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Synthesis of 1,6-hexanediyl-bis(semicarbazides) and 1,6-hexanediyl-bis(1,2,4-triazol-5-ones) and their antiproliferative and antimicrobial activity

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Abstract. A series of 1,6-bis(3-substituted 1,5-dihydro-5-oxo-4*H*-1,2,4-triazol--4-yl)hexanes **3a**–**g** were synthesized by the cyclization reaction of 1,6-bis{[(2--substituted hydrazinyl)carbonyl]amino}hexanes **2a**–**g** in alkaline medium. The new derivatives **3a**–**c** were screened *in vitro* for their antiproliferative and anticancer activity in human tumor cell lines derived from breast and lung carcinoma cells. Compounds **3a** (at a concentration of 0.18 mM), **3b** (at concentrations of 0.12 and 0.02 mM) and **3c** (at concentrations of 0.23 and 0.11 mM) were found to be the most effective against the lung cell line. Compound **3a** had the greatest antiproliferative effect on the breast carcinoma cell line. Representative compounds were established and evaluated as antimicrobial agents. All the tested derivatives showed minimum inhibitory concentrations (*MIC*) in the range 1.87–7.5 μg mL^{-1}. Compound **3b** was the most effective against *Candida albicans* (*MIC* 1.87 μg mL^{-1}).

Keywords: synthesis; semicarbazide; 1,2,4–triazole; biological activity.

INTRODUCTION

The synthesis of compounds containing a 1,2,4–triazole ring in their structure has attracted widespread attention, mainly in connection with their wide range of pharmacological properties. A variety of biological activities, such as antidepressant,[1,2] anticonvulsant,[3] antitumor[4] and antimicrobial[5,6] have been reported for mono-substituted 1,2,4–triazole systems. A great number of these derivatives display interesting anticancer activity.[7,8] It was reported that compounds having triazole moieties, such as vorozole, anastrozole and letrozole, appear to be very effective aromatase inhibitors and are very useful for preventing breast cancer.[9]

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Moreover, several compounds involving the triazole moiety and having diverse antibacterial and antifungal activities were reported.[10–12] 1,2,4-Triazol-3-ones have been prepared by different methods. One of the most common routes leading to the preparation of these compounds involves cyclodehydration of 1-acetylsemicarbazide with a variety of reagents, such as tris(formylamino)methane,[13] sodium hydroxide[14,15] and formic acid.[16] 4,5-Disubstituted and 2,4,5-trisubstituted 1,2,4-triazol-3-ones have been obtained in the reaction of amidrazones salts with isocyanate or urea performed in the melt.[17] Some bis(3,4-disubstituted 5--oxo-1,2,4-triazol-5-4-yl)alkanes were synthesized using esters of ethoxyalkylidene)hydrazinecarboxylic acids and diamines.[18]

On the other hand, it is known that semicarbazides, the key intermediates used in the synthesis of 1,2,4-triazol-3-one derivatives, are compounds with various pharmacological activities: antitubercular,[19] anticonvulsant[20] and antinociceptive.[21] It was found that some compounds possess antibacterial activity against Gram-positive bacteria, including staphylococci (coagulase-positive *Staphylococcus aureus* and coagulase-negative *Staphylococcus epidermidis*).[22] Semicarbazides possessing a heterocyclic ring show anticancer activity against human gastric carcinoma cell line.[23]

