Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(1):358-363
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, Antitumor, and Antimicrobial Evaluation of Some Novel Thioglucosyl Nucleosides

Adel A.-H. Abdel-Rahman[a,*] , Shafey G. Donia[b], Ashraf A. F. Wasfy[b], Aly A. ALY[b], and Amaal Y. El-Gazzar[b]

[a]*Department of Chemistry, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt.*
[b]*Department of Chemistry, Faculty of Science, Benha University, Benha, Egypt*

ABSTRACT

Some Novel thioglucosides of 2-(N-phthalimidomethyl)-4-chlorobenzylidene-5-imidazole derivatives were newly synthesized. The antitumor and antimicrobial activities of the prepared compounds were evaluated. The free hydroxyl thioglucosyl derivatives were the highly active compounds.

Keywords: *S*-Glycosides, *O*-glycosides, imidazolones, anti-hepatitis B virus, antimicrobial activity.

INTRODUCTION

Nucleoside analogues have occupied a significant position in the search for effective antiviral agents, owing to the fact that a large number of unnatural nucleoside derivatives have been shown to inhibit infection caused by viruses [1–6]. Thioglycosides have found their importance in enzyme inhibition studies due to their chemical and enzymatic stability, which is being greater than the corresponding *O*-glycoside-analogues [7-10]. They have also been found to be useful as inducers and ligands for affinity chromatrography of carbohydrate-processing enzymes and proteins [7-9]. Thioglycosides, as well, have been used as donors with excellent chemoselectivity in glycosylation processes in addition of being good acceptorsb [10]. Among these glycosyl donors are the glycosylthio heterocycles that can have heterocycles or be transformed into heterocycles with different functionalities in addition to their stability under variety of reaction conditions [7, 9]. In view of the above facts and as continuation of our program of identification of new candidates that may be valuable in design and synthesis of new active leads [5, 11-15] we report in the present work the synthesis and antimicrobial activity of new 2-(*N*-phthalimidomethyl)-4-chlorobenzylidene-5-imidazole derivatives, their oxadiazolyl, and acyclic *C*-analogues.

MATERIALS AND METHODS

Synthetic methods, analytical and spectral data

Melting points were determined with a *Kofler* block apparatus and are uncorrected. The IR spectra were recorded on a perkin-Elmer model 1720 FTIR spectrometer for KBr disc. NMR spectra were recorded on a varian Gemini NMR Spectrometer at 300 MHz for ^{1}H NMR with TMS as a standard. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F245. Elemental analyses were performed at the Microanalytical data centre at Faculty of science, Cairo University, Egypt.

2-{[4-(4-Chlorobenzylidene)]-1-[(3-mercapto-1*H*-1,2,4-triazol-5-ylmethyl)]-5-oxo-(4,5-dihydro-1*H*-imidazol-2-yl)methyl}isoindoline-1,3-dione (**1a**), 2-{[4-(4-chlorobenzylidene)]-1-[(3-mercapto-1*H*-1,2,4-triazol-5-ylmethyl)]-5-oxo-(4,5-dihydro-1-phenyl-1*H*-imidazol-2-yl)methyl} isoindoline-1,3-dione (**1b**), and 2-{{[4-(4-chlorobenzylidene)]-1-{[2-(2-mercapto-4-oxoquinazolin-3(4*H*)-yl]-2-oxoethyl}-5-oxo-(4,5-dihydro-1*H*-imidazol-2-yl)methyl}}isoindoline-1,3-dione (**4**) were prepared according to the reported procedure [16].

Chemistry

***General procedure for the preparation of O-acetylated thioglucosides* 2a,b *and* 5**

To a solution of **1a,b** or **4** [16] (10 mmol) and aqueous potassium hydroxide (1.12 g, 20 mmol) in dry acetone (20 ml), a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.11 g, 10 mmol) was dissolved in dry acetone (10 ml) and was added to the former solution. The reaction mixture was stirred at room temperature for 5 h. The solvent was evaporated under reduced pressure at 40 ^{o}C and the residue was washed with distilled water to remove the formed potassium bromide.

