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Design and synthesis of fluconazole/bile acid conjugate using click reaction

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Abstract—Novel fluconazole/bile acid conjugates were designed and their regioselective synthesis was achieved in very high yield via Cu(I) catalyzed intermolecular 1,3-dipolar cycloaddition. These new molecules showed good antifungal activity against *Candida* species.

1. Introduction

The incidence of life threatening fungal infections has tremendously increased in last two decades due to greater use of immunosuppressive drugs, prolonged use of broad-spectrum antibiotics, wide-spread use of indwelling catheters, and also in cancer and AIDS patients.[1] The presently marketed antifungal drugs are either highly toxic (amphotericin-B) or becoming ineffective due to appearance of resistant strains (flucytosine and azoles).[2] Azole antifungals are strong inhibitors of lanosterol 14α-demethylase, which is major component of fungal cell membrane.[3] Fluconazole (Fig. 1) is an orally effective, potent, and safe triazole based antifungal drug, with favorable pharmacokinetic characteristics and low toxicity.[4] Due to the emergence of new fungal pathogens, resistant to fluconazole, great efforts have been made to modify the chemical structure of fluconazole, in order to broaden its antifungal activity and increase its potency.[5]

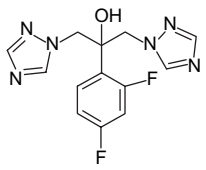
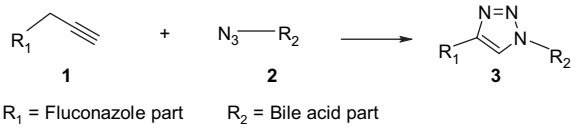


Figure 1. Fluconazole.

In recent years bile acid structures have become increasingly important in a number of fields, such as pharmacology, biomimetic, and supramolecular chemistry.[6] Bile acid transporters have been shown to accept and carry a variety of analogues that are derivatized at different positions of bile acids.[7] They have been used as absorption enhancers and as new cholesterol lowering agents.[7a] A common feature of bile acid derived antimicrobials is its potential to exhibit facially amphiphilic nature, due to polar hydroxyl groups on one face and nonpolar hydrophobic methyl groups on the other face.[8] Polyene macrolide amphotericin-B, peptide antimicrobial agent polymixin B, and squalamine in the cyclic form show such amphiphilicity and function as ionophores.[9]

Bioconjugation has recently emerged as a fast growing technology that affects almost every discipline of life science. It aims at the ligation of two or more molecules to form new complexes with the combined properties of its individual components.[10] In continuation of our work on bile acids,[11] we designed new bioconjugates **3** (Scheme 1) of bile acids



Scheme 1.

having amphiphilic nature as amphotericin-B and pharmacophore of fluconazole, linked together with 1,2,3-triazole, which may be viewed as an isoster of one of the 1,2,4-triazole component of fluconazole. 1,2,3-Triazole moieties are attractive connecting units, since they are stable to metabolic degradation and capable of hydrogen bonding, which can be favorable in binding of biomolecular targets and for solubility.[12] 1,2,3-Triazole moiety does not occur in nature, although the synthetic molecules containing 1,2,3-triazole unit shows diverse biological activities including antibacterial, herbicidal, fungicidal, antiallergic, and anti-HIV.[13]

Keywords: Bile acid; Fluconazole/bile acid conjugate; Click chemistry; Antifungal agent; 1,2,3-Triazole.
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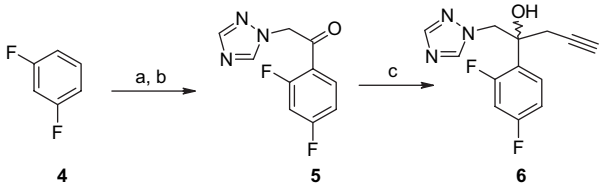
1,3-Dipolar cycloaddition of terminal acetylene and organic azides has been a method of choice for the synthesis of 1,2,3-triazoles.[14]

2. Results and discussion

In our approach to synthesize these new molecules, we considered performing Huisgen (click) reaction to connect fluconazole part containing terminal alkyne **1** and bile acid containing terminal azide **2**, in the presence of Cu(I) catalyst to form fluconazole/bile acid conjugate **3** (Scheme 1). Accordingly, we synthesized 2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)pent-4-yn-2-ol **6** by propargylation of the corresponding ketone **5**[5b] by using propargyl bromide and zinc dust to obtain racemic compound **6** (Scheme 2).

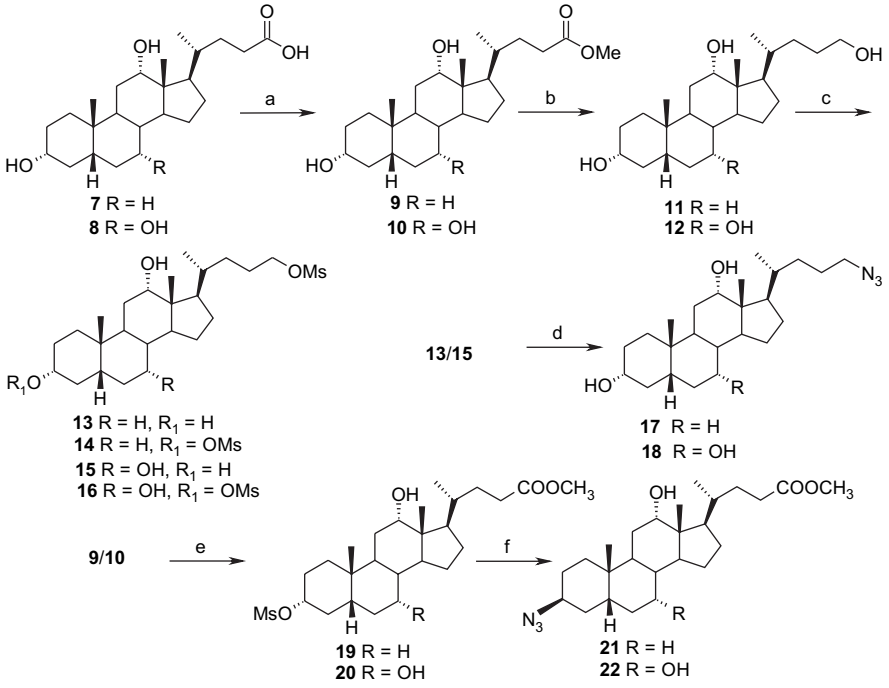


Scheme 2. Reagents and conditions: (a) $AlCl_3$, 1,2-dichloroethane, chloroacetyl chloride, 25 °C, 7 h; (b) 1,2,4-triazole, $NaHCO_3$, toluene, reflux, 4 h (overall 55% in a+b step); (c) Zn, propargyl bromide, DMF/THF, 25 °C, 5 h, 95%.