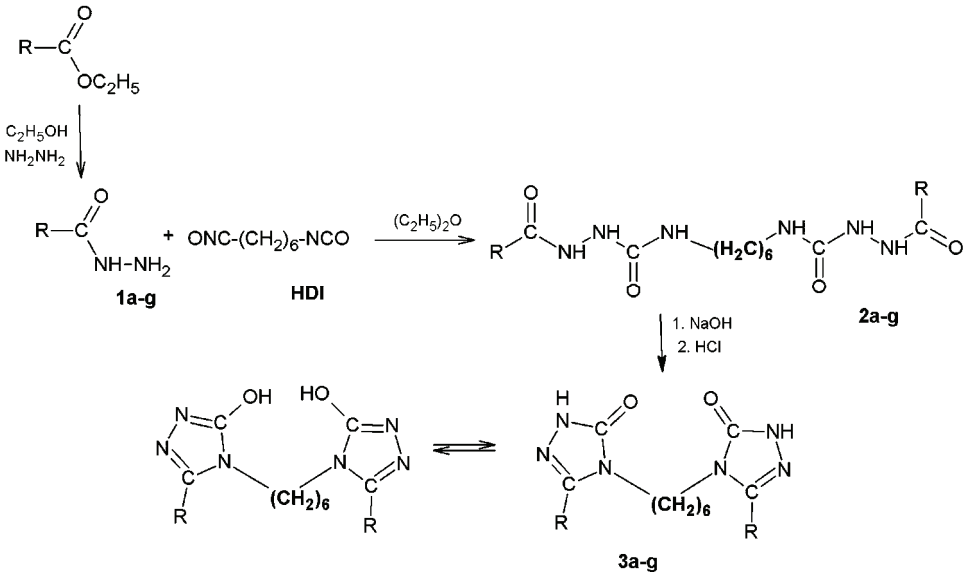
In view of the above-mentioned findings and in continuation of our research in the domain of heterocyclic compounds of the 1,2,4-triazole class with expected biological activity,[24,25] herein, the synthesis of some new bis-semicarbazides and their cyclization derivatives from the bis-1,2,4-triazole class with potential biological activity are described.

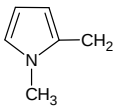
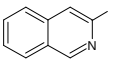
The newly obtained compounds were screened *in vitro* for their anticancer and antimicrobial activity.

RESULTS AND DISCUSSION

Synthesis

The carboxylic acid hydrazides **1a**–**g**, the key intermediates used for the synthesis of the 1,6-bis{[(2-substituted hydrazinyl)carbonyl]amino}hexanes **2a**–**g**, were synthesized according to a literature method.[26,27] New semicarbazide derivatives were obtained by the reaction of corresponding carboxylic acid hydrazide **1** with 1,6-hexamethylene diisocyanate (HDI). The reaction medium was diethyl ether and the process was realized at room temperature. Next, the obtained semicarbazides were subjected to cyclization in a 2 % solution of sodium hydroxide obtaining the corresponding 1,6-bis(3-substituted 1,5-dihydro-5-oxo-4*H*-1,2,4--triazol-4-yl)hexanes **3a**–**g**. The synthetic pathway followed for the preparation of the title compounds is presented in Scheme 1.



Comp.	R
a	CH_3
b	C_6H_5
c	
d	H
e	
f	
g	

Scheme 1. Synthesis of the 1,6-bis[(hydrazinylcarbonyl)amino)hexanes **2a**–**g** and of the 1,6-bis(5-oxo-1,2,4-triazol-4-yl)hexanes **3a**–**g**.

Characterization

The structures of the synthesized compounds were elucidated by ^{1}H-NMR, ^{13}C-NMR, MS and IR spectroscopy. Analytical and spectral data of the synthesized compounds are given in the Supplementary material.

The reaction products may exist in two different tautomeric forms (Scheme 1). The IR and ^{1}H-NMR data indicated that the obtained cyclization products exist in the keto form both in the solution and in the solid. In the IR spectra of the cyclic compounds containing the 1,2,4-triazole system, absorption bands of the

C=O group at 1683–1651 cm^{-1} were observed. ^{1}H-NMR spectra showed protons signals for the –N–C(O)–NH– group in the δ range 8.00–10.46 ppm.

Preliminary anticancer screening

The bis-1,2,4-triazol-3-one derivatives **3a**–**c** were evaluated for their antiproliferative and anticancer activity in two human cell lines derived from lung and breast carcinoma cells. One normal cell line was included in the cytotoxicity study – primary cell line of human skin fibroblasts (HSF). The results for each tested compound are reported as the growth inhibition percentage of the tested cells in comparison with the untreated cells. According to the data listed in Table I, compounds **3a**–**c** were found to be the most effective against human lung carcinoma cells *in vitro*. In the case of the human breast cancer cell line, a slight inhibition for compounds **3a** and **3b** was observed. A non-cytotoxic or stimulation effect of compound **3a** referring to normal cell line HSF and several-fold higher effect on the two observed carcinoma cell lines were ascertained. The investigated compounds exhibited dose-dependent effects. The most evident action was observed for all the examined compounds at a 50 μg mL^{-1} dose.

