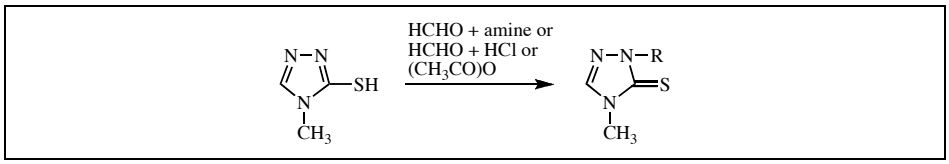
Synthesis, Structure and Investigations of Tuberculosis Inhibition Activities of New 4-Methyl-1-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione

Monika Wujec[1*], Marta Swatko-Ossor[2], Liliana Mazur[3], Zofia Rzaczynska[3] and Agata Siwek[1]

[1]Department of Organic Chemistry, Faculty of Pharmacy, Medical University, Staszica 6, 20-081 Lublin, Poland;+48815357373, e-mail: * monika.wujec@am.lublin.pl
[2]Department of Biochemistry, Medical University, Chodzki 1, Lublin, Poland; e-mail:marta.swatko@am.lublin.pl
[3]Department of General and Coordination Chemistry, Faculty of Chemistry, Maria Curie-Sklodowska University, 20-031 Lublin, Poland; e-mail: lmazur2@op.pl
Received April 30, 2008



By the reaction aminomethylation, chloromethylation and acylation of 4-methyl-4*H*-1,2,4-triazole-3-thiol, 4-methyl-1-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione **1**-**8** were obtained. Molecular structure of the obtained compounds was confirmed by an elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectra and additionally by X-ray analysis for **2**. Six new compounds **1**, **2**, **4-7** were tested for antibacterial activity against *Mycobacterium smegmatis*, *Mycobacterium phlei* and avirulent strain *Mycobacterium* H_{37}*Ra.*

J. Heterocyclic Chem., **45**, 1893 (2008).

INTRODUCTION

Tuberculosis (TB) has again become epidemic in many parts of the world. The increase in TB and other non-specific mycobacterial infections is associated with HIV, homelessness, drug abuse and immigration of persons with active infections. Another serious problem is the emergence of multidrug-resistant TB. Therefore new antituberculotic drugs and/or new derivatives of old drugs have been prepared and studied. A special interest has been focussed on five membered heterocyclic compounds used very often in the pharmacological and medicinal applications. The anti-tuberculosis activity of pyrrole [1-3], oxadiazole [4], imidazole [5], triazole [6-9] and other heterocyclic systems derivatives has been reported.

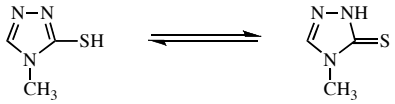
In this paper, we present the synthesis, structure and anti-tuberculosis activity of new 4-methyl-1-substituted-1-H-1,2,4-triazole-5(4*H*)-thione.

4-Methyl-4*H*-1,2,4-triazole-3-thiol is a starting material for the synthesis of new derivatives. This compound can exist in two tautomeric forms as shown in Scheme 1.

In various reactions this compound can give both S- or N-derivatives. In the previous paper S-derivatives were obtained, by the reaction with bromoacetate ester [10]. In this work 4-methyl-4*H*-1,2,4-triazole-3-thiol was subjected to following reactions: aminomethylation, chloromethylation and acylation (Scheme 2). Mechanism of this reaction have been reported earlier [11]. Some of the obtained compounds **1**, **2**, **4-7** were tested for anti-tuberculosis activity.

Scheme 1

Thiol/thione tautomerism forms of 4-methyl-4*H*-1,2,4-triazole-3-thiol.



Scheme 2

Synthesis 4-methyl-1-substituted-1*H*-1,2,4-triazole-5(4*H*)-thione.

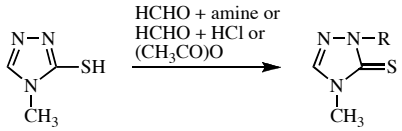


Table 1

Substituents of compounds **1-8.**

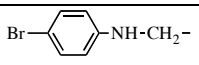
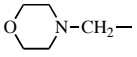
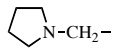
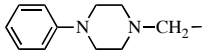
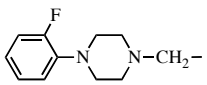
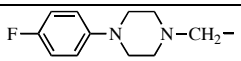
Compounds No	R
1	
2	
3	
4	
5	

Table 1 (continued)

Compounds No	R
6	
7	CH_2Cl
8	$COCH_3$

RESULTS AND DISCUSSION

The aminomethylation reactions were carried out in ethanolic solution with an equivalent amount of formalin by using 4-bromoaniline, morpholine, pyrrolidine, *N*-phenylpiperazine, 1-(2-fluorophenyl) piperazine, and 1-(4-fluorophenyl) piperazine as shown in the Scheme 2. The conditions of the reactions were established experimentally.

The obtained products were characterized by their elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectra. The signal of proton characteristic for the –NH-C=S group, found in the substrate, was not observed in the ^{1}H NMR spectra of new compounds.

Among the six tested compounds two of them (**2**,**7**) showed inhibitory activity from low to moderate. Compound **7** with chloromethyl group in position 1 showed the highest activity from all tested compounds with MIC = 128 mg/L against *Mycobacterium phlei*. Moreover 4-methyl-1-[1-(morpholin)methyl]-1*H*-1,2,4-triazole-5(4*H*)-thione (**2**) affected the growth of *Mycobacterium phlei* with MIC = 256 mg/L (Table 2).

Table 2

MICs and MBCs of the tested compounds.

Compound No	MIC(mg/L) MBC (mg/L)		
	M. phlei	*M. $H_{37}Ra$*	*M. smegmatis*
Rifampicin	16	16	128
	32	32	256
2	512	512	256
	1024	1024	512
7	128	256	256
	256	512	512

In conclusion, eight new 4-methyl-1-subtituted-1*H*-1,2,4-triazole-5(4*H*)-thione, with different substituents at the 1- position were synthesized. The synthesis showed an excellent average yield of over 87%, making these compounds readily available. Among tested compounds only triazoles with chloromethyl and morpholinemethyl group showed moderate antimicrobial activity against *Mycobacterium* spp. and can be consider for further microbiological tests.

X-ray Crystallography. The molecular structure of **2** was confirmed by X-ray analysis. Triclinic crystals of **2** are built up of two (A and B) symmetrically independent molecules. Numbering scheme and structural view with displacement parameters of molecules are shown in Fig. 1.

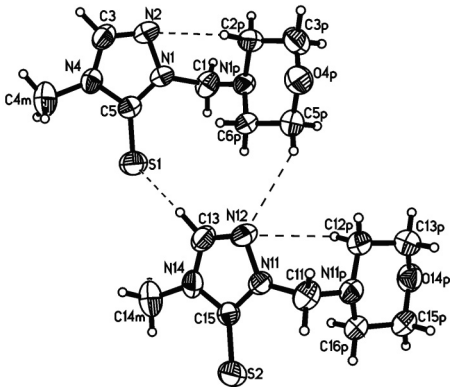


Figure 1. A view of the asymmetric unit of 2, showing the atomic numbering scheme. Displacement ellipsoids are drawn at the 50% probability level. Dashed lines indicate hydrogen bonds.

Selected bond distances are shown in Table 3.

Table 3

Selected bond lengths [Å] for 2.

N1–N2	1.377(3)	N11–N12	1.377(3)
N2–C3	1.282(3)	N12–C13	1.280(3)
C3–N4	1.350(4)	C13–N14	1.348(4)
N4–C5	1.366(3)	N14–C15	1.365(3)
N1–C5	1.348(3)	N11–C15	1.348(3)
N4–C4m	1.456(3)	N14–C14m	1.447(4)
C5–S1	1.665(3)	C15–S2	1.665(3)
N1–C1	1.472(3)	N11–C11	1.469(3)
C1–N1p	1.427(3)	C11–N11p	1.430(3)
N1p–C2p	1.457(3)	N11p–C12p	1.458(3)
N1p–C6p	1.453(3)	N11p–C16p	1.453(3)
O4p–C3p	1.406(4)	O14p–C13p	1.411(3)
O4p–C5p	1.409(3)	O14p–C15p	1.418(3)

Equivalent bond distances and angles for both molecules are equal within the experimental error, but they have different hydrogen-bonding patterns. The 1,2,4-triazole-5-thione system is planar in both molecules with deviations of S1 and S2 atoms from heterocyclic ring planes of 0.025(5) and 0.045(4) Å. At the same time the methyl C4m and C14m atoms are displaced out of the triazole rings by respectively 0.023(5) and 0.035(6) Å.

