Synthesis and biological evaluation of bile acid dimers linked with 1,2,3-triazole and bis-β-lactam†

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Received 30th May 2008, Accepted 18th July 2008
First published as an Advance Article on the web 29th August 2008
DOI: 10.1039/b809221d

We report herein the synthesis and biological evaluation of bile acid dimers **11–18** linked through 1,2,3-triazole and bis-β-lactam. The dimers **11–18** were synthesized using 1,3-dipolar cycloaddition reaction of diazido bis-β-lactams **3**, **4** and terminal alkynes **7–10** derived from cholic acid/deoxycholic acid in the presence of Cu(I) catalyst (click chemistry). These novel molecules were evaluated *in vitro* for their antifungal and antibacterial activity. Most of the compounds exhibited significant antifungal as well as antibacterial activity against all the tested fungal and bacterial strains. Moreover, their *in vitro* cytotoxicities towards HEK-293 and MCF-7 cells were also established.

Introduction

The azetidine-2-one (β-lactam) ring system is a common structural feature of a number of broad spectrum β-lactam antibiotics such as penicillins, cephalosporins, carbapenems, nocardicins and monobactams, which have been widely used as chemotherapeutic agents for treating microbial diseases.[1–3] It also shows many other interesting biological properties, such as cholesterol absorption inhibitors,[4] human cytomegalovirus protease inhibitors,[5] thrombin inhibitors,[6] antihyperglycemic,[7] anti-tumour,[8] anti-HIV,[9] anti-inflammatory and analgesic activities.[10] However, microorganisms have built up resistance against the most traditional β-lactam antibiotics due to the widespread overuse of antibiotics. Therefore, the phenomenon of bacterial resistance forces the continuous modification of structure of known active compounds and the development of new ones.

Azoles are the largest class of antifungal agents in clinical use.[11] 1,2,3-Triazole moieties are attractive connecting units, as they are stable to metabolic degradation and capable of hydrogen bonding, which can be favorable in binding of biomolecular targets and solubility.[12] The β-lactam ring system in combination with the 1,2,3-triazole moiety is present in a number of drugs such as the β-lactam antibiotic Tazobactam and the cephalosporine Cefatrizine.[13] The 1,2,3-triazole moiety does not occur in nature, although the synthetic molecules containing 1,2,3-triazole units show diverse biological activities such as anti-HIV,[14] anti-microbial,[15] selective β_3 adrenergic receptor agonist[16] and anti-allergic.[17]

Bile acids and their derivatives are pharmacologically interesting as potential carriers of liver specific drugs, absorption enhancers and cholesterol lowering agents.[18] A common feature of bile acid derived antimicrobials is their potential to exhibit facially amphiphilic nature, due to polar hydroxyl groups on one face and a nonpolar hydrophobic methyl group on the other.[19] These hydroxyl groups can have specific interactions, such as those found in inclusion compounds,[20] organogelators[21] and receptors[22] based on cholic acid. The polarity of the hydroxyl groups can be increased to emphasize the facial amphiphilicity of the steroid unit. The resulting facial amphiphiles have many applications in ion transport,[23] combinatorial chemistry,[24] vesical fusion[25] and improvement of membrane permeability.[26] This type of amphiphilicity can also be exhibited by polyene macrolide amphotericin B, peptide antimicrobial agent polymixin B, and squalamine in the cyclic form, which functions as an ionophore.[27] Furthermore, bile acids are imperative building blocks for the synthesis of dimers, oligomers and colaphanes[28,29] due to their rigid framework with multiple chiral centers. The dimers, oligomers and colaphanes were synthesised from bile acids and have a wide range of potential applications in pharmacology,[29a] membrane bilayer probes,[30] and ion complexation.[31] The synthesis of novel bile acid dimers containing 1,2,3-triazole as a linker have been reported from our laboratory.[32] More recently, Regen *et al.* have reported bile acid derived molecular umbrellas as anti-HIV and anti-HSV agents and molecular umbrella-assisted transport of an oligonucleotide across cholesterol-rich phospholipid bilayers.[33] Taking advantage of the amphiphilic topology of bile acids, we have reported the synthesis of cholic acid and deoxycholic acid dimers, which were found to posses antifungal and antiproliferative activity.[34]

In continuation of our work on bile acid dimers, we report herein the synthesis of eight novel cholic acid and deoxycholic acid dimers **11–18** using both 1,2,3-triazole and bis-β-lactam as a linker and

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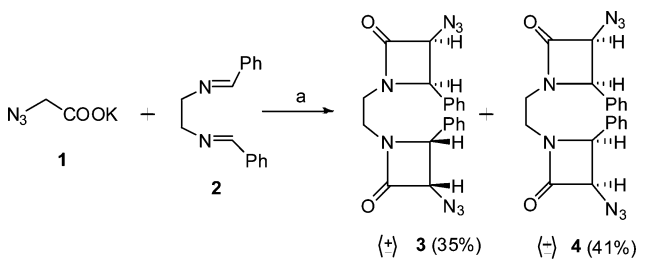
† CCDC reference numbers 690111 and 690112. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b809221d

studied their antimicrobial and cytotoxic activity. The syntheses of these novel cholic acid, deoxycholic acid dimers **11–18** linked with two unique pharmacophore units such as 1,2,3-triazole and β-lactam and the bioactivity data are reported herein for the first time.

Results and discussion

Chemistry

The Cu^{I}-catalysed variant of the Huisgen 1,3-dipolar cycloaddition of azide and alkynes affords regioselectively 1,4-disubstituted 1,2,3-triazoles with such efficiency and scope that the transformation has been described as "click" chemistry.[35] This unique transformation was independently discovered in 2002 by two groups, one led by Sharpless[36] in USA and the other in Denmark by Meldal.[37] Accordingly, our target molecules **11–18** were synthesized using the 1,3-dipolar cycloaddition reaction of bis-β-lactams **3**, **4** containing azide and cholic acids **7**, **9** and deoxycholic acids **8**, **10** containing terminal alkyne, in the presence of Cu(I) catalyst (click chemistry). Diazido bis-β-lactams **3** and **4** were prepared by the cycloaddition reaction (Staudinger)[38,39] using imine **2** and ketene derived from **1**. Thus, treatment of the potassium salt of azidoacetic acid **1** and imine **2** (prepared by using a literature[39] procedure) in the presence of triphosgene and triethylamine in anhydrous dichloromethane afforded a diastereomeric mixture of two diazido bis-β-lactams (Scheme 1). The mixture, after careful flash column chromatographic separation on silica gel, furnished diastereomeric compounds **3** and **4** in 35% and 41% yields respectively. The C_2-symmetric structure for bis-β-lactam **3** was assigned from ^{1}H NMR spectral analysis. The ^{1}H NMR spectrum of the compound **3** showed two doublets at δ 2.86 and 3.86 with geminal coupling of 11 Hz for the protons of the methylene groups joining two β-lactam rings. The *meso* structure was assigned to the other diastereomer **4** as the ^{1}H NMR spectrum of this compound showed two multiplets at δ 3.11 and 3.60 due to the non-equivalence of the two methylenes joining the two β-lactam rings. The structures of compounds **3** and **4** have been further confirmed unambiguously by X-ray crystal analysis (Fig. 1).



