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Research Article

Synthesis and Antimicrobial activity of steroidal C-20 tertiary alcohols with vinyl side chain

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Abstract: Synthesis of steroidal C-20 tertiary alcohols with vinyl side chain using Grignard reaction of steroidal ketones and vinyl magnesium bromide have been realized. These molecules were evaluated *in vitro* for their antifungal and antibacterial activities. Compounds **7**, **12** and **13** exhibits significant antifungal and compounds **6**, **7**, and **11-13** exhibits antibacterial activity against the tested strains.

1. Introduction

The recent discovery of many new sterols with novel side chain structures from marine and animal sources has focused attention on developing stereocontrolled methods to introduce these side chains onto tetracyclic steroidal starting materials.[1] An important problem that arises from this approach is the stereospecific control of the C-20 stereochemistry. Alkyl steroids constitute an important class of steroid compounds containing additional alkyl groups compared with the natural hormones. The introduction of the alkyl group enhances the physiological activity[2] and medicinal chemistry of steroids covers a large and interesting series of structures and biological activities.[3] The combination of a steroid molecule with structural elements possessing appropriate biological activities.[4] Among the many known analogues of steroids, the oxygenated sterols bind with the oxysterol receptors and so decrease the activity of HMG-CoA reductase. Most of these sterols oxygenated at the 3- and the 6-, 7-, 15-, 20-, 22-, or 25-positions.[5] Pregnenolone holds a central position in the biosynthetic route from cholesterol to the sex hormones. It is virtually inactive, but its acetate and hemisuccinate were given, in the early fifties, to arthritic patients on the assumption that pregnenolone would act as pro-drug for the expensive hydrocortisone.[6]

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The reaction of Grignard and other organometallic reagents with 20-ketones has been utilized by a number of investigators to construct the side chain in one- or in multistep sequences. In these reactions, chiral center at C-20 is created with mixture of epimers, the ratio of epimers depending greatly upon the structure of the steroids, particularly the nature of substituents near C-20 and the bulkiness of the reagent.[1,7-9]

Very recently, we have reported[10] the stereoselective synthesis and antimicrobial activity of steroidal C-20 tertiary alcohols with thiazole and pyridine side chain. In continuation of our work[11] on the synthesis and bioevaluation of various steroid derivatives, herein, we would like to report synthesis and antimicrobial activity of steroidal C-20 tertiary alcohols with vinyl side chain.

2. Results and Discussion

Commercially available[12] 16-dehydropregnenolone acetate **1**, on chemoselective catalytic hydrogenation with 10% palladium on charcoal in ethyl acetate, hydrolysis of acetate **2** with potassium hydroxide in aqueous methanol, followed by protection of 3β-hydroxy group in compound **3** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in *N*,*N*-dimethylformamide (DMF) afforded[13] compound **4** with an overall yield of 87% in three steps (Scheme 1).

The synthesis of compound **10** was achieved from 16-dehydropregnenolone acetate **1**. Treatment of compound **1** with KOH in *t*-butanol, followed catalytic hydrogenation of 3β-hydroxy compound **8** with 10% palladium on charcoal in ethanol afforded 5,6,16,17-tetrahydro 20-ketone **9** in 95% overall yield in two steps. The 3β-hydroxy group of **9** was transformed[14] to its TBDMS derivative **10** in excellent yield. The stereochemistry at C-5 and C-17 in compound **10** has been already reported by single X-ray crystal structure.[14] Compound **10** is an useful intermediate[15] for the synthesis of azasterols, which are the inhibitors of sterol 24-methyltransferase in *Leishmania species and Trypanosoma cruzi*.

Grignard reaction with vinyl magnesium bromide on steroidal ketones **4** and **10** in tetrahydrofuran yielded stereoselectively C(20*S*) compounds **5** and **11** in 98% and 97% yield respectively (Scheme 1). The major isomer was assigned the 20S configuration in keeping with the stereochemical preference reported for other Grignard reactions on C-20 steroidal ketones.[1,7-9] It can be mentioned here that, the products **5** and **11** can be purified by column chromatography over silica gel. The C(20*S*) tertiary alcohols **5** and **11** were assigned by spectroscopic data, in which the C-21 methyl protons in **5** was observed at 1.34 ppm and in **11** at 1.32 ppm in ^{1}H NMR spectrum. Deprotection of TBDMS group of compounds **5** and **11** with *n*-Bu_4NF in tetrahydrofuran resulted compounds **6** and **12** respectively in excellent yields. Selective acetylation of 3β-hydroxy group of **6** and **12** with acetic anhydride and catalytic amount of DMAP in pyridine yielded compounds **7** and **13** in 95% and 94% yield respectively.

3. Bioevaluation

Derivatives of 16-dehydropregnenolone displayed cytotoxic effects when added repeatedly to the human cancer cell culture media.[16] Antimigratory compounds that could be used to successfully combat migrating cancer cells have to be administered chronically to cancer patients.

The *in vitro* antifungal and antibacterial activity of the structurally promising steroidal derivatives against five fungal

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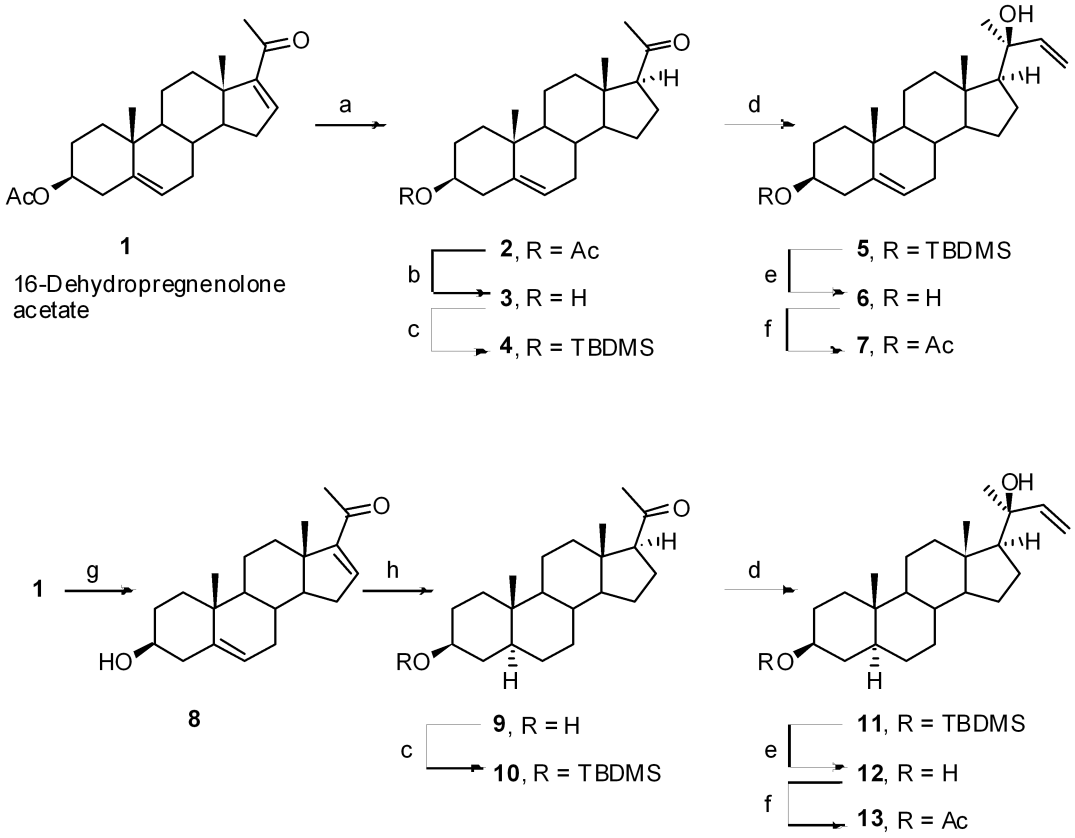

Scheme1. Reagents and conditions: (a) 10% Pd/C, H_2, EtOAc, 45 psi, 30 ºC, 12 h, 98%; (b) KOH, MeOH, H_2O, 30 ºC, 2 h, 97%; (c) TBDMSCl, Imidazole, DMF, 30 ºC, 10 h, **4** (92%) and **10** (97%); (d) CH_2=CHMgBr, THF, 0 ºC, 1 h, **5** (98%) and **11** (97%); (e) *n*-Bu_4NF, THF, 30 ºC, 18 h, **6** (93%) and **12** (95%); (f) Ac_2O, Pyridine, DMAP, 25 ºC, 2 h, **7** (95%) and **13** (94%); (g) KOH, *t*-BuOH, H_2O, 30 ºC, 10 h, 96%; (h) 10% Pd/C, H_2, EtOH, 55 psi, 30 ºC, 12 h, 99%.

strains and one strain of Gram-positive bacteria and one strain of Gram-negative bacteria was investigated using micro dilution method in comparison to the reference drugs amphotericin B, fluconazole, tetracycline and erythromycin. The results are shown in Table 1. The results clearly revealed that, all the tested compounds in the present study were found to have significant antifungal and antibacterial activity against the used strains.

The synthesized compounds **5-7** and **11-13** were tested in *vitro* for antifungal and antibacterial activity. The antifungal fungal strains *Candida albicans*, *Cryptococcus neoformans* (human pathogen), *Benjaminiella poitrasii*, *Yarrowia lipolytica* (saprophytes) and *Fusarium oxysporum* (plant pathogen). Most of the pathogen fungi *viz C. albicans* are dimorphic in nature. However, their use as model faces a number of problems of slow growth rate and difficulties in getting synchronous growth.[17] Therefore non pathogenic dimorphic fungus *B. poitrasii* was used as a model which exhibits a rapid and simple one-step process of yeast-mycelium transition in response to temperature and/or glucose change.[18]

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Table 1. *In vitro* antimicrobial activity of compounds **5-7** and **11-13**.

Compounds	Minimum Inhibitory concentration (MIC)[a] (μg/mL)						
	Fungal Strains					Bacterial Strains	
	CA	CN	BP	YL	FO	EC	SA
5	>64	>64	>64	>64	>64	32	64
6	>64	>64	32	64	64	32	**8**
7	>64	64	**16**	**16**	>64	16	**16**
11	>64	>64	>64	32	>64	32	**16**
12	>64	32	>64	**16**	32	**8**	**16**
13	64	>64	**16**	>64	>64	16	**16**
Ampho. B	2	16	16	16	16	-	-
Fluconazole	32	32	32	64	8	-	-
Tetracycline	-	-	-	-	-	8	16
Erythromycin	-	-	-	-	-	>64	32

Bold values in the table indicates that the compounds are significantly active than the standard drugs.

CA**,** *Candida albicans* (NCL1); CN**,** *Cryptococcus neoformans* (NCL2); BP**,** *Benjaminiella poitrasii* (NCL3); YL**,** *Yarrowia lipolytica* (NCL4); FO**,** *Fusarium oxysporum* (NCL5); EC**,** *Escherichia coli* (NCIM No. 2574); SA**,** *Staphylococcus aureus* (NCIM No. 2122).

[a]MIC (Minimum inhibitory concentration) was determined as 90% inhibition of growth with respect to the growth control.

Negative control, DMSO and THF (50:50), No inhibition.

As shown in Table 1, compound **7** have shown significant antifungal activity against *B. poitrasii* and *Y. lipolytica* with 16 μg/mL as a MIC value. Compounds **12** and **13** showed better activity against *Y. lipolytica* and *B. poitrasii* with reference to standard drug fluconazole respectively. While, all the compounds did not show any significant activity against human pathogens *C. albicans* and *C. neoformans* and also plant pathogen *F. oxysporum*. In case of antibacterial activity, almost all the compounds have shown moderate to good activity against Gram-positive *E. coli* and Gram-negative *S. aureus* bacterial strains. In particular, compound **12** have shown comparable activity against *E. coli* with reference drug tetracycline. Compound **6** showed higher activity against *S. aureus* with the reference drugs tetracycline and erythromycin. This indicates that, the hydroxy group at C-3 position in compounds **6** and **12** shows good antibacterial activity against *S. aureus* and *E. coli* respectively with reference drug tetracycline.

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4. Conclusions

A series of steroidal C-20 tertiary alcohols with vinyl side chain, using Grignard reagent vinyl magnesium bromide on C-20 ketones **4** and **10** have been realized. These compounds are characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, elemental analyses and evaluated for *in vitro* antifungal and antibacterial activities. Some of the compounds were found to be active against a limited panel of fungi and bacteria. In particular, compounds **7**, **12** and **13** were found to be the most effective analogs against the tested fungal strains and compounds **6**, **7**, **11**-**13** against the bacterial strains.

5. Experimental Section

5.1. General methods

All melting points were determined on Yanco Micro melting point apparatus and are uncorrected. Optical rotations were obtained on Bellingham and Stanly ADP-220 Polarimeter. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) using TLC aluminium sheets, silica gel 60-F_{254} precoated, Merck, Germany and locating the spots using UV light as the visualizing agent or spraying with ethanolic phosphomolybdic acid (PMA) solution followed by heating. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC-200 (200 MHz) at 200.13 and 50.32 or on a Bruker DRX-500 (500 MHz) spectrophotometer at 500.13 and 125.78, respectively. Chemical shifts are given in δ values relative to TMS (tetramethylsilane) as internal standard. IR spectra were recorded on Shimadzu 8400 series FTIR instrument and values are reported in cm^{-1} units. Specific rotations ([α]D) are reported in deg/dm and the concentration (c) is given in g/100 ml in the specific solvent. Mass spectra were recorded by either LC–MS or MS-TOF API QSTAR PULSAR spectrophotometer, samples introduced by infusion method using Electrospray Ionization Technique. Elemental analyses were performed by CHNS-O EA 1108-Elemental analyzer, Carloerba Instrument (Italy) or Elementor Vario EL (Germany) and were within ± 0.4% of calculated values.

5.1.1. 3β-Acetoxy-pregna-5-en-20-one (2)

To a solution of 16-dehydropregnenolone acetate **1** (3.56 g, 10 mmol) in ethyl acetate (100 mL) was added Pd/C catalyst (0.36 g, 10%) and hydrogenation was carried out at 45 psi pressure at room temperature (30 °C) for 12 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to obtain 3β-Acetoxy-pregna-5-en-20-one **2** (3.50 g, 98%) as a pale yellowish solid. Mp: 145-146 °C (Ethyl acetate-hexane). IR (nujol, cm^{-1}): 1730 ($OCOCH_3$), 1708 ($COCH_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 5.38 (d, 1H, *J* = 5 Hz, 6-H), 4.61 (m, 1H, 3-H), 2.54 (t, 1H, 17-H), 2.13 (s, 3H, $COCH_3$), 2.04 (s, 3H, $OCOCH_3$), 1.02 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 209.2 (C), 170.3 (C), 139.5 (C), 122.2 (CH), 73.6 (CH), 63.5 (CH), 56.7 (CH), 49.7 (CH), 43.8 (C), 38.6 (CH_2), 37.9 (CH_2), 36.9 (CH_2), 36.4 (C), 31.7 (CH), 31.6 (CH_2), 31.4 (CH_3), 29.6 (CH_2), 27.6 (CH_2), 24.3 (CH_2), 22. 7 (CH_2), 21.2 (CH_3), 20.9 (CH_2), 19.1 (CH_3), 13.1 (CH_3).

5.1.2. 3β-Hydroxy-pregna-5-en-20-one (3)

To a stirred solution of 3β-Acetoxy-pregna-5-en-20-one **2** (7.08 g, 20 mmol) in methanol (100 mL) was added aqueous

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solution of KOH (5.6 g, 100 mmol) in H_2O (5 mL). The reaction mixture was stirred for 2 h at 30 °C and methanol was removed under vacuuo. Usual workup with ethyl acetate yielded 3β-hydroxy-pregna-5-ene-20-one **3** (6.0 g, 96%) as a colourless solid. Mp: 192-194 ºC (Methanol). IR (nujol, cm^{-1}): 3520 (-OH), 1712 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 5.35 (d, 1H, *J* = 6 Hz, 6-H), 3.50 (m, 1H, 3-H), 2.13 (s, 3H, $COCH_3$), 1.01 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 209.6 (C), 140.8 (C), 121.2 (CH), 71.5 (CH), 63.6 (CH), 56.8 (CH), 49.9 (CH), 43.9 (C), 42.1 (CH_2), 38.7 (CH_2), 37.2 (CH_2), 36.4 (C), 31.8 (CH_3), 31. 7 (CH_2), 31.5 (CH_2), 31.4 (CH_2), 24.4 (CH_2), 22.7 (CH_2), 21.0 (CH_2), 19.2 (CH_3), 13.1 (CH_3). Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19; Found: C, 79.35; H, 10.49.

5.1.3. 3β-*tert*-Butyldimethylsilyloxy-pregna-5-ene-20-one (4)

tert-Butyldimethylsilyl chloride (5.4 g, 36 mmol) was added to a solution of 3β-hydroxy-pregna-5-ene-20-one **3** (5.188 g, 18 mmol) in dry DMF (120 mL). Imidazole (4.896 g, 72 mmol) was added to above solution and the reaction mixture was stirred at 30 °C for 10 h. The reaction was then quenched with crushed ice. The reaction mixture was extracted with dichloromethane (2 × 100 mL). The combined organic extracts were washed with brine (2 × 25 mL), dried over anhydrous Na_2SO_4, concentrated under reduced pressure to afford 3β-*tert*-butyldimethylsilyloxy-pregna-5-ene-20-one **4** (6.79 g, 92%) as a colourless solid. Mp: 160-162 °C (Ethyl acetate-Hexane). IR (nujol, cm^{-1}): 1712 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.33 (d, 1H, *J* = 5 Hz, 6-H), 3.49 (m, 1H, 3-H), 2.13 (s, 3H, $COCH_3$), 1.01 (s, 3H, 19-H_3), 0.90 (s, 9H, $SiCMe_3$), 0.64 (s, 3H, 18-H_3), 0.06 (s, 6H, $SiMe_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 209.3 (C), 141.6 (C), 120.9 (CH), 72.6 (CH), 63.8 (CH), 57.0 (CH), 50.2 (CH), 44.0 (C), 42.9 (CH_2), 39.0 (CH_2), 37.5 (CH_2), 36.7 (C), 32.1 (CH_2), 31. 9 (CH), 31.9 (CH_2), 31.4 (CH_3), 25.9 (3 x CH_3), 24.5 (CH_2), 22.9 (CH_2), 21.1 (CH_2), 19.4 (CH_3), 18.2 (C), 13.2 (CH_3), -4.6 (2 x CH_3). Mass: m/z 451.04 (M+H_2O). Anal. Calcd for $C_{27}H_{46}O_2Si$: C, 75.34; H, 10.69; Found: C, 75.07; H, 11.00.