The bond distances within the five-membered heterocyclic rings are not regular. The N2=C3 and N12=C13 distances (1.282(3) and 1.280(3) Å) are of double-bond character, whereas the remaining C–N and N–N endocyclic bonds have an intermediate character (Table 2), indicating delocalization of the electron cloud. The C5=S1 and C15=S2 distances are in accordance with values observed in other 1,2,4-triazole-5-thione systems [12]. The morpholine ring in both molecules exists in a chair conformation. The bond lengths observed within the six-membered systems are comparable with those reported for molecules of free morpholine [13]. The relative orientation of morpholine and triazole rings could

be described by for instance torsion angles N2–N1–C1–N1p/N12–N11–C11–N11p; their values (±89.4(3)°) indicate ±*sc* conformation. The bent conformation adopted by molecules of **2** in the solid state is additionally stabilized by weak C2p–H2p1···N2/ C12p–H12b···N12 intramolecular hydrogen bonds. In crystals of **2** the packing of molecules is stabilized by different weak intermolecular interactions (Table 4).

Table 4.

Hydrogen-bonding geometry [Å, °] for 2.

D–H…A	D–H	H…A	D…A	<DHA
C2p–H2p1…N2	0.97	2.803	3.233(3)	108
C12p–H12b…N12	0.97	2.830	3.244(3)	107
C5p–H5p1…N12	0.97	2.742	3.550(4)	141
C13–H13…S1	1.022	2.811	3.750(4)	153
C3–H3…S2$^{(i)}$	0.99	2.859	3.714(3)	145
C14m–H14c…O14p$^{(ii)}$	0.96	2.535	3.378(4)	147
Symmetry codes: (i) *x*, *y*+1, *z*-1; (ii) *x*, *y*+1, *z*				

EXPERIMENTAL

Chemistry. Melting points were determined in Fisher-Johns blocs and presented without any corrections. IR spectra were recorded in KBr using Specord IR-75 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 in DMSO-d_6 with TMS as internal standard. Chemicals were purchased from Lancaster or Merck Co. and used without further purification. Purity was checked by TLC on Merck Co. plates Aluminium oxide 60 F_{254} in a $CHCl_3$/C_2H_5OH(10:1) solvent system with UV visualization.

1-Aminomethyl-4-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives (1-6). 1.15 g (0.01 mole) of 4-methyl-4*H*-1,2,4-triazole-3-thiol was dissolved in 10 mL ethanol. Then 0.8g (0.01 mole) of 37% formaldehyde aqueous solution and 0.02 mole appropriate amine were added. The mixture was mixed carefully and kept at room temperature for 24 h (for compound **6** - 10 min). Next, the precipitate was filtered off, dried and crystallized from ethanol.

1-((4-Bromophenylamino)methyl)-4-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (1). Yield 2.69g (90 %). M.p. 127–129°C. For $C_{10}H_{11}N_4BrS$ (299.2) calculated: 40.14% C, 3.71% H, 18.73% N; found: 40.10% C, 3.69% H, 18.70% N. IR (KBr): 3300 (NH), 3120 (CH_{ar}), 2929, 1445 (CH_{al}), 1370 (C=S). ^{1}H NMR (DMSO-d_6): 3.46 (s, 3H, CH_3), 5.41, 5.43 (d, *J*= 7.4 Hz, 2H, CH_2), 6.84, 6.85, 6.87, 6.88 (dd, *J* = 2.15 Hz, *J*= 6.8 Hz, 2H, 2xCH_{ar}), 7.17 (s, 1H, NH), 7.21, 7.22, 7.23, 7.24 (dd, *J* = 2.08 Hz, *J* = 6.8 Hz, 2H, 2xCH_{ar}), 8.47 (s, 1H, CH). ^{13}C NMR 32.0 (CH_3), 56.6 (CH_2), 108.0, 145.3 (2xC_{ar}), 115.1 (2xCH_{ar}), 131.4 (2xCH_{ar}), 141.5 (CH), 165.6 (C=S).

4-Methyl-1-(morpholinomethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (2). Yield 1.91g (89 %). M.p. 116–118°C. For $C_8H_{14}N_4OS$ (214.3) calculated: 44.84% C, 6.59% H, 26.15% N; found: 44.72% C, 6.59% H, 26.20% N. IR (KBr): 3056 (CH_{al}), 1630 (C=N), 1550 (C–N), 1379 (C=S), 1258 (C–O–C). ^{1}H NMR (DMSO-d_6): 2.61, 2.63, 2.64 (t, *J* = 4.7 Hz, 4H, 2xCH_2), 3.48 (s, 3H, CH_3), 3.51, 3.53, 3.55 (t, *J* = 4.7 Hz, 4H, 2xCH_2), 4.98 (s, 2H, CH_2), 8.51 (s, 1H, CH). ^{13}C NMR 32.2 (CH_3), 50.2 (2xCH_2), 66.0 (2xCH_2), 68.7 (CH_2), 141.3 (CH), 167.3 (C=S).

4-Methyl-1-(pyrrolidin-1-ylmethyl]-1*H*-1,2,4-triazole-5(4*H*)-thione (3). Yield 1.71g (86 %). M.p. 89–91°C. For $C_8H_{14}N_4S$ (198.3) calculated: 48.46% C, 7.12% H, 28.26% N; found: 48.50% C, 7.08% H, 28.20% N. IR (KBr): 3024 (CH_{al}), 1620 (C=N), 1545 (C–N), 1358 (C=S). ^{1}H NMR (DMSO-d_6): 1.60, 1.61, 1.62 (t, *J* = 3.5 Hz, 4H, 2xCH_2), 2.72, 2.73, 2.74 (t, *J* = 2.4 Hz, 4H, 2xCH_2) 3.47 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 8.49 (s, 1H, CH). ^{13}C NMR 23.4 (2xCH_2), 32.1 (CH_3), 49.4 (2xCH_2), 64.6 (CH_2), 141.1 (CH), 167.1 (C=S).

4-Methyl-1-((phenylpiperazin-1-yl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (4). Yield 2.37g (82 %). M.p. 111–113°C. For $C_{14}H_{19}N_5S$ (289.4) calculated: 58.10% C, 6.62% H, 24.20% N; found: 58.02% C, 6.69% H, 24.10% N. IR (KBr): 3094 (CH_{ar}), 2980, 1421 (CH_{al}), 1671 (C=N), 1501 (C–N), 1364 (C=S). ^{1}H NMR (DMSO-d_6): 2.77-2.80 (m, 4H, 2xCH_2), 3.03-3.12 (m, 4H, 2xCH_2), 3.48 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 6.73-7.29 (m, 5H, 5xCH_{ar}), 8.52 (s, 1H, CH).

1-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-4-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (5). Yield 2.43g (79 %). M.p. 115–118°C. For $C_{14}H_{18}FN_5S$ (307.4) calculated: 54.70% C, 5.90% H, 22.78% N; found: 54.67% C, 5.83% H, 22.74% N. IR (KBr): 3102 (CH_{ar}), 2939, 1420 (CH_{al}), 1607 (C=N), 1516 (C–N), 1377 (C=S). ^{1}H NMR (DMSO-d_6): 2.77-2.80 (m, 4H, 2xCH_2), 3.04-3.06 (m, 4H, 2xCH_2), 3.49 (s, 3H, CH_3), 5.06 (s, 2H, CH_2), 6.84-7.01 (m, 4H, 4xCH_{ar}), 8.52 (s, 1H, CH).