Scheme 1 *Reagents and conditions:* (a) triphosgene, Et_3N, CH_2Cl_2, 0–25 °C, 15 h, (35% for **3** and 41% for **4**).

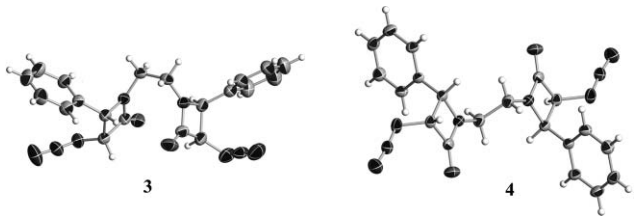
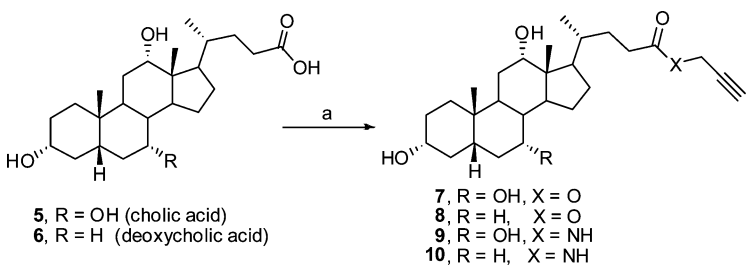


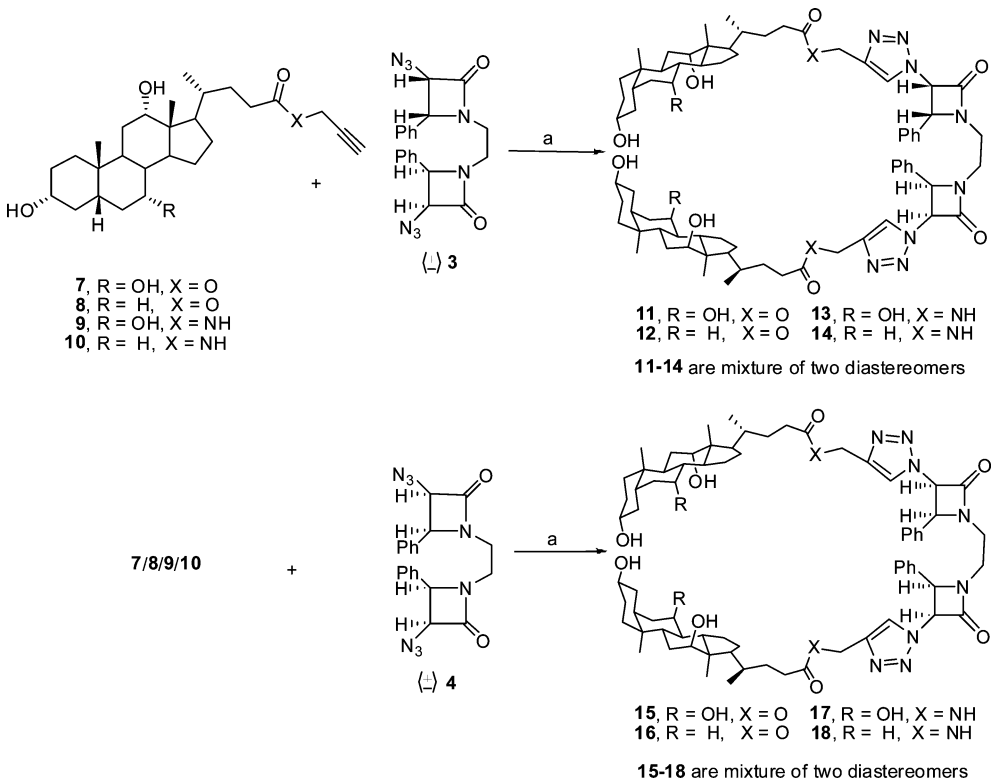
Fig. 1 Ortep view for compounds **3** and **4**.

Propargyl esters **7** and **8** were prepared by coupling propargyl alcohol with cholic acid **5** or deoxycholic acid **6** using EDC·HCl [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] as coupling agent in DMF at 0–25 °C with 88% and 90% yields respectively (Scheme 2). Using similar reaction conditions, amides **9** and **10** containing terminal alkyne functionality were prepared by coupling propargyl amine with cholic acid and deoxycholic acid in excellent yields.

Our next goal was to synthesise targeted cholic acid/deoxycholic acid dimers **11–18** linked with 1,2,3-triazole and bis-β-lactam. Reaction of propargyl ester **8** and bis-β-lactams **4** in *t*-BuOH–H_2O (7 : 3) with $CuSO_4{\cdot}5H_2O$, sodium ascorbate (click chemistry) at 70 °C for 32 h afforded the deoxycholic acid dimer linked with 1,2,3-triazole and bis-β-lactam **16** in 81% yield (Scheme 3). It has been well established that reaction under microwave conditions substantially decreased[40] the reaction time down to a few minutes as compared to several hours refluxing under thermal conditions. The cycloaddition reaction of propargyl esters **7**, **8** and amides **9**, **10** derived from cholic acid/deoxycholic acid with diazido bis-β-lactam **3** in DMF–H_2O (7 : 3) and $CuSO_4{\cdot}5H_2O$, sodium ascorbate under microwave irradiation for five minutes furnished a diasteriomeric mixture of novel dimers **11–14** in 93–95% yields. In a similar way, microwave irradiation of propargyl esters **7**, **8** and amides **9**, **10** with diazido bis-β-lactam **4** afforded a diasteriomeric mixture of dimeric compounds **15–18** in 92 to 94% yields. The combination of racemic core **3** and **4** with optically pure **7–10** afforded a diastereomeric mixture of dimers **11–18** in equal amounts. These diastereomers **11–18** were inseparable by flash column chromatography and also by crystallization.



Scheme 2 *Reagents and conditions:* (a) EDC·HCl, HOBt, propargyl alcohol/propargyl amine hydrochloride, Et_3N, DMF, 0–25 °C, 12 h.



Scheme 3 *Reagents and conditions:* (a) sodium ascorbate, $CuSO_4 \cdot 5H_2O$, DMF–H_2O (7 : 3), microwave (385 watt), 5 min, 92–95%.

Biological evaluation

Antimicrobial activity

All the newly synthesized dimers **11–18** were tested *in vitro* for antifungal and antibacterial activity. The antifungal activity was tested using the fungal strains *Candida albicans, Cryptococcus neoformans* (human pathogen), *Benjaminiella poitrasii*, *Yarrowia lipolytica* (saprophytes) and *Fusarium oxysporum* (plant pathogen). The antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus.* The MIC and IC_{50} values were determined using a standard broth microdilution technique described by NCCLS.[41] In comparison with the antimicrobial activity, amphotericin B and fluconazole were used as the reference antifungal agents, while tetracycline and ampicillin were used as the reference antibacterial agents. All the biological data of the tested compounds are depicted in Table 1 as MIC and IC_{50} values.

