SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL EVALUATION OF SOME NEW PYRROLE-2-CARBOXAMIDE DERIVATIVES

Yogesh D. Mane*

**Corresponding author:*

Shri Chhatrapati Shivaji College, Omerga, Dist-Osmanabad, M.S., India

Abstract

Some new pyrrole-2-carboxamide derivatives (**4a-j**) were synthesized by the coupling of 1-(4-chlorobenzyl)-1H-pyrrole-2-carboxylic acid with different aromatic and cycloaliphatic amines using EDC·HCl, HOBt and TEA in DMF and evaluated for their *in vitro* antibacterial and antifungal activity. Among the tested compounds, the most effective were **4a**, **4c**, **4f**, **4g**, **4h**, **4i** and **4j** with MIC value in the range of 1.02-6.35 µg/mL against Gram-negative bacterial strains. Carboxamide **4i** emerged as good antibacterial agent against *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* strains with respective MIC value of 1.02, 1.56, and 3.56 µg/mL. None of these compounds show significant antifungal activity.

Keywords: Pyrrole-2-carboxamide, Antibacterial and Antifungal activity

Introduction

Treatment of infectious disease is becoming difficult due the emerged resistance to the currently used antibiotics[1]. Drying of drug pipeline, Population, international travel, migration, immune-suppressed patients, and climate change are some of the factors that play a significant role in the emergence of drug resistance[2-6]. To suppress drug resistant pathogens, careful use of existing antimicrobial drugs and the design of novel drugs with different modes of action, e.g. linezolid[7-9], is essential[10-13].

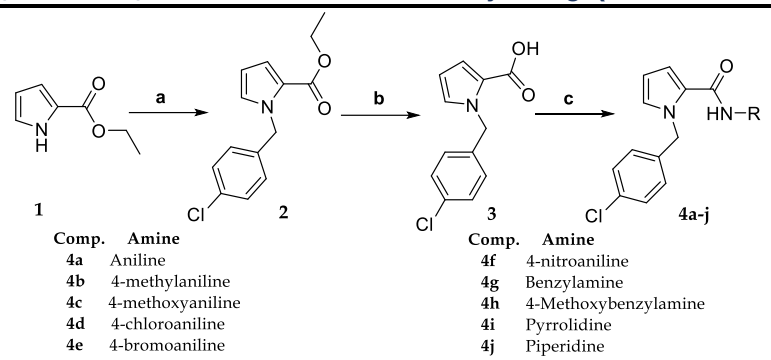
Pyrrole nucleus is present in many natural as well as synthetic products which exhibit fungicidal, antibiotic, anti-inflammatory[14], antitumor [15], cholesterol reducing activities[16]. Further, some pyrrole nucleus containing polymers find several industrial applications[17-23]. Compounds harboring pyrrole-2-carboxamide moiety exhibits insecticidal[24], antibiofilm[25], antibacterial[26], ATPase inhibitors of DNA gyrase[27], DNA binding, topoisomerase I inhibition[28], inducible nitric oxide synthase (nNOS and iNOS) inhibitors[29], antifungal[30], anticancer[31], JAK2 inhibitors[32], CB2 receptor antagonists[33], antitumor[34] activity.

By considering the potency of pyrrole-2-carboxamide, we decided to synthesis and evaluate some new pyrrole-2-carboxamides for their antibacterial and antifungal potency.

Results and discussion

Chemistry

Compounds **2** and **3** were prepared by the reported procedures[26c]. Carboxylic acid **3** on coupling substituted aryl and cycloaliphatic amines using EDC·HCl, HOBt and TEA in DMF affords the target carboxamides (**4a-j**) in good to excellent yields.



Scheme 1 Synthesis of pyrrole-2-carboxamides (**4a-j**)**:** (**a**) 4-Chlorobenzyl Chloride, Cs_2CO_3, DMF, 60 °C, 16 h; (**b**) LiOH, THF, H_2O, EtOH, Stirr, rt, 3.5 h; (**c**) Amine, EDC·HCl, HOBt, DIPEA, DMF, 0 °C to rt, 18-30 h.

Biological evaluation

Antimicrobial activity

Above synthesized carboxamides (**4a-j**) were screened for *in vitro* antibacterial activity, against *K. pneumoniae (ATCC 27736), E. coli (ATCC 9637), P. aeruginosa (ATCC BAA427) and S. Typhi (ATCC 19430)* using *Gentamicin* and *Ciprofloxacin* as internal standard and antifungal activity against fungi *C. albicans, C. neoformans, A. fumigatus, C. parapsilosis* (ATCC 22019) using *Fluconazole* and *Oxiconazole* as positive control and MIC were determined using the broth microdilution technique described by Clinical and Laboratory Standards Institute (CLSI) (Formerly NCCLS)[35-37]. The results of antibacterial and antifungal activity of the tested compounds (**4a-j)** are presented in **Table 1**.

Carboxamides **4a**, **4c**, **4f**, **4g, 4h, 4i** and **4j** show considerable antibacterial activity with MIC 1.02-6.35 µg/mL. Compounds **4a**, **4c**, **4f**, **4g** and **4h** inhibited *E. coli* considerably while compound **4i** inhibited *E. coli* almost as effectively as *Gentamicin* and *Ciprofloxacin* does. Compounds **4i** and **4j** also inhibited *K. pneumoniae* considerably with MIC values 1.02 and 3.39 µg/mL respectively. When compared commercial compounds as positive control, **4h** and **4i** acted identical with standard drugs, against *P. aeruginosa*. Among these compounds, **4i** had considerable to better antibacterial activity against *K. pneumonia*, *E. coli* and *P. aeruginosa* strains. None of the carboxamides shows any significant antifungal activity against the tested strains.

Table 1 *In vitro* antimicrobial activity of compounds (**4a-j**) against pathogenic Gram-Negative bacteria & fungi

Compd.	MIC (µg/mL)				MIC (µg/mL)			
	Klebsiella pneumoniae	*Escherichia coli.*	*P aeruginosa*	*Salmonella* Typhi	*C. albicans*	*C. neoformans*	*A. fumigatus*	*C. parapsilosis*
4a	25.25	6.25	12.01	>50	>50	>50	>50	>50
4b	12.05	12.01	12.50	>50	>50	>50	>50	>50
4c	3.25	6.35	12.25	>50	>50	>50	>50	>50
4d	12.50	12.25	12.50	>50	>50	>50	>50	>50
4e	25.25	12.25	12.01	>50	>50	>50	>50	>50
4f	12.50	6.35	25.25	>50	>50	>50	50	>50

4g	12.01	6.25	12.05	>50	>50	>50	>50	>50
4h	12.25	6.35	3.25	>50	>50	>50	>50	>50
4i	1.02	1.56	3.56	>50	>50	>50	>50	>50
4j	3.39	12.05	12.25	>50	>50	>50	25	>50
Gentamicin (Fluconazole)	0.25	1.25	3.025	1.65	(0.5)	(1.0)	(2.0)	(1.0)
Ciprofloxacin (Oxiconazole)	0.50	1.05	1.25	3.01	(0.03)	(1.4)	(2.0)	(0.01)

Experimental

Material and Methods

Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise specified. Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization/ slica gel (100-200 mesh) gravity column with suitable organic solvents. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR, ^{13}C NMR was determined in $CDCl_3$ and DMSO-d_6 solution on a Bruker Ac 200 or 400 MHz spectrometer.

Experimental procedures

General procedure for the synthesis of 1-(4-Chlorobenzyl)-1H-pyrrole-2-carboxamides (4a-j)

1-(4-chlorobenzyl)-1H-pyrrole-2-carboxylic acid **3** (0.68 mmol) was dissolved in DMF (10 mL) and cooled to 0^{o}C to rt. To that EDC·HCl (0.261 g, 1.36 mmol), HOBt (0.110 g, 0.816 mmol) and TEA (0.12 mL, 1.36 mmol) were added. The reaction mixture was stirred for 0.5 h and then appropriate Amine (0.816 mmol) was added and it was stirred for 18-30 h at room temperature. After completion of the reaction, the reaction mixture was poured in to water and the obtained solids **(4a-j)** (70-94 %) were filtered and washed thoroughly with water to afford the desired products. The product was confirmed by ^{1}H NMR, ^{13}C NMR & ES-MS.

*1-(4-chlorobenzyl)-N-phenyl-1H-pyrrole-2-carboxamide (**4a**)*: Off white crystal, yield 78 %, mp 241-243 ^{o}C, ^{1}H NMR (200 MHz, DMSO-d_6) δ_H: 9.1 (s, 1H), 7.8 (d, 1H), 7.6 (d, 1H), 7.5 (d, 2H), 7.3 (d, 2H), 7.1 (d, 2H), 7.1 (d, 3H), 6.7 (d, 1H), 5.4 (s, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 161.4, 136.2, 133.8, 130.8, 129.2, 128.3, 120.6, 118.3, 105.7, 54.2; MS (ESI) m/e = 311 $(M+H)^+$, 312 $(M+2)^+$

*1-(4-chlorobenzyl)-N-(p-tolyl)-1H-pyrrole-2-carboxamide (**4b**)*: Off white crystal, yield 80 %, mp 252-254 ^{o}C, ^{1}H NMR (200 MHz, DMSO-d_6) δ_H: 8.7 (s, 1H), 7.6 (d, 1H), 7.4 (d, 1H), 7.3-7.2 (m, 6H), 7.0 (d, 2H), 6.8 (d, 1H), 5.3 (s, 2H), 2.3 (s, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 162.2, 135.3, 134.2, 133.7, 130.1, 128.6, 122.7, 120.5, 109.4, 53.8, 22.4; MS (ESI) m/e = 325 $(M+H)^+$, 326 $(M+2)^+$

*1-(4-chlorobenzyl)-N-(4-methoxyphenyl)-1H-pyrrole-2-carboxamide (**4c**)*: Off white crystal, yield 84 %, mp 264-265 ^{o}C, ^{1}H NMR (200 MHz, DMSO-d_6) δ_H: 9.0 (s, 1H), 7.8 (d, 1H), 7.6 (d, 3H), 7.3 (d, 2H), 7.1 (d, 2H), 6.9 (d, 2H), 6.5 (d, 1H), 5.4 (s, 2H), 3.6 (s, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 160.6, 159.3,

136.4, 132.7, 131.9, 129.8, 128.4, 123.6, 120.9, 116.4, 109.5, 54.1, 53.6; MS (ESI) m/e = 341 $(M+H)^+$, 342 $(M+2)^+$

1-(4-chlorobenzyl)-N-(4-chlorophenyl)-1H-pyrrole-2-carboxamide ***(4d)***: Off white crystal, yield 76 %, mp 268-269 ^oC, ^1H NMR (200 MHz, DMSO-d_6) δ_H: 9.2 (s, 1H), 7.7 (d, 1H), 7.5 (d, 3H), 7.4 (d, 2H), 7.3 (d, 2H), 7.0 (d, 2H), 6.6 (d, 1H), 5.3 (s, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 161.8, 138.6, 134.8, 132.4, 130.2, 129.6, 128.4, 122.2, 120.7, 109.6, 54.8; MS (ESI) m/e = 344 $(M)^+$, 346 $(M+2)^+$ and 348 $(M+4)^+$

N-(4-bromophenyl)-1-(4-chlorobenzyl)-1H-pyrrole-2-carboxamide ***(4e)***: Off white crystal, yield 77 %, mp 276-278 ^oC, ^1H NMR (200 MHz, DMSO-d_6) δ_H: 9.1 (s, 1H), 7.8 (d, 1H), 7.6 (d, 3H), 7.5 (d, 2H), 7.3 (d, 2H), 7.1 (d, 2H), 6.7 (d, 1H), 5.4 (s, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 162.2, 137.6, 136.2, 132.8, 131.4, 129.7, 128.4, 124.3, 122.6, 120.8, 108.4, 54.5; MS (ESI) m/e = 388 $(M)^+$, 390 $(M+2)^+$ and 392 $(M+4)^+$

