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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

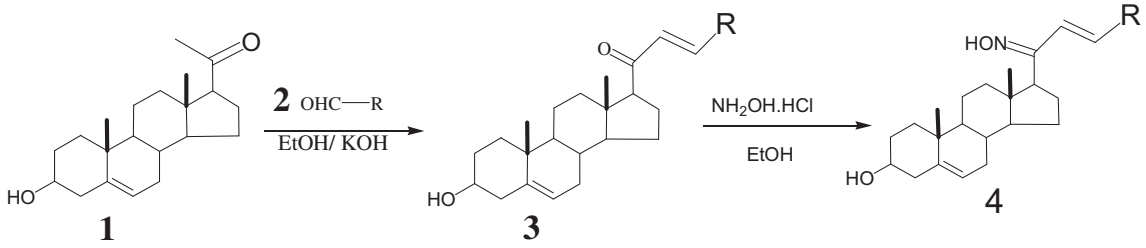
To a solution of pregnenolone 1 (0.316 g, 1 mmol, 1 eq.) in ethanol (10 ml) was added a conc. aq. solution of KOH (2 eq.). Then aldehyde 2 (1.2 eq.) was charged into the reaction mixture and the reaction mixture was stirred for 1–2 h at room temperature to get the corresponding benzylidine derivative 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.