As seen in Table 1, most of the synthesised dimers **11–18** showed moderate to good antifungal and antibacterial activity against all the tested fungal and bacterial strains. The activity of compounds **13** and **17** was higher than that of fluconazole against *C. albicans* with a MIC value of 16–25 μg mL^{-1}. The compounds **13** and **15–18** showed good antifungal activity against *C. neoformans* having a MIC value of 16–36 μg mL^{-1} higher or comparable to that of reference drug fluconazole. However, all the compounds **11–18** except **15** showed significant growth inhibitory activity against *B. poitrasii. Y. lipolytica* was adversely affected by **12**, **13** and **15–18**, and in particular, **15** and **16** were the most potent with low MIC values of 8–10 μg mL^{-1}. Compound **12** showed significant inhibitory effect with a MIC value of 16 μg mL^{-1}, comparable to that of amphotericin B against *F. oxysporum*, whereas compounds **11**, **13** and **17** also showed promising activity against *F. oxysporum.* Furthermore, among the dimers **11–18**, none of the compounds showed more or comparable antibacterial activity against *E. coli* than tetracycline or ampicillin; compounds **11**, **13**, **15** and **18** were found to be moderately active with MIC values of 16–22 μg mL^{-1}. However, only compound **13** showed comparable activity to that of tetracycline and ampicillin against *S. aureus* with a MIC value of 16 μg mL^{-1}. From the overall activity results, it was observed that the ester or amide linkage did not affect the activity of the compounds.

Cytotoxic activity

The cytotoxicity of all the dimers **11–18** was assessed *in vitro* against human embryonic kidney (HEK-293) and human mammary adenocarcinoma (MCF-7) cell lines. Bile acid derivatives are known to promote proliferation and metastasis of cells of cancer origin and inhibit the proliferation of cells of non-cancer origin.[42] Hence we tested the cytotoxicity of the synthesized compounds in two different cell lines, one of cancer origin and the other of non-cancer origin. HEK-293 cells are normal human embryonic kidney cells where as MCF-7 cells are human breast cancer derived. The cytotoxicity of the compounds **11–18** against HEK-293 and MCF-7 cells was evaluated using MTT assay.[43] The observed IC_{50} values of all the evaluated compounds are shown in Table 2. Among them, compound **11** was the most toxic to both cell lines, with IC_{50} values of 10 and 80 μM; the rest of the compounds did not show

Table 1 *In vitro* antimicrobial activity of compounds **11–18**

	Inhibitory concentration/μg mL^{-1}													
	Fungal strains										Bacterial strains			
	CA		CN		BP		YL		FO		EC		SA	
Compound	MIC[a]	IC_{50}[b]	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
11	32	12	51	11	17	2	>128	32	19	9	16	10	47	11
12	64	33	>128	38	44	10	14	8	16	9	>128	57	37	17
13	16	8	18	5	32	21	35	16	25	8	22	9	16	7
14	>128	35	>128	43	43	9	>128	64	75	42	67	28	>128	53
15	85	17	17	4	94	36	10	6	64	42	19	7	32	16
16	64	41	32	4	41	12	8	4	31	10	47	19	64	29
17	25	10	36	11	12	4	16	4	27	8	80	29	77	22
18	67	29	16	6	41	15	17	8	32	10	18	11	>128	64
Ampho. B	2	0.5	27	10	23	8	15	9	16	8	—	—	—	—
Fluconazole	28	4	43	22	39	17	78	36	8	4	—	—	—	—
Tetracycline	—	—	—	—	—	—	—	—	—	—	9	3	16	8
Ampicillin	—	—	—	—	—	—	—	—	—	—	2	1	14	4

CA, *Candida albicans* (values were recorded after 48 h); CN, *Cryptococcus neoformans* (values were recorded after 72 h); BP, *Benjaminiella poitrasii*; YL, *Yarrowia lipolytica* (values were recorded after 24 h); FO, *Fusarium oxysporum* (values were recorded after 48 h); EC, *Escherichia coli*; SA, *Staphylococcus aureus* (values were recorded after 24 h). Negative control, DMSO (2.5% v/v), No inhibition. "–" Not tested.[a] MIC (minimum inhibitory concentration) was determined as 90% inhibition of growth with respect to the growth control. [b] IC_{50} was the concentration at which 50% growth inhibition was observed.

Table 2 Cytotoxicity of compounds **11–18**

	IC_{50}/μM	
Compound	HEK-293	MCF-7
11	10	80
12	>1000	>1000
13	100	>1000
14	500	>1000
15	500	1000
16	>1000	>1000
17	100	>1000
18	>1000	>1000

any cytotoxicity up to 500 μM concentration to both cell lines. Furthermore, it was observed that compounds **12**, **13**, **16** and **18** enhanced the proliferation of MCF-7 cells and not HEK-293 cells.

Conclusion

A series of novel bile acid dimers **11–18** linked through 1,2,3-triazole and bis-β-lactam have been synthesized using a Cu(I) catalysed cycloaddition reaction (click chemistry) of diazido bis-β-lactams **3–4** and terminal alkynes **7–10** derived from cholic acid/deoxycholic acid in excellent yield. These novel dimers **11–18** were evaluated for antifungal as well as antibacterial and cytotoxic activities. Most of the compounds demonstrated potent antimicrobial activity against all the strains tested. Among them, compounds **13** and **17** showed significant antifungal activity against human pathogen, *C. albicans* and compounds **13**, **15** and **18** showed appreciable antifungal activity against *C. neoformans* than reference drug fluconazole. In the case of plant pathogen *F. oxysporum*, compound **12** showed comparable inhibitory activity to amphotericin B. In particular, compounds **15** and **16** exhibited the most significant activity against *Y. lipolitica* with MIC values of 8–10 μg mL^{-1}. Compounds **11**, **13** and **15** derived from cholic acid were moderately active against *E. coli.* Additionally, only compound **13** exhibited comparable activity against *S. aureus* to that of reference drugs. Furthermore, except compound **11**, all other compounds (**12–18**) did not show any significant cytotoxicity to the tested cell lines. The synthesis of bile acid dimers linked with two pharmacophores, 1,2,3-triazole and β-lactam, can open up a new horizon for the control of human and plant pathogens.

Experimental section

General methods

All melting points were determined on a Yanco Micro melting point apparatus and are uncorrected. Optical rotations were obtained on a Bellingham and Stanly ADP-220 Polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using TLC aluminium sheets, silica gel 60-F_{254} precoated, Merck, Germany and spots were located using UV light as the visualizing agent or spraying with ethanolic phosphomolybdic acid (PMA) solution, followed by heating. Column chromatography was performed on silica gel. ^{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 MSL-300 at 300.13 and 75.47 or on a Bruker DRX-500 spectrometer 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 Schimadzu 8400 series FTIR instrument and values are reported in cm^{-1} units. Specific rotations ($[\alpha]_D$) are reported in deg dm^{-1} and the concentration (c) is given in g/100mL in the specific solvent. Mass spectra were recorded by either a LC-MS or MS-TOF API QSTAR PULSAR spectrometer, samples introduced by infusion method using electrospray ionisation technique. Elemental analyses were performed by a CHNS-O EA 1108-Elemental analyser, Carlo Erba Instrument (Italy) or Elementor Vario EL (Germany) and were within ±0.3% of calculated values.