5.1.4. 16-Dehydropregnenolone (8)

To a stirred solution of 16-dehydropregnenolone acetate **1** (5.340 g, 15 mmol) in *t*-butanol (125 mL) was added solution of KOH (4.2 g, 75 mmol) in H_2O (5 mL). The reaction mixture was stirred for 10 h at 30 °C and *t*-butanol was removed under reduced pressure, crushed ice was added to it. The solid was filtered and washed with cold water (5 x 25 mL). It was then dried to yield 16-dehydropregnenolone **8** (4.52 g, 96%) as a colourless solid. From this 100 mg was crystallized in 5 mL of solvent (Methanol-Dichloromethane 9:1). Mp: 214-215 ºC. $[\alpha]^{22}_D$ –27.27 (*c* 0.66, CH_3OH). IR (Nujol, cm^{-1}): 3390 (-OH), 1654 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.72 (t, 1H, *J* = 3Hz, 16-H), 5.37 (d, 1H, *J* = 5 Hz, 6-H), 3.53 (m, 1H, 3-H), 2.26 (s, 3H, $COCH_3$), 1.05 (s, 3H, 19-H_3), 0.92 (s, 3H, 18-H_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 196.7 (C), 155.4 (C), 144.2 (CH), 141.4 (C), 120.9 (CH), 71.6 (CH), 56.4 (CH), 50.5 (CH), 46.1 (C), 42.2 (CH_2), 37.1 (CH_2), 36.7 (CH), 34.7 (CH_2), 32.2 (CH_2), 31.6 (CH_2), 31.5 (CH_2), 30.2 (CH), 27.0 (CH_3), 20.6 (CH_2), 19.2 (CH_3), 15.7 (CH_3). MS (LCMS) m/z: 315 (M+1. Anal. Calcd for $C_{21}H_{30}O_2 \cdot 0.5CH_4O$: C, 78.13; H, 9.76; Found: C, 78.12; H, 9.78.

5.1.5. 3β-Hydroxy-5α,17α-pregna-20-one (9)

To a solution of 16-dehydropregnenolone **8** (1.572 g, 5 mmol) in ethanol (100 mL)

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was added Pd/C catalyst (0.157 g, 10%) and hydrogenation was carried out using Parr apparatus at 55 psi pressure at 30 °C for 12 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to obtain 3β-hydroxy-5α,17α-pregna-20-one **9** (1.576 g, 99%) as a colourless solid. From this 150 mg was crystallized in 10 mL of solvent (Methanol-Dichloromethane 9:1). Mp: 193-195 °C. $[\alpha]^{23}_{D}$ +90.78 (*c* 0.70, CH_3OH). IR (Nujol, cm^{-1}): 3388 (-OH), 1691 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 3.60 (m, 1H, 3-H), 2.11 (s, 3H, $COCH_3$), 0.81 (s, 3H, 19-H_3), 0.60 (s, 3H, 18-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 209.7 (C), 70.9 (CH), 63.7 (CH), 56.5 (CH), 54.1 (CH), 44.7 (CH), 44.1 (C), 38.9 (CH_2), 37.9 (CH_2), 36.9 (CH_2), 35.3 (C), 35.3 (CH), 31.9 (CH_2), 31.4 (CH_3), 31.2 (CH_2), 28.5 (CH_2), 24.3 (CH_2), 22.6 (CH_2), 21.1 (CH_2), 13.3 (CH_3), 12.2 (CH_3). MS (LCMS) m/z: 319 (M+1. Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.75; Found: C, 78.89; H, 11.03.

5.1.6. 3β-*tert*-Butyldimethylsilyloxy-5α,17α-pregna-20-one (10)

To a solution of 3β-hydroxy-5α,17α-pregna-20-one **9** (3.185 g, 10 mmol) in dry DMF (90 mL) under nitrogen atmosphere was added imidazole (1.020 g, 15 mmol) and *tert*-butyldimethylsilyl chloride (1.875 g, 12.5 mmol). The mixture was stirred at 30 °C for 10 h. The resulting suspension was quenched with cold water and extracted with diethyl ether (2 x 200 mL) and washed with saturated sodium bicarbonate (2 x 75 mL) and water (2 x 50 mL). The organic extracts were washed with brine (2 x 50 mL), dried over anhydrous Na_2SO_4. Removal of solvent under reduced pressure afforded crude compound **10** (4.340 g). Column chromatographic purification over silica gel using ethyl acetate-petroleum ether (3:97; Rf 0.4, 10% EA/PE) as an eluent afforded 3β-*tert*-butyldimethylsilyloxy-5α,17α-pregna-20-one **10** (4.197 g, 97%) as a colourless solid. From this 200 mg was crystallized in 10 mL of solvent (Methanol-Dichloromethane 9:1). Mp: 140-141 °C. $[\alpha]^{23}_{D}$ +70.17 (*c* 0.57, $CHCl_3$). IR (Nujol, cm^{-1}): 1708 (C=O). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 3.55 (m, 1H, 3-H), 2.11 (s, 3H, $COCH_3$), 0.89 (s, 9H, $SiCMe_3$), 0.80 (s, 3H, 19-H_3), 0.60 (s, 3H, 18-H_3), 0.05 (s, 6H, $SiMe_2$). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 209.6 (C), 72.0 (CH), 63.8 (CH), 56.7 (CH), 54.3 (CH), 45.0 (CH), 44.2 (C), 39.1 (CH_2), 38.6 (CH_2), 37.1 (CH_2), 35.5 (C), 35.4 (CH), 32.0 (CH_2), 31.9 (CH_2), 31.5 (CH_3), 28.6 (CH_2), 25.9 (3 x CH_3), 24.4 (CH_2), 22.7 (CH_2), 21.2 (CH_2), 18.2 (C), 13.4 (CH_3), 12.3 (CH_3), -4.6 (2 x CH_3). MS (LCMS) m/z: 433 (M+1), 455 (M+Na). Anal. Calcd for $C_{27}H_{48}O_2Si$: C, 74.93; H, 11.17; Found: C, 75.00; H, 11.37.

