Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 3065–3068

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antimicrobial Activity of New 3α-Hydroxy-23,24-bisnorcholane Polyamine Carbamates

Hong-Seok Kim,[a,*] Kyung-Chan Kwon,[a] Ki Soo Kim[a] and Cheol Hae Lee[b]

[a]*Department of Industrial Chemistry, Kyungpook National University, Taegu 702-701, South Korea*
[b]*Bioorganic Division, Korea Research Institute of Chemical Technology, Taejeon 305-600, South Korea*

Received 13 June 2001; accepted 15 September 2001

Abstract—3α-Hydroxy-23,24-bisnorcholane spermidine and spermine carbamates **2**–**7** have been synthesized and their antimicrobial and hemolytic activities were evaluated. They exhibited excellent in vitro activities especially against methicillin-resistant *Staphylococcus aureus*. © 2001 Elsevier Science Ltd. All rights reserved.

Recently, various polyamines conjugated to cholesterol, cholenic acid, and bile acids have been reported.[1] Some of these sterol–polyamine conjugates exhibit antimicrobial,[2–5] anti-trypanosomal activities,[6] and DNA binding affinity.[7–10] Squalamine, isolated from the dogfish shark, *Squalus acanthias*, displays potent activity against both Gram-positive and Gram-negative bacteria.[11–13] It also shows significant preclinical antitumor activity against human lung cancer by the antiangiogenic effects.[14,15] Due to the emergence of penicillin-resistant *Staphylococei*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus pneunomiae*, and vancomycin-resistant *Enterococcus* in hospitalized patients,[16–18] the sterol–polyamine conjugates as new classes of antibiotics have attracted much interest in recent years.

Recently, we[2] and others[3–5] synthesized and evaluated squalamine analogues which exhibited comparable antimicrobial activity against Gram-positive and Gram-negative bacteria to squalamine (**1**), a 5α-hydrido, 7α-hydroxyl, 24-sulfated cholestane steroid conjugated to a spermidine at C-3β. As a part of our continuing studies on SAR of sterol–polyamine conjugates, we have synthesized polyamine 22-carbamates of 3α-hydroxy-23,24-bisnorcholane to investigate the effects of carbamates in the steroidal backbone and number of charges along the polyamine backbone on their antimicrobial and hemolytic activities. In this paper, we describe the synthesis and evaluation of a new class of antimicrobial agents made by conjugating different polyamines to a series of 3-keto-23,24-bisnorcholan-22-ol.

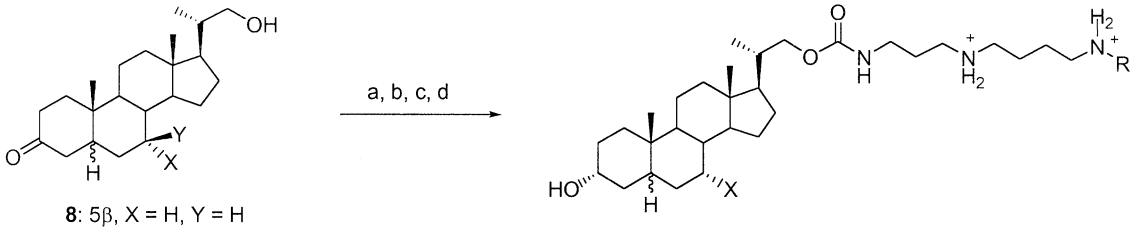
Six carbamates shown in Figure 1 were prepared to test the antimicrobial and hemolytic activities. Compound **2** and **6** have the 23,24-bisnor-5β-cholane frame except that **2** and **6** have spermidine and spermine moiety. Compounds **3**–**5** have the same 23,24-bisnor-5α-cholane spermidine carbamate with different substituent at C-7 on steroid, that is **4** and **5** have hydroxyl and fluoro group, respectively.

For the synthesis of 22-carbamate, the requisite steroid skeletons, 3-keto-23,24-bisnor-5β-chol-22-ol **8** and 5α-isomer **9** were prepared from 3-keto-23,24-bisnorchol-4-en-22-ol. Due to the difficulty in chromatographic separation of each isomer **8** and **9** obtained from hydrogenation of 3-keto-23,24-bisnorchol-4-en-22-ol in the presence of 5% Pd/C in ethyl acetate, the latter was protected by *tert*-butyldimethylsilyl chloride and hydrogenated. Chromatographic separation of the resulting hydrogenated mixture, followed by deprotection of each isomer with *p*-TSA produced **8** and **9** in 27 and 65%, respectively. 3-Keto-7α-fluoro-23,24-bisnor-5α-chol-22-ol **10** was synthesized by fluorination of 22-*tert*-butyldimethylsilyloxy-3-keto-23,24-bisnor-5α-chol-7β-ol with DAST (diethylaminosulfur trifluoride) and 3,7-diketo-23,24-bisnor-5α-chol-22-ol **11** was prepared by literature procedure.[2,19]

The 22-carbamates **2**–**7** were synthesized by coupling of the sterol with either protected spermidine or spermine as shown in Scheme 1. The activation of **8** with phosgene

*Corresponding author. Tel.: +82-53-950-5588; fax: +82-53-950-6594; e-mail: kimhs@knu.ac.kr

0960-894X/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(01)00632-1



8: 5β, X = H, Y = H
9: 5α, X = H, Y = H
10: 5α, X = F, Y = H
11: 5α, X, Y = O

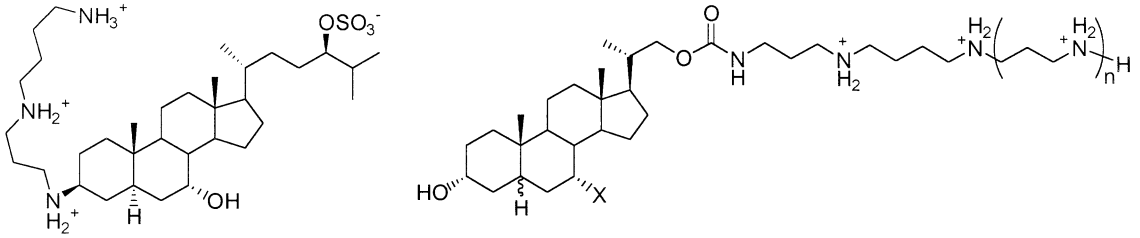
2: 5β, X = H, R = H, 35%
3: 5α, X = H, R = H, 66%
4: 5α, X = OH, R = H, 51%
5: 5α, X = F, R = H, 18%
6: 5β, X = H, R = $(CH_2)_3NH_3^+$, 42%
7: 5α, X = H, R = $(CH_2)_3NH_3^+$, 60%

Scheme 1. Synthesis of bisnorcholane polyamine carbamates: (a) $COCl_2$, DIPEA, CH_2Cl_2; (b) polyamine moiety, CH_2Cl_2; (c) K-Selectride, THF; (d) 10% HCl, MeOH.

solution (~ 20% in toluene) in the presence of diisopropylethylamine (DIEPA) gave bisnorcholanyl 22-chloroformate, followed by coupling of the latter with (N^1,N^5-di-*tert*-butoxycarbonyl)-1,8-diamino-5-azaoctane[20] to produce 23,24-bisnorcholane spermidine 22-carbamate. Reduction of the 22-carbamate with K-selectride,[21] followed by deprotection of Boc with 10% HCl provided 3α-hydroxy-23,24-bisnor-5β-cholane spermidine carbamate **2** in 35% overall yield. Similar coupling of 5α-analogues **9**, **10**, and **11** with (N^1,N^5-di-*tert*-butoxycarbonyl)-1,8-diamino-5-azaoctane, followed by subsequent K-selectride reduction and deprotection afforded **3**, **4**, and **5** in 66, 51, and 18% overall yields, respectively. The 3α-hydroxy-23,24-bisnorcholane spermine carbamates **6** and **7** were prepared by the same procedure for the preparation of **2** starting from compounds **8**, **9**, and (N^1,N^4,N^9-tri-*tert*-butoxycarbonyl)-1,12-diamino-4,9-diazadodecane[22,23] by three sequential steps in 42 and 60% overall yields, respectively. All of the 3α-hydroxy-23,24-bisnorcholane polyamine 22-carbamates were isolated as solid that gave satisfactory elemental analyses for C, H, and N. They also displayed the expected ^{1}H NMR and low-resolution chemical ionization mass spectra.

Minimum inhibitory concentrations (MICs) of the carbamates **2**–**7** were tested in triplicate against four strains of Gram-positive and six strains of Gram-negative bacteria as previously described.[2] Minimal hemolytic concentrations (MHCs) of **2**–**7** were determined in triplicate by literature procedure.[3] The results of in vitro MIC and MHC evaluation of **2**–**7** are detailed in Table 1. Both spermidine carbamates **2**–**5** and spermine carbamates **6**–**7** exhibited comparable and more potent antimicrobial activities to squalamine against the Gram-positive bacteria such as *Micrococcus luteus* 9341, *Staphylococcus aureus* 6538P, *Streptococcus equisimilis* 6580C and *Bacilius subtilis* 6633, and against Gram-negative bacteria including *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, *Salmonella typhimurium* 14028, and *Klebsiella pneumoniae* 10031. None of these carbamates exhibited good activity against *Proteus mirabilis* 25933 and *Serratia marcescens* 27117.

Steroid backbone appears to play an important role in the antimicrobial action of the sterol-polyamine conjugates. In general, the conjugate that has A,B-*cis* configuration is more potent than A,B-*trans* configuration. Thus, 5β-conjugate **2** was found to be more potent than 5α-conjugate **3** when evaluated against Gram-positive bacteria such as *M. luteus* 9341, *S. aureus* 6538P, *S. equisimilis*, and *B. subtilis* 6633. The conjugates **4**, **5** that have hydroxyl and fluoro group at the C-7α showed the enhanced activities against most of the tested strains except *S. typhimurium* and *K. pneumouniae*. Among the bisnorcholane spermidine 22-carbamate tested, **2** exhibited the strongest activity against *S. aureus* 6538P (MIC, 0.78 μg/mL). Bisnorcholane spermine carbamate **6** and **7** revealed comparable activity to the spermidine conjugate **2** and **3** against most of strains tested. Particularly, 3α-hydroxy-23,24-bisnor-5β-cholane spermine 22-carbamate **6** showed the strongest activity against *S. aureus* 6538P (MIC, 0.78 μg/mL) and *P. aeruginosa* 25933 (MIC, 3.13 μg/mL). Most of the 22-carbamates



1

2: 5β, X = H, n = 0; **3**: 5α, X = H, n = 0; **4**: 5α, X = OH, n = 0
5: 5α, X = F, n = 0; **6**: 5β, X = H, n = 1; **7**: 5α, X = H, n = 1.

Figure 1. The structures of squalamine (1) and bisnorcholane polyamine carbamates (**2**–**7**).

Table 1. In vitro antimicrobial and hemolytic activities of bisnorcholane polyamine carbamates **2**–**7**[a]

	MIC[b] (μg/mL)									MHC (μg/mL)
	Strains									
ATCC #	*E. coli* (25922)	*P. aeruginosa* (27853)	*P. mirabilis* (25933)	*S. marcescens* (27117)	*S. typhimurium* (14028)	*K. pneumoniae* (10031)	*M. luteus* (9341)	*S. aureus* (6538P)	*S. equisimilis* (6580C)	*B. subtilis* (6633)	
2	25	>100.00	>100.00	>100.00	12.5	3.13	1.56	0.78	3.13	3.13	25
3	>100.00	>100.00	>100.00	>100.00	12.5	1.56	3.13	3.13	12.5	6.25	25
4	50	25	>100.00	>100.00	>100.00	>100.00	3.13	3.13	12.5	6.25	100
5	12.5	12.5	>100.00	>100.00	>100.00	12.5	3.13	3.13	6.25	3.13	50
6	12.5	3.13	>100.00	>100.00	12.5	>100.00	1.56	0.78	1.56	1.56	25
7	6.25	25	>100.00	>100.00	>100.00	6.25	3.13	1.56	1.56	1.56	25
Squalamine	>100.00	>100.00	>100.00	>100.00	>100.00	12.5	3.13	6.25	3.13	3.13	ND[c]

[a]The MICs and MHCs were determined in triplicate for the experimental values.
[b]Activities against 10 strains compared to those of the squalamine determined in identical conditions.
[c]Not done.

tested had significant hemolytic activity. However, there was no correlation between MICs and MHCs, of individual compounds.

In summary, in vitro evaluation of 22-carbamates revealed the following trends: (1) the 5β-conjugate was more active than the 5α-conjugate; (2) introduction of substituent such as hydroxyl and fluoro group at C-7α in the ring B resulted in better antimicrobial activity; (3) both spermidine and spermine 22-carbamate showed similar activity; (4) The 22-carbamates exhibited hemolytic activity.

The results obtained suggest that the stereochemistry of 5-hydrido and substituent at C-7α of the steroid backbone are critical determinants of activity and the number of charges along the polyamine is a minor determinant of activity. Further detailed studies of the biological activity of these compounds will reveal their full potential.

Acknowledgements

This work was supported by a grant from the Ministry of Health and Welfare, Korea. The authors wish to thank H. D. Park and C. S. Lee in LGCI, Life Science R&D for the hemolytic activity.

References and Notes

1. For recent reviews, see: Karigiannis, G.; Papaioannou, D. *Eur. J. Org. Chem* **2000**, 1841.
2. Kim, H.-S.; Choi, B.-S.; Kwon, K.-C.; Lee, S.-O.; Kwak, H. J.; Lee, C. H. *Bioorg. Med. Chem.* **2000**, *8*, 2059.
3. Kikuchi, K.; Bernard, E. M.; Sadownik, A.; Regen, S. L.; Armstrong, D. *Antimicrob. Agents and Chemother.* **1997**, *41*, 1433.
4. Jones, S. R.; Kinney, W. A.; Zhang, X.; Jones, L. M.; Selinsky, B. S. *Steroids* **1996**, *61*, 565.
5. Sadownik, A.; Deng, G.; Janout, V.; Regen, S. L.; Bernard, E. M.; Kikuchi, K.; Armstrong, D. *J. Am. Chem. Soc.* **1995**, *117*, 6138.
6. Khabnadideh, S.; Tan, C. L.; Croft, S. L.; Kendrick, H.; Yardley, V.; Gilbert, I. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1237.
7. Walker, S.; Sofia, M. J.; Kakarla, R.; Kogan, N. A.; Wieriches, L.; Longley, C. B.; Bruker, K.; Axelrod, H. R.; Midha, S.; Babu, S.; Kahne, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1585.
8. Geall, A. J.; Al-Hadithi, D.; Blagbrough, I. S. *Chem. Commun.* **1998**, 2305.
9. Ajmani, P. S.; Attia, S.; Osifchin, M.; Legen, S.; Hughes, J. A. *Pharmazie* **1999**, *54*, 3.
10. Blagbrough, I. S.; Al-Hadithi, D.; Geall, A. J. *Tetrahedron* **2000**, *56*, 3439.
11. Wehrli, S. L.; Moore, K. S.; Roder, H.; Durell, S.; Zasloff, M. *Steroids* **1993**, *58*, 370.
12. Rao, M. N.; Shinnar, A. E.; Noecker, L. A.; Chao, T. L.; Feibush, B.; Snyder, B.; Sharkansky, I.; Sarkahian, A.; Zhang, X.; Jones, S. R.; Kinney, W. A.; Zasloff, M. *J. Nat. Prod.* **2000**, *63*, 631.
13. Moore, K. S.; Wehrli, S. L.; Roder, H.; Rogers, M.; Forrest, J. N.; McCrimmon, D. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1354.
14. Senior, K. *Drug Discov. Today* **2000**, *5*, 267.

15. Schiller, J. H.; Bitter, G. *Clin. Cancer Res.* **1999**, *5*, 4287.
16. Neu, H. C. *Science* **1992**, *257*, 1064.
17. Cohen, M. L. *Science* **1992**, *257*, 1050.
18. Muroi, H.; Kubo, I. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1925.
19. Kwon, K.-C. M. E. Thesis, Kyungpook National University, 2000.
20. Goodnow, R., Jr.; