European Journal of Medicinal Chemistry 44 (2009) 2597–2600

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and biological evaluation of some thiazolidinone derivatives of steroid as antibacterial agents

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ARTICLE INFO

Article history:
Received 31 March 2008
Received in revised form 20 July 2008
Accepted 1 September 2008
Available online 16 September 2008

Keywords:
Thiosemicarbazone
Thiazolidinone
Antibacterial activity
Amoxicillin

ABSTRACT

Steroidal thiazolidinone derivatives were prepared by the multi-step reactions of steroid. It is prepared from steroidal thiosemicarbazones with ethyl bromoacetate in dioxane. Steroidal thiosemicarbazones were prepared by the reaction of thiosemicarbazide with steroidal ketones. The structures of these compounds were elucidated by IR, ^{1}H NMR, Fab mass spectrometries and their purities were confirmed by elemental analyses. The antibacterial activity of these compounds was evaluated by the disk diffusion assay against two Gram-positive and two Gram-negative bacteria and then the minimum inhibitory concentration (MIC) of compounds was determined. The results showed that steroidal thiazolidinone derivatives are better in inhibiting the growth as compared to steroidal thiosemicarbazone derivatives of both types of the bacteria (Gram-positive and Gram-negative). Compounds **7** and **8** are better antibacterial agents as compared to standard drug Amoxicillin.

1. Introduction

The treatment of infectious diseases still remains an important and challenging problem. Despite search of novel antimicrobial agents is a field of current and growing interest. Many compounds have been synthesized with this aim, their clinical use has been limited by their relatively high risk of toxicity, bacterial resistance and/or pharmacokinetic deficiencies. A major research emphasis to counter this growing problem is the development of antimicrobials structurally unrelated to the existing molecules. One possibility to achieve this goal is the combination of a steroid molecule with structural elements possessing appropriate biological activities [1–3]. In addition, considerable attention has been focused on substituted thiosemicarbazone derivatives due to their interesting biological activity. Compounds with a thiosemicarbazone structure are known to possess tranquilizing, muscle relaxant, psychoanaleptic, hypnotic, ulcerogenic, antidepressant, antibacterial, antifungal and analgesic anti-inflammatory properties [4–11]. Steroidal thiosemicarbazones dramatically increase the diversity of certain biological properties [12–14]. The presence of thiazolidinone moiety in the structure of several naturally occurring molecules with important antibiotic, immunosuppressive and antitumor activities has been known for several years [15–18]. The aminothiazole ring system has found application in drug development for the treatment of HIV-infection, hypertension and inflammation [19]. Several thiazolidinone derivatives have been shown to exhibit excellent bactericidal [20] fungicidal [21,22] and anthelmintic [23] activities. Recently thiazolidinones have been synthesized and screened for possible antimicrobial activities [24,25]. Thiazolidinone as evident from the literature, it was noted that lot of research has been carried out on thiazolidinone derivatives have been antibacterial but no work has been done on steroidal (cholesterol) derivatives screening on bacterial. In this paper the steroidal thiazolidinone derivatives have been synthesized by the reaction of steroidal thiosemicarbazones and ethyl bromoacetate in dioxane. The activities of these compounds were screened *in vitro* against bacteria such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhimurium* and *Escherichia coli*.

2. Results and discussion

Thiosemicarbazones prepared by condensing the steroidal ketones with cyclopentyl thiosemicarbazide in the presence of catalytic amount of conc. HCl gave yield 63–69%. 3β-acetoxycholest-6-one [26], 3β-chloro-cholest-6-one [27], 5α-cholest-6-one [28] were prepared according to the published methods. The thiosemicarbazone derivatives were used as starting material for the preparation of thiazolidinone derivatives. The thiazolidinone derivatives were synthesized by the literature procedure [29] as indicated in Scheme 1. Thiosemicarbazones were refluxed with ethyl bromoacetate in dioxan for 12 h and after that solvent was removed under reduced pressure and crystallization was done in ethanol. All the compounds were soluble in DMSO and ethanol. The