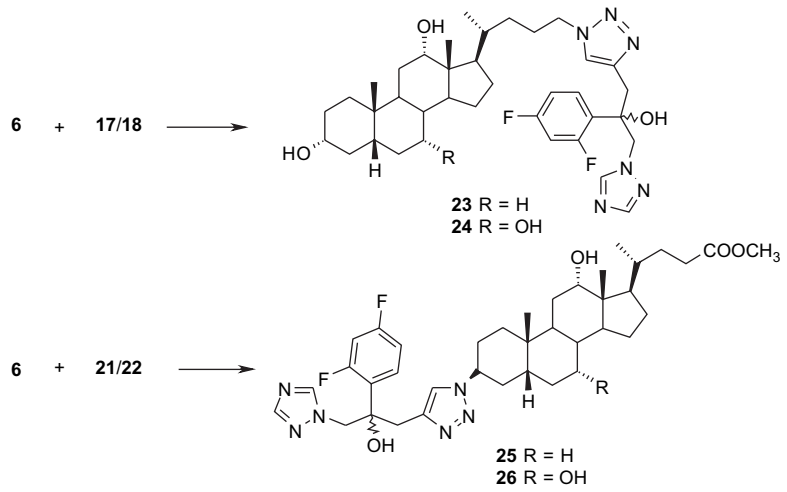
The compound **6** constitutes an alkyne component. In the proton NMR spectrum of compound **6**, the acetylenic proton was identified as triplet at δ 2.06 ppm and doublet of doublet at δ 2.87 ppm obtained due to β methylene. In the ^{13}C NMR spectrum it showed intricacies of various ^{19}F–^{13}C coupling as in fluconazole molecule.[15] The presence of acetylenic group was also evident from IR spectrum wherein the absorption due to acetylenic group was observed at 3307 cm^{-1}.

C-24-Azido bile acid derivatives **17** and **18** were synthesized from the corresponding mesyl compounds **13**[11a] and **15** (Scheme 3). IR of these compounds showed absorption due to azido group at 2100 cm^{-1}. In proton NMR spectrum, resonance corresponding to C-24-methylene group was observed at δ 3.24 ppm. Similarly, C-3-azido bile acid derivatives **21** and **22** were synthesized according to the literature procedures[16] with small modifications. The presence of azido group of the compounds **21** and **22** was also confirmed by IR spectrum. All the four compounds are well characterized by ^1H NMR, ^{13}C NMR, mass, and elemental analyses. The cycloaddition of **6** to azido compound **17** was attempted under previously reported conditions[11a] with copper sulfate and sodium ascorbate in *t*-BuOH/H_2O. This reaction failed at low temperature and at 50–60 °C, the reaction was slow and after 3 days the product **23** was obtained in 10% yield along with starting material. Although the result was encouraging, the reaction condition needed to be optimized. Among the various reaction conditions, microwave assisted Cu(I) catalyzed reaction was found to be suitable for this conjugation.[17] Under microwave irradiation compound **6** was reacted with C-24-azide **17** in DMF/H_2O using catalytic amount of Cu(I) to give fluconazole/bile acid conjugate **23** as a diastereomeric mixture in 92% yield (Scheme 4).

In the proton NMR spectrum of compound **23**, resonance corresponding to C-24-methylene protons was identified at δ 3.15 ppm and 3.48 ppm (two doublets) and the proton of 1,2,3-triazole was noticed at δ 7.19 ppm. Methylene at C-4 position of 1,2,3-triazole was identified at δ 4.20 ppm. In the IR spectrum, compound **23** showed absorption due to



Scheme 3. Reagents and conditions: (a) *p*-TsOH/MeOH, 25 °C, 24 h, 95–96%; (b) LAH/THF, 25 °C, 2 h, 93–97%; (c) (i) **11**, MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min or (ii) **12**, MsCl, pyridine, 0 °C, 10 min; (d) NaN_3, DMF, 60 °C, 3 h, 93–94%; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min; (f) NaN_3, DMF, 60 °C, 3 h, 90–91%.



Scheme 4. Reagents and conditions: $CuSO_4 \cdot 5H_2O$ (5 mol %), sodium ascorbate (40 mol %), DMF/H_2O (9:1), microwave, 5 min, 90–95%.

hydroxyl group at 3391 cm^{-1}. In addition, it gave satisfactory elemental analysis and in mass spectrum it showed molecular ion peak at 667.34 (M+1).

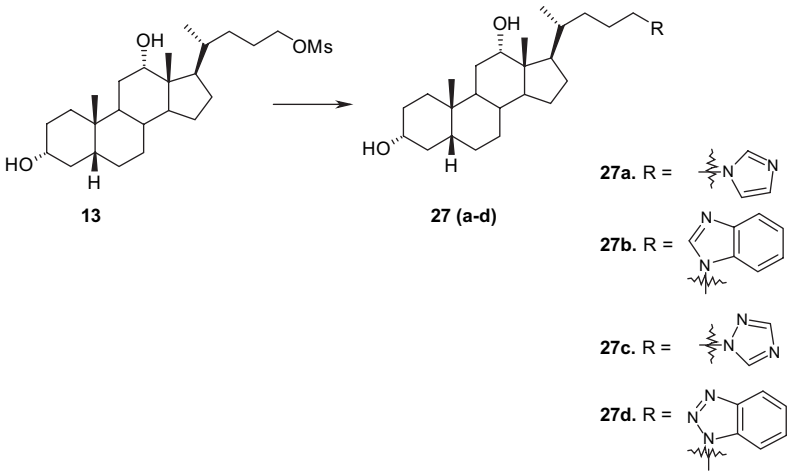
We then extrapolated the ligation protocol successfully to other bile acid derived azides, **18**, **21**, and **22** and synthesized fluconazole/bile acid conjugates **24**, **25**, and **26** (Scheme 4). All these fluconazole/bile acid bioconjugates showed very good antifungal activity against *Candida* species (Table 1).