3-{{(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-5-{[4-(4-chlorobenzylidene)]-[5-(oxo-(4,5-dihydro-1H-imidazol-1-yl)methyl]-2-(1,3-isoindolinon-2yl)}methyl}}-1H-1,2,4-triazole (**2a**)

White powder (7.12 g, 88%), mp 148-150 ^{o}C; IR (KBr, cm^{-1}): 3457 (NH), 1761, 1714 (2C=O), 1615 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.87, 1.89, 2.05, 2.10 (4s, 12H, 4CH_3CO), 4.02 (m, 1H, H-5′), 4.10-4.14 (m, 2H, H-6′, 6″), 4.20 (s, 2H, CH_2), 4.26 (s, 2H, CH_2), 4.90-4.93 (m, 1H, H-4′), 5.18-5.25 (m, 1H, H-3′), 5.34 (t, *J* = 9.6 Hz, 1H, H-2′), 5.70 (d, *J* = 10.2 Hz, 1H, H-1′), 6.79 (s, 1H, CH), 7.20-7.30 (m, 4H, Ar-H), 7.65-7.80 (m, 4H, Ar-H), 13.50 (brs, 1H, NH) ppm. EI-MS: *m/z* 564 809 [M^{+}]. Anal. Calcd. For $C_{36}H_{33}ClN_6O_{12}S$; C, 53.43; H, 4.11; N, 10.39. Found: C, 53.30; H, 4.00; N, 10.11.

3-{{(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-5-{[4-(4-chlorobenzylidene)]-[5-(oxo-(4,5-dihydro-1H-imidazol-1-yl)methyl]-2-(1,3-isoindolinon-2yl)}methyl}}-1-phenyl-1H-1,2,4-triazole (**2b**)

White powder (8.23 g, 93%), mp 220-222 ^{o}C; IR (KBr, cm^{-1}): 1759, 1717 (2C=O), 1615 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.89, 1.95, 2.06, 2.12 (4s, 12H, 4CH_3CO), 4.09-4.12 (m, 1H, H-5′), 4.16-4.25 (m, 6H, H-6′, 6″, 2CH_2), 4.95-4.99 (m, 1H, H-4′), 5.22 (m, 1H, H-3′), 5.36 (t, *J* = 9.6 Hz, 1H, H-2′), 5.76 (d, *J* = 10.2 Hz, 1H, H-1′), 6.75 (s, 1H, CH), 7.18-7.30 (m, 4H, Ar-H), 7.39-7.66 (m, 5H, Ar-H), 7.71-7.94 (m, 4H, Ar-H) ppm. Anal. Calcd. For $C_{42}H_{37}ClN_6O_{12}S$; C, 56.98; H, 4.21; N, 9.49. Found: C, 56.80; H, 4.09; N, 9.22.

2-{{(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-3-{[4-(4-chlorobenzylidene)]-[5-oxo-(4,5-dihydro)-1H-imidazol-1-oxoethyl]-2-(1,3-isoindolinon-2yl)}methyl}}-quinazolin-4(3H)-one (**5**)

White powder (8.68 g, 95%), mp 177-179 ^{o}C; IR (KBr, cm^{-1}): 1712 (C=O), 1616 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.85, 1.88, 2.07, 2.12 (4s, 12H, 4CH_3CO), 4.07-4.16 (m, 7H, H-5′, H-6′, 6″, 2CH_2), 4.88-4.90 (m, 1H, H-4′), 5.18-5.22 (m, 1H, H-3′), 5.33 (t, *J* = 9.6 Hz, 1H, H-2′), 5.73 (d, *J* = 10.2 Hz, 1H, H-1′), 6.90 (s, 1H, CH), 7.16-7.25 (m, 4H, Ar-H), 7.30-7.60 (m, 4H, Ar-H), 7.70-8.00 (m, 4H, Ar-H) ppm. EI-MS: *m/z* 564 913/914 [M^{+}]. Anal. Calcd. For $C_{43}H_{36}ClN_5O_{14}S$; C, 56.49; H, 3.97; N, 7.66. Found: C, 56.33; H, 3.86; N, 7.50.