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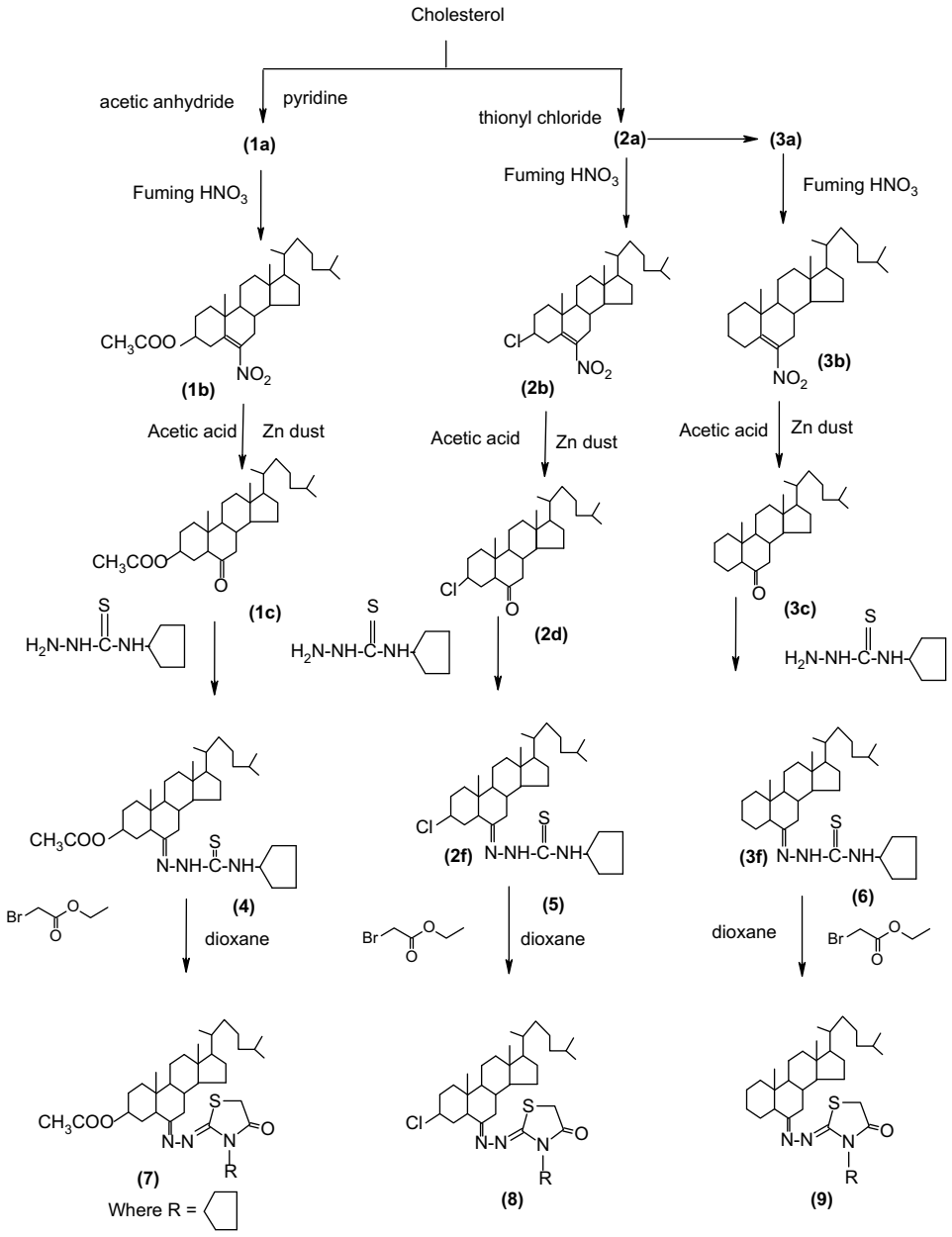
0223-5234/$ – see front matter © 2008 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmech.2008.09.004



Scheme 1. Schematic diagram showing the synthesis of compounds **7**–**9**. Where compound (**1a**): 3β-acetoxycholest-5-ene, compound (**2b**): 3β-chlorocholest-5-ene, compound (**3a**): cholest-5-ene.

structures of all the compounds were established by means of their IR, ^{1}H NMR, FAB mass spectra and the elemental analyses were carried out to check the purity of the compounds.