TABLE I. *In vitro* inhibition of the growth of normal and cancer cells by compounds **3a**–**c**; the examined concentration of compounds: I – a concentration of 100 μg mL^{-1}, which corresponds to a concentration of 0.35 (**3a**), 0.25 (**3b**) and 0.23 mM (**3c**); II – a concentration of 50 μg mL^{-1}, which corresponds to a concentration of 0.18 (**3a**), 0.12 (**3b**) and 0.11 mM (**3c**); III – a concentration of 10 μg mL^{-1}, which corresponds to a concentration of 0.04 (**3a**), 0.02 (**3b**) and 0.02 mM (**3c**)

Cell	Time of incubation h	Growth inhibition factor, *GI* / %								
		Compound								
		3a			**3b**			**3c**		
		Dose								
		I	II	III	I	II	III	I	II	III
Human skin fibroblast HSF	24	0	0	0	5	5	0	0	0	5
	48	0	0	0	5	5	5	0	5	10
	72	0	0	0	5	5	5	5	5	10
Human lung cancer cell line A549	24	0	22	0	5	5	10	10	10	0
	48	0	25	0	5	20	10	25	25	0
	72	5	25	5	5	20	10	25	25	5
Human breast cancer cell line T47D	24	5	5	5	0	0	0	0	5	5
	48	5	10	10	0	10	10	5	5	5
	72	0	10	10	5	10	0	0	0	5

Antimicrobial activity

Selected compounds, **2a**–**c** and **3a**–**c**, were screened for their possible antimicrobial activities against *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 700603, *Enterococcus faecalis* ATCC 2912, *Escherichia coli* ATCC 25822, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC

90028, and clinical isolates of *C. albicans*, *C. parapsilosis*, *C. kefyr*, and *C. tropicalis*. The antimicrobial susceptibility to the tested compounds was determined by *MIC* (minimum inhibitory concentration) values and compared to amphotericin B and meropenem as standard drugs. All the studied bacterial species were classified as resistant to the standard meropenem, *MIC* > 1.0 μg mL^{-1},[28] and the studied species *Candida* spp. as resistant to the standard amphotericin B, *MIC* > > 16.0 μg mL^{-1}.[29] Compounds **3a** and **3b** at concentrations 3.75 and 1.87μg mL^{-1}, respectively, were active against the tested *C. albicans* (clinical isolates, **3b**), *C. albicans* ATCC 90028 (**3b**) and *C. tropicalis* (**3a**, Table II). The other compounds were also found to inhibit growth of *Candida* spp. at a concentration of 7.5 μg mL^{-1}. All the tested derivatives showed *in vitro* effectiveness against the five reference species: *S. aureus* ATCC 25923, *K. pneumoniae* ATCC 700603, *E. faecalis* ATCC 2912, *E. coli* ATCC 25822 and *P. aeruginosa* ATCC 27853 at a concentration of 7.5 μg mL^{-1}.

TABLE II. Minimum inhibitory concentration (*MIC* / μg mL^{-1}) of compounds **2a–c** and **3a–c**; Ca* – *C. albicans* ATCC 90028, Ca - *C. albicans*, Cp - *C. parapsilosis*, Ck - *C. kefyr*, Ct- *C. tropicalis*, Sa- *S. aureus* ATCC 25923, Kp - *K. pneumoniae* ATCC 700603, Ef - *E. faecalis* ATCC 2912, Ec – *E. coli* ATCC 25822, Pa- *P. aeruginosa* ATCC 27853, ST - Standard: *amphotericin B, **meropenem

Compound	Microorganism									
	Ca*	Ca	Cp	Ck	Ct	Sa	Kp	Ef	Ec	Pa
2a	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
2b	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
2c	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
3a	7.5	7.5	7.5	7.5	3.75	7.5	7.5	7.5	7.5	7.5
3b	1.87	1.87	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
3c	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
ST	<0.5*	<0.5*	<0.5*	<0.5*	<0.5*	0.05**	0.05**	5.0**	0.5**	0.1**