1-((4-(4-Fluorophenyl)piperazin-1-yl)methyl)-4-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (6). Yield 2.67 g (87 %). M.p. 112–113°C. For $C_{14}H_{18}FN_5S$ (307.4) calculated: 54.70% C, 5.90% H, 22.78% N; found: 54.69% C, 5.68% H, 22.70% N. IR (KBr): 3099 (CH_{ar}), 2978, 1420 (CH_{al}), 1620 (C=N), 1526 (C–N), 1370 (C=S). ^{1}H NMR (DMSO-d_6): 2.68-2.83 (m, 4H, 2xCH_2), 2.96-2.99 (m, 4H, 2xCH_2), 3.50 (s, 3H, CH_3), 5.14 (s, 2H, CH_2), 6.88-7.21 (m, 4H, 4xCH_{ar}), 8.53 (s, 1H, CH). ^{13}C NMR 32.2 (CH_3), 49.1 (2xCH_2), 49.7 (2xCH_2), 68.4 (CH_2), 115.3 (2xCH_{ar}), 117.3 (2xCH_{ar}), 141.5 (CH), 154.4, 157.6 (2xC_{ar}), 166.2 (C=S).

1-(Chloromethyl)-4-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione (7). 0.01 Mole of 4-methyl-4*H*-1,2,4-triazole-3-thiol, 0.8g (0.01 mole) of 37% formaldehyde aqueous solution and 0.02 mole 36% HCl were mixed. After 15 min the product was collected by filtration, dried and crystallized from ethanol. This compoud is known [14].

1-(4-Methyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)-ethanone (8). 4-Methyl-4*H*-1,2,4-triazole-3-thiol (0.01 mole) and 5 mL acetic anhydride were refluxed for 3 h. After cooling the precipitate was collected by filtration, dried and crystallized from ethanol. Yield 1.41 g (90 %). M.p. 165–167°C. For $C_5H_7N_3OS$ (157.2) calculated: 38.20% C, 4.49% H, 26.73% N; found: 38.12% C, 4.49% H, 26.50% N. IR (KBr): 3080 (CH_{al}), 1752 (C=O), 1605 (C=N), 1560 (C–N), 1345 (C=S). ^{1}H NMR (DMSO-d_6): 2.69 (s, 3H, CH_3), 3.44 (s, 3H, CH_3), 8.62 (s, 1H, CH). ^{13}C NMR 24.4 (CH_3), 31.8 (CH_3), 142.5 (CH), 167.4 (C=S), 167.7 (C=O).

Crystal Structure Determination. *Crystal data for compound 2*: $C_8H_{14}N_4OS$, M_r = 214.29, space group *P*(-1), *a* = 8.765(3) Å, *b* = 10.798(5) Å, c = 12.575(5) Å, α = 67.31(4)°, β = 80.12(3)°, γ = 78.63(3)°, V = 1070.5(8) Å^3, Z = 4, d_{calc} = 1.330 g cm^{-3}, colorless prism, 0.56 × 0.36 × 0.09 mm, μ = 0.278 mm^{-1}.

Single-crystal diffraction data were measured at room temperature in the $\omega/2\theta$ mode on the Oxford Diffraction Xcalibur diffractometer using graphite-monochromated Mo K_α radiation (λ = 0.71073 Å). The stability of intensities was

monitored by measurement of 3 standards every 100 reflections. Crystal structure was solved by direct methods using SHELXS97 [15] program and refined by the full-matrix least-squares on F^2 using the SHELXL97 [16] program. All non-hydrogen atoms were refined with anisotropic displacement parameters. H-atoms bonded to C(3) and C(13) atoms were located from a difference Fourier map and their positional parameters were not refined. The remaining hydrogen atoms were positioned geometrically and allowed to ride on their parent atoms, with U_{iso}(H) = 1.2 U_{eq}(C). Final discrepancy factors are R_1 = 0.0666, wR_2 = 0.1022 for $I > 2\sigma(I)$, GOOF = 0.977, $\Delta\rho_{min, max}$ = –0.24/0.21 e Å^{-3}.

CCDC 676833 contains the supplementary crystallographic data for this paper. Copies of the data can be obtained free of charge from CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Antimycobacterial activity. Antimycobacterial activity of the compounds was evaluated against mycobacterium strains: *Mycobacterium smegmatis*, *Mycobacterium phlei* and *Mycobacterium* H_{37}*Ra* using the broth dilution method for determination of the Minimum Inhibitory Concentration (MIC) to inhibit growth of the microorganism and Minimum Bactericidal Concentration (MBC) as well [17].