***General procedure for the preparation of free hydroxyl thioglucosides* 3a,b *and* 6**

A solution of **2a,b** or **5** (1 mmol) in methanol and ammonia solution (5:5) was stirred at room temperature for 4 h. The solvent was evaporated under reduced pressure and the residue was dissolved in absolute ethanol (10 ml) and left over night. The formed precipitates were filtered off and dried well.

3-{{(β-D-Glucopyranosylsulfanyl)-5-{[4-(4-chlorobenzylidene)]-[5-(oxo-(4,5-dihydro-1H-imidazol-1-yl)methyl]-2-(1,3-isoindolinon-2yl)}methyl}}-1H-1,2,4-triazole (**3a**)

White powder (0.54 g, 85%), mp 188-190 ^{o}C; IR (KBr, cm^{-1}): 3411-2922 (OH), 1709 (C=O), 1628 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.39-3.44 (m, 3H, H-5′, H-6′,6″), 3.91-3.99 (m, 2H, H-3′,H-4′), 4.22-4.28 (m, 5H, H-2′, 2CH_2), 4.72 (brs, 1H, OH), 4.84 (brs, 1H, OH), 5.22 (brs, 1H, OH), 5.25 (brs, 1H, OH), 5.74 (d, *J* = 9.8 Hz, 1H, H-1′), 6.82 (s, 1H, CH), 7.23-7.35 (m, 4H, Ar-H), 7.60-7.75 (m, 4H, Ar-H), 13.40 (brs, 1H, NH) ppm. EI-MS: *m/z* 564 641 [M^{+}]. Anal. Calcd. For $C_{28}H_{25}ClN_6O_8S$; C, 52.46; H, 3.93; N, 13.11. Found: C, 52.31; H, 3.76; N, 13.00.

3-{{(β-D-Glucopyranosylsulfanyl)-5-{[4-(4-chlorobenzylidene)]-[5-(oxo-(4,5-dihydro-1H-imidazol-1-yl)methyl]-2-(1,3-isoindolinon-2yl)}methyl}}-1-phenyl-1H-1,2,4-triazole (**3b**)

White powder (0.62 g, 87%), mp 245-247 ^{o}C; IR (KBr, cm^{-1}): 3399-2927 (OH), 1684 (C=O), 1597 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.40-3.49 (m, 3H, H-5′, H-6′,6″), 3.90-4.02 (m, 2H, H-3′,H-4′), 4.26-4.33 (m, 5H, H-2′, 2CH_2), 4.76 (brs, 2H, 2OH), 5.27 (brs, 2H, 2OH), 5.70 (d, *J* = 9.8 Hz, 1H, H-1′), 6.78 (s, 1H, CH), 7.20-7.30 (m,

4H, Ar-H), 7.40-7.60 (m, 5H, Ar-H), 7.71-7.90 (m, 4H, Ar-H) ppm. Anal. Calcd. For $C_{34}H_{29}ClN_6O_8S$; C, 56.94; H, 4.08; N, 11.72. Found: C, 56.82; H, 3.96; N, 11.59.

2-{{β-D-Glucopyranosylsulfanyl)-3-{[4-(4-chlorobenzylidene)]-[5-oxo-(4,5-dihydro)-1H-imidazol-1-oxoethyl]-2-(1,3-isoindolinon-2yl)}methyl}}-quinazolin-4(3H)-one (**6**)
White powder (0.69 g, 93%), mp 263-265 ^{o}C; IR (KBr, cm^{-1}): 3425-2922 (OH), 1630 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.45-3.53 (m, 3H, H-5′, H-6′,6″), 3.97-4.09 (m, 6H, H-3′,H-4′, 2CH$_2$), 4.22-4.25 (m, 1H, H-2′,), 4.74 (brs, 2H, 2OH), 5.32 (brs, 2H, 2OH), 5.77 (d, *J* = 9.8 Hz, 1H, H-1′), 6.75 (s, 1H, CH), 7.15-7.20 (m, 4H, Ar-H), 7.41-7.53 (m, 5H, Ar-H), 7.71-7.84 (m, 4H, Ar-H) ppm. EI-MS: *m/z* 564 746/748 [M^{+}]. Anal. Calcd. For $C_{35}H_{28}ClN_5O_{10}S$; C, 56.20; H, 3.50; N, 9.10. Found: C, 56.34; H, 3.78; N, 9.39.

Antitumor activity
The antitumor activity of the newly synthesized compounds was investigated against Ehrlich Ascites Carcinoma cells (EAC). These cells were maintained by weekly intraperitoneal transplantation of 2.5 x 10^6 cells in female Swiss albino mice. The tumor is characterized by a moderately rapid growth, which leads to the death of the mice in about 20 days due to the distal metastasis. EAC is of mammary origin; as spontaneous breast cancer served as the original tumor from which an ascites variant was obtained [17]. Ascites fluid was withdrawn under aseptic conditions (ultraviolet laminar flow system) from the peritoneal cavity of tumor bearing mice by needle aspiration after 7 days of EAC cells inoculation. To adjust the number of EAC cells/ml, tumor cells obtained were diluted several times with normal saline. EAC viable cells were counted by trypan blue exclusion method where, 10 µl trypan blue (0.05%) was mixed with 10 µl of the cell suspension. Within 5 min, the mixture was spread onto haemocytometer, covered with a cover slip and then the cells were examined under microscope. Dead cells are blue stained but viable cells are not [18]. Cell suspension was adjusted to contain 2.5 x 10^6 viable cells/ml. EAC cells, RPMI medium, drugs, and DMSO were added in sterile test tubes according to trypan blue exclusion method [18]. The cells were incubated for 1 and 24 h at 37 ^{o}C under a constant over lay of 5% CO_2. EAC viable cells were counted by trypan blue exclusion using haemocytometer as mentioned above. The cell surviving fraction was calculated from the relation *T/C*; where, *T* and *C* represent the number of viable cells in a unit volume and the number of total (viable dead) cells in the same unit volume, respectively (Table 2).

Table 1. In vitro antitumor activity of the tested compounds.

Compound	IC50 (µg/ml)	Compound	IC50 (µg/ml)
Doxorubicin	38	**3a**	42
1a	68	**3b**	41
1b	70	**4**	62.5
2a	52	**5**	50
2b	51	**6**	44