5.1.7. (20*S*)-3β-*tert*-Butyldimethylsilyloxy-24-norcholesta-5,22-diene-20-ol (5)

A preliminary calcinated three-necked flask was charged in a stream of argon with 0.120 g of magnesium turnings, a few crystals of iodine were added, the flask was heated, 20 mL of tetrahydrofuran was added. The mixture was cooled to 0 °C, and a solution of vinyl bromide in tetrahydrofuran (0.8 mL, 5.5 M) was added dropwise under stirring. A solution of 3β-*tert*-Butyl-dimethylsilyloxy-pregna-5-en-20-one **4** (0.864 g, 2 mmol) in 10 mL of tetrahydrofuran then added. The reaction mixture was stirred at the same temperature for 2 h. The reaction was quenched with saturated solution of ammonium chloride (10 mL) and THF was removed under reduced pressure. The residue was extracted with ethyl acetate (2 x 100 mL). The organic layer was washed with water (2 x 25 mL), brine (2 x 25 mL), dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure to afford crude compound **5** (0.938 g). Flash colum

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chromatographic purification over silica gel using ethyl acetate-petroleum ether (1:99, Rf 0.4, 10% EA/PE) as eluent afforded pure (20*S*)-3β-*tert*-butyldimethylsilyloxy-24-norcholesta-5,22-diene-20-ol **(5)** (0.898 g, 98%) as a colourless solid. 50 mg of this was crystallized in 10 mL of solvent (Ethyl acetate-Petroleum ether, 1:9). Mp: 148-150 °C (*Lit*.[7c] 157-158 °C). IR (Nujol, cm^{-1}): 3313 (-OH).^{1}H NMR (200 MHz, $CDCl_3$) δ = 5.99 (dd, 1H, *J* = 10.7 and 17.3 Hz, 22-H), 5.32 (d, 1H, *J* = 4.8 Hz), 5.15 (dd, 1H, *J* = 1.3 and 17.2 Hz, 23-H), 4.96 (dd, 1H, *J* = 1.4 and 10.8 Hz, 23-H), 3.48 (m, 1H, 3-H), 1.34 (s, 3H, 21-H_3), 1.00 (s, 3H, 19-H_3), 0.89 (s, 9H, $SiCMe_3$), 0.83 (s, 3H, 18-H_3), 0.06 (s, 6H, $SiMe_2$).^{13}C NMR (50 MHz, $CDCl_3$) δ = 146.1 (CH), 141.5 (C), 121.0 (CH), 110.2 (CH_2), 75.7 (C), 72.6 (CH), 59.4 (CH), 56.8 (CH), 50.1 (CH), 42.8 (CH_2), 42.8 (C), 40.2 (CH_2), 37.3 (CH_2), 36.5 (C), 32.0 (CH_2), 31.8 (CH_2), 31.3 (CH), 28.7 (CH_3), 25.9 (3 x CH_3), 23.8 (CH_2), 23.2 (CH_2), 20.9 (CH_2), 19.4 (CH_3), 18.2 (C), 13.8 (CH_3), -4.6 (2 x CH_3). MS (LCMS) m/z: 481 (M+Na). Anal. Calcd for $C_{29}H_{50}O_2Si$: C, 75.92; H, 10.98; Found: C, 75.60; H, 11.27.

5.1.8. (20*S*)-3β-*tert*-Butyldimethylsilyloxy-24-norcholesta-5α-22-ene-20-ol (11)

Experimental procedure was the same as the procedure described for compound **5**. Yield: 97%. Mp: 140-142 °C (Ethyl acetate-Petroleum ether). IR (Nujol, cm^{-1}): 3382 (-OH). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 5.98 (dd, 1H, *J* = 10.8 and 17.3 Hz, 22-H), 5.14 (dd, 1H, *J* = 1.2 and 17.3 Hz, 23-H), 4.96 (dd, 1H, *J* = 1.1 and 10.7 Hz, 23-H), 3.55 (m, 1H, 3-H), 1.32 (s, 3H, 21-H_3), 0.89 (s, 9H, $SiCMe_3$), 0.80 (s, 6H, 19-H_3 and 18-H_3), 0.05 (s, 6H, $SiMe_2$). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 146.1 (CH), 110.1 (CH_2), 75.7 (C), 72.1 (CH), 59.5 (CH), 56.5 (CH), 54.4 (CH), 45.0 (CH), 43.0 (C), 40.4 (CH_2), 38.6 (CH_2), 37.1 (CH_2), 35.4 (C), 34.8 (CH), 32.0 (CH_2), 31.9 (CH_2), 28.7 (CH_3), 28.7 (CH_2), 25.9 (3xCH_3), 23.7 (CH_2), 23.1 (CH_2), 21.0 (CH_2), 18.2 (C), 13.9 (CH_3), 12.3 (CH_3), -4.6 (2 x CH_3). MS (LCMS) m/z: 483 (M+Na). Anal. Calcd for $C_{29}H_{52}O_2Si$: C, 75.59; H, 11.37; Found: C, 75.31; H, 11.45.

5.1.9. General procedure for deprotection of TBDMS group in compounds 5 and 11

To the solution of 3β-*tert*-butyldimethylsilyloxy compounds **5** or **11** (1 mmol) in dry tetrahydrofuran (10 mL), 1M *n*-tetrabutylammonium fluoride in tetrahydrofuran (2 mL, 2 mmol) was added. The reaction mixture was stirred at 30 °C for 12 h and then quenched with aqueous ammonium chloride. Tetrahydrofuran was removed under vaccuo and the reaction mixture was extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with brine (2 × 25 mL), dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure to afford crude 3-OH compounds. Column chromatographic purification over silica gel using ethyl acetate-petroleum ether as an eluent gave pure compounds.

