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Synthesis of steroidal and nonsteroidal vicinal heterocyclic alcohols, *N*-(1-cycloalkenyl)heterocycles and their antibacterial studies

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ABSTRACT

A solvent free steroidal and nonsteroidal epoxide ring opening reaction by nitrogen containing heterocycles under microwave irradiation is described. Some of the epoxide ring opening compounds were converted to their corresponding *N*-(1-cycloalkenyl)heterocycles *via* an acid catalyzed dehydration reaction. The antimicrobial activities of the epoxide ring opening compounds and *N*-(1-cycloalkenyl)heterocyclic compounds were tested by agar diffusion assay. Compounds **6**, **9**–**12**, **24** and **27** showed moderate inhibition against the growth of pathogenic bacteria *Escherichia coli*, *Pseudomonas syringae*, *Bacillus subtilis*, *Proteus vulgaris* and *Staphylococcus aureus*.

1. Introduction

The incorporation of a heterocycle in the steroid backbone often alters the biological activity of the steroidal molecule which serves as a platform for developing a new pharmaceutical compound for various applications [1,2]. Vicinal imidazolyl alcohols and their derivatives are very important class of compounds due to their wide range of biological activities [3]. For example, non steroidal vicinal imidazolyl alcohols such as ornidazole, metronidazole (**I**, Fig. 1) and secnidazole (**II**, Fig. 1) are effective antiprotozoal drugs. At the same time different heterocycle substituted steroidal compounds having vicinal hydroxyl group are found to have different biological activities. In Fig. 1, steroidal compounds such as 2β-piperazinyl-5α-androstane-3α,17β-diols (**III**) and 2,3-dihydrowithaferin A-3β-triazoles (**IV**) show antiproliferative potency and cytotoxic activity respectively [4,5].

On the other hand, both nonsteroidal and steroidal *N*-(1-cycloalkenyl)heterocycles are important classes of compounds which have potential biological activity, for instance nonsteroidal compounds 1-[2-(methylsulfanyl)-1-cyclopentenyl]imidazole (**VIII**, Fig. 1) and 1-[2-(2-thienyl)-1-cyclopentenyl]imidazole (**IX**, Fig. 1) are reported as neuronal injury inhibitors [6] whereas steroidal *N*-(1-cycloalkenyl)heterocycles such as abiraterone (**V**), abiraterone acetate (**VI**) and VN/124-1 (**VII**) are potent CYP17 inhibitors [7,8] (Fig. 1).

As a part of our ongoing research on development of new methodology for the synthesis of novel steroidal and nonsteroidal heterocyclic compounds [9], herein we report a new solvent free green strategy [10] for the steroidal and nonsteroidal epoxide ring opening reaction by imidazoles, benzimidazole for the synthesis of hydroxy-(1*H*-imidazol-yl)/hydroxy-(1*H*-benzimidazol-yl) steroids and 2-(1*H*-imidazol-1-yl)/2-(1*H*-benzimidazol-1-yl)cyclohexanols under microwave irradiation. We extended our methodology for the epoxide ring opening reactions with other nitrogen containing heterocycles also. Further, these epoxide ring opening steroidal and nonsteroidal β-hydroxy heterocyclic compounds were converted to the corresponding *N*-(1-cycloalkenyl)heterocycles *via* an acid catalyzed dehydration reaction in good yields. We also report *in vitro* antimicrobial activities of these compounds against five pathogenic strains, they are, *Escherichia coli* (ATCC 8739), *Proteus vulgaris* (MTCC 426), *Pseudomonas syringae* (MTCC 673), *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 29213) species.

2. Experimental

2.1. General methods

Melting points were measured with a Buchi B-540 melting point apparatus and are uncorrected. IR spectra were recorded on

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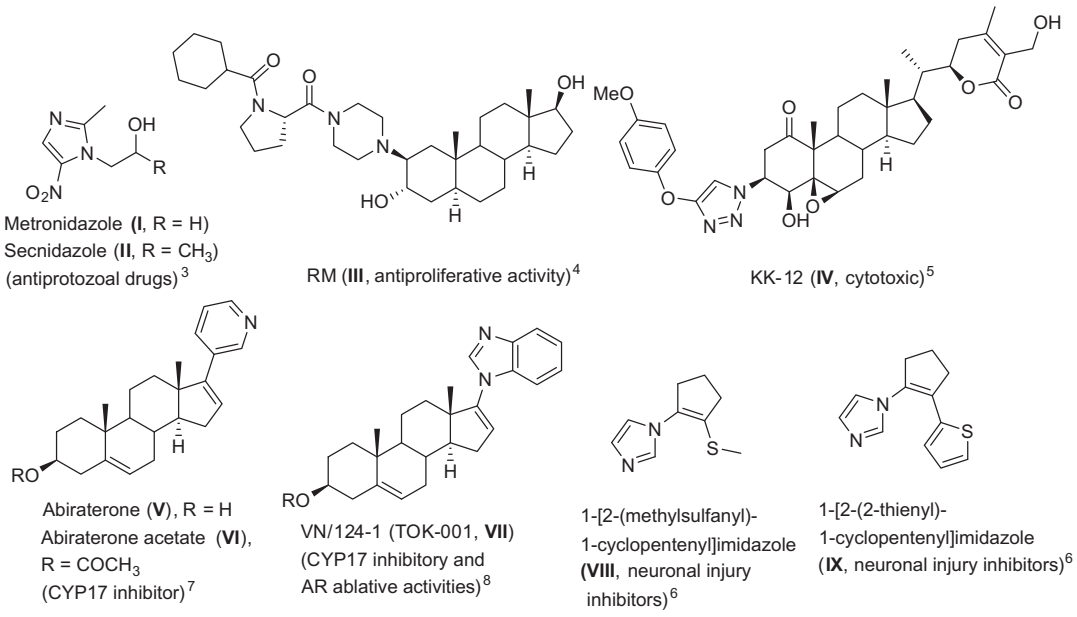


Fig. 1. Example of biologically important steroidal/nonsteroidal heterocyclic alcohol and *N*-(1-cycloalkenyl)heterocycles.

Elmer FT-IR-2000 spectrometer using KBr pellets or on a thin film using chloroform. NMR spectra were recorded on Avance DPX 300 MHz FT-NMR spectrometer or Bruker Avance III 500 MHz FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on Trace DSQ GCMS instrument. All the commercially available reagents were used as received. All experiments were monitored by thin layer chromatography (TLC). TLC was performed on pre-coated silica gel plates (Merck). Column chromatography was performed on silica gel (60–120 mesh, Merck Chemicals). All microwave reactions were carried out in a Synthos 3000 (Anton Paar) microwave reactor.

2.2. Chemical synthesis

2.2.1. Conversion of cholesteroyl acetate (**1**) to 3α,4β-epoxy-5-en-cholest-7-one (**4**)

*2.2.1.1. Preparation of 3β-acetoxy-cholest-5-en-7-one (**2**)[11].* To a solution of cholesteroyl acetate (**1**, 10.0 g, 23.34 mmol) and $RuCl_3 \cdot H_2O$ (60 mg) in cyclohexane (40 mL), 70% *tert*-butyl hydroperoxide (20 mL) was added at room temperature for 1 h and the reaction mixture was stirred for another 5 h. The reaction mixture was then treated with saturated $NaHCO_3$ solution, washed with water, extracted with EtOAc and dried over Na_2SO_4. The crude product obtained after removal of the EtOAc layer was purified by silica gel column chromatography using ethyl acetate/hexane (1:9) as the eluent to afford pure keto compound **2**. Yield 8.26 g (80%); R_f = 0.5 (EtOAc/hexane = 1:9); m.p. 158–160 °C. IR (KBr, cm^{-1}) 2949, 1732, 1670, 1466, 1376, 1248, 758; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H), 0.65–2.55 (m, 29H), 0.86 (d, *J* = 6.3 Hz, 6H), 1.21 (s, 3H), 1.61 (s, 3H), 4.77–4.66 (m, 1H), 5.70 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.3, 18.9, 21.2, 21.3, 22.6, 22.8, 23.8, 27.3, 28.0, 26.3, 28.6, 35.7, 36.0, 36.2, 37.7, 38.3, 38.6, 39.5, 43.1, 45.4, 49.8, 49.9, 54.7, 72.2, 126.7, 163.9, 170.3, 202.0; MS (EI, *m*/*z*) = 382.3 [$M^+ - CH_3COOH$]; Anal. Calcd for $C_{29}H_{46}O_3$: C 78.68; H 10.47; Found: C 78.70; H 10.69.

*2.2.1.2. Preparation of cholesta-3,5-dien-7-one (**3**)[12].* Compound **2** (8.0 g, 18.09 mmol) was refluxed with hydrochloric acid (20 mL) in methanol for 1 h. The residue obtained after removal of the solvent was washed with water, neutralised with $NaHCO_3$, extracted with DCM and dried over Na_2SO_4. The crude product obtained after removal of the DCM layer was purified by silica gel column chromatography using ethyl acetate/hexane (0.5:9.5) as the eluent to afford steroidal 7-keto 3,5-diene **3**. Yield 6.36 g (92%); R_f = 0.7 (EtOAc/hexane = 1:9); m.p. 117–119 °C. IR (KBr, cm^{-1}): 2945, 1712, 1580, 1250; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.71 (s, 3H), 0.85–2.36 (m, 24H), 0.88 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.4 Hz, 3H), 1.11 (s, 3H), 5.60 (s, 1H), 6.06–6.22 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 16.6, 18.9, 21.2, 22.6, 22.9, 23.4, 23.9, 26.4, 26.5, 28.0, 28.6, 32.8, 35.8, 36.3, 39.0, 39.5, 43.4, 46.0, 49.6, 50.7, 54.9, 124.2, 127.7, 136.6, 160.7, 202.3. MS (EI, *m*/*z*) = 382; Anal. Calcd. for $C_{27}H_{42}O$: C 84.75; H 11.06; found: C 84.77; H 11.28.

*2.2.1.3. Preparation of 3α,4α-epoxy-5-en-cholest-7-one (**4**)[13].* To a stirring solution of compound **3** (6.2 g, 16.22 mmol) in chloroform (15 mL), *m*-CPBA (4.2 g, 24.33 mmol) was added at 0 °C. The reaction mixture was then stirred for 4 h at room temperature and after completion of the reaction, as indicated by TLC, 4% Na_2SO_3 was added into it and the whole reaction mixture was kept stirring for another 4 h. The reaction mixture was then extracted with $CHCl_3$ washed with water, brine and dried over Na_2SO_4. The crude product obtained after removal of the solvent was purified by silica gel column chromatography using ethyl acetate/hexane (1:9) as the eluent to afford pure steroidal epoxide **4**. Yield 4.84 g (75%); R_f = 0.5 (EtOAc/hexane = 1:9); m.p. 128–129 °C. IR (KBr, cm^{-1}): 2952, 1670, 1462, 1381; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.69 (s, 3H), 0.85–2.36 (m, 24H), 0.88 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.08 (s, 3H), 3.39–3.49 (m, 2H), 6.04 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.7, 18.8, 20.7, 21.2, 22.6, 22.8, 23.8, 26.2, 26.4, 28.0, 28.6, 35.6, 35.8, 36.2, 38.8, 39.5, 43.4, 46.5, 49.7, 50.5, 52.0, 52.4, 54.8, 131.4, 160.2, 201.3. MS (EI, *m*/*z*) = 398; Anal. calcd. for $C_{27}H_{42}O_2$: C 81.35; H 10.62; found: C 81.33; H 10.59.

*2.2.1.4. Preparation of 3β-acetoxy-cholest-5,6-epoxide (**13**:**14** = 4:1)[14].* To a stirring solution of Cholesterol acetate **1** (3.0 g, 7.0 mmol) in chloroform (15 mL), *m*-CPBA (1.81 g, 10.5 mmol) was added at 0 °C. The reaction mixture was then stirred for 6 h at room temperature and after completion of the reaction, as indicated by TLC, 4% Na_2SO_3 was added into it and the whole reaction

mixture was kept stirring for another 4 h. The reaction mixture was then extracted with $CHCl_3$ washed with water, brine and dried over Na_2SO_4. The crude product obtained after removal of the solvent was purified by silica gel column chromatography using ethyl acetate/hexane (1:9) as the eluent to afford a mixture of α and β steroidal epoxides **13** and **14** (α/β = 4:1). Yield 2.27 g (73%); m.p. 94–97 °C. IR (KBr, cm^{-1}): 2952, 1725; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.61 (s, 2.4H), 0.64 (s, 0.6H), 0.82–2.32 (m, 40H), 2.02 (s, 2.4H), 2.03 (s, 0.6H), 2.84–2.91 (m, 0.8H), 3.04–3.12 (m, 0.2H), 4.72–4.79 (m, 0.2H), 4.88–5.02 (m, 0.8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.8, 11.9, 15.9, 17.0, 18.6, 20.6, 21.3, 21.9, 22.6, 22.8, 23.79, 23.84, 24.0, 24.2, 27.2, 28.0, 28.07, 28.14, 28.8, 29.7, 29.9, 32.1, 32.5, 36.7, 38.0, 39.4, 39.5, 39.8, 42.3, 42.4, 51.0, 55.8, 56.2, 56.8, 59.2, 62.5, 63.6, 65.2, 71.3, 71.4, 170.2, 170.6; MS (EI, *m*/*z*) = 398 [M^+].

2.2.2. General procedure for the preparation of steroidal and nonsteroidal vicinal heterocyclic alcohols (**5**–**12**, **15**, **16**, **18**–**22**)

The epoxide **4** (0.5 mmol) and *N*-containing heterocycle (0.5 mmol) were mixed intimately and the mixture was irradiated in a closed vessel in a Synthos 3000 microwave reactor at 600 Watt (140 °C and 12 bar) for 6–16 min. The residue obtained was purified by silica gel column chromatography using EtOAc/hexane as the eluent to afford the vicinal heterocyclic alcohols.

*2.2.2.1. 3β-(1H-Imidazolo)-4α-hydroxy-5-en-cholest-7-one (**5**).* Thick yellow gum, yield 69%; IR ($CHCl_3$, cm^{-1}): 3421, 2952, 1672, 1451, 756; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.67 (s, 3H), 0.83–2.66 (m, 25H), 0.85 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.41 (s, 3H), 2.65–2.67 (m, 1H), 4.34 (br s, 1H), 5.83 (s, 1H), 6.90 (s, 1H), 7.11 (s, 1H), 7.58 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.7, 17.3, 18.8, 20.5, 22.6, 22.8, 23.8, 26.4, 28.2, 28.5, 29.6, 32.9, 35.7, 35.9, 38.6, 39.2, 39.5, 43.2, 45.8, 50.6, 52.3, 54.7, 66.7, 66.9, 74.8, 132.5, 133.8, 134.5, 162.7, 202.1. MS (EI, *m*/*z*) = 466 (M^+). Anal. calcd. for $C_{30}H_{46}N_2O_3$: C, 77.21; H, 9.93; N, 6.00; found: C, 77.45; H, 9.71; N, 6.37.

*2.2.2.2. 3β-(4-Nitro-1H-imidazolo)-4α-hydroxy-5-en-cholest-7-one (**6**).* Thick yellow gum, yield 62%; IR ($CHCl_3$, cm^{-1}): 3424, 2950, 1672, 754; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.69 (s, 3H), 0.86–2.64 (m, 25H), 0.84 (d, *J* = 6.4 Hz, 6H), 0.90 (d, *J* = 6.6 Hz, 3H), 1.42 (s, 3H), 2.65–2.68 (m, 1H), 4.36 (s, 1H), 5.84 (s, 1H), 8.20 (s, 1H), 8.23 (s, 1H); MS (EI, *m*/*z*) = 511 (M^+). Anal. calcd. for $C_{30}H_{45}N_3O_4$: C, 70.42; H, 8.86; N, 8.21; found: C, 70.52; H, 8.86; N, 8.35.

*2.2.2.3. 3β-(4-Formyl-1H-imidazolo)-4α-hydroxy-5-en-cholest-7-one (**7**).* Thick brown gum, yield 66%; IR ($CHCl_3$, cm^{-1}): 3421, 2954, 1676, 752; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.70 (s, 3H), 0.85–2.64 (m, 25H), 0.85 (d, *J* = 6.4 Hz, 6H), 0.92 (d, *J* = 6.6 Hz, 3H), 1.40 (s, 3H), 2.66–2.68 (m, 1H), 4.37 (s, 1H), 5.86 (s, 1H), 7.54 (s, 1H), 7.61 (s, 1H), 9.93 (s, 1H); MS (EI, *m*/*z*) = 494 (M^+). Anal. calcd. for $C_{31}H_{46}N_2O_3$: C, 75.26; H, 9.37; N, 5.66; found: C, 75.60; H, 9.71; N, 5.43.

*2.2.2.4. 3β-(1H-Benzo[d]imidazolo)-4α-hydroxy-5-en-cholest-7-one (**8**).* Thick yellow gum, yield 65%; IR ($CHCl_3$, cm^{-1}): 3429, 2955, 1678; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.70 (s, 3H), 0.84–2.64 (m, 25H), 0.84 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.6 Hz, 3H), 1.40 (s, 3H), 2.66–2.68 (m, 1H), 4.35 (s, 1H), 5.87 (s, 1H), 7.13–7.91 (m, 5H); MS (EI, *m*/*z*) = 516 (M^+). Anal. calcd. for $C_{34}H_{48}N_2O_2$: C, 79.02; H, 9.36; N, 5.42; found: C, 78.82; H, 9.30; N, 5.67.

*2.2.2.5. 3β-Piperidino-4α-hydroxy-5-en-cholest-7-one (**9**).* Brown solid; m.p. 117–119 °C; yield 68%; IR ($CHCl_3$, cm^{-1}): 3418, 2929, 1670, 1466, 1382, 757; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.66 (s, 3H), 0.83–2.60 (m, 35H), 0.86 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.01 (s, 3H), 2.63 (br s, 1H), 4.34 (s, 1H), 5.79 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.7, 18.8, 20.7, 21.2, 22.6, 22.8, 23.8, 26.2, 26.4, 28.0, 28.6, 29.7, 35.6, 35.7, 36.1, 38.8, 39.5, 43.4, 46.5, 49.7, 50.5, 52.0, 52.4, 54.8, 131.4, 160.3, 201.4; MS (EI, *m*/*z*) = 483 $(M)^+$, 465 $(M-18)^+$. Anal. calcd. for $C_{32}H_{53}NO_2$: C, 79.45; H, 11.04; N, 2.90; found: C, 79.38; H, 11.26; N, 2.71.

*2.2.2.6. 3β-Morpholino-4α-hydroxy-5-en-cholest-7-one (**10**).* Yellow solid; m.p. 121–123 °C; yield 74%; IR ($CHCl_3$, cm^{-1}): 3422, 2951, 1669, 1453, 756; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.67 (s, 3H), 0.84–2.66 (m, 25H), 0.86 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.42 (s, 3H), 2.38–2.42 (m, 4H), 2.66 (d, *J* = 2.0 Hz, 1H), 3.68 (t, *J* = 6.0 Hz, 4H), 4.33 (s, 1H), 5.81 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.9, 17.4, 18.9, 20.6, 22.6, 22.8, 23.8, 26.3, 28.0, 28.5, 29.7, 32.9, 35.7, 36.1, 38.6, 39.2, 39.5, 43.1, 45.8, 50.6, 52.3, 54.8, 66.7, 66.9, 74.7, 132.7, 162.6, 202.2; MS (EI, *m*/*z*) = 485 $(M)^+$. Anal. calcd. for $C_{31}H_{51}NO_3$: C, 76.65; H, 10.58; N, 2.88 found: C, 76.48; H, 10.66; N, 2.92.

*2.2.2.7. 3β-Thiomorpholino-4α-hydroxy-5-en-cholest-7-one (**11**).* Yellow thick oil; yield 69%; IR ($CHCl_3$, cm^{-1}): 3423, 2954, 1665, 1457, 754; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.68 (s, 3H), 0.85–2.64 (m, 33H), 0.85 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.5 Hz, 3H), 1.40 (s, 3H), 2.65 (d, *J* = 2.0 Hz, 1H), 4.36 (s, 1H), 5.84 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.7, 17.4, 18.9, 20.4, 22.6, 22.8, 23.8, 26.3, 28.5, 28.6, 29.5, 32.9, 35.7, 36.1, 38.7, 38.9, 39.7, 43.6, 45.8, 50.6, 52.3, 55.2, 58.3, 66.7, 66.9, 74.7, 132.9, 162.8, 202.4; MS (EI, *m*/*z*) = 501 $(M)^+$. Anal. calcd. for $C_{31}H_{51}NO_2S$: C, 74.20; H, 10.24; N, 2.79. found: C, 74.13; H, 10.35; N, 2.98.

*2.2.2.8. 3β-Tetrahydroisoquinolino-4α-hydroxy-5-en-cholest-7-one (**12**).* White solid; m.p. 194–196 °C; yield 77%; IR ($CHCl_3$, cm^{-1}): 3395, 2924, 1670, 1461, 1374, 1156, 771; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.67 (s, 3H), 0.83–2.60 (m, 29H), 0.86 (d, *J* = 6.5 Hz, 6H), 0.91 (d, *J* = 6.5 Hz, 3H), 1.08 (s, 3H), 2.84 (d, *J* = 2.1 Hz, 1H), 3.41–3.48 (m, 2H), 4.51 (s, 1H), 5.87 (s, 1H), 7.01–7.83 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 14.1, 17.7, 18.8, 21.2, 22.6, 22.7, 22.8, 26.2, 26.4, 28.0, 28.6, 29.7, 35.6, 35.7, 36.1, 38.8, 39.4, 43.4, 46.5, 49.7, 50.5, 54.8, 126.2, 127.8, 129.7, 129.9, 131.4, 160.2, 201.4; MS (EI, *m*/*z*) = 531 $(M)^+$. Anal. calcd. for $C_{36}H_{53}NO_2$: C, 81.30; H, 10.05; N, 2.63; Found: C, 81.38; H, 9.97; N, 2.89.

*2.2.2.9. 3β-Acetoxy-5α-hydroxy–cholest-6β-(1H-imidazole) (**15**).* This compound was further recrystallized from ethanol. White solid, m.p. 225–228 °C, yield 75%; IR ($CHCl_3$, cm^{-1}): 3205, 2945, 1729, 1381, 1245, 771; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.74 (s, 3H), 0.75 (s, 3H), 0.87–2.20 (m, 28H), 0.87 (d, *J* = 6.5 Hz, 6H), 0.92 (d, *J* = 6.4 Hz, 3H), 2.05 (s, 3H), 2.83 (br s, 1H), 3.97 (d, *J* = 5.7 Hz, 1H), 5.13–5.24 (m, 1H), 7.01 (s, 1H), 7.08 (s, 1H), 7.70 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.2, 15.6, 18.5, 21.0, 21.4, 22.5, 22.7, 23.8, 24.0, 27.9, 28.1, 30.6, 30.9, 32.4, 32.9, 35.7, 36.0, 38.3, 38.5, 39.4, 39.6, 42.7, 45.0, 55.9, 56.0, 62.1, 70.2, 76.6, 119.9, 128.0, 137.7, 171.0. MS (EI, *m*/*z*) = 512.4 (M^+). Anal. calcd. for $C_{32}H_{52}N_2O_3$: C, 74.95; H, 10.22. N, 5.46; found: C, 74.81; H, 10.28; N, 5.84.

*2.2.2.10. 3β-Acetoxy-5α-hydroxy-cholest-6β-(1H-benzo[d]imidazole) (**16**).* This compound was further recrystallized from ethanol. White solid, m.p. 157–159 °C, yield 71%; IR ($CHCl_3$, cm^{-1}): 3402, 2937, 1727, 1381, 1248, 771; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.80 (s, 3H), 0.87–2.52 (m, 28H), 0.87 (d, *J* = 6.5 Hz, 6H), 0.94 (d, *J* = 6.2 Hz, 3H), 1.01 (s, 3H), 1.95 (s, 3H), 3.41 (br s, 1H), 4.36 (d, *J* = 6.7 Hz, 1H), 5.11–5.18 (m, 1H), 7.25–7.30 (m, 2H), 7.45 (m, 1H), 7.80 (m, 1H), 8.39 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.5, 17.1, 18.4, 18.6, 21.2, 21.4, 22.6, 22.8, 24.0, 26.4, 28.0, 28.2, 32.2, 32.9, 33.8, 35.8, 36.2, 37.7, 38.7, 39.5, 39.8, 42.8, 44.8, 56.3, 58.4, 60.4, 71.0, 77.1, 111.2, 120.1, 122.2, 122.9, 136.0, 142.6, 142.9,

171.3. MS (EI, m/z) = 562.4 (M^+). Anal. calcd. for $C_{36}H_{54}N_2O_3$: C, 76.82; H, 9.67; N, 4.98. Found: C, 76.89; H, 9.74; N, 4.73.

*2.2.2.11. Trans-2-(1H-imidazol-1-yl)cyclohexanol (**18**).* White solid, m.p. 134–136 °C, yield 79%; IR ($CHCl_3$, cm^{-1}): 3447, 2923, 1437, 1260, 1119; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.22–2.14 (m, 8H), 3.52–3.74 (m, 2H), 4.94 (br s, 1H), 6.83 (s, 1H), 6.92 (s, 1H), 7.36 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.3, 25.0, 32.3, 34.4, 63.8, 72.7, 117.3, 127.9, 136.2; MS (EI, m/z) = 166 (M^+). Anal. calcd. for $C_9H_{14}N_2O$: C, 65.03; H, 8.49; N, 16.85; Found: C, 65.06; H, 8.52; N, 16.88.

*2.2.2.12. Trans-2-(4-nitro-1H-imidazol-1-yl)cyclohexanol (**19**).* Yellow oil, yield 75%; IR ($CHCl_3$, cm^{-1}): 3443, 2925, 1262, 1120; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.84–2.20 (m, 8H), 3.29–3.82 (m, 2H), 7.47 (s, 1H), 7.79 (s, 1H); MS (EI, m/z) = 211 (M^+). Anal. calcd. for $C_9H_{13}N_3O_3$: C, 51.18; H, 6.20; N, 19.89; Found: C, 51.40; H, 6.28; N, 19.63.

*2.2.2.13. Trans-1-(2-hydroxycyclohexyl)-1H-imidazole-4-carbaldehyde (**20**).* Colorless liquid, yield 76%; IR ($CHCl_3$, cm^{-1}): 3451, 2923, 1723, 1434, 1262; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.88–2.19 (m, 8H), 3.67–3.82 (m, 2H), 7.72 (s, 1H), 7.87 (s, 1H), 9.66 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.2, 24.9, 31.8, 34.1, 64.5, 72.9, 125.0, 139.2, 141.2, 184.9; MS (EI, m/z) = 194 (M^+). Anal. calcd. for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42; Found: C, 61.96; H, 7.18; N, 14.40.

*2.2.2.14. Trans-2-(1H-benzimidazol-1-yl)cyclohexanol (**21**).* Brown solid, m.p. 163–165 °C. yield 78%; IR ($CHCl_3$, cm^{-1}): 3090, 2926, 1615, 1494, 1458, 1253, 1075, 742; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.42–1.60 (m, 3H); 1.74–1.93 (m, 3H), 2.03–2.09 (m, 1H), 2.23–2.29 (m, 1H), 3.92–4.08 (m, 2H), 7.14 (t, J = 7.3 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.50 (m, 2H), 7.60 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.5, 25.3, 31.9, 34.4, 62.5, 72.4, 110.6, 119.5, 122.2, 122.7, 133.8, 140.8, 142.8; MS (EI, m/z) = 216 (M^+). Anal. calcd. for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95; Found: C, 72.21; H, 7.48; N, 12.99.

*2.2.2.15. Trans-2-(1H-pyrazol-1-yl)cyclohexanol (**22**).* White solid, m.p. 65–66 °C; yield 80%; IR ($CHCl_3$, cm^{-1}): 3447, 2931, 1245, 1123; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29–1.35 (m, 3H), 1.70–1.77 (m, 3H), 2.00–2.07 (m, 2H), 3.69–3.85 (m, 2H), 4.07 (br s, 1H), 6.17 (d, J = 2 Hz, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.43 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.1, 24.8, 31.2, 33.6, 66.9, 72.9, 105.0, 128.5, 139.1; MS (EI, m/z) = 166 (M^+). Anal. calcd. for $C_9H_{14}N_2O$: C, 65.03; H, 8.49; N, 16.85; Found: C, 65.25; H, 8.58; N, 16.99.

*2.2.3. Preparation of N-(1-cycloalkenyl)heterocycles (**6**)*

A stirring solution of vicinal hydroxy compound (200 mg) and catalytic amount of sulfuric acid in acetic acid (0.5 mL) was heated at 80 °C for 4–6 h. Acetic acid was removed in vacuo and ethylacetate (30 mL) and saturated solution of $NaHCO_3$ were added into the residue. The ethylacetate layer was washed with brine and the residue obtained after removal of the ethylacetate layer was purified by silica gel column chromatography using EtOAc/hexane as the eluent to afford the steroidal/nonsteroidal *N*-(1-cycloalkenyl)heterocycle derivative.

*2.2.3.1. 3-(1H-Imidazolo)-3,5-dien-cholest-7-one (**23**).* Yellow solid; m.p. 182–186 °C; yield 88%; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.72 (s, 3H), 0.80–2.50 (m, 24H), 0.85 (d, J = 6.7 Hz, 6H), 0.93 (d, J = 6.4 Hz, 3H), 1.22 (s, 3H), 5.09 (s, 1H), 6.25 (t, J = 4.2 Hz, 1H), 6.88 (s, 1H), 7.10 (s, 1H), 7.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 12.6, 14.1, 16.6, 18.9, 21.4, 22.6, 22.7, 22.8, 23.8, 26.2, 28.0, 28.5, 29.4, 29.7, 31.9, 32.4, 35.7, 36.1, 37.3, 39.5, 43.2, 45.7, 49.1, 50.2, 54.8, 114.1, 120.3, 122.4, 128.6, 133.4, 154.2, 156.5, 201.7. IR ($CHCl_3$, cm^{-1}): 2926, 1662, 1466, 772; MS (EI, m/z) = 448 $[M]^+$. Anal. calcd. for $C_{30}H_{44}N_2O$: C, 80.31; H, 9.88; N, 6.24; Found: C, 80.44; H, 9.87; N, 6.39.

*2.2.3.2. 3-(4-Nitro-1H-imidazolo)-3,5-dien-cholest-7-one (**24**).* Yellow solid; m.p. 90–92 °C; yield 83%; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.72 (s, 3H), 0.80–2.59 (m, 24H), 0.86 (d, J = 6.7 Hz, 6H), 0.93 (d, J = 6.4 Hz, 3H), 1.23 (s, 3H), 5.31 (s, 1H), 6.54 (t, J = 4.1 Hz, 1H), 8.22 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.9, 13.0, 18.7, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 28.5, 29.0, 29.7, 35.7, 35.8, 36.2, 38.3, 39.5, 42.4, 51.1, 53.7, 56.2, 56.3, 114.3, 120.8, 122.6, 129.2, 134.4, 156.1, 156.0, 201.2; IR ($CHCl_3$, cm^{-1}): 2930, 1672, 1466; MS (EI, m/z) = 493 $[M^+]$. Anal. calcd. for $C_{30}H_{43}N_3O_3$: C, 72.99; H, 8.78; N, 8.51; found: C, 72.79; H, 8.61; N, 8.64.

*2.2.3.3. 3-(4-Formyl-1H-imidazolo)-3,5-dien-cholest-7-one (**25**).* Yellow solid; m.p. 108–110 °C; yield 75%; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.72 (s, 3H), 0.81–2.55 (m, 24H), 0.86 (d, J = 6.6 Hz, 6H), 0.93 (d, J = 6.4 Hz, 3H), 1.27 (s, 3H), 5.33 (s, 1H), 6.33 (t, J = 4.3 Hz, 1H), 7.53 (d, J = 0.8 Hz, 1H), 7.58 (d, J = 1.0 Hz, 1H), 9.90 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 16.7, 18.9, 21.4, 22.6, 22.8, 23.8, 28.0, 28.5, 29.7, 31.0, 32.2, 35.7, 36.1, 37.3, 38.6, 39.5, 43.2, 45.8, 49.0, 50.1, 54.8, 122.4, 125.3, 132.8, 134.6, 139.0, 142.2, 155.7, 186.0, 201.3; IR ($CHCl_3$, cm^{-1}): 2952, 1671, 1536, 1466; MS (EI, m/z) = 476 $[M^+]$. Anal. calcd. for $C_{31}H_{44}N_2O_2$: C, 78.11; H, 9.30; N, 5.88; found: C, 78.14; H, 9.33; N, 5.91.

*2.2.3.4. 3-(1H-Benzo[d]imidazolo)-3,5-dien-cholest-7-one (**26**).* Brown solid; m.p. 126–128 °C; yield 88%; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.73 (s, 3H), 0.80–2.60 (m, 24H), 0.86 (d, J = 6.6 Hz, 6H), 0.93 (d, J = 6.5 Hz, 3H), 1.25 (s, 3H), 5.21 (s, 1H), 6.38 (s, 1H), 7.10–7.86 (m, 4H), 7.87 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 16.9, 18.9, 22.6, 22.8, 26.2, 28.0, 29.7, 35.8, 36.2, 38.7, 39.5, 42.8, 51.8, 56.0, 56.6, 114.1, 120.5, 121.9, 122.6, 122.8, 129.2, 134.4, 156.2, 156.7, 201.7; IR ($CHCl_3$, cm^{-1}): 3418, 2929, 1670, 1466, 1382, 757; MS (EI, m/z) = 498 $[M]^+$. Anal. calcd. for $C_{34}H_{46}N_2O$: C, 81.88; H, 9.30; N, 5.62; found: C, 81.98; H, 9.21; N, 5.75.

*2.2.3.5. 3-(Morpholino)-3,5-dien-cholest-7-one (**27**).* Thick yellow liquid; yield 68%; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.67 (s, 3H), 0.80–2.45 (m, 24H), 0.86 (d, J = 6.6 Hz, 6H), 0.92 (d, J = 6.4 Hz, 3H), 1.41(s, 3H), 2.46 (t, J = 4.0 Hz, 4H), 3.67 (t, J = 4.2 Hz, 4H), 5.27 (s, 1H), 5.79 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.0, 17.4, 18.9, 22.6, 22.7, 22.8, 22.9, 26.3, 28.0, 29.7, 35.7, 36.1, 38.5, 38.9, 39.0, 39.5, 43.0, 45.9, 50.0, 52.3, 54.8, 66.7, 71.9, 123.9, 132.8, 159.7, 201.8; IR ($CHCl_3$, cm^{-1}): 2952, 1672, 1457, 1355, 1177, 923; MS (EI, m/z) = 467 $[M]^+$. Anal. calcd. for $C_{31}H_{49}NO_2$: C, 79.60; H, 10.56; N, 2.99; found: C, 79.81; H, 10.42; N, 3.27.

*2.2.3.6. 3β-Acetyl-6-(1H-imidazolo)-cholest-5-en (**28**).* Yellow solid; m.p. 99–102 °C; yield 81%; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.71 (s, 3H), 0.80–2.40 (m, 28H), 0.85 (d, J = 6.6 Hz, 6H), 0.92 (d, J = 6.5 Hz, 3H), 1.24 (s, 3H), 2.00 (s, 3H), 4.47–4.55 (m, 1H), 6.85 (s, 1H), 7.10 (s, 1H), 7.43 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.8, 18.7, 19.6, 21.2, 22.5, 22.8, 23.8, 24.1, 27.4, 28.0, 28.1, 31.9, 35.7, 36.1, 36.9, 37.4, 39.5, 42.3, 49.5, 56.0, 56.2, 72.8, 119.2, 129.0, 129.3, 136.7, 136.9, 170.2. IR ($CHCl_3$, cm^{-1}): 2928, 1733, 1584, 1020; MS (EI, m/z) = 494.4 $[M]^+$. Anal. calcd. for $C_{32}H_{50}N_2O_2$: C, 77.68; H, 10.19; N, 5.66; found: C, 77.51; H, 10.04; N, 5.84.

*2.2.3.7. 3β-Acetyl-6-(1H-benzo[d]imidazolo)-cholest-5-en (**29**).* Yellow solid; m.p. 105–108 °C; yield 83%; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.70 (s, 3H), 0.80–2.42 (m, 28H), 0.85 (d, J = 6.6 Hz, 6H), 0.92 (d, J = 6.5 Hz, 3H), 1.23 (s, 3H), 2.11 (s, 3H), 4.74–4.85 (m, 1H),

7.00–7.23 (m, 3H), 7.64–7.68 (m, 1H), 7.70 (s, 1H); IR ($CHCl_3$, cm^{-1}): 2948, 1733, 1462, 1225; MS (EI, *m*/*z*) = 544.4 $[M]^+$. Anal. calcd. for $C_{36}H_{52}N_2O_2$: C, 79.36; H, 9.62; N, 5.14; found: C, 79.42; H, 9.67; N, 5.06.

*2.2.3.8. 1-(Cyclohex-1-en-1-yl)-1H-imidazole (**30**).* Brown oil, yield 79%; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.64–1.72 (m, 2H), 1.78–1.86 (m, 2H), 2.17–2.22 (m, 2H), 2.41–2.43 (m, 2H), 5.83 (s, 1H), 7.07 (s, 1H), 7.09 (s, 1H), 7.66 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.6, 22.3, 24.0, 27.3, 116.4, 116.5, 129.2, 133.7, 134.5; IR ($CHCl_3$, cm^{-1}): 2927, 1661, 773; MS (EI, *m*/*z*) = 148 (M^+). Anal. calcd. for $C_9H_{12}N_2$: C, 72.94; H, 8.16; N, 18.90; found: C, 72.98; H, 8.01; N, 18.72.

*2.2.3.9. 1-(Cyclohex-1-en-1-yl)-1H-pyrazole (**31**).* Brown liquid, yield 72%; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.61–1.71 (m, 2H), 1.80–1.89 (m, 2H), 2.18–2.27 (m, 2H), 2.53–2.60 (m, 2H), 6.11–6.13 (m, 1H), 6.31–6.32 (m, 1H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.61 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.8, 22.4, 24.1, 25.9, 105.9, 113.8, 125.8, 136.5, 139.7; IR ($CHCl_3$, cm^{-1}): 2926, 1657, 768; MS (EI, *m*/*z*) = 148 (M^+). Anal. calcd. for $C_9H_{12}N_2$: C, 72.94; H, 8.16; N, 18.90; found: C, 72.81; H, 8.11; N, 18.58.