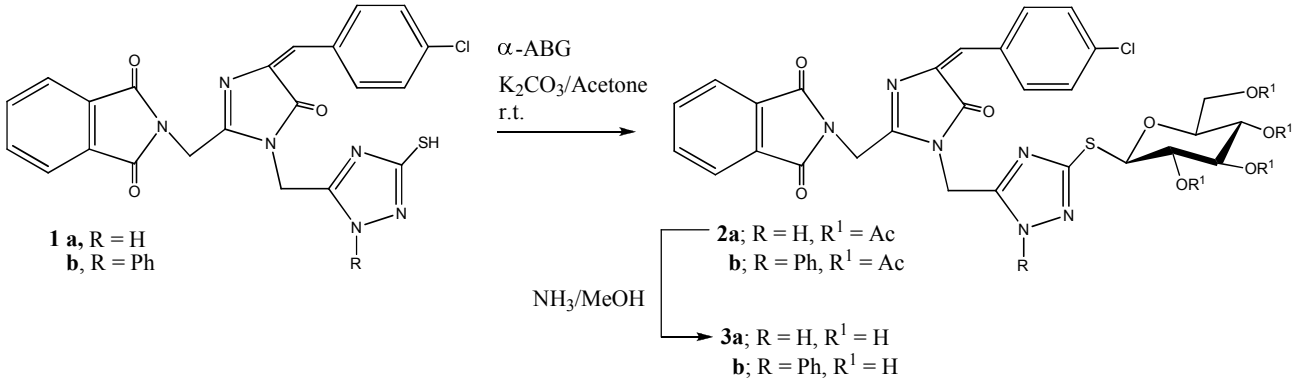
Antimicrobial activity
The agar diffusion method reported by Cruickshank *et al* [19] was used for the screening process. The bacteria and fungi were maintained on nutrient agar and Czapek's-Dox agar media, respectively. The assay medium flasks containing 50 mL of nutrient agar for bacteria and Czapek's-Dox agar medium for fungi respectively were allowed to reach 40-50 ^{o}C to be inoculated with 0.5 ml of the test organism cell suspension. The flasks were mixed well and poured each into a Petri dish (15 x 2 cm) and allowed to solidify. After solidification, holes (0.6 cm diameter) were made in the agar plate by the aid of a sterile cork poorer (diameter 6 mm). The synthesized target compounds were dissolved each in 2 ml DMSO. In these holes, 100 µl of each compound was placed using an automatic micropipette. The Petri dishes were left at 5 °C for 1 h to allow diffusion of the samples through the agar medium and retard the growth of the test organism. Plates were incubated at 30 ^{o}C for 24 h for bacteria and 72 h of incubation at 28 ^{o}C for fungi. DMSO showed no inhibition zones. The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin [20, 21] (50 µg/ml) and fusidic acid [22] (50 µg/ml) were used as standard for antibacterial and antifungal activity respectively. The observed zones of inhibition are presented in Table 2.

Table 2. In vitro antimicrobial activity by agar diffusion method of the tested compounds

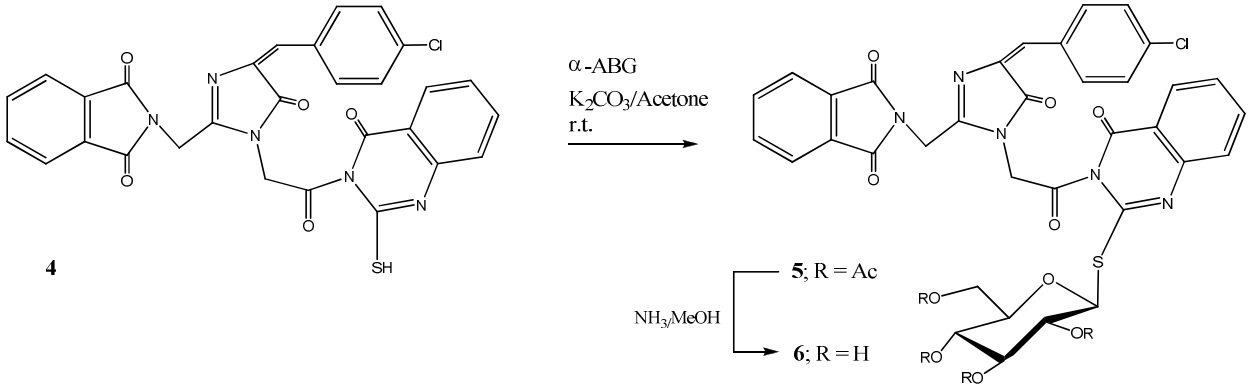
Compound No.	Zone of Inhibition (mm) of Microorganisms			
	Bacillus subtilis	*Escherichia coli*	*Candida albicans*	*Aspergillus flavus*
Penicillin	50	45	17	46
1a	20	17	8	22
1b	13	12	9	13
2a	30	25	12	9
2b	28	18	13	34
3a	41	40	15	41
3b	38	35	14	42
4	15	9	9	17
5	34	28	13	37
6	42	38	14	42

RESULTS AND DISCUSSION

In this investigation, when **1a,b** and/or **4** [16] were allowed to react with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in dry acetone and in the presence of anhydrous potassium carbonate gave the corresponding acetylated thioglucosides **2a,b**, and **5** in 88-95% yields. The assignments of sugar protons were based on the chemical shift equivalences to the assigned structures of related sugar thioglycosides [23,24]. Thus, the ^{1}H-NMR spectra of the glucosides **2a,b**, and **5** showed signals corresponding to the acetyl-methyl signals and the sugar protons in addition to the aromatic protons. The anomeric protons appeared at δ 5.70, 5.76, and 5.73 ppm with coupling constants *J* = 10.2 Hz for **2a,b**, and **5**, respectively, indicating the β-orientation of the thioglycosidic bond (Scheme 1 and 2).



Scheme 1. Sythesis of thioglucosides **2** and **3**.



Scheme 2. Synthesis of thioglucosides **5** and **6**.

Deprotection of the acetyl groups was carried out by using ammonia in methanol at room temperature to afford the corresponding free hydroxyl thioglucosides **3a,b**, and **6** in 85-93% yields The structures of the free hydroxyl glucosides were confirmed by IR, 1H-NMR, mass spectra, and elemental analysis (see experimental part).

The antitumor efficacy of the compounds against ESC cell lines was demonstrated compared with doxorubicin. The obtained results revealed that compounds **3a,b** and **6** were the most active derivatives among the series of tested compounds and affected the EAC cell viability on a dose dependent manner whereas other compounds exhibited little or no activity. The effective dose calculated as IC_{50}, which correspond to the compound concentration resulted in 50% mortality in the total cells count and presented in (Table 1). The free hydroxyl thioglucosides **3a,b** and **6** displayed the highest activity with IC_{50} values 42, 41, and 44 µg/ml, respectively, followed by compounds **1a,b**, **2a,b**, and **4**.

The synthesized compounds were screened *in vitro* for their antimicrobial activities [19-22] against *Escherichia coli* NRRL B-210 (Gram -ve bacteria), *Bacillus subtilis* NRRL B-543 (Gram +ve bacteria), *Aspergillus flavus* and *Candida albicans* NRRL Y-477 (Fungi). The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Tetracycline was used as standard for the antimicrobial activity and the observed zone of inhibition is presented in Table 2. The results indicated generally that tested compounds did not show high activity against bacteria under test (*Escherichia coli* and *Bacillus subtilis*) while some compounds revealed high activity against fungi. The free hydroxyl thioglucosides **3a,b** and **6** were the most active against the microorganisms followed by the *O*-acetylated thioglucosides **2a,b** and **5**. The heterocyclic bases **1a,b** and **4** showed the low activity against the microorganisms in comparison with the protected and deprotected thioglucosides.

Structure Activity Relationship (SAR) Studies

The antimicrobial activity results and structure activity relationship indicated that the attachment of thioglucoside moiety to triazole and/or oxadiazoline ring system resulted in increase of antimicrobial and antitumor activities. Furthermore, the free hydroxyl thioglucosides showed higher activity than the corresponding acetylated analogues.

CONCLUSION

In conclusion, the antimicrobial as well as antitumor screening suggests that all the newly synthesized compounds showed good to very good activity. Hence the fact that the compounds prepared in this study are chemically unrelated to the current medication, suggests that further work with similar analogues is clearly warranted.

REFERENCES

[1] M. Bretner, T. Kulilowski, J. M. Dzik, M. Balinska, W. Rode, D. G. Shugar, *J. Med. Chem.*, **1993**, 36, 3611.
[2] T. Hiromichi, B. Mansori, U. Masouri, T. Hideaki, S. Kouichi, N. Issei, S. Shiro, T. W. Richared, D. C. Erik, M. Todashi, *J. Med. Chem.*, **1991**, 34, 1394.
[3] G. H. Elgemeie, S. R. El-Ezbawy, H. A. El-Aziz, *Synth. Commun.*, **2001**, 31, 3453.
[4] W. A. El-Sayed, O. M. Ali, M. M. Hathoot, A. A.-H. Abdel-Rahman, *Z. Naturforsh*, **2010**, 65c, 22.
[5] W. A. El-Sayed, I. F. Nassar, A. A.-H. Abdel-Rahman, *J. Heterocyclic Chem.*, **2011**, 48, 135.
[6] A. A. Fadda, A. A.-H. Abdel-Rahman, W. A. El-Sayed, T. A. Zidan, F. A. Badria, *Chem. Heterocyclic Compounds*, **2011**, 47, 856.
[7] A. A.-H. Abdel-Rahman, E. S. H. El Ashry, R. R. Schmidt, *Carbohydr. Res.*, **2002**, 337, 195.
[8] E. S. H. El Ashry, L. F. Awad, A. I. Atta, *Tetrahedron*, **2006**, 62, 2943.
[9] E. S. H. El Ashry, L. F. Awad, H. M. Abdel Hamid, A. I. Atta, *Synthetic Commun.*, **2006**, 36, 2769.
[10] E. S. H. El Ashry, N. Rashed, A. H. Shobier, *Pharmzie*, **2000**, 55, 251; *Pharmzie*, **2000**, 55, 331; *Pharmzie*, **2000**, 55, 403.
[11] W. A. El-Sayed, M. M. M. Ramiz, A. A.-H. Abdel-Rahman, *Monatsh. Chem.*, **2008**, 139, 1499.
[12] W. A. El-Sayed, N. M. Fathi, W. A. Gad, E. S. H. El-Ashry, *J. Carbohyd. Chem.*, **2008**, 27, 357.
[13] W. A. El-Sayed, A. A.-H. Abdel-Rahman, M. M. M. Ramiz, *Z. Naturforsch.*, **2009**, 64c, 323.
[14] W. A. El-Sayed, I. F. Nassar, A. A.-H. Abdel-Rahman, *Monatsh. Chem.*, **2009**, 140, 365.
[15] W. A. El-Sayed, A. E. Rashad, S. M. Awad, M. M. Ali, , *Nucleosides Nucleotides & Nucleic Acids*, **2009**, 28, 261.
[16] A. A. Aly, S. G. Donia, A. A. F. Wasfy, M. M. Azab, A. Y. El-Gazzar, *Egyptian J. Chem.*, **2008**, 51(5), 715.

[17] R. Cruickshank, J. P. Duguid, B. P. Marion, R. H. A. Swain, Medicinal Microbiology, twelfth ed., vol. II, Churchill Livingstone, London, **1975**, 196.
[18] R. Dahiya, *Sci. Pharm.*, **2008**, 76, 217.
[19] H. C. Su, K. Ramkissoon, J. Doolittle, M. Clark, J. Khatun, A. Secrest, M. C. Wolfgang, M. C. Giddings, *Antimicrob. Agents Chemother.*, **2010**, 4626.
[20] T. F. Poyner, B. K. Dass, *J. Eur. Acad. Dermatol. Venereol.*, **1996**, 7, S23.
[21] M. Gupta, U. K. Mazumder, R. S. Kumar, T. S. Kumar, *Acta Pharmacol. Sin.*, **2004**, 25, 1070.
[22] D. A. Ribeiro, M. E. Marques, D. M. Salvadori, *Braz. Dent. J.*, **2006**, 17, 228.
[23] W. A. El-Sayed, A. A.-H. Abdel-Rahman, M. M. M. Ramiz, *Z. Naturforsch.*, **2009**, 64c, 323.
[24] W. A. El-Sayed, I. F. Nassar, A. A.-H. Abdel-Rahman, *Monatsh. Chem.*, **2009**, 140, 365.