Procedure for the synthesis of β-lactams 3 and 4

A solution of triphosgene (0.741 g, 2.5 mmol), in anhydrous CH_2Cl_2 (20 mL), was added slowly to a mixture of the potassium salt of azidoacetic acid **1** (0.695 g, 5 mmol), imine **2** (0.472 g, 2 mmol) and triethylamine (2 mL, 15 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. After the addition, the reaction mixture was allowed to warm up to room temperature (25 °C) and was stirred for 15 h. The reaction mixture was then washed with water (25 mL), saturated sodium bicarbonate solution (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated to get the crude product as a diastereomeric mixture. Flash column chromatographic purification over silica gel using ethyl acetate–petroleum ether (12 : 88) as an eluent afforded pure β-lactam **3** (0.281 g, 35%). Further elution with same solvent system yielded pure β-lactam **4** (0.329 g, 41%).

1,2-Bis[3′-azido-4′-phenylazetidin-2′-one-1′-yl]ethane 3. Mp 123–124 °C; (Found: C, 59.87; H, 4.62; N, 28.09. Calc. for $C_{20}H_{18}N_8O_2$: C, 59.69; H, 4.51; N, 27.85%.); IR ν_{max} ($CHCl_3$)/(cm^{-1}) 2115 (N_3), 1762 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.46–7.21 (10H, m, Ar-H), 5.17 (2H, d, J = 5.1), 4.91 (2H, d, J = 5.1), 3.84 (2H, d, J = 11.2), 2.86 (2H, d, J = 11.2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.8, 132.6, 129.1, 128.8, 127.7, 68.6, 59.9, 37.8. MS (LCMS) m/z: 425 (M + Na)$^+$.

1-[3′-Azido-4′-phenylazetidin-2′-one-1′-yl]-2-[3″-azido-4″-phenylazetidin-2″-one-1″-yl]ethane 4. Mp 162–163 °C; (Found: C, 59.57; H, 4.29; N, 28.13. Calc. for $C_{20}H_{18}N_8O_2$: C, 59.69; H, 4.51; N, 27.85%.); IR ν_{max} ($CHCl_3$)/(cm^{-1}) 2115 (N_3), 1762 (CO); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.48–7.28 (10H, m, Ar-H), 4.79 (2H, d, J = 4.9), 4.74 (2H, d, J = 4.9,), 3.55–3.69 (2H, m), 3.04–3.18 (2H, m); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 164.7, 132.3, 129.3, 128.9, 127.9, 68.1, 60.9, 38.6. MS (LCMS) m/z: 425 (M + Na)$^+$.

X-Ray crystal structure determination for 3 and 4

Single crystals of compounds **3** and **4** were obtained from an ethyl acetate–light petroleum ether mixture (30 : 70). X-Ray diffraction data were collected on a Bruker SMART APEX CCD diffractometer with graphite-monochromatized (Mo Kα = 0.71073 Å) radiation at room temperature (297 K).†

Crystallographic data for 3. ($C_{20}H_{18}N_8O_2$): M = 402.42, crystal dimensions 0.58 × 0.32 × 0.17 mm3, monoclinic, space group P $2_1/c$, a = 13.838(11), b = 6.873(6), c = 22.068(19) Å, β = 96.710(15)°; V = 2085(3) Å^3; Z = 4; ρ_{calcd} = 1.282 gcm^{-3}, μ (Mo-K$_\alpha$) = 0.089 mm^{-1}, F(000) = 840, $2\theta_{max}$ = 50.00°, 9849 reflections collected, 3667 unique, 2786 observed ($I > 2\sigma$ (I)) reflections, 298 refined parameters, R value 0.0541, $wR2$ = 0.1327 (all data R = 0.0699, $wR2$ = 0.1433), S = 1.068, minimum and maximum transmission 0.9503 and 0.9851; maximum and minimum residual electron densities 0.155 and –0.142 e Å^{-3}.

Crystallographic data for 4. ($C_{20}H_{18}N_8O_2$): M = 402.42, crystal dimensions 0.78 × 0.15 × 0.13 mm^3, monoclinic, space group P $2_1/c$, a = 13.196(8), b = 5.751(4), c = 13.632(8) Å, β = 109.453(9)°; V = 975.4(10) Å^3; Z = 2; ρ_{calcd} = 1.370 gcm^{-3}, μ (Mo-K$_\alpha$) = 0.095 mm^{-1}, F(000) = 420, $2\theta_{max}$ = 50.00°, 8813 reflections collected, 1715 unique, 1538 observed ($I > 2\sigma$ (I)) reflections, 136 refined parameters, R value 0.0359, $wR2$ = 0.0896 (all data R = 0.0399, $wR2$ = 0.0930), S = 1.070, minimum and maximum transmission 0.9296 and 0.9878; maximum and minimum residual electron densities 0.149 and –0.128 e Å^{-3}.

All the data were corrected for Lorentzian, polarization and absorption effects using Bruker's SAINT and SADABS programs. SHELX-97 (G. M. Sheldrick, SHELX-97 program for crystal structure solution and refinement, University of Gottingen, Germany, 1997) was used for structure solution and full-matrix least-squares refinement on F^2. Hydrogen atoms for both the structures were placed in a geometrically idealized position with C–H = 0.93 Å (for Ar–H), C–H= 0.98 Å (for methyne-H) and C–H = 0.97 Å (for methylene-H) and constrained to ride on their parent atoms with U_{iso}(H) = 1.2U_{eq}(C).

General procedure for the synthesis of alkyne compounds 7–10

EDC·HCl (1.5 equiv) and HOBt (0.5 equiv) were added to a solution of cholic acid/deoxycholic acid (1 equiv) and propargyl alcohol/propargyl amine (1.1 equiv) in dry DMF (5 mL) under argon at 0 °C. After the addition, the reaction mixture was allowed to warm up to 25 °C and was stirred for 12 h. The reaction was quenched by adding crushed ice and extracted three times with ethyl acetate. The combined extracts were washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo.* The resulting crude product was purified by column chromatography to give pure alkyne compounds **7–10**.

Propargyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate 7. Yield: 88%; mp 112–114 °C; $[\alpha]_D^{27}$ +26.15 (c 1.3, $CHCl_3$); (Found: C, 72.35; H, 9.23. Calc. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48%.); IR ν_{max} ($CHCl_3$)/(cm^{-1}) 3396, 3307, 1737; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.68 (2H, d, J = 2.5 Hz, OCH_2), 3.96 (1H, bs, CH-12), 3.84 (1H, bs, CH-7), 3.45 (1H, m, CH-3), 2.47 (1H, t, J = 2.5 Hz, alkyne CH), 0.98 (3H, d, J = 5.8 Hz, CH_3-21), 0.89 (3H, s, CH_3-19), 0.68 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.4, 77.7, 74.7, 73.0, 71.7, 68.3, 51.6, 46.8, 46.3, 41.4, 39.3, 35.1, 34.7, 30.9, 30.6, 30.2, 29.6, 28.0, 27.4, 26.1, 23.1, 22.3, 17.2, 12.3; MS (LCMS) m/z: 469 (M + Na)$^+$.

Propargyl 3α,12α-dihydroxy-5β-cholan-24-oate 8. Yield: 90%; mp 160–161 °C; $[\alpha]_D^{27}$ +43.5 (c 1.5, $CHCl_3$); (Found: C, 75.11; H, 9.62. Calc. for $C_{27}H_{42}O_5$: C, 75.31; H, 9.83%.); IR ν_{max} ($CHCl_3$)/(cm^{-1}) 3382, 3307, 1737; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.68 (2H, d, J = 2.2 Hz, OCH_2), 3.98 (1H, bs, CH-12), 3.55–3.64 (1H, m, CH-3), 2.47 (1H, t, J = 2.2 Hz, alkyne CH), 0.97 (3H, d, J = 5.9 Hz, CH_3-21), 0.91 (3H, s, CH_3-19), 0.67 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2, 77.7, 74.6, 72.9, 71.5, 51.6, 48.1, 47.1, 46.4, 42.0, 36.3, 35.9, 35.2, 35.1, 34.0, 33.5, 30.9, 30.7, 30.2, 28.5, 27.4, 27.1, 26.06, 23.6, 22.9, 17.1, 12.6; MS (LCMS) m/z: 453.14 (M + Na)$^+$.

***N*-Propargyl-3α,7α,12α-trihydroxy-5β-cholan-24-amide 9.** Yield: 89%; mp 276–278 °C; $[\alpha]_D^{27}$ +22.9 (c 1.31, $CHCl_3$); (Found: C, 72.93; H, 9.51; N, 2.92. Calc. for $C_{27}H_{43}NO_4$: C, 72.77; H, 9.73; N, 3.14%.); IR ν_{max} ($CHCl_3$)/(cm^{-1}) 3309, 1652; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.43 (1H, t, J = 5.2 Hz, CONH), 4.02–4.06 (2H, m, NH-CH_2), 3.97 (1H, bs, CH-12), 3.84 (1H, bs, CH-7), 3.38–3.51 (1H, m, CH-3), 2.84 (3H, s, OH), 2.24 (1H, t, J = 2.5 Hz, alkyne CH), 0.99 (3H, d, J = 5.8 Hz, CH_3-21), 0.89 (3H, s, CH_3-19), 0.68 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.2, 80.0, 73.1, 71.8, 71.1, 68.4, 46.2, 41.4, 39.3, 35.3, 35.2, 34.7, 34.6, 32.4, 31.4,

30.2, 28.9, 28.0, 27.5, 26.2, 23.2, 22.4, 17.4, 12.4; MS (LCMS) *m/z*: 446.19 $(M + 1)^+$, 468.16 $(M + Na)^+$.

***N*-Propargyl-3α,12α-dihydroxy-5β-cholan-24-amide 10.** Yield: 92%; mp 184 °C; $[\alpha]_D^{27}$ +51.76 (*c* 0.97, $CHCl_3$); (Found: C, 75.26; H, 9.87; N, 3.45. Calc. for $C_{27}H_{43}NO_3$: C, 75.48; H, 10.09; N, 3.26%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3475, 3446, 3238, 3209, 1633; ^1H NMR ($CDCl_3$ + $DMSOD_6$, 200 MHz) δ 6.80 (1H, bs, CONH), 3.88–3.92 (2H, m, NH-CH_2), 3.85 (1H, bs, CH-12), 3.41–3.55 (1H, m, CH-3), 2.14 (1H, t, $J = 2.5$ Hz, alkyne CH), 0.87 (3H, d, $J = 6.1$ Hz, CH_3-21), 0.79 (3H, s, CH_3-19), 0.56 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$ + $DMSOD_6$, 50 MHz) δ 174.5, 79.3, 72.7, 71.0, 70.7, 47.7, 46.3, 46.0, 41.8, 35.6, 35.0, 34.9, 33.8, 33.2, 32.3, 31.2, 29.4, 28.4, 28.2, 27.2, 26.8, 25.8, 23.4, 22.7, 16.6, 12.3; MS (LCMS) *m/z*: 430.21 $(M + 1)^+$, 452.19 $(M + Na)^+$.

General procedure for the synthesis of dimers 11–18

The alkynes of cholic acid/deoxycholic acid (1 equiv) and the diazido bis-β-lactams **3** or **4** (0.5 equiv) were dissolved in DMF–H_2O 7 : 3 (10 mL). To this solution, $CuSO_4 \cdot 5H_2O$ (0.05 equiv) and sodium ascorbate (0.5 equiv) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 5 min at 385 watt. It was then cooled to room temperature, quenched with crushed ice and extracted with ethyl acetate. The extract was washed with water and brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography to obtain dimers **11–18**.

Dimer 11. Mp 167–168 °C; (Found: C, 68.45; H, 7.71; N, 8.89. Calc. for $C_{74}H_{102}N_8O_{12}$: C, 68.60; H, 7.94; N, 8.65%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3411, 1766, 1731; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (1H, s, triazole-H), 7.47 (1H, s, triazole-H), 7.21 (10H, bs, Ar-H), 6.16 (1H, d, $J = 4.8$, Hz), 6.13 (1H, d, $J = 5.0$, Hz), 5.56 (2H, d, $J = 4.8$ Hz), 4.97 (4H, s, -OCH_2), 4.11 (2H, t, $J = 9.3$ Hz), 3.94 (2H, bs, CH-12), 3.84 (2H, d, $J = 13.6$ Hz, CH-7), 3.40 (2H, bs, CH-3,), 3.13 (2H, t, $J = 11.8$ Hz), 0.94 (6H, s, CH_3-19), 0.86 (6H, d, $J = 7.8$ Hz, CH_3-21), 0.64 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.7, 163.4, 142.4, 130.9, 128.8, 128.4, 126.6, 124.0, 72.6, 71.3, 68.7, 67.9, 60.4, 56.5, 46.4, 46.0, 41.1, 39.0, 38.9, 38.6, 34.9, 34.4, 34.2, 30.7, 30.3, 29.6, 27.7, 27.2, 25.9, 22.8, 22.1, 16.8, 12.1; MS (LCMS) *m/z*: 1295 $(M + 1)^+$, 1318 $(M + Na)^+$.

Dimer 12. Mp 157–159 °C; (Found: C, 70.07; H, 7.94; N, 9.02. Calc. for $C_{74}H_{102}N_8O_{10}$: C, 70.34; H, 8.14; N, 8.87%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3434, 1768, 1730; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.43 (2H, s, Ar-H), 7.20–7.25 (10H, m, Ar-H), 6.15–6.12 (2H, m), 5.55 (2H, d, $J = 5.1$ Hz), 4.97 (4H, s, -OCH_2), 4.12 (2H, d, $J = 11.1$ Hz), 3.97 (1H, bs, CH-12), 3.66–3.55 (2H, m, CH-3), 3.13 (2H, d, $J = 11.3$ Hz), 2.35–2.16 (4H, m, -CH_2CO), 0.94 (6H, d, $J = 5.8$ Hz, CH_3-21), 0.91 (6H, s, CH_3-19), 0.66 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.7, 163.6, 142.7, 131.2, 129.1, 128.7, 126.8, 123.9, 72.9, 71.5, 69.1, 60.6, 56.9, 48.1, 47.0, 46.4, 42.0, 38.7, 36.3, 35.9, 35.2, 35.0, 34.0, 33.5, 31.0, 30.9, 30.6, 30.3, 28.6, 27.4, 27.0, 26.1, 23.6, 23.0, 17.2, 12.6; MS (LCMS) *m/z*: 1286 $(M + Na)^+$.