1-(4-chlorobenzyl)-N-(4-nitrophenyl)-1H-pyrrole-2-carboxamide ***(4f)***: Off white crystal, yield 72 %, mp 280-282 ^oC, ^1H NMR (200 MHz, DMSO-d_6) δ_H: 9.2 (s, 1H), 8.1 (d, 2H), 8.0 (d, 3H), 7.6 (d, 1H), 7.3 (d, 2H), 7.1 (d, 2H), 6.6 (d, 1H), 5.5 (s, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 161.6, 145.8, 136.4, 132.1, 129.4, 128.5, 125.2, 120.7, 108.6, 54.2; MS (ESI) m/e = 356 $(M+H)^+$, 357 $(M+2)^+$

N-benzyl-1-(4-chlorobenzyl)-1H-pyrrole-2-carboxamide ***(4g)***: Off white crystal, yield 84 %, mp 254-256 ^oC, ^1H NMR (200 MHz, DMSO-d_6) δ_H: 8.9 (s, 1H), 8.0 (d, 1H), 7.7 (d, 1H), 7.6 (d, 1H), 7.3-7.2 (m, 7H), 7.1 (d, 2H), 6.5 (d, 1H), 5.4 (s, 2H), 3.8 (s, 2H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 160.2, 138.6, 136.8, 132.5, 129.1, 127.4, 126.2, 114.8, 108.4, 54.1, 44.2; MS (ESI) m/e = 325 $(M+H)^+$, 326 $(M+2)^+$

1-(4-Chlorobenzyl)-N-(4-methoxybenzyl)-1H-pyrrole-2-carboxamide ***(4h)***: Off white crystal, yield 87 %, mp 242-244 ^oC, ^1H NMR (200 MHz, DMSO-d_6) δ_H: 8.5 (t, 1H), 7.3 (d, 2H), 7.0 (t, 5H), 6.8 (d, 3H), 6.0 (t, 1H), 5.5 (s, 2H), 4.3 (d, 2H), 3.4 (s, 3H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 161.1, 158.0, 138.6, 131.9, 131.5, 128.6, 126.6, 113.5, 107.3, 55.0, 50.1, 41.12; HRMS, m/z, Calcd = 354.1135, Found 355.1203 $(M+H)^+$.

(1-(4-chlorobenzyl)-1H-pyrrol-2-yl)(pyrrolidin-1-yl)methanone ***(4i)***: Off white crystal, yield 86 %, mp 232-234 ^oC, ^1H NMR (200 MHz, DMSO-d_6) δ_H: 8.0 (d, 1H), 7.8 (d, 1H), 7.7 (d, 1H), 7.4 (d, 2H), 7.2 (d, 2H), 6.8 (d, 1H), 5.5 (s, 2H), 3.4 (m, 4H), 2.0 (m, 4H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 163.4, 136.2, 132.9, 129.7, 128.3, 114.6, 108.5, 56.4, 48.6, 26.2; MS (ESI) m/e = 389 $(M+H)^+$, 390 $(M+2)^+$

(1-(4-chlorobenzyl)-1H-pyrrol-2-yl)(piperidin-1-yl)methanone ***(4j)***: Off white crystal, yield 88 %, mp 238-239 ^oC, ^1H NMR (200 MHz, DMSO-d_6) δ_H: 7.8 (d, 1H), 7.6 (d, 1H), 7.3 (d, 2H), 7.1 (d, 2H), 6.7 (d, 1H), 5.4 (s, 2H), 3.7 (m, 4H), 1.7-1.6 (m, 6H); ^{13}C NMR (50 MHz, DMSO-d_6) δ_C: 163.6, 136.4, 132.6, 129.5, 127.1, 114.2, 108.1, 53.8, 46.4, 26.2, 24.6; MS (ESI) m/e = 303 $(M+H)^+$, 304 $(M+2)^+$