Assignments of selected characteristic IR band positions provide significant indication for the formation of the cyclized thiazolidinone analogues of thiosemicarbazones. All the compounds showed ν (C═N) stretch at 1562–1572 cm^{-1} due to the ring closure. In addition, the absorption band at 1162–1185 cm^{-1} was attributed to the ν (C–N) stretch vibrations. The compounds showed intense bands at 624–642 cm^{-1} due to ν (C–S) stretch, which also confirm the formation of thiazole ring in all the compounds.

Further evidence for the formation of thiazolidinone compounds was obtained from the ^{1}H NMR spectra, which provide diagnostic tools for the positional elucidation of the protons. Assignments of the signals are based on the chemical shifts and intensity patterns. The thiazole protons of all the compounds are shown as singlet in the range 3.80–4.21 ppm.

Characteristic peaks were observed in the mass spectra of compounds **7**–**9**, which followed the similar fragmentation pattern. The spectrum of compound **7** showed a molecular ion peak ($M^{+\bullet}$) at m/z 626, compound **8** showed a molecular ion peak ($M^{+\bullet}$) at m/z 601/603 and compound **9** showed a molecular ion peak ($M^{+\bullet}$) at m/z 569. Further fragmentation pattern of these compounds is given in the in Section 3.

2.1. Antibacterial activity

The compounds (**4**–**9**) were tested for their antibacterial activities by disc-diffusion method [30] using nutrient broth medium [contained (g/L): beef extract 3 g; peptone 5 g; pH 7.0]. The Gram-positive bacteria and Gram-negative bacteria utilized in this study consisted of *S. aureus*, *S. pyogenes*, *S. typhimurium* and *E. coli*. In the disc-diffusion method, sterile paper discs (05 mm) impregnated with compound dissolved in dimethylsulfoxide (DMSO) at

concentration 100 μg/mL were used. Then, the paper discs impregnated with the solution of the compound tested were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35 °C for 24 h. After incubation, the growth inhibition zone are shown in Table 1. The thiazolidinone derivatives were further checked by MIC method. The results are presented in Table 2. The molecular structure of these active compounds showed enhanced activity. The distinct differences in the antibacterial property of these compounds further justify the purpose of this study. The importance of such work lies in the possibility that the new compound might be more efficacious drugs against bacteria for which a thorough investigation regarding the structure–activity relationship, toxicity and in their biological effects which could be helpful in designing more potent antibacterial agents for therapeutic use.

3. Experimental

The entire chemicals were purchased from Aldrich Chemical Company (U.S.A) and were used without further purification. The reactions were monitored by precoated aluminium silica gel 60F 254 thin layer plates procured from Merck (Germany). All melting points were measured with a capillary apparatus and are uncorrected. All the compounds were routinely checked by IR, ^{1}H NMR and mass spectrometries. IR spectra were recorded in KBr on a Perkin–Elmer model 1620 FTIR spectrophotometer. ^{1}H NMR spectra were recorded at ambient temperature using a Brucker spectroscopin DPX-300 MHz spectrophotometer in $CDCl_3$ and DMSO. The following abbreviations were used to indicate the peak multiplicity s- singlet, d- doublet, t- triplet, m- multiplet. FAB mass spectra were recorded on a JEOL SX102 mass spectrometer using Argon/Xenon (6 kV, 10 mB) gas. Column chromatography was performed on silica gel (Merck). Anhydrous sodium sulfate was used as a drying agent for the organic phase.