2.3. Biology

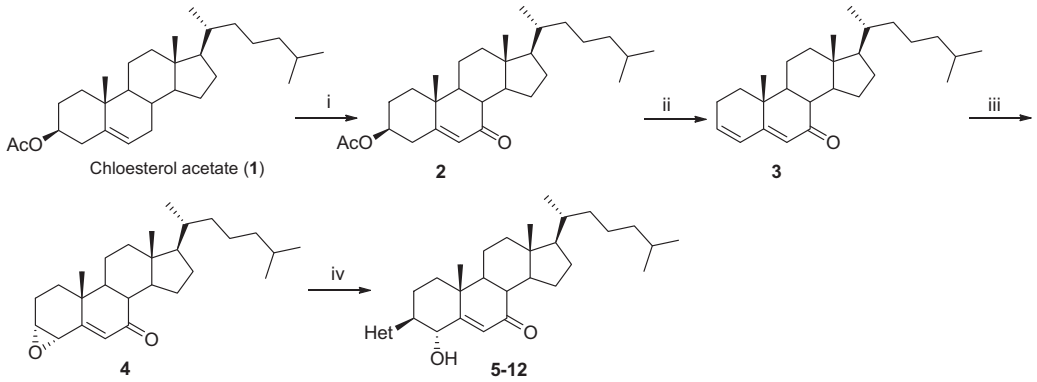
The antibacterial assay was performed following standard protocols and using test pathogens *E. coli*, *P. syringae*, *B. subtilis*, *P. vulgaris* and *S. aureus*. The antibacterial activity of all the compounds was tested using agar diffusion assay method [15] by observing presence of inhibition zone (in mm) around the well. All tested bacteria were grown in Mueller Hinton broth (MHB) at 37 °C for 24 h. Whole experiments were carried out in a laminar flow to strictly maintain aseptic conditions. 100 μL of fresh inoculum from each culture was added on the plates and spread using sterilized spreader. Wells were punctured on freshly spread bacterial culture on MHA using sterile cork borer. The solution of test compound was prepared in DMSO solvent (Stock concentration 25 mg/mL) and the bored wells were filled with test compound (50 μg). Four wells were bored on each plate, each filled with same compound and two plates for each test compound were taken. All the plates were incubated at 37 °C for 24 h. Growth inhibition of test organisms was measured with standard scale, the mean values of inhibition zones were taken and the data were compared with standard drug kanamycin acid sulphate.

3. Results and discussion

3.1. Chemistry

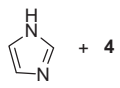
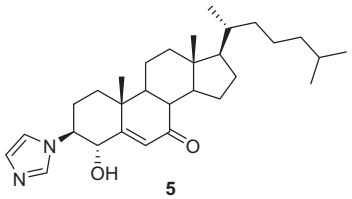
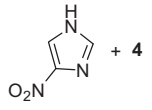
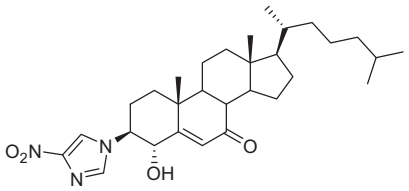
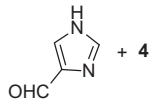
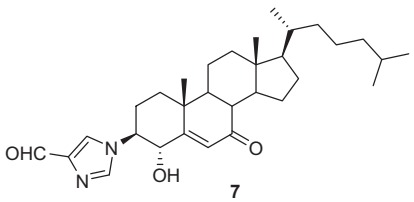
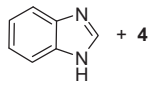
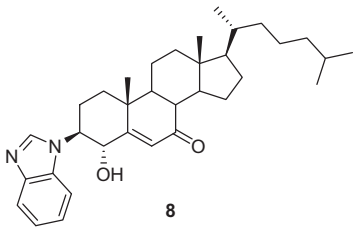
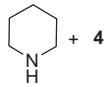
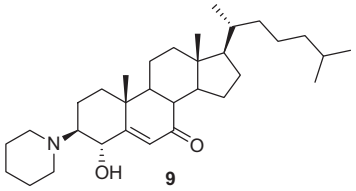
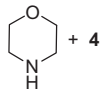
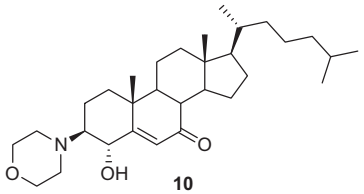
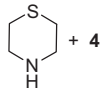
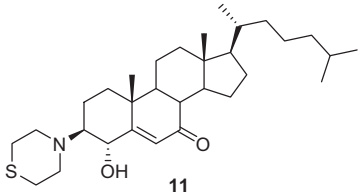
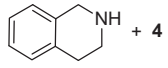
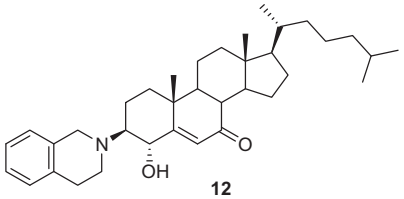
In general, epoxide rings can be opened by different nucleophiles in presence of acidic or basic catalysts [16]. Epoxide ring opening by the nucleophiles imidazoles and benzimidazoles has medicinal importance because the products 1-(2-hydroxyalkyl)imidazoles and 1-(2-hydroxyalkyl)benzimidazoles obtained are biologically important compounds due to their antifungal properties [17]. Epoxide ring opening with imidazole nucleophiles can be performed using LiBr [3], strong base [18], Lewis acid [$Yb(OTf)_3$] [19] and same epoxide ring opening with benzimidazole can be performed under strong basic conditions [18]. In addition, the synthesis of pharmaceutically important compounds *N*-(1-cyclohex-1-enyl)imidazole (**30**) and *N*-(1-cyclohex-1-enyl)pyrazole (**31**) was carried out by Katritzky and co-workers via elimination of benzotriazole or 5-phenyltetrazole from the corresponding 1-[1-(heterocycyl)cycloalkyl]-benzotriazoles or 1-[1-(heterocycyl) cyclohexyl]-5-phenyltetrazole [20]. Moreover, Ogata and co-workers found *N*-(1-cyclohex-1-enyl)imidazole (**30**) as a byproduct in the preparation of 1,1-*bis*-(1*H*-imidazol-1-yl)cyclohexane [21].

We started the synthesis of epoxide **4a** from commercially available cholesterol acetate (**1**) via allylic oxidation, acid catalyzed elimination and epoxidation pathway [11–13] (Scheme 1). This epoxidation afforded only α-epoxide **4** due to the presence of angular methyl group at C-10 position of the steroid moiety [13]. Finally, the microwave irradiation of an equimolar mixture of epoxide **4** and imidazole in a closed vessel in a Synthos 3000 microwave reactor at 600 Watt (140 °C and 12 bar) for 6 min afforded compound 3-(1*H*-imidazol-yl)-4-hydroxy-5-en-cholest-7-one (**5**) in 69% yield (Table 1, entry 1). Similarly, microwave reaction of steroidal epoxide **4** with different imidazole derivatives and benzimidazole afforded 4α-hydroxy-3β-heterosteroids **6–8** (Table 1, entry 2–4). This reaction condition was also effective for opening of steroidal epoxide with different *N*-containing heterocycles. For example, the ring opening reaction of steroidal epoxide **4** with different heterocycles such as pyrrolidine, morpholine, thiomorpholine and tetrahydroisoquinoline under the above reaction condition afforded 4α-hydroxy-3β-heterosteroids **9–12** in good yields (Table 1, entry 5–8). On the other hand, cholesterol acetate (**1**) on epoxidation with *m*CPBA provided a mixture of epoxides **13** (α) and **14** (β) (**13**/**14** = 4:1) (Scheme 2) [14]. The α and β ratio of compounds **13** and **14** was determined by the integration of the C-6 proton signals in the ^1H NMR spectra of the crude mixture of epoxides **13** and **14** (δ = 2.84–2.91 for the α-epoxide and