For the comparison of biological activity we synthesized bile acid azoles conjugates **27a–d** containing imidazole, benzimidazole, triazole, and benzotriazole at C-24 position from C-24-monomesyl compound **13** (Scheme 5). However,

Table 1. In vitro antifungal activity for compounds **23**, **24**, **25**, **26**, and **27a–d**

Compound	Inhibitory concentration in μg/mL against											
	1		2		3		4		5		6	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
23	3.12	2.11	12.5	11.34	6.25	6.11	12.5	10.58	>50	>50	6.25	5.82
24	6.25	4.58	50	44.76	25	12.34	25	24.82	>50	>50	6.25	5.16
25	6.25	5.72	25	22.23	25	23.34	25	22.48	>50	>50	6.25	5.48
26	6.25	3.44	50	48.42	3.12	2.64	25	21.46	>50	>50	3.12	2.18
27a	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
27b	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
27c	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
27d	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Amphotericin-B	0.12	0.09	0.06	0.04	0.12	0.08	0.12	0.09	0.5	0.38	0.12	0.11
Fluconazole	0.5	0.13	1.0	0.46	2.0	1.06	1.0	0.63	2.0	1.06	1.0	0.21

1. *Candida albicans*, 2. *Cryptococcus neoformans*, 3. *Sporothrix schenckii*, 4. *Trichophyton mentagrophytes*, 5. *Aspergillus fumigatus*, 6. *Candida parapsilosis* (ATCC-22019).



Scheme 5. Reagents and conditions: **RH**, NaH, DMF, 25 °C, 12–20 h, 75–95%.

all these compounds showed very weak antifungal activity (Table 1).

3. Conclusion

In summary we have designed and synthesized fluconazole/bile acid conjugates at C-3 and C-24 positions of bile acids under microwave assisted Cu(I) catalyzed cycloaddition reaction. This reaction gave fluconazole/bile acid conjugates, linked with 1,4-disubstituted 1,2,3-triazole regioselectively, in excellent yield and in less reaction time. These new molecules showed very good antifungal activity against *Candida albicans*, *Sporothrix schenckii*, and *Candida parapsilosis* having MIC ranging from 3.12 to 6.25 μg/mL. It is thought that in this biological activity, bile acid part acts as a drug carrier and fluconazole part acts as an inhibitor of 14α-demethylase enzymes in fungal cell. Work to clarify the role of bile acid is underway in our laboratory. This is the first report of use of 'click chemistry' for the modification of fluconazole.

4. Experimental

4.1. General experimental techniques and apparatus

TLC was performed on precoated silica gel F_{254} plates (0.25 mm; E. Merck) and product(s) and starting material(s) were detected by either viewing under UV light or treating with an ethanolic solution of phosphomolybdic acid or anisaldehyde spray followed by heating. Column chromatography was performed on neutral deactivated aluminum oxide. Optical rotations were obtained on Bellingham & Stanley ADP-220 Polarimeter. Specific rotations ($[\alpha]_D$) are reported in deg/dm and the concentration (*c*) is given in g/100 mL in the specified solvent. Infrared spectra were recorded in $CHCl_3$ as a solvent on Schimadzu 8400 series FTIR instrument. ^{1}H NMR spectra were recorded on a Brucker AC-200 and 400 spectrometers at 200.13 and 400.13 MHz and ^{13}C NMR spectra were recorded on a Brucker AC-200 at 50.32 MHz. The chemical shifts are given in parts per million relative to tetramethylsilane. Mass spectra were recorded on LC–MS/MS-TOF API QSTAR PULSAR spectrometer, and samples were introduced by infusion method using Electrospray Ionisation Technique. Elemental analyses were performed by CHNS-O EA 1108-Elemental analyser, Carloerba Instrument (Italy) or Elementar vario EL (Germany) and were within ±0.4% of calculated values. Melting points were determined on a Thermonik Campbell melting point apparatus and were uncorrected. Microwave irradiation was carried out in an open glass vessel using a domestic microwave oven (800 W, BPL-make). Standard work up: after extraction of all the reactions, the organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4 and concentrated in vacuum.

4.2. Synthesis of terminal acetylene

4.2.1. 1-(2,4-Difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl) ethanone (5). Compound **5** was synthesized from **4** using literature procedure.[5b] White solid, yield 55% (overall in a+b step); mp=104–106 °C (lit.[5b] 103–105 °C); IR (cm^{-1}): 1703, 1614, 1593; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.59 (d, *J*=3.54 Hz, 2H), 6.93–7.10 (m, 2H), 7.99–8.11 (m, 2H), 8.21 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 58.2 (dd, $^4J_{CF}$=14.09 Hz), 104.8 (dd, $^2J_{CF}$=25.66 Hz), 112.9 (dd, $^2J_{CF}$=21.63 Hz, $^4J_{CF}$=3.02 Hz), 118.8 (dd, $^2J_{CF}$=14.09 Hz, $^4J_{CF}$=3.52 Hz), 132.9 (dd, $^3J_{CF}$=4.52, 10.81 Hz), 144.8, 151.7, 163.0 (dd, $^1J_{CF}$=256.38 Hz, $^3J_{CF}$=13.08 Hz), 166.6 (dd, $^1J_{CF}$=259.9 Hz, $^3J_{CF}$=12.58 Hz), 187.6 (d, $^3J_{CF}$=5.53 Hz). Anal. Calcd for $C_{10}H_7F_2N_3O$: C, 53.82; H, 3.16; F, 17.03; N, 18.83. Found: C, 54.10; H, 3.02; F, 16.87; N, 18.71; MS (LC–MS) *m/z*: 224.57 (M+1), 246.57 (M+23 for Na).

4.2.2. 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazole-1-yl)-pent-4-yn-2-ol (6). The ketone **5** (0.500 g, 2.24 mmol) and propargyl bromide (4 mL, 6.73 mmol) were dissolved in a mixed solvent DMF/THF 1:1 (10 mL). To this well stirred solution, activated zinc dust (washed with 2% HCl and water and dried in vacuum) (0.439 g, 6.73 mmol) was slowly added at room temperature.[18] After 5 min exothermic reaction brought itself to reflux, which was allowed to attain 25 °C. The whole reaction mixture was then stirred for 5 h at 25 °C. Ice-cold HCl solution (5%) was added to the reaction mixture and it was extracted with EtOAc, and washed with water and brine. Solvent was evaporated under reduced pressure to afford crude product, which was purified by column chromatography on silica gel (5% MeOH/DCM) to produce compound **6** (0.551 g) as a white solid.

Yield 95%; mp=145–146 °C; IR (cm^{-1}): 3272, 3137; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.06 (t, *J*=2.65 Hz, 1H), 2.86 (dd, *J*=16.01, 2.65 Hz, 1H ABX pattern), 2.92 (dd, *J*=18.01, 2.65 Hz, 1H ABX pattern), 4.13 (br s, 1H, OH), 4.72 and 4.82 (two d, *J*=14.01 Hz, 2H AB pattern), 6.73–6.87 (m, 2H), 7.50–7.59 (m, 1H), 7.87 (s, 1H), 8.20 (s, 1H); ^{13}C NMR ($CDCl_3$+CD_3OD, 50 MHz): δ 29.1 (d, $^4J_{CF}$=5.03 Hz), 56.3 (d, $^4J_{CF}$=5.03 Hz), 71.9, 73.4 (d, $^3J_{CF}$=4.53 Hz), 78.1, 103.9 (dd, $^2J_{CF}$=26.17, 27.17 Hz), 111.1 (dd, $^2J_{CF}$=20.63 Hz, $^4J_{CF}$=3.52 Hz), 124.2 (dd, $^2J_{CF}$=13.08 Hz, $^4J_{CF}$=3.52 Hz), 129.6 (dd, $^3J_{CF}$=6.04, 9.56 Hz), 144.2, 150.4, 158.6 (dd, $^1J_{CF}$=246.58 Hz, $^3J_{CF}$=12.08 Hz), 162.6 (dd, $^1J_{CF}$=249.59 Hz, $^3J_{CF}$=12.08 Hz). Anal. Calcd for $C_{13}H_{11}F_2N_3O$: C, 59.31; H, 4.21; F, 14.43; N, 15.96. Found: C, 59.45; H, 4.13; F, 14.31; N, 15.87; MS (LC–MS) *m/z*: 264.06 (M+1), 286.05 (M+23 for Na).

4.3. Synthesis of terminal azide

4.3.1. Synthesis of methyl 3α,12α-dihydroxy-5β-cholane-24-oate (9) and methyl 3α,7α,12α-trihydroxy-5β-cholane-24-oate (10). Compounds **9** and **10** were synthesized in overall good yield starting from cholic acid **7** and deoxycholic acid **8** using the literature procedure.[11e]

4.3.1.1. Methyl 3α,12α-dihydroxy-5β-cholane-24-oate (9). White solid; mp=82–105 °C (lit.[19a] 70–108 °C); IR (cm^{-1}): 3385, 1728; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.68 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.98 (d, *J*=6.36 Hz, 3H, CH_3-21), 3.62 (m, 1H, CH-3), 3.67 (s, 3H), 3.98 (br s, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.1, 23.0, 23.6, 26.0, 27.1, 27.4, 28.4, 29.6, 30.1,

30.8, 31.0, 33.4, 34.0, 35.2, 35.9, 36.2, 42.0, 46.3, 47.0, 48.0, 51.4, 71.4, 72.9, 174.7.

4.3.1.2. Methyl 3α,7α,12α-trihydroxy-5β-cholane-24-oate (10). White solid; mp=155–156 °C (lit.[19b] 155–156 °C); IR (cm^{-1}): 3376, 1731; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H, CH_3-18), 0.89 (s, 3H, CH_3-19), 0.98 (d, *J*=5.69 Hz, 3H, CH_3-21), 3.57 (m, 1H, CH-3), 3.67 (s, 3H), 3.89 (br s, 1H, CH-7), 4.01 (br s, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.8, 17.7, 22.8, 23.6, 26.6, 27.7, 28.5, 30.0, 31.4, 31.5, 35.2, 35.2, 35.7, 35.8, 39.9, 39.9, 42.0, 42.0, 46.8, 47.3, 51.8, 68.8, 72.2, 73.4, 175.2.

4.3.2. Synthesis of 3α,12α,24-trihydroxy-5β-cholane (11) and 3α,7α,12α,24-tetrahydroxy-5β-cholane (12). Compounds **11** and **12** were synthesized in overall good yield starting from methyl esters of cholic acid **9** and deoxycholic acid **10** using the literature procedure.[19c]

4.3.2.1. 3α,12α,24-Trihydroxy-5β-cholane (11). White solid; mp=123–124 °C (lit.[19d] 107–114 °C, lit.[19e] 123 °C); IR (cm^{-1}): 3257; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H), 0.91 (s, 3H), 0.99 (d, *J*=6.87 Hz, 3H), 3.61 (m, 3H), 4.00 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.7, 23.1, 23.7, 26.2, 27.2, 27.6, 28.5, 29.4, 30.4, 31.8, 33.6, 34.1, 35.3, 35.4, 36.1, 36.4, 42.1, 46.5, 47.5, 48.2, 63.4, 71.8, 73.3.

4.3.2.2. 3α,7α,12α,24-Tetrahydroxy-5β-cholane (12). Mp=236–238 °C (lit.[19c] 236.5–238 °C); IR, ^1H NMR, and ^{13}C NMR spectroscopic data are consistent that reported in literature.[19c]

4.3.3. Synthesis of 3α,12α-dihydroxy 24-mesyloxy-5β-cholane (13) and 3α,24-dimesyloxy-12α-hydroxy-5β-cholane (14). To a solution of **11** (2.000 g 5.28 mmol) in dry CH_2Cl_2 (20 mL) was added triethylamine (1.5 mL, 0.56 mmol) at 0 °C. Methane sulfonyl chloride (0.53 mL, 6.86 mmol in 10 mL CH_2Cl_2) was added dropwise in 10 min at 0 °C, and ice was added to the reaction mixture immediately after addition was complete. The reaction mixture was extracted with CH_2Cl_2. Organic layer was washed with $NaHCO_3$, water, and brine. Solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (0.5% MeOH/CH_2Cl_2) to obtain pure products **13** (1.805 g) and **14** (0.615 g).