EXPERIMENTAL

All chemicals were purchased from Merck or Alfa-Aesar and used without further purification. Melting points (m.p.) were determined in a Fisher-Johns block and are not corrected. The IR spectra were recorded in KBr discs using a Specord IR-75 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were generally recorded at room temperature on a Bruker AC 200F instrument (300 MHz) in DMSO-d_6 with TMS as the internal standard. The mass spectra were obtained using an AMD-604 mass spectrometer with a 70 eV electron beam. The purity of the obtained compounds was checked by TLC on aluminum oxide 60 F_{254} plates (Merck) in $CHCl_3/C_2H_5OH$ (10:1 and 10:2) solvent systems with UV or iodine visualization.

*General procedure for the synthesis of 1,6-bis{[(2-substituted hydrazinyl)carbonyl]amino}-hexanes **2a–g***

A mixture of an appropriate carboxylic acid hydrazide **1** (20 mmol) and 1,6-hexametylene diisocyanate (1.68 g, 10 mmol), and 10 mL of diethyl ether was kept at room tempera-

ture for 24 h. Then, the formed compound was filtered off, washed with diethyl ether and crystallized from ethanol.

*General procedure for the synthesis of 1,6-bis(3-substituted 1,5-dihydro-5-oxo-4*H*-1,2,4-triazol-4-yl)hexanes **3a–g***

The appropriate semicarbazide **2** (10 mmol) was placed in a round-bottomed flask equipped with reflux and 40–50 mL of a 2 % sodium hydroxide solution was added. The flask was heated for 10 h. After cooling, the solution was neutralized with dilute hydrochloric acid. The precipitate was filtered off and then crystallized from ethanol.

Proliferation of tumor cells assay

The synthesized compounds **3a**–**c** were evaluated for their anticancer activity in human tumor cell lines derived from lung and breast carcinoma cells. The studies was performed on A549 (ECACC 86012804 human lung epithelial) cells and T47D (ECACC 85102201 human breast epithelial). The influence of the newly synthesized triazoles on human skin fibroblast cells (HSF) was also determined. The cell lines were incubated at 10^4 cells per mL density on microtiter plates. The tested compounds were then added at three examined concentrations: 10, 50 and 100 μg mL^{-1}, and the cultures were incubated under standard conditions (37 °C, 5 % CO_2 and 90 % humidity) for 24, 48 and 72 h. The determinations were realized by 5-bromo-2'-deoxyuridine (BrdU) labeling[30,31] and detection kit (Roche) on an ELISA reader (BIO-TEC Instruments, USA). The viability of normal and carcinoma cells were evaluated spectrophotometrically. The results of all spectrophotometric measurements were registered as percent growth inhibition or growth stimulation. All experiments were repeated in triplicate.

Antifungal screening

Clinical isolates of *Candida albicans*, *C. parapsilosis*, *C. kefyr* and *C. tropicalis* and *C. albicans* ATCC 90028, all susceptible to amphotericin B (as determined by ATB fungitest-bioMerieux), were tested for their susceptibility to the newly obtained compounds. Stock solutions were prepared in dimethyl sulfoxide (DMSO), at a working concentration of 300 mg mL^{-1}. Final dilutions were prepared in RPMI 1640 medium (Sigma) buffered to pH 7.0, 0.165 M 4-morpholinepropanesulfonic acid (MOPS) buffer (Sigma) in 96-well plates (NUNC). The concentration of the *Candida* species in the final inoculums was $(1.5\pm1.0)\times10^3$ cells mL^{-1} RPMI 1640. All strains were incubated for 48 h at 35 °C.[32-34] As controls, DMSO and amphotericin B were used. The results were obtained spectrophotometrically (570 nm) and compared to the control results.[35]

Antibacterial screening

Antibacterial susceptibility was determined on following strains: *S. aureus* ATCC 25923, *K. pneumoniae* ATCC 700603, *E. faecalis* ATCC 2912, *E. coli* ATCC 25822 and *P. aeruginosa* ATCC 27853. Dilutions of the tested compounds were prepared from the stock solutions in DMSO. The susceptibilities of the bacterial strains 1.0 x 10^3 cells mL^{-1} to the tested compounds were determined by the CLSI microlitre broth dilution method,[22,36] performed in 96-well plates (NUNC) – 200 μL per well, at 35 °C for 24 h. As the control, meropenem at the same dilutions in DMSO was used for bacterial strains cultured under the same conditions. The results were obtained spectrophotometrically at 570 nm.