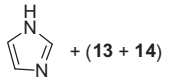
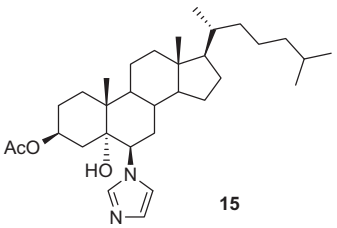
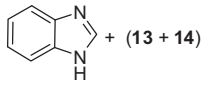
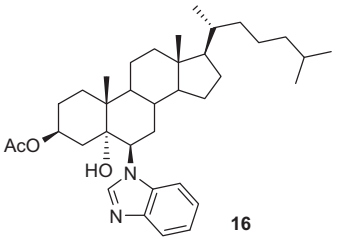
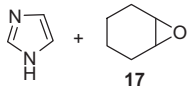
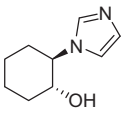
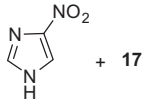
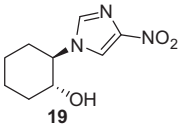
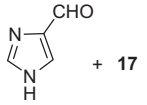
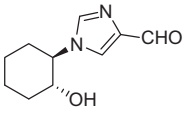
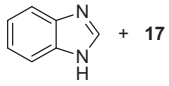
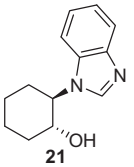
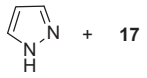
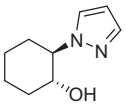
Scheme 1. Reagents and conditions: (i) ^tBuOOH, $RuCl_3$, cyclohexane, rt, 4 h; (ii) HCl, MeOH, reflux, 1 h; (iii) *m*CPBA, $CHCl_3$, rt, 5 h; (iv) *N*-heterocycles, microwave, neat, 6–16 min.

Table 1
Opening of steroidal/nonsteroidal epoxide rings by *N*-containing heterocycles.

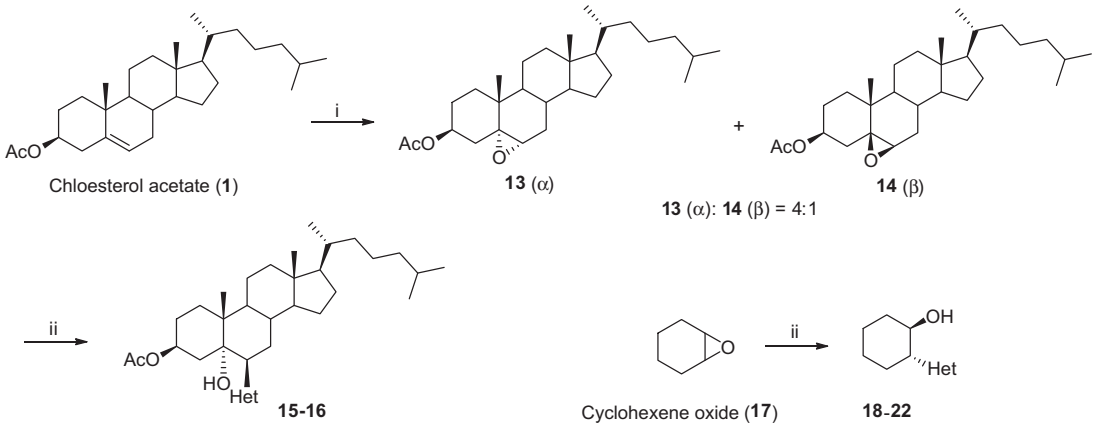
Entry	*N*-Heterocycle + epoxide	Vicinal heterocyclic alcohol	Time (min)	Yield[a] (%)
1	 + **4**	 **5**	6	69
2	 + **4**	 **6**	6	62
3	 + **4**	 **7**	6	66
4	 + **4**	 **8**	6	65
5	 + **4**	 **9**	10	68
6	 + **4**	 **10**	10	74
7	 + **4**	 **11**	10	69
8	 + **4**	 **12**	10	77

(*continued on next page*)

Table 1 (*continued*)

Entry	*N*-Heterocycle + epoxide	Vicinal heterocyclic alcohol	Time (min)	Yield[a] (%)
9	 + (**13** + **14**)	 **15**	10	75[b]
10	 + (**13** + **14**)	 **16**	10	71[b]
11	 **17**	 **18**	10	79
12	 + **17**	 **19**	10	75
13	 + **17**	 **20**	10	76
14	 + **17**	 **21**	16	78
15	 + **17**	 **22**	10	80

[a] Referring to the amount of product isolated by chromatography.
[b] Recrystallized from ethanol.



Scheme 2. Reagents and conditions: (i) *m*-CPBA, $CHCl_3$, rt, 5 h; (ii) *N*-heterocycles, microwave, neat, 10 min, recrystallization.

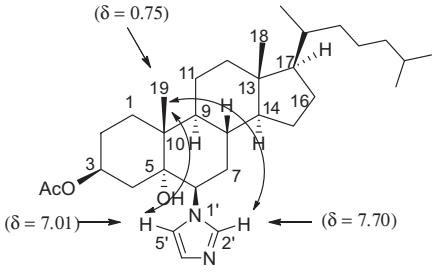
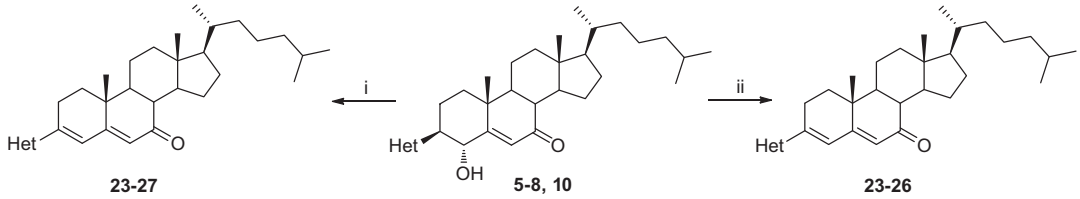


Fig. 2. Key NOESY correlations and relative stereochemistry of compound **15**.

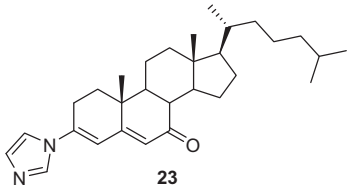
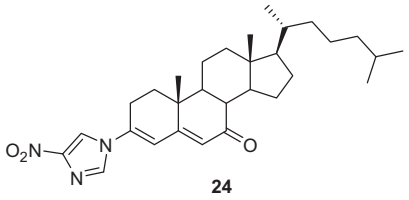
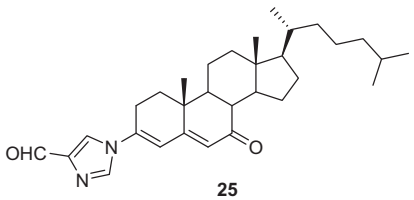
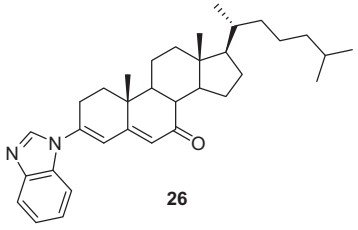
δ = 3.04–3.12 for the β-epoxide) [22]. When equimolar amount of mixture of epoxides **13** and **14** was treated with imidazole and benzimidazole by following above condition under microwave we obtained a diastereomeric mixture of imidazolyl and benzimidazolyl alcohols, which on recrystalliztion in ethanol provided pure imidazolyl alcohol **15** and benzimidazolyl alcohol **16** (Table 1, entries 9 and 10). In the NOESY spectrum of compound **15**, the presence of correlation of CH_3-19 protons with H-2′ and H-5′ also indicated that imidazole group of **15** was β oriented (Fig. 2). Similarly, microwave reaction of nonsteroidal epoxide **17** with different imidazole derivatives, benzimidazole and pyrazole provided compounds **18**–**22** in good yield (Table 1, entries 11–15).

