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Synthesis and antimicrobial activity of β-lactam–bile acid conjugates linked via triazole

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Abstract—Synthesis of novel 1,2,3-triazole-linked β-lactam–bile acid conjugates **17**–**24** using 1,3-dipolar cycloaddition reaction of azido β-lactam and terminal alkyne of bile acids in the presence of Cu(I) catalyst (click chemistry) have been realized. These molecules were evaluated in vitro for their antifungal and antibacterial activities. Most of the compounds exhibited significant antifungal and moderate antibacterial activity against all the tested strains.

β-Lactams are a large class of antibiotics characterized by the presence of an azetidine-2-one ring, which is the core of biological activity.[1] The azetidine-2-one (β-lactam) ring system is a common structural feature of a number of broad spectrum β-lactam antibiotics, like penicillins, cephalosporins, carbapenems, nocardicins and monobactams, which have been widely used as chemotherapeutic agents for treating microbial diseases.[2] It also shows many other interesting biological properties, such as cholesterol absorption inhibitors,[3] human cytomegalovirus protease inhibitors,[4] thrombin inhibitors,[5] antihyperglycemic,[6] anti-tumour,[7] anti-HIV,[8] anti-inflammatory, analgesic activities[9] and serine-dependent enzyme inhibitors.[10] However, microorganisms have built up resistance against the most traditional β-lactam antibiotics due to the wide-spread 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] 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 antibacterial, herbicidal, fungicidal, antiallergic, and anti-HIV.[13]

Bile acids have been considered very useful in the preparation of new pharmaceutical drugs because of their inherent chemical and biological properties.[14] They are pharmacologically interesting as potential carriers of liver-specific drugs, absorption enhancers and cholesterol lowering agents.[15] 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 nonpolar hydrophobic methyl group on the other.[16] 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 inophores.[17] Several cholic acid-derived facial amphiphiles have been reported[18] to improve the permeability of membranes including bacterial cell wall. Isolation of two naturally occurring steroidal β-lactams from the plants *Pachysandra terminalis*[19] and *Pachysandra procumbens*[20] has been reported. Steroidal β-lactam, isolated from *P. procumbens*, is known as antiestrogen-binding site inhibitory agent. There are two reports for synthetically prepared β-lactam on steroid and the biological activity data of these compounds have not been revealed.[21] In our group, cholic acid- and

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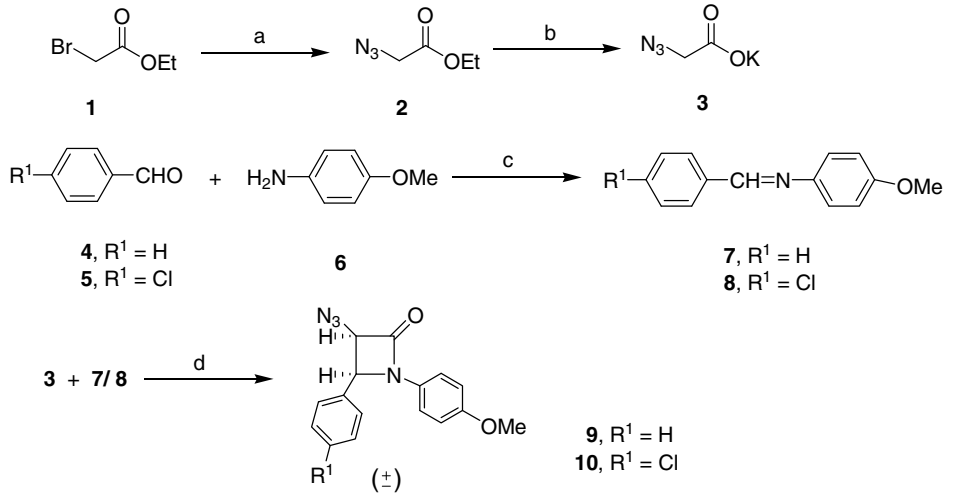
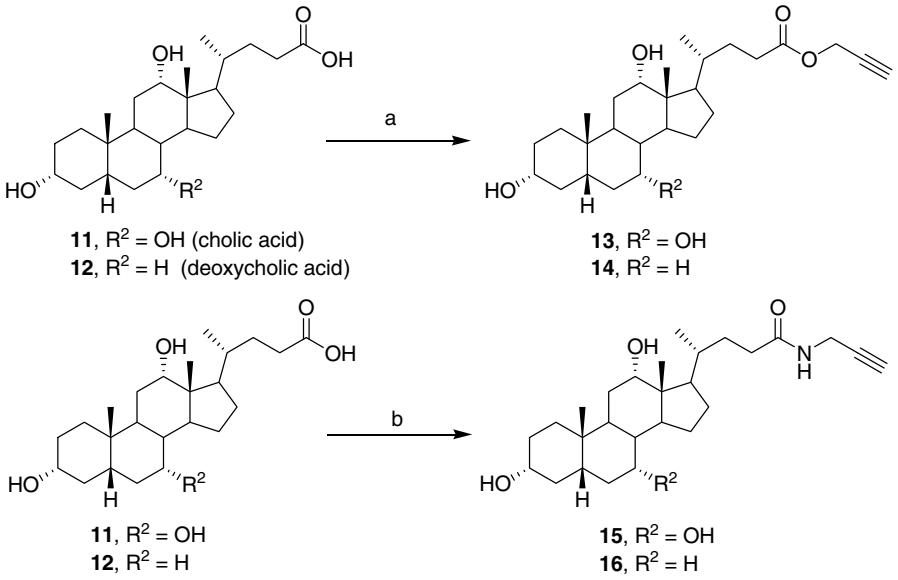
deoxycholic acid-based antimicrobials have been prepared as bile acid amides derived from chiral amino alcohols.[22] Flucanazole/bile acid conjugates have been reported recently from our laboratory with good antifungal activity.[23] In continuation of our recent work on bile acids,[22,24] we report herein the synthesis of 1,2,3-triazole-linked β-lactam-cholic acid/deoxycholic acid conjugates **17**–**24** and have studied their antibacterial and antifungal properties. This is the first report of synthesis and biological activity of the triazole-linked β-lactam on cholic acid/deoxycholic acid.

Target molecules **17**–**24** were synthesized using 1,3-dipolar cycloaddition reaction of β-lactams containing azide **9** and **10** and bile acids containing terminal alkyne **13**–**16**, in the presence of Cu(I) catalyst (click chemistry). Accordingly, the synthesis of azido β-lactams **9** and **10** was started from ethyl bromoacetate **1** (Scheme 1). Compound **1** on treatment with sodium azide afforded ethyl azidoacetate **2**. Subsequent saponification of ester functionality of compound **2** with KOH in methanol gave the potassium salt of azidoacetic acid **3** with 92% overall yield in two steps. The azido β-lactams **9** and **10** were prepared by using the ketene–imine cycloaddition (Staudinger)[25] reaction of **3** and imines **7** or **8** (prepared from the aldehydes **4** or **5** with the amine **6** in excellent yields) in the presence of triphosgene and triethylamine in anhydrous dichloromethane with 81% and 77% yields, respectively. Compounds **9** and **10** were obtained as racemic mixture and no attempt was made to resolve these in optically pure form.

Propargyl esters **13** and **14** were prepared by coupling propargyl alcohol with cholic acid **11** or deoxycholic acid **12** 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,



Scheme 1. Reagents and conditions: (a) NaN_3, Bu_4NBr, CH_2Cl_2/H_2O (1:1), 25 °C, 36 h, 98%; (b) KOH, MeOH, 25 °C, 4 h, 94%; (c) anhydrous $MgSO_4$, CH_2Cl_2, 25 °C, 12 h, (97% for **7** and 95% for **8**); (d) Triphosgene, Et_3N, CH_2Cl_2, 0–25 °C, 15 h, (81% for **9** and 77% for **10**).



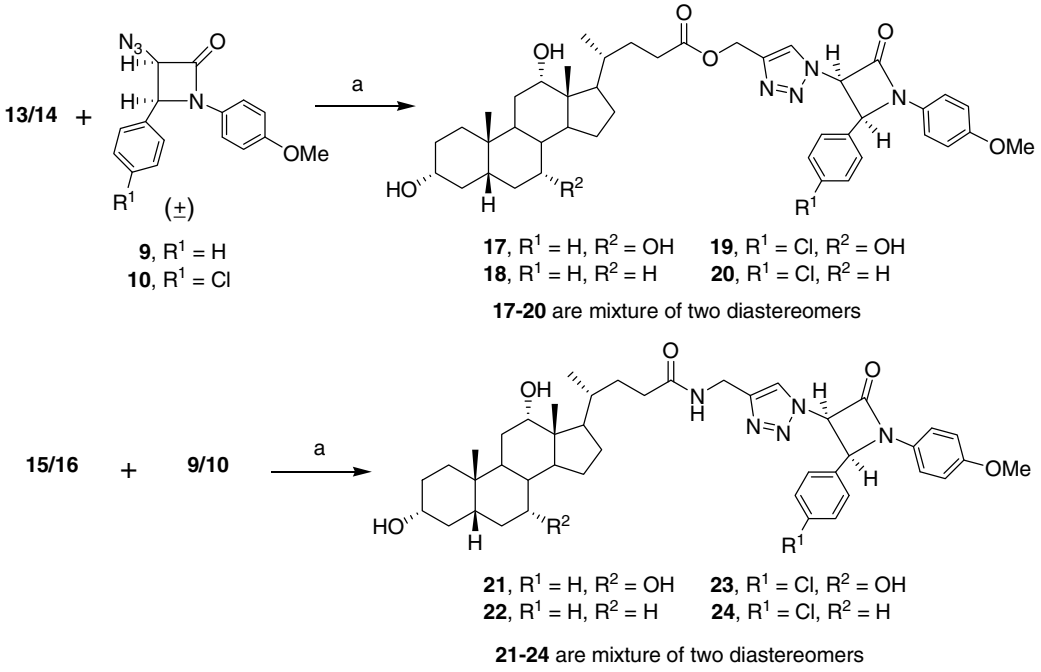
Scheme 2. Reagents and conditions: (a) EDC·HCl, HOBt, Propargyl alcohol, DMF, 0–25 °C, 12 h, 88% for **13** and 90% for **14**; (b) EDC·HCl, HOBt, Propargyl amine hydrochloride, Et_3N, DMF, 0–25 °C, 11 h, 89% for **15** and 92% for **16**.

amides **15** and **16** containing terminal alkyne functionality were prepared by coupling propargyl amine with cholic acid and deoxycholic acid in excellent yields.

Our next target was to synthesise 1,2,3-triazole-linked β-lactam–bile acid conjugates **17**–**24**. The cycloaddition reaction of propargyl esters **13** and **14** with azido β-lactams **9** and **10** in the presence of Cu(I) catalyst (click chemistry)[23] under microwave irradiation furnished diastereomeric mixture of novel conjugates **17**–**20** in excellent yields (Scheme 3). In a similar way, exposure of propargyl amides **15** and **16** with azido β-lactams **9** and **10** afforded diastereomeric mixture of hitherto unknown compounds **21**–**24** with 95–97% yields. The combination of racemic core **9** and **10** with optically pure **13**–**16** afforded diastereomeric mixture of triazole-linked β-lactam–bile acid conjugates **17**–**24** in equal amounts. These diastereomers were inseparable by flash column chromatography and also by crystallization.

All the newly synthesized azido β-lactams **9**, **10**, steroidal alkynes **13**–**16** and 1,2,3-triazole-linked β-lactam–bile acid conjugates **17**–**24** were tested in vitro for antifungal and antibacterial activity. The antifungal activity was tested using NCL isolate 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.[26] Therefore nonpathogenic 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.[27] The antibacterial activity was evaluated against *Escheirchia coli* and *Staphylococcus aureus*. The MIC and IC_{50} values were determined using standard broth microdilution technique described by NCCLS.[28] 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.

From the biological data (Table 1), it was observed that azido β-lactams **9**, **10** and steroidal alkynes **13**–**16** were almost inactive against all the tested strains. The MIC value for all these compounds was >128 μg/mL. As seen in Table 1 most of the β-lactam–bile acid conjugates **17**–**24** generally showed potent antifungal and antibacterial activity against all the tested fungal and bacterial strains. The activity of compounds **20**–**22** and **24** was higher or comparable to that of fluconazole against *C. albicans* with MIC value of 16–32 μg/mL. The compounds **17**, **19**, **20**, **22** and **23** showed good antifungal activity against *C. neoformans* having MIC value of 32 μg/mL comparable to that of reference drug fluconazole. However, the growth inhibitory activity of compound **24** was more potent than the reference drug amphotericin B and fluconazole against *B. poitrasii,* and also the compounds **17**–**19** and **23** showed significant activity against *B. poitrasii* with MIC value of 32 μg/mL. *Y. lipolytica* was adversely affected by **18**, **19**, **21**, **23** and **24**, and in particular, **18** was the most potent with a low MIC value of 4 μg/mL. The compounds **18** and **20** showed significant inhibitory effect with MIC value of 16 μg/mL comparable to that of amphotericin B against *F. oxysporum*. Furthermore, compounds **19** and **22** showed good antibacterial activity against *E. coli* having MIC value of 16 μg/mL. The compounds **17**,



Scheme 3. Reagents: (a) Sodium ascorbate, $CuSO_4 \cdot 5H_2O$, DMF/H_2O (7:3), microwave (385 W), 5 min, 95–97%.

Table 1. In vitro antimicrobial activity of compounds **9**, **10** and **13–24**

Compound	Inhibitory concentration (μg/mL)													
	Fungal strains										Bacterial strains			
	CA		CN		BP		YL		FO		EC		SA	
	MIC[a]	IC_{50}[b]	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
9	>128	64	>128	>128	>128	>128	>128	128	>128	64	>128	64	>128	>128
10	>128	64	>128	64	>128	>128	>128	>128	>128	64	>128	128	>128	>128
13	>128	128	>128	>128	>128	>128	>128	64	>128	128	>128	128	>128	128
14	128	32	>128	64	>128	128	>128	128	>128	64	>128	128	>128	128
15	>128	>128	>128	>128	>128	>128	>128	64	>128	128	>128	128	>128	64
16	>128	64	>128	128	>128	128	>128	>128	>128	64	>128	>128	>128	128
17	64	8	32	16	32	8	64	16	64	32	64	32	32	16
18	128	64	>128	32	32	16	4	2	16	8	32	8	>128	64
19	128	16	32	8	32	17	8	4	>128	64	16	8	32	16
20	32	8	32	8	>128	32	64	32	16	8	64	32	128	32
21	32	16	128	16	64	32	16	4	32	16	32	16	32	16
22	32	16	32	8	64	32	128	32	64	16	16	8	128	32
23	64	32	32	8	32	8	16	16	128	32	32	16	64	32
24	16	8	64	16	8	4	32	8	32	8	64	32	>128	64
Ampho. B	2	0.5	16	8	16	8	16	8	16	8	—	—	—	—
Fluconazole	32	4	32	16	32	16	64	32	8	4	—	—	—	—
Tetracycline	—	—	—	—	—	—	—	—	—	—	8	4	16	8
Ampicillin	—	—	—	—	—	—	—	—	—	—	2	1	16	4

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).
Negative control, DMSO, no inhibition.
[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.

19, **21** and **23** derived from cholic acid having 7-hydroxy showed moderate antibacterial activity against *S. aureus*. However, the compounds **18**, **20**, **22**, and **24** derived from deoxycholic acid in the absence of 7-hydroxy were less active against *S. aureus* with MIC value of $\geqslant$128 μg/mL. From the overall activity results, it was observed that the ester or amide linkage and chloro substituent on phenyl ring of β-lactam part did not affect the activity of the compounds.

In conclusion, a series of novel 1,2,3-triazole-linked β-lactam–bile acid conjugates were synthesized using Cu(I) catalyzed cycloaddition reaction of azido β-lactams and terminal alkynes derived from cholic acid/deoxycholic acid in excellent yields and their antimicrobial activities were evaluated. Compounds **17–24** demonstrated potent antimicrobial activity against all the strains tested. The compound **24** showed very good antifungal activity with MIC value of 16 μg/mL against *C. albicans* and 8 μg/mL against *B. poitrasii*. In particular, compound **18** exhibited the maximum activity with MIC values of 4 μg/mL against *Y. lipolytica*. Additionally, only the compounds **17**, **19**, **21** and **23** derived from cholic acid were moderately active against *S. aureus*. This is the first report of synthesis and biological activity of the triazole-linked β-lactam–bile acid conjugates.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.102.

References and notes

1. Broccolo, F.; Cainelli, G.; Caltabiano, G.; Cocuzza, C. E. A.; Fortuna, C. G.; Galletti, P.; Giacomini, D.; Musumarra, G.; Musumeci, R.; Quintavalla, A. *J. Med. Chem.* **2006**, *49*, 2804, and references cited therein.
2. Halve, A. K.; Bhadauria, D.; Dubey, R. *Bioorg. Med. Chem. Lett* **2007**, *17*, 341, and references cited therein.
3. Rosenblum, S. B.; Huynh, T.; Afonso, A.; Davis, H. R., Jr.; Yumibe, N.; Clader, J. W.; Burnett, D. A. *J. Med. Chem* **1998**, *41*, 973.
4. Borthwick, A. D.; Weingarte, G.; Haley, T. M.; Tomaszewski, M.; Wang, W.; Hu, Z.; Bedard, J.; Jin, H.; Yuen, L.; Mansour, T. S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 365.
5. Han, W. T.; Trehan, A. K.; Wright, J. J. K.; Federici, M. E.; Seiler, S. M.; Meanwell, N. A. *Bioorg. Med. Chem.* **1995**, *3*, 1123.
6. Goel, R. K.; Mahajan, M. P.; Kulkarni, S. K. *J. Pharm. Pharm. Sci* **2004**, *7*, 80.
7. Veinberg, G.; Shestakova, I.; Verma, M.; Kenepe, I.; Lukevics, E. *Bioorg. Med. Chem. Lett* **2004**, *14*, 147.
8. Sperka, T.; Pitlik, J.; Bagossi, P.; Tozser, J. *Bioorg. Med.Chem. Lett.* **2005**, *15*, 3086.
9. Saturnino, C.; Fusco, B.; Saturnino, P.; De Martino, G.; Rocco, F.; Lanceolat, J. C. *Biol. Pharm. Bull.* **2000**, *23*, 654.
10. (a) Konaklieva, M. I. *Curr. Med. Chem.-Anti-Infect. Agents* **2002**, *1*, 215; (b) Clemente, A.; Domingos, A.; Grancho, A. P.; Iley, J.; Moreira, R.; Neres, J.; Palma, N.;

Santana, A. B.; Valente, E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1065.
11. Odds, F. C.; Brown, A. J. P.; Gow, N. A. R. *Trends Microbiol.* **2003**, *11*, 272.
12. (a) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. *Chem. Res. Toxicol.* **2002**, *15*, 269; (b) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366.
13. (a) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, C. J.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953; (b) Wamhoff, H. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; pp 669–732; (c) Buckle, D. R.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1986**, *29*, 2262; (d) Alvarez, R.; Velazquez, S.; San-Felix, A.; Aquaro, S.; De Clercq, E.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185.
14. (a) Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem.* **2004**, 3385; (b) Salunke, D. B.; Hazra, B. G.; Pore, V. S. *Curr. Med. Chem.* **2006**, *13*, 813.
15. Enhsen, A.; Kramer, W.; Wess, G. *Drug Discov. Today* **1998**, *3*, 409.
16. Stein, T. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3943.
17. Savage, P. B. *Expert Opin. Investig. Drugs* **2000**, *9*, 263.
18. Li, C.; Peters, A. S.; Meridith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961.
19. Kikuchi, T.; Uyeo, S. *Chem. Pharm. Bull.* **1977**, *15*, 549.
20. Chang, L. C.; Bhat, K. P. L.; Pisha, E.; Kennelly, J. E.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1998**, *61*, 1257.
21. (a) Bose, A. K.; Anjaneyulu, B.; Bhattacharya, S. K.; Manhas, M. S. *Tetrahedron* **1967**, *23*, 4769; (b) Ugi, I.; Domling, A.; Kehagia, K. *Tetrahedron* **1995**, *51*, 9519.
22. 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.
23. Pore, V. S.; Aher, N. G.; Kumar, M.; Shukla, P. K. *Tetrahedron* **2006**, *62*, 11178, and references cited therin.
24. (a) Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem.* **2004**, *47*, 1589; (b) Salunke, D. B.; Hazra, B. G.; Gonnade, R. G.; Bhadbhade, M. M.; Pore, V. S. *Tetrahedron* **2005**, *61*, 3605; (c) Salunke, D. B.; Ravi, D. S.; Pore, V. S.; Mitra, D.; Hazra, B. G. *J. Med. Chem.* **2006**, *49*, 2652.
25. Krishnaswamy, D.; Govande, V. V.; Gumaste, V. K.; Bhawal, B. M.; Deshmukh, A. R. A. S. *Tetrahedron* **2002**, *58*, 2215.
26. Deshpande, M. V. *Recent Trends Mycoses* **1998**, 55.
27. Ghormade, V.; Deshpande, M. V. *Naturwissenschaften* **2000**, *87*, 236.
28. (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.