Dimer 13. Mp 195–196 °C; (Found: C, 68.41; H, 8.23; N, 10.66. Calc. for $C_{74}H_{104}N_{10}O_{10}$: C, 68.70; H, 8.10; N, 10.83%.); IR ν_{max} (Nujol)/(cm^{-1}) 3369, 1758, 1650; ^1H NMR ($CDCl_3$ + CD_3OD, 500 MHz) δ 7.53 (2H, s, triazole-H), 7.21 (10H, bs, Ar-H), 6.12 (2H, bs, unresolved splitting), 5.55 (2H, s, unresolved splitting), 4.19 (4H, s, -$NHCH_2$), 4.08 (2H, t, $J = 9.8$ Hz, N-CH_2), 3.94 (2H, bs, CH-12), 3.83 (2H, bs, CH-7), 3.42–3.37 (2H, m, CH-3,), 3.17–3.14 (2H, m, N-CH_2), 0.96 (6H, d, $J = 5.5$ Hz, CH_3-21), 0.89 (6H, s, CH_3-19), 0.66 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.6, 163.5, 144.7, 130.8, 128.7, 128.2, 126.7, 123.2, 72.6, 71.2, 68.7, 67.8, 60.6, 46.1, 45.9, 41.2, 41.1, 39.0, 38.7, 38.6, 35.0, 34.9, 34.3, 34.1, 33.8, 32.3, 31.2, 29.4, 27.6, 27.1, 25.9, 22.8, 22.0, 16.7, 12.0; MS (LCMS) *m/z*: 1316 $(M + Na)^+$.

Dimer 14. Mp 177–178 °C; (Found: C, 70.37; H, 8.08; N, 11.29. Calc. for $C_{74}H_{104}N_{10}O_8$: C, 70.45; H, 8.31; N, 11.10%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3392, 1766, 1658; ^1H NMR ($CDCl_3$ + CD_3OD, 500 MHz) δ 7.21 (10H, bs, Ar-H), 6.21 (2H, bs, unresolved splitting), 5.56 (2H, bs, unresolved splitting), 4.10 (4H, s, -$NHCH_2$), 3.96 (2H, s, CH-12), 3.58 (2H, bs, CH-3), 3.14–3.00 (4H, m, -NCH_2), 0.96 (6H, bs, CH_3-21), 0.90 (6H, s, CH_3-19), 0.67 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$ + CD_3OD 125 MHz): δ 163.7, 130.8, 128.8, 128.3, 126.7, 72.6, 71.0, 70.0, 59.9, 47.7, 46.4, 46.1, 41.7, 38.5, 35.7, 35.6, 35.1, 34.9, 33.8, 33.1, 31.2, 29.5, 28.2, 27.2, 26.8, 25.8, 23.4, 22.7, 16.8, 12.3; MS (LCMS) *m/z*: 1284 $(M + Na)^+$, 1285 $(M + Na + 1)^+$.

Dimer 15. Mp 173–175 °C; (Found: C, 68.74; H, 7.83; N, 8.57. Calc. for $C_{74}H_{102}N_8O_{12}$: C, 68.60; H, 7.94; N, 8.65%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3454, 1770, 1731; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.67 (1H, s, triazole-H), 7.49 (1H, s, triazole-H), 7.30–7.27 (10H, m, Ar-H), 6.23–6.16 (2H, m), 5.38 (2H, bs, unresolved splitting), 5.0 (4H, bs, -OCH_2), 3.95 (2H, bs, CH-12), 3.84 (2H, bs, CH-7), 3.76–3.47 (6H, m, 2 × CH-3, 2 × -NCH_2), 0.95 (6H, bs, CH_3-21), 0.88 (6H, s, CH_3-19), 0.67 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.8, 163.2, 131.1, 129.2, 128.6, 127.0, 72.7, 71.4, 68.7, 68.1, 61.7, 56.7, 2 × 46.6, 46.1, 41.3, 40.3, 39.2, 35.1, 34.5, 34.3, 30.8, 30.5, 29.8, 27.8, 27.3, 26.0, 22.8, 22.2, 17.0, 12.2; MS (LCMS) *m/z*: 1317 $(M + Na)^+$.

Dimer 16. Mp 145–146 °C; (Found: C, 70.26; H, 8.11; N, 8.96. Calc. for $C_{74}H_{102}N_8O_{10}$: C, 70.34; H, 8.14; N, 8.87%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3442, 1766, 1731; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.62 (2H, s, triazole-H), 7.32–7.22 (10H, m, Ar-H), 6.15–6.13 (2H, m), 5.37–5.33 (2H, m), 5.0 (4H, s, -OCH_2), 3.97 (1H, bs, CH-12), 3.83–3.43 (6H, m, 2 × CH-3, 2 × -NCH_2), 0.95 (6H, bs, CH_3-21), 0.91 (6H, s, CH_3-19), 0.67 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.6, 163.1, 142.5, 131.2, 129.1, 128.6, 127.0, 124.1, 72.3, 71.3, 68.7, 61.8, 56.8, 47.9, 46.8, 46.3, 41.9, 40.3, 36.2, 35.8, 35.1, 35.0, 33.9, 33.3, 30.8, 30.5, 30.1, 28.5, 27.3, 27.0, 26.0, 23.5, 22.9, 17.0, 12.5; MS (LCMS) *m/z*: 1285 $(M + Na)^+$.

Dimer 17. Mp 199–200 °C; (Found: C, 68.46; H, 8.31; N, 10.54. Calc. for $C_{74}H_{104}N_{10}O_{10}$: C, 68.70; H, 8.10; N, 10.83%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3500, 1758, 1650; ^1H NMR ($CDCl_3$ + $DMSOD_6$, 400 MHz) δ 7.60 (1H, s, triazole-H), 7.51 (1H, s, triazole-H), 7.27–7.25 (10H, m, Ar-H), 6.17–6.13 (2H, m), 5.38–5.35 (2H, m), 4.21 (4H, bs, -$NHCH_2$), 3.90 (1H, bs, CH-12), 3.82–3.77 (4H, m, 2 × CH-7, N-CH_2), 3.48–3.43 (2H, m, CH-3), 3.39–3.33 (2H, m, N-CH_2), 0.98 (6H, d, $J = 5$ Hz, CH_3-21), 0.88 (6H, s, CH_3-19), 0.66 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$ + CD_3OD, 125 MHz): δ 174.5, 163.2, 144.6, 131.1, 129.0, 128.4, 127.0, 123.2, 72.7, 71.3, 68.5, 67.9, 61.6, 46.0, 41.3, 39.9, 39.1, 35.1, 34.5, 34.3, 32.3, 31.3,

29.7, 29.4, 27.8, 27.2, 26.0, 22.9, 22.2, 17.0, 12.1; MS (LCMS) *m/z*: 1316 $(M + Na)^+$.

Dimer 18. Mp 180–181 °C; (Found: C, 70.61; H, 8.15; N, 11.22. Calc. for $C_{74}H_{104}N_{10}O_8$: C, 70.45; H, 8.31; N, 11.10%.); IR ν_{max} $(CHCl_3)/(cm^{-1})$ 3373, 1768, 1658; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.58 (1H, s, triazole-H), 7.47 (1H, s, triazole-H), 7.27–7.24 (10H, m, Ar-H), 6.72–6.63 (2H, m, -NH), 6.12 (2H, bs, unresolved splitting), 5.34 (2H, bs, unresolved splitting), 4.27 (4H, bs, $-NHCH_2$), 3.96 (1H, bs, CH-12), 3.77 (2H, bs, $N-CH_2$), 3.59–3.45 (4H, m, $2\times$ CH-3, $N-CH_2$), 0.95 (6H, bs, CH_3-21), 0.91 (6H, s, CH_3-19), 0.67 (6H, s, CH_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.3, 163.0, 144.7, 131.0, 128.9, 128.3, 126.9, 123.1, 72.6, 71.0, 68.6, 61.4, 47.6, 46.3, 46.1, 41.7, 39.9, 35.8, 35.6, 35.1, 33.8, 33.1, 31.2, 29.7, 28.2, 27.2, 26.9, 25.9, 23.4, 22.8, 16.9, 12.3; MS (LCMS) *m/z*: 1284 $(M + Na)^+$.

Antimicrobial activity: materials and methods

Human pathogens *C. albicans* and *C. neoformans*; saprophytes *B. poitrasii* and *Y. lipolytica* were maintained on YPG (yeast extract, 0.3%; peptone, 0.5%; and glucose, 1%) agar slants. *F. oxysporum* (plant pathogen) was maintained on PDA (potato, 20%; dextrose, 2%) agar slants at 28 °C. *E. coli* and *S. aureus* were maintained on nutrient agar (NA; beef extract, 0.3%; peptone, 0.5%; sodium chloride, 0.5%) agar slants. Strains of *C. albicans, C. neoformans, Y. lipolytica* and *B. poitrasii* were inoculated in YPG broth. *C. albicans, C. neoformans* and *Y. lipolytica* were incubated at 28 °C whereas *B. poitrasii* was incubated at 37 °C for 24 h. *F. oxysporum* was inoculated in potato dextrose and incubated at 28 °C for 48 h whereas bacterial strains *E. coli* and *S. aureus* were inoculated in NA broth for 24 h. Compounds **11–18** were solubilized in DMSO (2.5% v/v) and stock solutions of 1.28 mg mL^{-1} were prepared. Amphotericin B, fluconazole, tetracycline and ampicillin were also dissolved in DMSO, and were used as a positive control.

MIC and IC_{50} determination

In vitro antifungal and antibacterial activity of the newly synthesized compounds were studied against the fungal strains *viz.*, *C. albicans*, *C. neoformans*, *B. poitrasii*, *Y. lipolytica, F. oxysporum* strains and bacterial strains *E. coli*, and *S. aureus*, respectively to find out MIC (minimum inhibitory concentration) and IC_{50} (50%, inhibition of growth). All the experiments were done in triplicate under similar experimental conditions. MIC and IC_{50} of the synthesized compounds were determined according to a standard broth microdilution technique as per NCCLS guidelines. Testing was performed in U bottom 96 well tissue culture plates in YPG, PD broth for fungal strains and nutrient broth for bacterial strains. The concentration range of tested compounds and standard was 0.25 to 128 μg mL^{-1}. The plates were incubated at 28 °C for all the microorganisms (except at 37 °C for *B. poitrasii*). Absorbances at 600 nm were 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 and IC_{50} was the concentration at which 50% growth inhibition was observed.

Antiproliferative activity. Materials and methods: cell culture

Human embryonic kidney (HEK-293) and human mammary adenocarcinoma (MCF-7) cell lines were grown in a monolayer in nutrient media DMEM (Dulbecco's Modified Eagle's Media) supplemented with fetal bovine serum (10%), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) (all from Invitrogen Life Technologies, MD). The cells were grown at 37 °C in the presence of 5% CO_2.

MTT cell proliferation assay

HEK-293 and MCF-7 cells were plated at a density of 10^4 cells per well in 96-well tissue culture plates. Cells were allowed to adhere for 24 h at 37 °C. Stock solutions of all the compounds were prepared in DMSO at a concentration of 10 mM and diluted to the required concentration. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was dissolved (5 mg mL^{-1}) in DMEM (without phenol red) and filtered through a 0.22 μm filter before use. The cells were treated with various concentrations (0, 1, 10, 100 and 1000 μM) of compounds dissolved in DMSO for an additional 48 h, in triplicate. In the control wells, nutrient medium with a corresponding concentration of DMSO only was added to the cells. Thereafter, the drug containing medium was replaced with 50 μL media containing 1 mg mL^{-1} MTT and incubated for 4 h at 37 °C. Medium was then aspirated off from the formazan crystals, which were then solubilized in 100 μL of acidified isopropanol. The optical density was read on a microplate reader at 570 nm using 630 nm as a reference filter against a blank prepared from cell free wells. Absorbance given by cells treated with the carrier DMSO alone was taken as 100% cell growth. All assays were performed in triplicate.

Acknowledgements

NSV thanks CSIR, New Delhi, for the award of a Senior Research Fellowship. An Emeritus Scientist Scheme awarded to BGH by the CSIR, New Delhi is gratefully acknowledged.

References

1 A. K. Halve, D. Bhadauria and R. Dubey, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 341–345.
2 C. D. Risi, G. P. Pollini, A. C. Veronese and V. Bertolasi, *Tetrahedron Lett.*, 1999, **40**, 6995–6998.
3 *The Organic Chemistry of* β-*Lactams*, ed. G. I. Georg, VCH, New York, 1993.
4 S. B. Rosenblum, T. Huynh, A. Afonso, H. R. Davis, N. Yumibe, J. W. Clader and D. A. Burnett, *J. Med. Chem.*, 1998, **41**, 973–980.
5 A. D. Borthwick, G. Weingarte, T. M. Haley, M. Tomaszewski, W. Wang, Z. Hu, J. Bedard, H. Jin, L. Yuen and T. S. Mansour, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 365–370.
6 W. T. Han, A. K. Trehan, J. J. K. Wright, M. E. Federici, S. M. Seiler and N. A. Meanwell, *Bioorg. Med. Chem.*, 1995, **3**, 1123–1143.
7 R. K. Goel, M. P. Mahajan and S. K. Kulkarni, *J. Pharm. Pharm. Sci.*, 2004, **7**, 80–83.
8 G. Veinberg, I. Shestakova, M. Verma, I. Kenepe and E. Lukevics, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 147–150.
9 T. Sperka, J. Pitlik, P. Bagossi and J. Tozser, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3086–3090.
10 C. Saturnino, B. Fusco, P. Saturnino, G. De Martino, F. Rocco and J. C. Lanceolat, *Biol. Pharm. Bull.*, 2000, **23**, 654–656.
11 F. C. Odds, A. J. P. Brown and N. A. R. Gow, *Trends Microbiol.*, 2003, **11**, 272–279.