Conclusion

Some new pyrrole-2-carboxamides (**4a-j**) were prepared by the coupling of 1-(4-chlorobenzyl)-1H-pyrrole-2-carboxylic acid with different aromatic and cycloaliphatic amines using EDC·HCl, HOBt and TEA in DMF. The antimicrobial activity of these compounds (**4a-j**) was studied against Gram-negative bacteria and fungi. The results exhibit that compounds **4a**, **4c**, **4c**, **4f, 4g, 4h, 4i** and **4j** bear considerable antibacterial activity. Carboxamide **4i** emerged as good antibacterial agent against *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* strains with respective MIC value of 1.02, 1.56, and 3.56 µg/mL. None of these compounds show significant antifungal activity.

Acknowledgments

The authors are grateful to the Department of Postgraduate Studies and Research in Chemistry, Shri Chhatrapati Shivaji College, Omerga for providing laboratory facilities, Indian Institute of Chemical Technology, Hyderabad for spectral data.

References

01 ECDC EMEA Joint Technical Report, The bacterial challenge: time to react, EMEA576176, 2009.

02 Tenover, F. C.; McDonald, L. C.; *Curr Opin Infect Dis*, **2005**, *18*, 300.

03 Pfeltz, R. F.; Wilkinson, B. J.; *Curr Drug Targets Infect Disord*, **2004**, *4*, 273.

04 Roberts, M. C.; *Curr Drug Targets Infect Disord*, **2004**, *4*, 207.

05 Dessen, A.; Di Guilmi, A. M.; Vernet, T.; Dideberg, O.; *Curr Drug Targets Infect Disord*, **2001**, *1*, 63.

06 De Luca D'Alessandro E.; Giraldi, G.; *Clin Term*, **2011**, *162*, 93.

07 Torres-Viera, C.; Dembry, L. M.; *Curr Opin Infect Dis*, **2004**, *17*, 541.

08 Anstead, G. M.; Owens, A. D.; *Curr Opin Infect Dis*, **2004**, *17*, 549.

09 Ford, C. W.; Zurenko, G. E.; Barbachyn, M. R.; *Curr Drug Targets Infect Disord*, **2001**, *1*, 181.

10 Nina, S; *Clin Infect Dis*, **2001**, *33*, 1692.

11 Fridkin, S. K.; Jarvis, W. R.; *Clin Microbiol Rev*, **1996**, *9*, 499.

12 Ablordeppey, S. Y.; Fan, P.; Ablordeppey, J. H.; Mardenborough, L.; *Curr Med Chem*, **1999**, *6*, 1151.

13 Beck-Sague, C. M.; Jarvis, W. R.; *J Infect Dis*, **1993**, *167*, 1247.

14 Wilkerson, W. W.; Copeland, R. A.; Covington, M.; Trzaskos, J. M.; *J Med Chem*, **1995**, *38*, 3895.

15 Lee, H.; Lee, J.; Lee, S.; Shin, Y.; Jung, W.; Kim, J. H.; Park, K.; Kim, K.; Cho, H. S.; Ro, S.; *Bioorg Med Chem Lett*, **2001**, *11*, 3069.

16 Wurz, R. P.; Charette, A. B.; *Org Lett*, **2005**, *7*, 2313.

17 Piliego, C.; Holcombe, T. W.; Douglas, J. D.; Woo, C. H.; Beaujuge, P. M.; Frechet, J. M.; *J Am Chem Soc*, **2010**, *132*, 7595.