CONCLUSIONS

In the present study, novel series of 1,6-bis{[(2-substituted hydrazinyl)carbonyl]amino}hexanes and 1,6-bis(3-substituted 1,5-dihydro-5-oxo-4*H*-1,2,4-triazol-

-4-yl)hexanes were synthesized and characterized. The new derivatives **3a**–**c** were screened *in vitro* for their antiproliferative and anticancer activity in human tumor cell lines derived from breast and lung carcinoma cells. Compounds **3a** (at a concentration of 0.18 mM), **3b** (at concentrations of 0.12 and 0.02 mM) and **3c** (at concentrations of 0.23 and 0.11 mM) were found to be the most effective against the lung cell line. Compounds **2a**–**c** and **3a**–**c** were screened for their antimicrobial activities. All the tested derivatives showed *MIC* values in range 1.87–7.5 μg mL^{-1}.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

СИНТЕЗА 1,6-ХЕКСАНДИИЛ-БИС(СЕМИКАРБАЗИДА) И 1,6-ХЕКСАНДИИЛ-БИС-(1,2,4,-ТРИАЗОЛ-5-ОНА) И ЊИХОВА АНТИПРОЛИФЕРАТИВНА И АНТИБАКТЕРИЈСКА АКТИВНОСТ

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Синтетисана је серија 1,6-бис(3-супституисаних 1,5-дихидро-5-оксо-4*H*-1,2,4-триазол-4--ил)хексана **3a**–**g** реакцијом циклизације 1,6-бис{[(2-супституисаних хидразинил)карбо-нил]}хексана **2a**–**g** под базним условима. Испитана је *in vitro* антипролиферативна активност нових деривата **3a**–**3c** према ћелијским линијама хуманих тумора дојке и плућа. Утврђено је да су једињењења **3a** (при концентрацији 0,18 mM), **3b** (при концентрацијама 0,12 и 0,02 mM) и **3c** (при концентрацијама 0,23 и 0,11 mM) најактивнија према ћелијској линији рака плућа. Једињење **3a** је најактивније према ћелијској линији рака дојке. Репрезентативним једињењима испитана је антимикробна активност. Сва испитана једињења показују *MIC* вредности у опсегу 1,87–7,5 μg/mL. Једињење **3b** је најактивније према *C. albicans* (*MIC* = = 1,87 μg/mL).

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REFERENCES

1. D. L. Temple, US 4386091 (1983) (*CA* **99** 19529)
2. A. Catanese, E. Lisciari, *Boll. Chim. Farm.* **109** (1970) 369
3. Ş. G. Küçükgüzel, G. Rollas, O. Ötük-Saniş, I. Bayrak, T. Altuğ, J. P. Stables *Farmaco* **59** (2004) 893
4. B. S. Holla, B. Veerendra, M. K. Shivananada, B. Poojary, *Eur. J. Med. Chem*. **38** (2003) 759
5. S. Rolas, N. Kalyonuoglu, D. Sur-Altiner, Y. Yegenoglu, *Pharmazie* **48** (1993) 308
6. N. Gülerman, S. Rollas, M. Kiraz, A. C. Ekinci, A Vidin, *Farmaco* **52** (1997) 691
7. O. Bekircan, M. Kucuk, B. Kahveci, H. Bektas, *Z. Naturforsch.* **63b** (2008) 130
8. K. Sztanke, T. Tuzimski, J. Rzymowska, K. Pasternak, M. Kandefer-Szerszeń, *Eur. J. Med. Chem.* **43** (2008) 404