12 (*a*) D. K. Dalvie, A. S. Kalgutkar, S. C. Khojasteh-Bakht, R. S. Obach and J. P. O'Donnell, *Chem. Res. Toxicol.*, 2002, **15**, 269–299; (*b*) W. S. Horne, M. K. Yadav, C. D. Stout and M. R. Ghadiri, *J. Am. Chem. Soc.*, 2004, **126**, 15366–15367.
13 M. Journet, D. Cai, J. J. Kowal and R. D. Larsen, *Tetrahedron Lett.*, 2001, **42**, 9117–9118.
14 R. Alvarez, S. Velazquez, A. San-Felix, S. Aquaro, E. De Clercq, C. F. Perno, A. Karlsson, J. Balzarini and M. J. Camarasa, *J. Med. Chem.*, 1994, **37**, 4185–4194.
15 M. J. Genin, D. A. Allwine, D. J. Anderson, M. R. Barbachyn, D. E. Emmert, S. A. Garmon, D. R. Graber, K. C. Grega, J. B. Hester, D. K. Hutchinson, J. Morris, R. J. Reischer, C. W. Ford, G. E. Zurenko, C. J. Hamel, R. D. Schaadt, D. Stapert and B. H. Yagi, *J. Med. Chem.*, 2000, **43**, 953–970.
16 L. L. Brockunier, E. R. Parmee, H. O. Ok, M. R. Candelore, M. A. Cascieri, L. F. Colwell, L. Deng, W. P. Feeney, M. J. Forrest, G. J. Hom, D. E. MacIntyre, L. Tota, M. J. Wywratt, M. H. Fisher and A. E. Weber, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2111–2114.
17 D. R. Buckle, C. J. M. Rockell, H. Smith and B. A. Spicer, *J. Med. Chem.*, 1984, **27**, 223–227.
18 A. Enhsen, W. Kramer and G. Wess, *Today*, 1998, **3**, 409–418.
19 T. M. Stein and S. H. Gellman, *J. Am. Chem. Soc.*, 1992, **114**, 3943–3950.
20 V. Bertolasi, O. Bortolini, M. Fogagnolo, G. Fantin and P. Pedrini, *Tetrahedron: Asymmetry*, 2001, **12**, 1479–1483.
21 (*a*) H. M. Willemen, T. Vermonden, A. T. M. Marcelis and E. J. R. Sudholter, *Eur. J. Org. Chem.*, 2001, 2329–2335; (*b*) K. Nakano, Y. Hiswikawa, K. Sada, M. Miyata and K. Hanabusa, *Chem. Lett.*, 2000, 1170–1171.
22 J. Tamminen and E. Kolehmainen, *Molecules*, 2001, **6**, 21–46, and references cited therein.
23 (*a*) P. Bandyopadhyay, V. Janout, L. Zhang and S. L. Regen, *J. Am. Chem. Soc.*, 2001, **123**, 7691–7696; (*b*) Y. Kobuke and T. Nagatani, *Chem. Lett.*, 2000, 298–299.
24 J. F. Barry, A. P. Davis, M. N. Perez-Payan, M. R. J. Elsegood, R. F. W. Jackson, C. Gennari, U. Piarulli and M. A. Gude, *Tetrahedron Lett.*, 1999, **40**, 2849–2852.
25 Y. R. Vandenburg, B. D. Smith, M. N. Perez-Payan and A. P. Davis, *J. Am. Chem. Soc.*, 2000, **122**, 3252–3253.
26 C. Li, A. S. Peters, E. L. Meredith, G. W. Allman and P. B. Savage, *J. Am. Chem. Soc.*, 1998, **120**, 2961–2962.
27 P. B. Savage, *Expert Opin. Invest. Drugs*, 2000, **9**, 263–272.
28 (*a*) J. Tamminen, E. Kolehmainen, M. Haapala and J. Linnanto, *Synthesis*, 2000, 1464–1468; (*b*) P. S. Pandey, R. Rai and R. B. Singh, *Tetrahedron*, 2002, **58**, 355–362; (*c*) A. P. Davis and R. S. Wareham, *Angew. Chem., Int. Ed.*, 1999, **38**, 2978–2996.
29 (*a*) L. Yuexian and J. R. Dias, *Chem. Rev.*, 1997, **97**, 283–304 and references cited therein; (*b*) A. P. Davis, *Chem. Soc. Rev.*, 1993, 243–253.
30 J. T. Groves and R. Neumann, *J. Am. Chem. Soc.*, 1989, **111**, 2900–2909.
31 R. P. Bonar-Law and J. K. M. Sanders, *Tetrahedron Lett.*, 1992, **33**, 2071–2074.
32 N. G. Aher and V. S. Pore, *Synlett*, 2005, 2155–2158.
33 (*a*) B. Jing, V. Janout, B. C. Herold, M. E. Klotman, T. Heald and S. L. Regen, *J. Am. Chem. Soc.*, 2004, **126**, 15930–15931; (*b*) V. Janout, B. Jing and S. L. Regen, *J. Am. Chem. Soc.*, 2005, **127**, 15862–15870.
34 D. B. Salunke, B. G. Hazra, V. S. Pore, M. K. Bhat, P. B. Nahar and M. V. Deshpande, *J. Med. Chem.*, 2004, **47**, 1591–1594.
35 V. D. Bock, H. Hiemstra and J. H. V. Maarseveen, *Eur. J. Org. Chem.*, 2006, 51–68.
36 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
37 W. C. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
38 D. Krishnaswamy, V. V. Govande, V. K. Gumaste, B. M. Bhawal and A. R. A. S. Deshmukh, *Tetrahedron*, 2002, **58**, 2215–2225.
39 K. Karupaiyan, V. G. Puranik, A. R. A. S. Deshmukh and B. M. Bhawal, *Tetrahedron*, 2000, **56**, 8555–8560.
40 D. T. S. Rijkers, G. W. Van Esse, R. Merkx, A. J. Brouwer, H. J. F. Jacobs, R. J. Pieters and R. M. J. Liskamp, *Chem. Commun.*, 2005, 4581–4583 and references cited therein.
41 (*a*) National Committee for Clinical Laboratory Standards. 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 Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming Filamentous Fungi: Proposed Standard. Document M38-P; National Committee for Clinical Laboratory Standards: 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 edn; National Committee for Clinical Laboratory Standards: Villanova, PA, 2000, M7-A5.
42 (*a*) R. Pai, A. S. Tarnawski and T. Tran, *Mol. Biol. Cell*, 2004, **5**, 2156–2163; (*b*) T. Lamireau, M. Zoltowska, E. Levy, I. Yousef, J. Rosenbaum, B. Tuchweber and A. Desmoulière, *Life Sci.*, 2003, **72**, 1401–1411; (*c*) H. S. Yoon, J. H. Rho, K. W. Yoo, W. C. Park, S. H. Rho, Y. H. Choi, H. Suh, N. D. Kim, K. S. Yoo and Y. H. Yoo, *Curr. Eye Res.*, 2001, **22**, 367–374.
43 D. Sladowski, S. J. Steer, R. H. Clothier and M. Balls, *J. Immunol. Methods*, 1993, **157**, 203–207.