3.1. Synthesis of thiosemicarbazones: a general method

Steroidal thiosemicarbazones were synthesized (Scheme 1) by refluxing the solution of cyclopentyl thiosemicarbazide (0.03 mol) and the alcoholic solution of steroidal ketones (0.03 mol) in ethanol in the presence of few drops of HCl at 80 °C for 5 h with continuous stirring. After cooling the compounds were filtered and recrystallized from methanol.

3.1.1. 3β-Acetoxycholest-6-one cyclopentyl thiosemicarbazone (**4**)

Yields: 64%; Mp. 186–188 °C; Anal. Calcd. for $C_{35}H_{59}N_3O_2S$: C, 71.79; H, 10.08; N, 7.17. Found: C, 70.08; H, 9.25; N, 6.58; IR (ν_{max}; cm^{-1} KBr): 3385 (N–H), 1738 ($OCOCH_3$), 1625 (weak, C=C), 1586 (C=N), 1158 (C=S); ^{1}H NMR δ: 9.82 (s, 1H, N–H), 7.32 (d, 1H, –NH), 4.5 (Br, m, w1/2 = 17 Hz, C3α-H, axial), 4.7 (m, 8H, $-CH_2$) 2.4 (s, 3H, $OCOCH_3$), δ 1.22 (C10–CH_3), 0.75 (C13–CH_3) 0.95, 0.89 (other methyl protons); MS ($M^{+\bullet}$) at *m/z* 586, 527 (M-AcO), 517 (M-C_5H_9), 502 (M-$C_5H_{10}N$), 458 (M-$C_6H_{10}NS$), 443 (M-$C_6H_{11}N_2S$).

Table 1
Antibacterial activity of steroidal derivatives positive control (Amoxicillin) and negative control (DMSO) measured by the halo zone test (unit, mm).

Compounds	Corresponding effect on microorganisms			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
4	12.6 ± 0.5	11.4 ± 0.4	10.4 ± 0.4	13.3 ± 0.4
5	11.5 ± 0.6	10.2 ± 0.3	11.2 ± 0.3	11.7 ± 0.4
6	09.4 ± 0.3	10.2 ± 0.2	10.4 ± 0.4	12.3 ± 0.5
7	18.5 ± 0.7	21.4 ± 0.4	19.6 ± 0.4	22.8 ± 0.7
8	20.4 ± 0.5	21.4 ± 0.3	19.4 ± 0.5	22.5 ± 0.6
9	14.5 ± 0.5	15.2 ± 0.6	13.6 ± 0.5	14.2 ± 0.6
Amoxicillin	17.0 ± 0.5	18.2 ± 0.4	17.2 ± 0.8	20.0 ± 0.2
DMSO	–	–	–	–

Table 2
Minimum inhibition concentration (MIC) of steroidal thiazolidinone derivatives, positive Amoxicillin control.

Strain	MIC (μg mL^{-1})			
	Compounds			Positive control
	7	**8**	**9**	
S. aureus	64	32	128	32
S. pyogenes	32	32	128	32
S. typhimurium	64	64	128	32
E. coli	32	64	128	32

3.1.2. 3β-Chloro-cholest-6-onecyclopentyl thiosemicarbazone (**5**)

Yields: 69%; Mp. 174–176 °C; Anal. Calcd. for $C_{33}H_{56}N_3SCl$: C, 70.46; H, 9.96; N, 7.47. Found: C, 69.32; H, 8.47; N, 6.35; IR (ν_{max}; cm^{-1} KBr): 3385 (N–H), 1570 (C=N), 1165 (C=S), 718 (C–Cl); ^{1}H NMR: δ 9.84 (s, 1H, NH), 7.6 (d, 1H), 4.6 (br, m, 1H, w1/2 = 15 Hz, C3α axial), 4.4 (m, 8H, $-CH_2$) 1.22 (C10–CH_3), 0.72 (C13–CH_3), 1.02, 0.87 (remaining methyl protons); Mass spectra ($M^{+\bullet}$) at *m/z* 561/563, 528 (M-Cl), 492/494 (M-C_5H_9), 477/478 (M-$C_5H_{10}N$), 433/435 (M-$C_6H_{10}NS$), 418/420 (M-$C_6H_{11}N_2S$).