9. M. Clemons, R.E. Coleman, S. Verma, *Cancer Treat. Rev*. **30** (2004) 325
10. N. Singhal, P.K Sharma, R. Dudhe, N. Kumar, *J. Chem. Pharm. Res.* **3** (2011) 126
11. A. Demirbas, D. Sahin, N. Demirbas, S. A. Karaoglu, *Eur. J. Med. Chem*. **44** (2009) 2896
12. H. Bektaş, N. Karaali, D. Şahin, A. Demirbaş, Ş.A. Karaoglu, N. Demirbaş, *Molecules* **15** (2010) 2427
13. M. Dobosz, *Annales UMCS, Lublin, Sectio AA* **34** (1979) 163
14. M. Dobosz, A. Pachuta-Stec, E. Tokarzewska-Wielosz, E. Jagiełło-Wójtowicz, *Acta Pol. Pharm.* **57** (2000) 205
15. M. Wujec, M. Pitucha, M. Dobosz, *Heterocycles* **68** (2006) 779
16. M. Koch, DE 3114314 (1983) (*CA* **98** 72110g)
17. A. Stunder, DE 3114349 (1983) (*CA* **98** 198249t)
18. A. A. Ikizler, K. Sancak, *Collect. Czech. Chem. Commun*. **60** (1995) 903
19. O. V. Fedorowa, G. G. Mordovskoi, G. L. Rusinov, I. G. Ovchinnikova, M. N. Zueva, M. A. Kravchenko, O. N. Chupakhin, *Pharm. Chem*. **32** (1998) 2
20. F. Azam, I.A. Alksakas, S.L. Khokra, O. Prakash, *Eur. J. Med. Chem* **44** (2009) 203
21. S. V. Andurkar, C. Beguin, J. P. Stables, H. Kohn, *J. Med. Chem*. **44** (2001) 1475
22. M. Pitucha, Z. Karczmarzyk, U. Kosikowska, A. Malm, *Z. Naturforsch*. **66b** (2011) 505
23. Z. Cui, Y. Ling, B. Li, Y. Li, C. Rui, J. Cui, Y. Shi, X. Yang, *Molecules* **15** (2010) 4267
24. M. Pitucha, B. Polak, R. Świeboda, U. Kosikowska, A. Malm, *Z. Naturforsch*. **64b** (2009) 570
25. M. Pitucha, A. Chodkowska, M. Maciejewski, E. Jagiełło-Wójtowicz, A. Pachuta-Stec, *Monatsh. Chem*. **141** (2010) 199
26. M. Dobosz , M. Pitucha, M. Wujec *Acta Pol. Pharm*. **53** (1996) 31
27. M. Pitucha, M. Wujec, M. Dobosz, *Annales UMCS, Sectio AA* **59** (2004) 144
28. D. J. Diekema, S. A. Messer, L. B. Boyken, R. J. Hollis, J. Kroeger, S. Tendolkar, M. A. Pfaller. *J. Clin. Microbiol.* **47** (2009) 3170
29. Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing, Twentieth Informational Supplement*, M100-S20-U, Wayne, PA, 2010
30. J. P. Magaud, I. Sarget, D.Y. Mason, *J. Immunol. Methods* **106** (1988) 95
31. D. Muris, S. Varon, M. Manthorpe, *Anal. Biochem*. **185** (1990) 377
32. M. P. Arevalo, A. J. Carrillo-Munoz, J. Salgado, D. Cardenes, S. Brio, G. Quindos A. Espinel-Ingroff , *J. Antimicrob. Chemother*. **51** (2003) 163
33. M. R. Capoor, D. Nair, M. Dep, P. K. Verma, L. Srivastava, P. Aggarwaj, *Jpn. J. Infect. Dis*. **58** (2005) 344
34. F. Sabatelli, P. A. Mann, C. A. Mendrick, C. C. Norris, R. Hare, D. Loebenberg, T. A. Black, P. M. McNicholas, *Antimicrob. Agents. Chemother*. **50** (2006) 2009
35. K. L. Threse, R. Bagyalakshim, H. N. Madhavan, P. Deepa, *Indian J. Med. Microbiol*. **20** (2002) 160
36. Clinical and Laboratory Standards Institute (CLSI), National Committee for Clinical Laboratory Standards (NCLS), *Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically*, Approved standard M7-A6, 6th ed., Wayne, PA, 2003.