5.1.9.1. (20*S*)-24-Norcholesta-5,22-diene-3,20-diol (6)

Yield: 94%. Mp: 135-137 °C (Ethyl acetate). IR (Nujol, cm^{-1}): 3363 (-OH). ^{1}H NMR (200 MHz, $CDCl_3$) δ = 5.98 (dd, 1H, *J* = 10.8 and 17.3 Hz, 22-H), 5.35 (d, 1H, *J* = 5.1 Hz), 5.14 (dd, 1H, *J* = 1.2 and 17.3 Hz, 23-H), 4.96 (dd, 1H, *J* = 1.4 Hz and 10.7 Hz, 23-H), 3.52 (m, 1H, 3-H), 1.33 (s, 3H, 21-H_3), 1.01 (s, 3H, 19-H_3), 0.83 (s, 3H, 18-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 146.1 (CH), 140.8 (C), 121.5 (CH), 110.2 (CH_2), 75.7 (C), 71.7 (CH), 59.4 (CH), 56.7 (CH), 50.0 (CH), 42.8 (CH_2), 42.8 (C), 40.1 (CH_2), 37.2 (CH_2), 36.5 (C), 31.7 (CH_2), 31.6 (CH_2), 31.3 (CH), 28.7

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(CH_3), 23.8 (CH_2), 23.1 (CH_2), 20.9 (CH_2), 19.4 (CH_3), 13.8 (CH_3). MS (LCMS) m/z: 367 (M+Na). Anal. Calcd for $C_{23}H_{36}O_2$: C, 80.18; H, 10.53; Found: C, 79.97; H, 10.76.

5.1.9.2. (20*S*)-24-Norcholesta-5α-22-ene-3,20-diol (12)

Yield: 95%. Mp: 159-161 °C (Methanol). IR (Nujol, cm^{-1}): 3286 (-OH). ^1H NMR (200 MHz, $CDCl_3$) δ = 5.98 (dd, 1H, *J* = 10.8 and 17.3 Hz, 22-H), 5.14 (dd, 1H, *J* = 1.2 and 17.3 Hz, 23-H), 4.96 (dd, 1H, *J* = 1.1 and 10.7 Hz, 23-H), 3.59 (m, 1H, 3-H), 1.32 (s, 3H, 21-H_3), 0.81 (s, 6H, 19-H_3 and 18-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 146.1 (CH), 110.1 (CH_2), 75.7 (C), 71.2 (CH), 59.5 (CH), 56.5 (CH), 54.3(CH), 44.8 (CH), 43.0 (C), 40.4 (CH_2), 38.1 (CH_2), 36.9 (CH_2), 35.4 (C), 34.8 (CH), 31.9 (CH_2), 31.5 (CH_2), 28.7 (CH_3), 28.6 (CH_2), 23.7 (CH_2), 23.1 (CH_2), 21.0 (CH_2), 13.9 (CH_3), 12.3 (CH_3). MS (LCMS) m/z: 369 (M+Na). Anal. Calcd for $C_{23}H_{38}O_2{\cdot}CH_4O$: C, 75.19; H, 11.11; Found: C, 75.89; H, 11.32.

5.1.10. General procedure for selective acylation of 3-OH group in compounds 6 and 12

To the solution of 3β,20(*S*)-dihydroxy compounds **6** or **11** (0.5 mmol) in dry pyridine (2 mL), was added acetic anhydride (0.1 mL, 1 mmol) and catalytic amount of *N*,*N*-dimethylaminopyridine (0.012 g, 0.1 mmol). The reaction mixture was stirred at 25 °C for 2 h, quenched with crushed ice and extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with brine (2 × 10 mL), dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure resulted crude compounds. Column chromatographic purification over silica gel using ethyl acetate-petroleum ether as an eluent afforded pure products.

5.1.10.1. (20*S*)-3β-Acetoxy-24-norcholesta-5,22-diene-20-ol (7)

Yield: 94%. Mp: 152-153 °C (Methanol). IR (Nujol, cm^{-1}): 3452 (-OH), 1720 (-$OCOCH_3$). ^1H NMR (200 MHz, $CDCl_3$) δ = 5.99 (dd, 1H, *J* = 10.7 and 17.3 Hz, 22-H), 5.37 (d, 1H, *J* = 4.6 Hz), 5.15 (dd, 1H, *J* = 1.4 and 17.3 Hz, 23-H), 4.96 (dd, 1H, *J* = 1.3 and 10.7 Hz, 23-H), 4.63 (m, 1H, 3-H), 2.04 (s, 3H, -$OCOCH_3$), 1.34 (s, 3H, 21-H_3), 1.02 (s, 3H, 19-H_3), 0.84 (s, 3H, 18-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 170.5 (C), 146.01 (CH), 139.6 (C), 122.4 (CH), 110.2 (CH_2), 75.6 (C), 73.9 (CH), 59.3 (CH), 56.6 (CH), 49.9 (CH), 42.7 (C), 40.0 (CH_2), 38.0 (CH_2), 36.9 (CH_2), 36.5 (C), 31.7 (CH_2), 31.2 (CH), 28.7 (CH_3), 23.7 (CH_2), 23.1 (CH_2), 21.4 (CH_3), 20.8 (CH_2), 19.2 (CH_3), 13.7 (CH_3). MS (LCMS) m/z: 409 (M+Na). Anal. Calcd for $C_{25}H_{38}O_3$: C, 77.68; H, 9.91; Found: C, 77.52; H, 10.15.

5.1.10.2. (20*S*)-3β-Acetoxy-24-norcholesta-5α-22-ene-20-ol (13)

Yield: 93%. Mp: 181-183 °C (Methanol). IR (Nujol, cm^{-1}): 3207 (-OH), 1718 (-$OCOCH_3$). ^1H NMR (200 MHz, $CDCl_3$) δ = 5.98 (dd, 1H, *J* = 10.4 and 17.3 Hz, 22-H), 5.14 (dd, 1H, *J* = 1.4 and 17.3 Hz, 23-H), 4.95 (dd, 1H, *J* = 1.4 and 10.7 Hz, 23-H), 4.68 (m, 1H, 3-H), 2.03 (s, 3H, -$OCOCH_3$), 1.32 (s, 3H, 21-H_3), 0.82 (s, 3H, 19-H_3), 0.81 (s, 3H, 18-H_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 146.1 (CH), 110.1 (CH_2), 75.7 (C), 71.2 (CH), 59.5 (CH), 56.5 (CH), 54.3 (CH), 44.8 (CH), 43.0 (C), 40.4 (CH_2), 38.1 (CH_2), 36.9 (CH_2), 35.4 (C), 34.8 (CH), 31.9 (CH_2), 31.5 (CH_2), 28.7 (CH_3), 28.6 (CH_2), 23.7 (CH_2), 23.1 (CH_2), 21.0 (CH_2), 13.9 (CH_3), 12.3 (CH_3). MS (LCMS) m/z: 411 (M+Na). Anal. Calcd for $C_{23}H_{38}O_2{\cdot}CH_4O$: C, 75.19; H, 11.11; Found: C, 75.89; H, 11.32.