In the next step, the microwave induced dehydration reaction of compound **5** was studied. We observed that increasing the time of the epoxide opening reaction from 6 to 25 min for the synthesis of compounds **5**–**8** afforded the water eliminated compounds **23**–**26** in moderate yields (**23** = 66%, **24** = 55%, **25** = 56% and **26** = 61%, Scheme 3). Surprisingly, all of our attempts to carry out the dehydration reaction of all other compounds by increasing the time of the reaction met with failure. Then we found that stirring a solution of compound **5** and catalytic amount of sulfuric acid in acetic



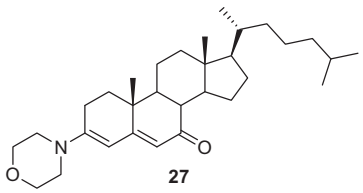
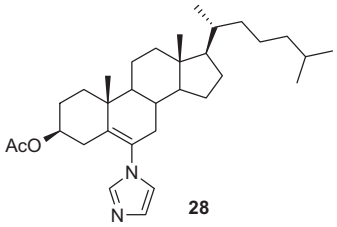
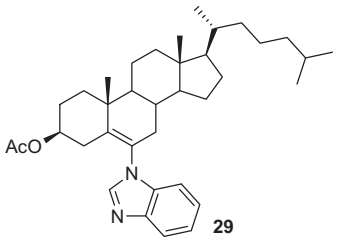
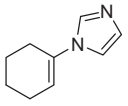
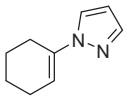
Scheme 3. Reagents and conditions: (i) H_2SO_4 (cat.), CH_3COOH, 80 °C, 4; (ii) *N*-hyterocycles, microwave(140 °C and 12 bar), neat, 25 min.

Table 2
Synthesis of steroidal and nonsteroidal *N*-(1-cycloalkenyl)heterocycles.

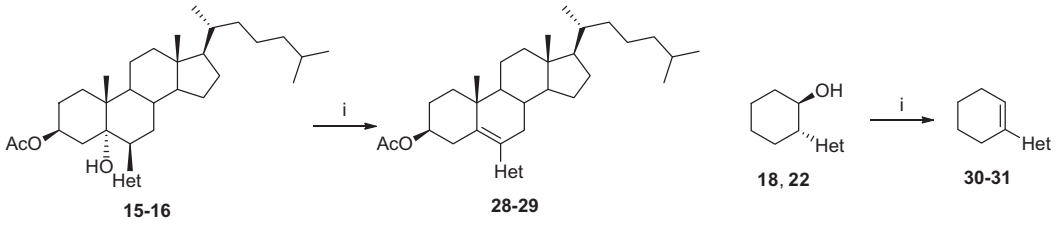
Entry	Vicinal heterocyclic alcohol	Steroidal/nonsteroidal *N*-(1-cycloalkenyl)heterocycles	Time (h)	Yield[a] (%)
1	**5**	 **23**	4	88
2	**6**	 **24**	4	83
3	**7**	 **25**	4	75
4	**8**	 **26**	4	88

(*continued on next page*)

Table 2 (*continued*)

Entry	Vicinal heterocyclic alcohol	Steroidal/nonsteroidal *N*-(1-cycloalkenyl)heterocycles	Time (h)	Yield[a] (%)
5	**10**	 **27**	6	68
6	**15**	 **28**	4	81
7	**16**	 **29**	4	83
8	**18**	 **30**	4	79
9	**22**	 **31**	6	72

[a] Referring to the amount of product isolated by chromatography.



Scheme 4. Reagents and conditions: (i) H_2SO_4 (cat.), CH_3COOH, 80 °C, 4–6 h.

acid at 80 °C for 4 h also provided compound **23** in good yield (Table 2, 88%, entry 1).

This reaction condition was efficiently used for the dehydration reactions of other hydroxyl compounds **6**–**8**, **10**, **15**–**16**, **18** and **22** to obtain steroidal and nonsteroidal *N*-(1-cycloalkenyl)heterocycles **24**–**31** in good yields (Table 2, entries 2–9), although the dehydration reaction of compounds **10** and **22** took longer time (6 h) to provide dehydrated compounds **27** and **31** respectively. (see Scheme 4).

3.2. Biology

The antimicrobial activity of compounds **5**–**12**, **15**–**16**, **18**–**31** was evaluated and compared with standard drug kanamycin acid sulphate (Table 3). As shown in Table 3 only few of the synthesized steroidal *N*-heterocyclic derivatives (**6**, **9**–**12**, **24** and **27**) showed moderate *in vitro* antimicrobial activity against the tested microorganisms. The steroidal derivatives **9**, **10** and **11** were effective in inhibiting all the tested bacterial strains. The tetrahydroisoquinoline substituted steroidal derivative **12** showed inhibition against three Gram-negative bacterial strains *E. coli*, *P. syringe* and *P. vulgaris*. Among the steroidal imidazoles only nitro group substituted steroidal imidazole **6** showed inhibition against *S. aureus* and *B. subtilis*. Removal of hydroxyl group of this steroidal derivative led to decrease in the antimicrobial activity of the compounds as seen for compounds **24** and **27**. Compound **24** showed inhibition only against bacterial strain *S. aureus* whereas compound **27** showed inhibition only against three Gram-negative bacterial strains

Table 3
Antibacterial screening data of Michael adducts.

Compound	Zone of inhibition[a,b] (mm) Bacterial strains				
	E. coli	*P. syringe*	*P. vulgaris*	*S. aureus*	*B. subtilis*
6	–	–	–	18.0 ± 0.5	12.06 ± 0.6
9	15.75 ± 0.5	12.10 ± 0.6	13.94 ± 0.5	10.19 ± 0.5	14.19 ± 0.6
10	12.13 ± 0.6	16.13 ± 0.4	16.0 ± 0.6	10.06 ± 0.7	14.19 ± 0.8
11	10 ± 0.4	13.93 ± 0.7	13.12 ± 0.9	10.88 ± 0.6	13.06 ± 0.9
12	12 ± 0.6	12.06 ± 0.8	14.75 ± 0.8	–	–
24	–	–	–	14.0 ± 0.8	–
27	10.3 ± 0.5	12.06 ± 0.8	9.81 ± 0.9	–	–
Kanamycin	32.19 ± 0.7	35.88 ± 0.7	32.06 ± 0.7	32.94 ± 0.6	28.75 ± 0.4

[a] Zone of inhibitions less than 10 mm are not shown in the table.
[b] Kanamycin (50 μg/well) was used as positive reference, synthesized compounds (50 μg/well).

E. coli, P. syringe and P. vulgaris. Compounds **5**, **7**–**8**, **15**–**16**, **18**–**23**, **25**–**26** and **28**–**31** were not effective against any of the strains tested (data not shown).

4. Conclusion

In conclusion, a new solvent-free and catalyst-free green method has been developed for the steroidal and nonsteroidal epoxide ring opening reaction by nitrogen containing heterocycles under microwave irradiation. Moreover, we converted some of these epoxide opening products to their corresponding *N*-(1-cycloalkenyl)heterocycles by acid catalyzed dehydration reaction. All the epoxide opening and dehydrated compounds were screened *in vitro* for antibacterial activities against a panel of various bacterial strains. It was observed from the data that some of the epoxide opening and dehydrated compounds showed moderate inhibition activity against tested bacterial strains, indicating that these compounds are promising antibacterial compounds for further research.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2014.03.011.

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