S. epidermidis	⩽128	⩽64	⩽64	⩽128	⩽128	⩽64	⩽128	⩽256	⩽64	⩽128
P. vulgaris	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
P. aeruginosa	⩽256	⩽128	⩽256	⩽128	⩽256	⩽512	⩽256	⩽128	⩽256	⩽512
Fungi										
A. niger	⩽128	⩽64	⩽128	⩽64	⩽128	⩽128	–	⩽256	⩽64	⩽128
P. chrysogenum	⩽128	⩽256	⩽128	⩽256	⩽128	⩽256	⩽256	–	⩽128	⩽256

Bacillus subtilis (MTCC 619), *Staphylococcus epidermidis* (MTCC 435), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 424).A. niger (MTCC 1344), P.chrysogenum (MTCC 947)

Table gives the MIC data obtained after treating different bacterial and fungal strains against test doses of the different steroidal chalcones and the values are reported in terms of μg.

Table 3
Antioxidant activity (DPPH assay data) of compounds **4a–4j**.

Compound	% inhibition				
	25 μg/ml	50 μg/ml	75 μg/ml	100 μg/ml	125 μg/ml
4a	6.14 ± 0.02	9.43 ± 0.03	12.17 ± 0.02	19.22.±0.01	24.23 ± 0.01
4b	10.87 ± 0.04	13.64 ± 0.01	18.43 ± 0.06	23.19 ± 0.02	27.51 ± 0.03
4c	8.99 ± 0.05	14.18 ± 0.06	28.90 ± 0.06	33.63 ± 0.08	40.29 ± 0.06
4d	9.56 ± 0.01	15.75 ± 0.09	26.61 ± 0.09	40.89 ± 0.06	51.27 ± 0.08
4e	13.41 ± 0.04	21.68 ± 0.01	28.17 ± 0.08	38.56 ± 0.03	56.59 ± 0.09
4f	12.56 ± 0.06	19.96 ± 0.02	24.41 ± 0.04	37.42 ± 0.01	48.80 ± 0.04
4g	11.24 ± 0.01	16.81 ± 0.06	22.18 ± 0.01	34.76 ± 0.03	45.85 ± 0.01
4h	16.88 ± 0.08	22.22 ± 0.07	32.28 ± 0.02	41.23 ± 0.08	58.24 ± 0.01
4i	11.15 ± 0.0	15.20 ± 0.0	22.39 ± 0.0	30.13 ± 0.0	38.13 ± 0.04
4j	12.78 ± 0.01	16.43 ± 0.04	20.21 ± 0.03	27.61 ± 0.09	35.72 ± 0.05
Control	–	–	–	–	–
Standard	26.73 5 μg/ml	48.63 10 μg/ml	56.46 15 μg/ml	84.10 20 μg/ml	96.27 25 μg/ml

1. Values represent the mean ± standard error mean (SEM) of three experiments.
2. (–): No inhibition, standard: ascorbic acid.

evaluated through their ability to quench the DPPH• using ascorbic acid as reference. Among them compounds (**4e** and **4h**) exhibited good antioxidant properties, with the strongest being observed in compound **4h**. However all the synthesized compounds were less potent than ascorbic acid as the reference. From the results it can be interpreted that the varying substituents on the aromatic ring showed marked effects on the antioxidant property of these compounds. However, bulkiness of the substituents on the aromatic nucleus showed enhanced potency of the oximes, this may be attributed to the field effects or can be associated with the steroid nucleus. The potencies for the antioxidant activity of the test compounds to the reference drug are shown in Table 3.

5. Conclusion

A series of novel D-ring substituted oximes of chalconyl derivatives of Pregnenolone was synthesized and screened for antioxidant activity using DPPH scavenging method and antimicrobial activity against a panel of various bacterial and fungal strains. Among them compounds (**4e** and **4h**) exhibited good antioxidant properties against ascorbic acid. The antibacterial and antifungal and their MIC screening data revealed that some newly generated compounds are potential antimicrobial agents. Thus the idea of appending the oxime moiety to steroidal nucleus so as to combine the beneficial effects in a single structure by expecting some biological activities like antibacterial and antifungal proved to be successful. In conclusion, the present study showed that the synthesized compounds can be used as template for future development through modification and derivatization to design more potent and selective antioxidant and antimicrobial agents.

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