3.1.3. Cholest-6-onecyclopentyl thiosemicarbazone (**6**)

Yields: 63%; Mp.156–158 °C; Anal. Calcd. for $C_{33}H_{57}N_3S$: C, 75.14; H, 10.81; N, 7.96. Found: C, 74.95; H, 9.26; N, 6.95; IR (ν_{max}; cm^{-1} KBr): 3386 (NH), 1628 (C=C), 1568 (C=N) 1185 (C=S), 2928 (C–H); ^{1}H NMR: δ 9.7 (s, 1H, NH), 5.9 (d, 1H, NH), 0.07 (C10–CH_3), 0.66 (C13–CH_3), 0.90 and 0.82 (remaining proton); Mass spectra ($M^{+\bullet}$) at *m/z* 528, 459 (M-C_5H_9), 444 (M-$C_5H_{10}N$), 400 (M-$C_6H_{10}NS$), 385 (M-$C_6H_{11}N_2S$).

3.2. General procedure for the syntheses of the steroidal thiazolidinone

To a suspension of the steroidal thiosemicarbazone (**4**–**6**) (1.5 mmol) in dioxane (20 mL), an equivalent amount of ethyl bromoacetate (1.5 mmol) was added. The reaction mixture was heated under reflux for 12 h, concentrated to approximately half its volume and allowed to cool to room temperature. The separated solid product was filtered, washed with ethanol, dried and crystallized from ethanol.

3.2.1. 3β-Acetoxycholest-6-diazo(4-thiazolidinon)cholestane (**7**)

Yields: 73%; Mp.148–152 °C; Anal. Calcd. for $C_{37}H_{59}N_3SO_3$: C, 71.04; H, 9.42; N, 6.72. Found: C, 69.32; H, 9.46; N, 5.85; IR (ν_{max}; cm^{-1} KBr): 2936 (C–H), 722 ($OCOCH_3$), 1682 (C=O), 572 (C=N), 1185 (C–N), 624 (C–S); ^{1}H NMR: δ 4.6 (m, 10H, $-CH_2$), 4.5 (br, m, 1H, w1/2 = 15 Hz, C3α axial), 4.21 (s, 2H, thiazole-H), 2.3 (s, 3H, $OCOCH_3$), 1.20 (C10–CH_3), 0.76 (C13–CH_3), 0.98 and .88 (remaining proton); Mass spectra ($M^{+\bullet}$) at *m/z* 626, 567 (M-AcO), 598 (M-CO), 584 (M-C_2H_2O), 557 (M-C_5H_9), 552 (M-C_2H_2OS), 443 (M-$C_8H_{11}N_2SO$).

3.2.2. 3β-Chloro-cholest-6-diazo(4-thiazolidinon)cholestane (**8**)

Yields: 75%; Mp. 134–136 °C; Anal. Calcd. for $C_{35}H_{56}N_3SOCl$: C, 69.76; H, 9.30; N, 6.72. Found: C, 68.50; H, 8.88, N, 6.62; IR (ν_{max}; cm^{-1} KBr): 2922 (C–H), 1568 (C=N), 1162 (C–N), 715 (C–Cl), 632 (C–S); ^{1}H NMR (DMSO-d_6) (δ): 4.72 (br, m, 1H, w1/2 = 15 Hz axial C3α-H), 4.11 (s, 2H, thiazole-H) 1.20 (C10–CH_3), 0.74 (C10–CH_3), 0.88, 1.08 (other methyl protons); Mass spectra ($M^{+\bullet}$) at *m/z* 601/603, 567 (M-Cl), 587/589 (M-CH_2), 573/575 (M-CO), 559/561

(M-C_2H_2O), 532/534 (M-C_5H_9), 527/529 (M-C_2H_2OS), 418/420 (M-$C_8H_{11}N_2SO$).

3.2.3. 6-Diazo(4-thiazolidinon)cholestane (**9**)

Yields: 68%; Mp. 116–118 °C; Anal. Calcd. for ($C_{35}H_{57}N_3SO$): C, 74.07; H, 10.05; N, 7.40. Found: C, 73.52; H, 9.35; N, 6.80; IR (ν_{max}; cm^{-1} KBr): 2935 (C–H), 1562 (C═N), 1178 (C–N), 1672 (C═O), 642 (C–S); ^1H NMR (DMSO-d_6) (δ): 3.8 (s, 2H, thiazole-H) 1.22, (C10–CH_3), 0.69 (C10–CH_3), 0.82, 1.10 (other methyl protons); Mass spectra ($M^{+\bullet}$) at m/z 569, 555 (M-CH_2), 541 (M-CO), 527 (M-C_2H_2O), 500 (M-C_5H_9), 495 (M-C_2H_2OS), 386 (M-$C_8H_{11}N_2SO$).

3.3. Organism culture and in vitro screening

Antibacterial activity was done by the disk diffusion method with minor modifications. *S. aureus*, *S. pyogenes*, *S. typhimurium* and *E. coli* were sub-cultured in BHI medium and incubated for 18 h at 37 °C, and then the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^{-5} CFU mL^{-1}: 10 μL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40 °C and poured onto an agar plate in a laminar flow cabinet. Five paper disks (05 mm in diameter) were fixed onto nutrient agar plate. 1 mg of each test compound was dissolved in 100 μL DMSO to prepare stock solution and from stock solution different concentrations 10, 20, 25, 50, and 100 μg/μL of each test compound were prepared. These compounds of different concentrations were poured over disk plate onto it. Amoxicillin (30 μg/disk) was used as standard drug (positive control). DMSO poured disk was used as negative control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. Table 1 reports the inhibition zones (mm) of each compound and the controls. The minimum inhibitory concentration (MIC) was evaluated by the macro dilution test using standard inoculums of 10^{-5} CFU mL^{-1}. Serial dilutions of the test compounds, previously dissolved in dimethylsulfoxide (DMSO) were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 μg/mL to each tube was added 100 μL of a 24 h old inoculum. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h, at 37 °C, and the results obtained are presented in (Table 2). Tests using DMSO and Amoxicillin as negative and positive controls.

4. Conclusion

This research examined the antibacterial activities of these compounds were carried out against culture of bacteria. The biological behavior of these compounds revealed that chloro and acetoxy substituents on the 3β-position of the steroidal thiazolidinone ring increased the antibacterial activity. Among all the six compounds compounds **7** and **8** showed better antibacterial activity than their respective drug.

Acknowledgment

Authors are thankful to Head, Department of Chemistry, Punjabi University Patiala, for providing laboratory facilities for research.