4.3.3.1. 3α,12α-Dihydroxy 24-mesyloxy-5β-cholane (13). White solid; mp=78 °C; $[\alpha]_D^{26}$ +93.04 (*c* 0.2, $CHCl_3$); IR (cm^{-1}): 3419, 1416, 1448 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, *J*=6.26 Hz, 3H, CH_3-21), 3.00 (s, 3H), 3.60 (m, 1H, CH-3), 3.97 (s, 1H, CH-12), 4.19 (t, *J*=6.66 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.4, 23.0, 23.6, 25.9, 26.0, 27.0, 27.5, 28.6, 30.3, 31.4, 33.5, 34.0, 35.0, 35.2, 35.9, 36.3, 37.3, 42.0, 46.4, 47.3, 48.1, 70.5, 71.6, 73.0. Anal. Calcd for $C_{25}H_{44}O_5S$: C, 65.75; H, 9.71; S, 7.02. Found: C, 65.45; H, 9.53; S, 7.28; MS (LC–MS) *m/z*: 457.19 (M+1), 479.13 (M+23 for Na).

4.3.3.2. 3α,24-Dimesyloxy-12α-hydroxy-5β-cholane (14). White solid; mp=67–68 °C; $[\alpha]_D^{27}$ +43.25 (*c* 2.7, $CHCl_3$); IR (cm^{-1}): 3566; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H, CH_3-18), 0.92 (s, 3H, CH_3-19), 1.00 (d, *J*=6.26 Hz, 3H, CH_3-21), 3.00 (s, 3H), 3.01 (s, 3H), 4.00 (s, 1H, CH-12), 4.21 (t, *J*=6.66 Hz, 2H), 4.65 (m, 1H, CH-3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.4, 22.7, 23.4, 25.7, 25.8, 26.6, 27.4, 27.5, 28.5, 31.2, 33.1, 33.4, 33.7, 34.7, 34.9, 35.7, 37.2, 38.7, 41.9, 46.3, 47.1, 47.9, 70.6, 72.7, 82.7. Anal. Calcd for $C_{26}H_{46}O_7S_2$: C, 58.39; H, 8.67; S, 11.99. Found: C, 58.22; H, 8.39; S, 12.16; MS (LC–MS) *m/z*: 557.32 (M+23 for Na).

4.3.4. Synthesis of 3α,7α,12α-trihydroxy 24-mesyloxy-5β-cholane (15) and 3α,24-dimesyloxy-7α,12α-dihydroxy-5β-cholane (16). To a solution of **12** (0.394 g, 1 mmol) in dry pyridine (5 mL), methane sulfonyl chloride (0.12 mL, 1.5 mmol) was added to the reaction mixture. After 10 min, ice was added and it was extracted with EtOAc. Organic layer was washed with cold water, cold-HCl (5%), water, and brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (10% MeOH/CH_2Cl_2) to obtain pure **15** (0.329 g) and pure **16** (0.125 g).

4.3.4.1. 3α,7α,12α-Trihydroxy 24-mesyloxy-5β-cholane (15). White solid; mp=79–81 °C; $[\alpha]_D^{26}$ +68.49 (*c* 0.9, $CHCl_3$); IR (cm^{-1}): 3408; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H, CH_3-18), 0.89 (s, 3H, CH_3-19), 0.99 (d, *J*=6.44 Hz, 3H, CH_3-21), 3.01 (s, 3H), 3.44 (m, 1H, CH-3), 3.84 (s, 1H, CH-7), 3.97 (s, 1H, CH-12), 4.21 (t, *J*=6.57 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.3, 17.5, 20.8, 22.3, 23.1, 25.8, 26.2, 27.5, 28.0, 29.6, 30.1, 31.3, 34.5, 34.7, 35.1, 37.4, 39.2, 39.3, 41.3, 41.5, 46.3, 46.9, 68.4, 70.8, 71.9, 73.1. Anal. Calcd for $C_{25}H_{44}O_6S$: C, 63.52; H, 9.38; S, 6.78. Found: C, 63.21; H, 9.12; S, 6.53; MS (LC–MS) *m/z*: 473.91 (M+1), 495.89 (M+23 for Na).

4.3.4.2. 3α,24-Dimesyloxy-7α,12α-dihydroxy-5β-cholane (16). White solid; mp=82–84 °C; $[\alpha]_D^{25}$ +29.85 (*c* 0.8, $CHCl_3$); IR (cm^{-1}): 3434; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, *J*=6.44 Hz, 3H, CH_3-21), 2.99 (s, 3H), 3.01 (s, 3H), 3.87 (br s, 1H), 4.00 (s, 1H, CH-12), 4.21 (t, *J*=6.69 Hz, 2H), 4.51 (m, 1H, CH-3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.3, 17.5, 22.1, 22.7, 23.0, 26.3, 27.4, 27.8, 28.0, 31.3, 34.2, 34.4, 34.7, 35.1, 35.9, 37.2, 38.7, 39.2, 41.3, 41.6, 46.3, 47.1, 68.0, 70.7, 72.8, 83.0. Anal. Calcd for $C_{26}H_{46}O_8S_2$: C, 56.70; H, 8.42; S, 11.64. Found: C, 56.84; H, 8.26; S, 11.81; MS (LC–MS) *m/z*: 573.51 (M+23 for Na).

4.3.5. Synthesis of 3α,12α-dihydroxy 24-azido-5β-cholane (17) and 3α,7α,12α-trihydroxy 24-azido-5β-cholane (18). To a solution of **13** (0.300 g, 0.66 mmol) in dry DMF (10 mL), sodium azide (0.214 g, 3.28 mmol) was added and stirring was continued at 60–65 °C for 3–5 h. The reaction mixture was allowed to cool to room temperature. It was then poured into ice-cold water (30 mL) and extracted with EtOAc. The organic extract was washed with cold water and brine. Solvent was evaporated under reduced pressure to afford crude product **17**, which was purified by column chromatography on silica gel (10% EtOAc/hexane) to produce pure compound **17** as a white solid (0.247 g).

4.3.5.1. 3α,12α-Dihydroxy 24-azido-5β-cholane (17). White solid, yield 94%; mp=126 °C; $[\alpha]_D^{28}$ +40.57 (*c* 1.4,

$CHCl_3$); IR (cm^{-1}): 2090, 3409; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, J=6.65 Hz, 3H, CH_3-21), 3.24 (t, J=7.05 Hz, 2H), 3.62 (m, 1H, CH-3), 4.00 (br s, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.6, 23.1, 23.6, 25.6, 26.1, 27.1, 27.5, 28.7, 30.5, 32.9, 33.7, 34.1, 35.3, 36.1, 36.5, 42.2, 46.5, 47.5, 48.3, 51.9, 71.7, 73.2. Anal. Calcd for $C_{24}H_{41}N_3O_2$: C, 71.42; H, 10.24; N, 10.41. Found: C, 71.19; H, 10.46; N, 10.38; MS (LC–MS) *m/z*: 404.88 (M+1), 426.83 (M+23 for Na).

4.3.5.2. 3α,7α,12α-Trihydroxy 24-azido-5β-cholane (18). Compound **18** was prepared by similar procedure from compound **15**. White solid, yield 93%; mp=160 °C; $[\alpha]_D^{25}$ +31.46 (*c* 0.8, $CHCl_3$); IR (cm^{-1}): 2098, 3410; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H), 0.89 (s, 3H), 0.99 (d, J=6.32 Hz, 3H), 3.25 (t, J=6.57 Hz, 2H), 3.52 (m, 1H), 3.87 (br s, 1H), 4.00 (br s, 1H), 4.50 (br s, 3H, OH); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.6, 22.4, 23.1, 25.6, 26.2, 27.6, 28.1, 30.2, 32.8, 34.6, 34.7, 35.3, 35.3, 39.3, 39.4, 41.4, 41.5, 46.3, 47.1, 51.9, 68.4, 71.8, 73.1. Anal. Calcd for $C_{24}H_{41}N_3O_3$: C, 68.70; H, 9.85; N, 10.01. Found: C, 68.65; H, 9.69; N, 9.94; MS (LC–MS) *m/z*: 420.24 (M+1), 442.23 (M+23 for Na).

4.3.6. Synthesis of methyl-3α-mesyloxy-12α-hydroxy-5β-cholane-24-oate (19) and methyl-3α-mesyloxy-7α-12α-dihydroxy-5β-cholane-24-oate (20). Compounds **19** and **20** were prepared from compounds **9** and **10** by using similar procedure as used for the preparation of compound **13**.

4.3.6.1. Methyl-3α-mesyloxy-12α-hydroxy-5β-cholane-24-oate (19). White solid, yield 89%; mp=62–63 °C; $[\alpha]_D^{27}$ +45.77 (*c* 4.1, $CHCl_3$); IR (cm^{-1}): 1728, 3549; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, J=6.06 Hz, 3H), 2.99 (s, 3H), 3.65 (s, 3H), 3.98 (br s, 1H), 4.64 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.3, 16.8, 22.5, 23.3, 25.6, 26.5, 27.1, 27.3, 28.3, 30.5, 30.6, 32.9, 33.1, 33.5, 34.5, 34.8, 35.5, 38.4, 41.7, 46.1, 46.7, 47.6, 51.1, 72.3, 82.5, 174.3. Anal. Calcd for $C_{26}H_{44}O_6S$: C, 64.43; H, 9.15; S, 6.62. Found: C, 64.58; H, 9.02; S, 6.73; MS (LC–MS) *m/z*: 485.23 (M+1), 507.22 (M+23 for Na).

4.3.6.2. Methyl-3α-mesyloxy-7α-12α-dihydroxy-5β-cholane-24-oate (20). White solid, yield 87%; mp=83–85 °C; $[\alpha]_D^{28}$ +29.98 (*c* 0.9, $CHCl_3$); IR (cm^{-1}): 1728, 3460; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H), 0.91 (s, 3H), 0.99 (d, J=6.06 Hz, 3H), 2.99 (s, 3H), 3.67 (s, 3H), 3.88 (br s, 1H), 4.00 (br s, 1H), 4.51 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.2, 22.1, 23.0, 26.3, 27.4, 27.8, 28.0, 30.7, 30.9, 34.1, 34.4, 34.7, 35.1, 35.9, 38.7, 39.3, 41.3, 41.6, 46.4, 47.0, 51.4, 68.0, 72.8, 82.9, 174.7. Anal. Calcd for $C_{26}H_{44}O_7S$: C, 62.37; H, 8.86; S, 6.40. Found: C, 62.23; H, 8.92; S, 6.23; MS (LC–MS) *m/z*: 501.07 (M+1), 523.17 (M+23 for Na).

4.3.7. Synthesis of methyl-3β-azido-12α-hydroxy-5β-cholane-24-oate (21) and methyl-3β-azido-7α,12α-dihydroxy-5β-cholane-24-oate (22). The compounds **19** and **20** were reacted with NaN_3 (3 equiv) in DMF for 4 h at 80–90 °C to give compounds **21** and **22**.[20]

4.3.7.1. Methyl-3β-azido-12α-hydroxy-5β-cholane-24-oate (21). White solid, yield 91%; mp=127–128 °C (lit.[16a] 128 °C); $[\alpha]_D^{27}$ +41.34 (*c* 0.8, $CHCl_3$); IR (cm^{-1}): 1728, 2102, 3503; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H), 0.94 (s, 3H), 0.97 (d, J=6.20 Hz, 3H), 3.66 (s, 3H), 3.94 (br s, 1H), 3.99 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.3, 23.5, 23.5, 24.5, 25.9, 26.4, 27.4, 28.8, 30.1, 30.5, 30.8, 31.0, 33.2, 34.4, 35.0, 35.8, 37.2, 46.5, 47.3, 48.3, 51.4, 58.7, 73.1, 174.6. Anal. Calcd for $C_{25}H_{41}N_3O_3$: C, 69.57; H, 9.57; N, 9.74. Found: C, 69.47; H, 9.90; N, 9.66; MS (LC–MS) *m/z*: 432.26 (M+1), 454.25 (M+23 for Na).

4.3.7.2. Methyl-3β-azido-7α,12α-dihydroxy-5β-cholane-24-oate (22). White solid, yield 90%; mp=169–170 °C (lit.[16b] 157 °C); $[\alpha]_D^{27}$ +22.45 (*c* 1.16, MeOH) (lit.[16b] +23.7); IR (cm^{-1}): 1728, 2098, 3439; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.70 (s, 3H), 0.93 (s, 3H), 0.97 (d, J=6.06 Hz, 3H), 3.67 (s, 3H), 3.86–3.89 (br s, 2H), 3.99 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.2, 22.7, 23.2, 24.5, 26.0, 27.4, 28.3, 30.4, 30.8, 31.0, 33.0, 34.2, 35.1, 35.2, 36.7, 39.3, 41.7, 46.5, 47.2, 51.5, 58.7, 68.4, 73.0, 174.7. Anal. Calcd for $C_{25}H_{41}N_3O_4$: C, 67.08; H, 9.23; N, 9.39. Found: C, 67.21; H, 9.18; N, 9.31; MS (LC–MS) *m/z*: 448.24 (M+1), 470.22 (M+23 for Na).

4.4. General procedure for cycloaddition (23–26)

The alkyne **6** (1 equiv) and the azide **17**, **18**, **21**, or **22** (1.3 equiv) were dissolved in DMF/H_2O 4:1 (5 mL). To this solution, $CuSO_4 \cdot 5H_2O$ (0.05 equiv) and sodium ascorbate (0.40 equiv) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 5 min at 415 W. The reaction mixture was cooled, ice was added, and it was then extracted with EtOAc. The extract was washed with water and brine. Solvent was evaporated under reduced pressure and crude product was purified by column chromatography on silica gel using 5% MeOH/CH_2Cl_2 system to obtain fluconazole/bile acid conjugates **23**, **24**, **25**, or **26** linked with 1,4-disubstituted 1,2,3-triazole.

4.4.1. 3α,12α-Dihydroxy-24-[(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)]-5β-cholane (23). White solid, yield 95%; mp=169–171 °C; IR (cm^{-1}): 1597, 1618, 3391; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.65 (s, 3H), 0.90–0.93 (6H), 3.15 and 3.48 (two doublets, J=14.90 Hz, 2H, adjacent to C-4 end of 1,2,3-triazole), 3.62 (m, 1H), 3.95 (br s, 1H), 4.20 (t, J=6.82 Hz, 2H), 4.53–4.74 (two doublets, J=14.15 Hz, 2H), 5.55 (1H, OH), 6.70–6.80 (m, 2H), 7.19 (br s, 1H), 7.33–7.46 (m, 1H), 7.81 (br s, 1H), 8.16 (br s, 1H). Anal. Calcd for $C_{37}H_{52}F_2N_6O_3$: C, 66.64; H, 7.86; F, 5.70; N, 12.60. Found: C, 66.81; H, 7.77; F, 5.51; N, 12.55; MS (LC–MS) *m/z*: 667.34 (M+1).

4.4.2. 3α,7α,12α-Trihydroxy-24-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)-5β-cholane (24). White solid, yield 93%; mp=118–121 °C; IR (cm^{-1}): 1597, 1618, 3404; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.65 (s, 3H), 0.88 (s, 3H), 0.93 (d, J=6.52 Hz, 3H), 3.17 (d, J=14.15 Hz, 1H adjacent to 1,2,3-triazole), 3.42–3.49 (m, 2H, C3-H and 1H adjacent

to 1,2,3-triazole), 3.83 (br s, 1H), 3.94 (br s, 1H), 4.19 (m, 2H), 4.58 and 4.72 (two doublets, J=14.06 Hz, 2H, adjacent to 1,2,4-triazole), 6.69–6.78 (m, 2H), 7.20 (br s, 1H), 7.42 (m, 1H), 7.83 (br s, 1H), 8.20 (br s, 1H). Anal. Calcd for $C_{37}H_{52}F_2N_6O_4$: C, 65.08; H, 7.68; F, 5.56; N, 12.31. Found: C, 64.93; H, 7.66; F, 5.39; N, 12.45; MS (LC–MS) *m/z*: 683.29 (M+1), 705.27 (M+23 for Na).

4.4.3. Methyl-3β-[(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)]-12α-hydroxy-5β-cholane-24-oate (25). White solid, yield 90%; mp=183–185 °C; IR (cm^{-1}): 1618, 1728, 3362; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 0.80 (s, 3H), 0.96 (d, J=5.93 Hz, 3H), 3.19 and 3.54 (two doublets, J=16.29 Hz, 2H, adjacent to 1,2,3-triazole), 3.65 (s, 3H), 4.00 (br s, 1H), 4.54 (br s, 1H), 4.73 (br s, 2H, adjacent to 1,2,4-triazole), 6.63–6.80 (m, 2H), 7.23–7.35 (m, 2H), 7.86 (br s, 1H), 8.46 (br s, 1H). Anal. Calcd for $C_{38}H_{52}F_2N_6O_4$: C, 65.68; H, 7.54; F, 5.47; N, 12.09. Found: C, 65.43; H, 7.67; F, 5.24; N, 11.93; MS (LC–MS) *m/z*: 695.35 (M+1), 717.31 (M+23 for Na).

4.4.4. Methyl-3β-[(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)]-7α-12α-dihydroxy-5β-cholane-24-oate (26). White solid, yield 91%; mp=172–174 °C; IR (cm^{-1}): 1618, 1730, 3420; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.69 (s, 3H), 0.79 (s, 3H), 0.99 (d, J=6.27 Hz, 3H), 3.12 and 3.51 (two doublets, J=14.35 Hz, 2H, adjacent to 1,2,3-triazole), 3.66 (s, 3H), 3.87 (br s, 1H), 4.00 (br s, 1H), 4.46 (br s, 1H, C3-H), 4.63–4.72 (two doublets, J=14.30 Hz, 2H, adjacent to 1,2,4-triazole), 6.66–6.75 (m, 2H), 7.21 (br s, 1H), 7.36 (br s, 1H), 7.83 (br s, 1H), 8.30 (br s, 1H). Anal. Calcd for $C_{38}H_{52}F_2N_6O_5$: C, 64.21; H, 7.37; F, 5.35; N, 11.82. Found: C, 64.10; H, 7.29; F, 5.19; N, 11.77; MS (LC–MS) *m/z*: 711.29 (M+1), 733.26 (M+23 for Na).

4.5. General procedure for 27a–d

Azole compound **RH** (2 mmol) and NaH (3 mmol) were stirred in dry DMF (3 mL) at 0 °C for 20 min. To this mixture compound **13** (1 mmol) in DMF (2 mL) was added dropwise at 0 °C. The reaction mixture was allowed to warm to 25 °C and stirred for 24 h at this temperature. Ice was added to the reaction mixture and it was extracted with EtOAc. The extract was washed with water and brine, and solvent was evaporated under reduced pressure to afford crude product, which was purified by column chromatography on silica gel (5% MeOH/CH_2Cl_2) to produce compounds **27a–d** in 75–95% yield.