18 Katritzky, A. R.; Barczynski, P.; Musumarra, G.; Pisano, D.; Szafran, M.; *J Am Chem Soc*, **1989**, *111*, 7.

19 Etherton, B. P.; Krishnamurti, R.; Nagy, S. US Patent No 5, 554, 775, **1996**.

20 Rigoberto, A.; Pemites, R. B. US Patent App 13/179,515, **2011**.

21 Kaledkowski, A.; Trochimczuk, A. W.; *Rea Fun Poly*, **2006**, *66*, 740.

22 Gelling, V. J.; Wiest, M. M.; Tallman, D. E.; Bierwagen, G. P.; Wallace, G. G.; *Prog Org Coat*, **2001**, *43*, 149.

23 Rieth, R. D.; Mankad, N. P.; Calimano, E.; Sadighi, J. P.; *Org Lett*, **2004**, *6*, 3981.

24 Li, Y.; Zhang, P.; Ma, Q.; Song, H.; Liu,Y.; Wang, Q.; *Bioorg Med Chem Lett*, **2012**, *22*, 6858.

25 Rane, R. A.; Sahu, N. U.; Shah, C. P.; *Bioorg Med Chem Lett*, **2012**, *22*, 7131.

26 (a) Khalaf, A. I.; Anthony, N.; Breen, D.; Donoghue, G.; Mackayb, S. P.; Scott, F. J.; Suckling, C. J.; *Eur J Med Chem*, **2011**, *46* 5343; (b) Anthony, N. G.; Breen, D.; Donoghue, G.; Khalaf, A. I.; Mackay, S. P.; Parkinson, J. A.; Suckling, C. J.; *Org Biomol Chem*, **2009**, *7*, 1843; (c) Mane, Y. D.; Survarse, S. M.; Biradar, D. O.; Jawalw, B. H.; Khade, B. C.; *J. Heterocyclic Chem.*, **2017**, *54(5)*, 2627-2634

27 Zidar, N.; Tomasic T.; Macut, H.; Sirc, A.; Brvar, M.; Montalvao, S.; Tammela, P.; Ilas, J.; Kikelj, D.; *Eur J Med Chem*, **2016**, *117*, 197.

28 Hotzel, C.; Marotto, A.; Pindur, U.; *Eur J Med Chem*, **2003**, *38*, 189.

29 Lopez Cara, L. C. M.; Camacho, E.; Dora Carrion, M.; Tapias, V.; Gallo, M. A.; Escames, G.; Acuna-Castroviejo, D.; Espinosa, A.; Entrena, A.; *Eur J Med Chem*, **2009**, *44*, 2655.

30 Wang, M. Z.; Xu, H.; Liu, T. W.; Feng, Q.; Yu, S. J.; Wang, S. H.; Li, Z. M.; *Eur J Med Chem*, **2011**, *46*, 1463.

31 Dyson, L.; Wright, A. D.; Young, K. A.; Sakoff, J. A.; McCluskey, A.; *Bioorg Med Chem*, **2014**, *22*, 1690.

32 Brasca, M. G.; Gnocchi, P.; Nesi, M.; Amboldi, N.; Avanzi, N.; Bertrand, J.; Bindi, S.; Canevari, G.; Casero, D.; Ciomei, M.; Colombo, N.; Cribioli, S.; Fachin, G.; Felder, E. R.; Galvani, A.; Isacchi, A.; Motto, I.; Panzeri, A.; Donati, D., *Bioorg Med Chem*, **2015**, *23*, 2387.

33 (a) Osman, N. A.; Ligresti, A.; Klein, C. D.; Allara, M.; Rabbito, A.; Di Marzo, V.; Abouzid, K. A.; Abadi, A. H.; *Eur J Med Chem*, **2016**, *122*, 619; (b) Ragusa, G.; G_omez-Canas Maria, Morales, P.; Hurst, D. P.; Deligia, F.; Pazos, R.; Pinna, G. A.; Fernandez-Ruiz, J.; Goya, P.; Reggio, P. H.; Jagerovic, N.; Garcia-Arencibia, M.; Murineddu, G.; *Eur J Med Chem*, **2015**, *101*, 651.

34 Wang, M.; Ye, C.; Liu, M.; Wu, Z.; Li, L.; Wang, C.; Liu, X.; Guo, H.; *Bioorg Med Chem Lett*, **2015**, *25*, 2782.

35 CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9, Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, **2012**.

36 CLSI, Reference Method for Broth Dilution Antifungal Susceptibility Testing Filamentous Fungi, Approved Standard, 2nd ed., CLSI document M38-A2, Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, **2008**.

37 CLSI, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, 2nd ed., NCCLS document M27-A2, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, **2002**.