MIC was defined as the highest dilution of compound that inhibited growth judged by lack of turbidity in the tube. Inocula for susceptibility testing were from a suspension equivalent to a McFarland No. 1 standard.

Turbidity of the suspension was measured using nefelometer BD PhoenixSpec, Becton, Dickinson and Company USA.

The tubes containing the drugs concentration in Mueller-Hinton broth (Oxoid Ltd. England), between 2 – 1024 mg/L were inoculated with the microorganisms cultures, then incubated at 37°C. The inoculated tube kept in 4°C overnight was used as the standard for the determination of complete inhibition.

MBC (Minimum Bactericidal Concentration) is the highest dilution showing at least 99% inhibition. All tubes not showing visible growth were subculturated by spreading a loopful evenly over a quarter of the plate on Mueller-Hinton Agar and incubated overnight at 37°C.

REFERENCES

[1] Biava, M.; Fioravanti, R.; Porretta, G. C.; Deidda, D.; Maullu, C.; Pompei, R. *Biorg. Med. Chem. Lett*. **1999**, *9*, 2983.

[2] Biava, M.; Porretta, G. C.; Deidda, D.; Pompei, R.; Tafo, A.; Manetti, F. *Biorg. Med. Chem.* **2003**, *11*, 515.

[3] Sbardella, G.; Mai, A.; Artico, M.; Loddo, R.; Setzu, M. G.; La Colla, P. *Biorg. Med. Chem. Lett*. **2004**, *14*, 1537.

[4] Kücükgüzel, S. G.; Oruc, E. E.; Rollas, S.; Sahin, F.; Özbek, A. *Eur. J. Med. Chem*. **2002**, *37*, 197.

[5] Jackson, C. J.; Lamb, D. C.; Kelly, D. E.; Kelly, S. L. *FEMS Microbiol. Lett.* **2000**, *192*, 159.

[6] Kücükgüzel, I.; Kücükgüzel, S. G.; Rollas, S.; Kirgiz, M. *Biorg. Med. Chem. Lett.* **2001**, *11*, 1703.

[7] Ulusoy, N.; Gürsoy, A.; Ötük, G. *Il Farmaco* **2001**, *56*, 947.

[8] Dabak, K.; Sezer, Ö.; Akar, A.; Anaç, O. *Eur. J. Med. Chem.* **2003**, *38*, 215.

[9] Klimesova, V.; Zahaiska, L.; Waisser, K.; Kaustova, J.; Möllmann, U. *Il Farmaco* **2004**, *59*, 279.

[10] Maliszewska-Guz, A.; Wujec, M.; Pitucha, M.; Dobosz, M.; Chodkowska, A.; Jagiełło-Wójtowicz, E.; Mazur, L.; Kozioł, A. E. *Collect. Czech. Chem. Commun*. **2005**, *70*, 51.

[11] Wujec, M.; Paneth, P. *J. Phys. Org. Chem.* **2007**, *20*, 1043.

[12] Allen, F. H. *Acta Cryst.* **2002**, *B58*, 380.

[13] Parkin, A.; Oswald, T. D. H.; Parsons, S. *Acta Cryst.* **2004**, *B60*, 219.

[14] Timmler, H.; Hammann, I.; Wegler, R. Brit. Patent 1161381, **1969**.

[15] Sheldrick, G. M. *SHELXS97*, *Program for a Crystal Structure Solution*; University of Göttingen, Göttingen, Germany, **1997**.

[16] Sheldrick, G. M. *SHELXL97*, *Program for the Refinement of a Crystal Structure from Diffraction Data,* University of Göttingen, Göttingen, Germany, **1997.**

[17] John, D. T.; James, H. J. *Antimicrobial Susceptibility Testing : General Considerations.* In *Manual of Clinical Microbiology* 7th Edition, Murray, P. R.; Baron, E. J.; Pfaller, M. A.; Tenover, F. C.; Yolken, R. American Society for Microbiology, Washington DC, **1999**, 1469-1473.