References

[1] R.K. Pettit, G.D. Cage, G.R. Pettit, Liebman, Int. J. Antimicrob Agents 15 (2000) 299–304.
[2] P.B. Savage, Eur. J. Org. Chem. 5 (2002) 759–768.
[3] P.B. Savage, C. Li, U. Taotafa, B. Ding, Q. Guan, FEMS Microbiol. Lett. 217 (2002) 1–7.
[4] I.H. Hall, S.Y. Cheen, B.J. Barnes, X.D. Wext, Met. Based Drugs 6 (1999) 143–147.
[5] E. Bermejo, R. Carballo, A. Castineiras, R. Dominguez, A.E. Liberta, C. Maichle-Mossmer, D.X.Z. Wext, Naturforsch 54 (1999) 777–787.
[6] J.M. Perez, A.I. Matesanz, A. Marin-Ambite, P. Navarro, C. Alonso, P. Souza, J. Inorg. Biochem. 75 (1999) 255–261.
[7] K.H. Reddy, P.S. Reddy, P.R. Babu, J. Inorg. Biochem. 77 (1999) 169–176.
[8] F.P. Kelly, A.M.Z. Slawin, A. Soriano-Rama, J. Chem. Soc., Dalton Trans. (1996) 53–59.
[9] D.X. West, S.B. Pardhye, P.B. Sonawane, Struct. Bond. 76 (1991) 1–50.
[10] A.E. Liberta, D.X. West, BioMetals 5 (1992) 121–126.
[11] D.X. West, A.E. Liberta, S.B. Padhye, R.C. Chikate, P.B. Sonawane, A.S. Kumbhar, R.G. Yerande, Coord. Chem. Rev. 123 (1993) 49–71.
[12] M. Merlani, L.S. Amiranashvili, M.G. Davitishvili, E.P. Kemertelidze, K. Papdopoulos, E. Yannakopoulos, Chem. Nat. Compd. 42 (2006) 194–197.
[13] M. Merlani, E.P. Kemertelidze, K. Papadopoulos, N. Men'Shova, Russ. J. Bioorg. Chem. 30 (2004) 497–501.
[14] A. Omar, M.E. Mohsen, S.M. El-Khawass, A.B. Makar, N.M. Bakry, T.T. Daabees, Pharmazie 33 (1978) 577–580.
[15] J. Mulzer, A. Mantoulider, E. Ohler, Tetrahedron Lett. 38 (1997) 7725–7728.
[16] A. Badorc, M.F. Bordes, P. DeCointel, P. Savi, A. Lale, M. Petitou, J.P. Maffrand, J.M. Herbert, J. Med. Chem. 40 (1997) 3393–3401.
[17] H.M. Vagdevi, V.P. Vaidya, K.P. Latha, B. Padmashali, Ind. J. Pharm. Sci. 68 (2006) 719–725.
[18] M. Rzasa, H.A. Shea, D. Romo, J. Am. Chem. Soc. 120 (1998) 591–592.
[19] P.C. Kearney, M. Fernandz, J.A. Flygare, J. Org. Chem. 63 (1988) 196–200.
[20] P.S. Kenderekar, R.F. Siddiqui, P.S. Patil, S.R. Bhusare, R.P. Pawar, Ind. J. Pharm. Sci. 65 (2003) 313–315.
[21] I.R. Siddiqui, P.K. Singh, J. Agric. Food Chem. 51 (2003) 7062–7065.
[22] H.L. Liu, Z. Li, T. Anthonsen, Molecules 5 (2000) 1055–1061.
[23] H.M. Vagdevi, V.P. Vaidya, K.P. Latha, B. Padmashali, Indian J. Pharm. Sci. 68 (2006) 719–725.
[24] A.R. Kulkarni, V.H. Kulkarni, J. Keshavayya, V.I. Hukkeri, H.W. Sung, Macromol. Biosci. 5 (2005) 490–493.
[25] J.V. Galietta, A.S. Verkman, J. Biol. Chem. 277 (2002) 29224–29230.
[26] R.K. Callow, V.H.T. James, J. Chem. Soc. (1956) 4744–4748.
[27] A.H. Millurn, E.V. Truter, J. Chem. Soc. (1956) 1736–1739.
[28] G.W. Dauben, K.H. Takemura, J. Am. Chem. Soc. 75 (1953) 6302–6304.
[29] A.A. Bekhit, H.M.A. Ashour, Y.S.A. Ghany, A.A. Bekhit, A. Baraka Eur. J. Cancer, 2008, Med. Chem. 43 (2008) 456–463.
[30] W.A. Bauer, M.W. Kirby, C.J. Sherris, M. Turck, Am. J. Clin. Pathol. 45 (1966) 493–496.