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5.2. Antimicrobial Activity: Materials and Methods

Candida albicans, *Cryptococcus neoformans* (human pathogen), *Benjaminiella poitrasii* and *Yarrowia lipolytica* (non pathogen) strains were maintained on YPG (yeast extract, 0.3%, peptone, 0.5%, and glucose, 1%) agar slants. *Fusarium oxysporum* (plant pathogen) strain was maintained on PDA (potato, 20% dextrose, 2%) agar slants at 28 ^{o}C. *Escherichia coli* (NCIM No. 2574) and *Staphylococcus aureus* (NCIM No. 2122) were maintained on NA (beef extract, 0.3%, peptone, 0.5%, sodium chloride, 0.5%) slants. Strains of *C. albicans, C. neoformans, Y. lipolytica* were inoculated in YPG broth at 28 ^{o}C and *B. poitrasii* at 37 ^{o}C for 24 h respectively, *F. oxysporum* in potato dextrose at 28 ^{o}C for 48 h whereas bacterial strains *E. coli* and *S. aureus* in NA broth for 24 h. Compounds **5-7,** and **11-13** were solubilized in DMSO and THF (50:50), and stock solutions of 1.28 mg/mL were prepared. Amphotericin B, Fluconazole, Tetracycline and Erythromycin were also dissolved in DMSO and THF (50:50), and were used as a positive control.

5.3. MIC determination

In *vitro* antifungal and antibacterial activity of newly synthesized compounds were studied against fungal strains *viz*., *C. albicans*, *C. neoformans*, *B. poitrasii*, *Y. lipolytica*, *F. oxysporum* and bacterial strains *E. coli* (NCIM No. 2574), and *S. aureus* (NCIM No. 2122) respectively to find out MIC (Minimum Inhibitory Concentration). All the experiments were done in triplicate under similar experimental conditions. MIC of the synthesized compounds were determined according to standard broth microdilution technique as per NCCLS guidelines.[19] Testing was performed in U bottom 96 well tissue culture plates in YPG, PDA for fungal strains and NA for bacterial strains. The concentration range of tested compounds and standard was 64-0.5 µg/ml. The plates were incubated at 28 °C for all the microorganisms except for *B. poitrasii* at 37 °C, absorbance at 600 nm was recorded to assess the inhibition of cell growth after 24 h for *B. poitrasii* and *Y. lipolytica*, 48 h for *C. albicans* and *F. oxysporum*, 72 h for *C. neoformans* and 24 h for bacterial cultures. MIC was determined as 90% inhibition of growth with respect to the growth control was observed.

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6. References

1. (a) Piatak, D. M.; Wicha, J. *Chem. Rev.* **1978**, *78*, 199-241; (b) Redpath, J.; Zeelan, F. J. *Chem. Soc. Rev*. **1983**, *12*, 75-98; (c) Kameritskii, A. V.; Reshetova, I. G.; Chernoburova, E. I. *Chem. Nat. Comp*. **1988**, *24*, 1-22; (d) Zhabinskii, V. N.; Ol'khovik, V. K.; Khripach, V. A. *Russ. J. Org. Chem.* **1996**, *32*, 305-344; (e) Taber, D. F.; Jiang, Q.; Chen, B.; Zhang, W.; Campbell, C. L. *J. Org. Chem.* **2002**, *67*, 4821-4827; (f) Gorobets, E.; Stepanenko, V.; Wicha, J. *Eur. J. Org. Chem.* **2004**, 783-799; (g) Chapelon, A.-S.; Moraleda, D.; Rodriguez, R.; Ollivier, C.; Santelli, M. *Tetrahedron* **2007**, *63*, 11511-11616.
2. Zeelen, F. J. In *Advances in Drug Research*, Testa, B.; Meyer, U.A., Eds.; Academic Press: London, **1994**, *25*, 87-102; (b) Morgan, B. P.; Moynihan, M. S. In *Kirk-Othmer Encyclopedia of Chemical Technology*, Kroschwitz, J. I.; Howe-Grant, M., Eds.; John Wiley & Sons: New York, **1997**, *22*, 851-921.
3. Akhrem, A. A.; Hyukhina, T. V.; Titov, Y. A. *Russ. Chem. Rev.* **1969**, *38*, 850-857.
4. (a) Petit, R. K.; Cage, G. D.; Petit, G. R. Liebman, J. A. *Antimicrob. Agents* **2000**, *15*, 299-304; (b) Savage, P. B. *Eur. J. Org. Chem.* **2002**, *5*, 759-768; (c) Savage, P. B.; Li, C.; Taotafa, U.; Ding, B.; Guan, Q. *FEMS Microbiol. Lett*. **2002**, *217*, 1-7.
5. (a) Kandutsch, A. A.; Chen, H. W.; Heiniger, H.-J. *Science* **1978**, *201*, 498 –501; (b) Chorvat, R. J.; Desai, B. N.; Radak, S. E.; McLaughlin, K.

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Chemistry & Biology Interface, 2011, 1, 2, 198-208

T.; Miller, J. E.; Jett, C.; Rohrbacher, E. *J. Med. Chem.* **1985**, *28*, 194-200; (c) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839-1895; (d) Janowski, B. A.; Willy, P. J.; Devi, T. R.; Falck, J. R.; Mangelsdorf, D. J. *Nature* **1996**, *383*, 728-731; (e) Schroepfer, Jr. G. J. *Physiological Rev*. **2000**, *80*, 361-554; (f) Massey, J. B.; Pownall, H. J. *Biochemistry* **2006**, *45*, 10747-10758; (g) Carvalho, J. F. S.; Cruz Silva, M. M.; Moreira, J. N.; Simoes, S.; Sae Melo, M. L. *J. Med. Chem.* **2009**, *52*, 4007-4019; (h) Carvalho, J. F. S.; Cruz Silva, M. M.; Moreira, J. N.; Simoes, S.; Sae Melo, M. L. *J. Med. Chem*. **2010**, *53*, 7632-7638.

6. Zeelen, F. J. *Medicinal Chemistry of Steroids*, Elsevier Science B. V: New York, **1990.**
7. (a) Hikino, H.; Okuyama, T.; Arihara, S.; Hikino, Y.; Takemoto, T.; Mori, H.; Shibata, K. *Chem. Pharm. Bull*. **1975**, *23*, 1458-1479; (b) Nagahisa, A.; Orme-Johnson, W. H.; Wilson, S. R. *J. Am. Chem. Soc*. **1984**, *106*, 1166-1167; (c) Drew, J.; Lettelier, M.; Morand, P.; Szabo, A. G. *J. Org. Chem*. **1987**, *52*, 4047-4052 and references cited theirin.
8. (a) Litvinovskaya, R. P.; Drach, S. V.; Khripach, V. A. *Russ. J. Org. Chem.* **2004**, *40*, 1450-1455; (b) Litvinovskaya, R. P.; Drach, S. V.; Masalova, E. N.; Khripach, V. A. *Russ. J. Org. Chem.* **2004**, *40*, 1456-1461.
9. (a) Narwid T. A.; Cooney, K. E.; Uskokovic, M. R. *Helv. Chim. Acta* **1974**, *57*, 771-781; (b) Kametani, T.; Tsubuki, M.; Nemoto, H. *Tetrahedron Lett.* **1981**, *22*, 2373-2374; (c) Kametani, T.; Tsubuki, M.; Nemoto, H. *J. Chem. Soc. Perkin Trans.1* **1981**, 3077-3082; (d) Kametani, T.; Katoh, T.; Fujio, J.; Nogiwa, I.; Tsubuki, M.; Honda, T. *J. Org. Chem.* **1988**, *53*, 1982-1991; (e) Kim. B. J.; Morita, H. *Chem. Lett*. **2000**, 42-43; (f) Jogireddy, R.; Rullkotter, J.; Christoffers, J. *Synlett* **2007**, 2847-2850.
10. Shingate, B. B.; Hazra, B. G.; Salunke, D. B.; Pore, V. S.; Shirazi, F.; Deshpande, M. V. *Eur. J. Med. Chem*. **2011**, *46*, 3681-3689.
11. (a) Hazra, B. G.; Pore, V. S.; Dey, S. K.; Datta, S.; Darokar, P. M.; Saikia, D.; Khanuja, S. P. S.; Thakur, A. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 773-777; (b) Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem*. **2004**, *47*, 1591-1594; (c) Salunke, D. B.; Hazra, B. G.; Pore, V. S. *Curr. Med. Chem*. **2006**, *13*, 813-847; (d) Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Chavan P. S.; Deshpande, M. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2043-2047; (e) Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Deshpande, M. V.; Kadreppa, S.; Chattopadhyay, S.; Gonnade, R. G. *Org. Biomol. Chem*. **2008**, *6*, 3823-3830; (f) Bavikar, S. N.; Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Dodd, R. H.; Thierry, J.; Shirazi, F.; Deshpande, M. V.; Kadreppa, S.; Chattopadhyay, S. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 5512-5517.
12. The plant genus *dioscoria floribunda*, which is cultivated in many parts of India, is an abundant source of diosgenin. 16-Dehydropegnenolone acetate **1** is prepared from commercially available diosgenin following Marker's procedure (Marker, R. E.; Wagner, R. B.; Ulshafer, P. R.; Wittbecker, E. L.; Goldsmith, D. P. J.; Rouf, C. H. *J. Am. Chem. Soc*. **1947**, *69*, 2167-2230).
13. (a) Hazra, B. G.; Basu, S.; Bahule, B. B.; Pore, V. S.; Vyas, B. N.; Ramraj, V. M. *Tetrahedron* **1997**, *53*, 4909-4920; (b) Shingate, B. B.; Hazra, B. G.; Pore, V. S.; Gonnade, R. G.; Bhadbhade, M. M. *Chem. Commun*. **2004**, 2194-2195.
14. (a) Shingate, B. B.; Hazra, B. G.; Pore, V. S.; Gonnade, R. G.; Bhadbhade, M. M. *Tetrahedron Lett*. **2006**, *47*, 9343-9347; (b) Shingate, B. B.; Hazra, B. G.; Pore, V. S.; Gonnade, R. G.; Bhadbhade, M. M. *Tetrahedron* **2007**, *63*, 5622-5635.
15. Magaraci, F.; Jimenz, C. J.; Rodrigues, J. C. F.; Braga, M. V.; Yardley, V.; Luca-Fradley, K.; Croft, S. L.; Souza, W.; Ruiz-Prez, L. M.; Urbina, J.; Pacanowska, D. G.; Gilbert, J. H. *J. Med. Chem.* **2003**, *46*, 4714-4727.
16. (a) Braekman, J. C.; Van Quaquebeke, E.; Ingrassia, L.; Dewelle, J.; Kiss, R.; Darro, F. Patent WO2004/055039, **2004**; (b) Braekman, J. C.; Ingrassia, L.; Nshimyumukiza, P.; Van Quaquebeke, E.; Dewelle, J.; Van den Hove, L.; Kiss, R.; Darro, F. Patent WO2005/058934, **2005**.
17. Deshpande, M. V. *Recent Trends Mycoses* **1998**, 55-63.
18. Ghormade, V.; Deshpande, M. V. *Naturwissenschaften* **2000**, *87*, 236-240.
19. (a) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast, Approved Standard. Document M27-A; National Committee for Clinical Laboratory Standards: Wayne, PA, USA, **1997**; (b) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming Filamentous Fungi: Proposed Standard. Document M38-P; National Committee for Clinical Laboratory Standard: Wayne, PA, USA, **1998**; (c) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard, 5th ed.; NCCLS: Villanova, PA, **2000**; M7–A5.