4.5.1. 3α,12α-Dihydroxy-24-(1*H*-imidazol-1-yl)-5β-cholane (27a). White solid, yield 75%; mp=215–216 °C; $[\alpha]_D^{25}$ +50.30 (*c* 0.7, $CHCl_3$); IR (cm^{-1}): 3300, 1510; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, J=6.26 Hz, 3H), 3.62 (m, 1H), 3.90 (t, J=7.44 Hz, 2H), 3.98 (br s, 1H), 6.91 (br s, 1H), 7.06 (br s, 1H), 7.48 (br s, 1H); ^{13}C NMR ($CDCl_3$+CD_3OD, 50 MHz): δ 12.3, 17.1, 22.7, 22.7, 23.4, 25.9, 26.9, 27.3, 27.4, 28.4, 29.6, 32.4, 33.3, 33.9, 35.0, 35.0, 35.8, 41.9, 46.7, 47.4, 47.7, 48.6, 71.1, 72.6, 118.8, 128.1, 136.5. Anal. Calcd for $C_{27}H_{44}N_2O_2$: C, 75.65; H, 10.35; N, 6.54. Found: C, 75.77; H, 10.21; N, 6.43; MS (LC–MS) *m/z*: 429.30 (M+1), 451.26 (M+23 for Na).

4.5.2. 3α,12α-Dihydroxy-24-(1*H*-benzo[*d*]imidazol-1-yl)-5β-cholane (27b). White solid, yield 91%; mp=118 °C; $[\alpha]_D^{27}$ +42.77 (*c* 1.0, $CHCl_3$); IR (cm^{-1}): 3346, 1643, 1616; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.65 (s, 3H), 0.90 (s, 3H), 0.95 (d, J=6.60 Hz, 3H), 3.60 (m, 1H), 3.96 (br s, 1H), 4.13 (t, J=6.60 Hz, 2H), 7.26–7.32 (m, 2H), 7.38–7.41 (m, 1H), 7.79–7.82 (m, 1H), 7.90 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.5, 17.4, 23.0, 23.5, 26.0, 26.4, 27.0, 27.4, 28.7, 30.4, 32.9, 33.5, 34.0, 35.1, 35.2, 35.9, 36.3, 42.0, 45.4, 46.3, 47.0, 48.1, 71.4, 72.8, 109.5, 120.1, 121.9, 122.7, 133.7, 142.7, 143.5. Anal. Calcd for $C_{31}H_{46}N_2O_2$: C, 77.78; H, 9.69; N, 5.85. Found: C, 77.64; H, 9.54; N, 5.77; MS (LC–MS) *m/z*: 479.26 (M+1), 501.20 (M+23 for Na).

4.5.3. 3α,12α-Dihydroxy-24-(1*H*-1,2,4-triazol-1-yl)-5β-cholane (27c). White solid, yield 95%; mp=196–197 °C; $[\alpha]_D^{26}$ +45.33 (*c* 0.7, $CHCl_3$); IR (cm^{-1}): 3421; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.66 (s, 3H), 0.90 (s, 3H), 0.98 (d, J=6.26 Hz, 3H), 3.61 (m, 1H), 3.97 (br s, 1H), 4.13 (t, J=6.65 Hz, 2H), 7.94 (s, 1H), 8.05 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.1, 22.8, 23.4, 25.9, 26.2, 26.9, 27.4, 28.3, 26.6, 32.2, 33.3, 33.8, 35.0, 35.0, 35.7, 35.7, 41.8, 46.1, 46.8, 47.8, 50.1, 71.2, 72.7, 142.6, 151.0. Anal. Calcd for $C_{26}H_{43}N_3O_2$: C, 72.68; H, 10.09; N, 9.78. Found: C, 72.49; H, 10.00; N, 9.67; MS (LC–MS) *m/z*: 430.49 (M+1), 452.44 (M+23 for Na).

4.5.4. 3α,12α-Dihydroxy-24-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-5β-cholane (27d). White solid, yield 92%; mp=98–101 °C; $[\alpha]_D^{26}$ +47.82 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 3417; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.65 (s, 3H), 0.90 (s, 3H), 0.95 (d J=6.59 Hz, 3H), 3.61 (m, 1H), 3.95 (br s, 1H), 4.61 (t, J=7.33 Hz, 2H), 7.34–7.40 (m, 1H), 7.46–7.55 (m, 2H), 8.06 (d, J=8.06 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.5, 23.0, 23.6, 26.1, 26.4, 27.1, 27.4, 28.6, 30.4, 32.8, 33.6, 34.0, 35.0, 35.2, 36.0, 36.4, 42.1, 46.4, 47.2, 48.1, 48.6, 71.6, 73.0, 109.2, 119.9, 126.0, 127.0, 132.9, 144.2. Anal. Calcd for $C_{30}H_{45}N_3O_2$: C, 75.11; H, 9.46; N, 8.76. Found: C, 75.26; H, 9.39; N, 8.71; MS (LC–MS) *m/z*: 480.53 (M+1), 502.49 (M+23 for Na).

4.6. Biological evaluation procedure

4.6.1. MIC and IC_{50} determination. Minimum inhibitory concentration of compounds was tested according to standard microbroth dilution technique as per NCCLS guidelines.[21] Briefly, testing was performed in flat bottom 96 well tissue culture plates (CELLSTAR® Greiner bio-one GmbH, Germany) in RPMI 1640 medium buffered with MOPS (3-[*N*-morpholino]propanesulfonic acid) (Sigma Chem. Co., MO, USA) for fungal strains and in Muller Hinton broth (Titan Biotech Ltd, India) for bacterial strains. The concentration range of tested compounds was 50–0.36 and 32–0.0018 μg/mL for standard compounds. The plates were incubated in a moist chamber at 35 °C and absorbance at 492 nm was recorded on VersaMax microplate reader (Molecular Devices, Sunnyvale, USA) after 48 h for *C. albicans* and *C. parapsilosis*, 72 h for *Aspergillus fumigatus*, *S. schenckii*, and *Cryptococcus neoformans*, and 96 h for *Trichophyton mentagrophytes*. MIC was determined as 90% inhibition of growth with respect to the growth control and IC_{50} was the concentration at which 50% growth

inhibition was observed by using SOFTmax Pro 4.3 Software (Molecular Devices, Sunnyvale, USA).

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Supplementary data

^{1}H NMR, ^{13}C NMR, DEPT, and spectral chart of compounds **5**, **6**, **13**–**26**, and **27a**–**d**, and LC–MS of compounds **23**–**26**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.09.021.

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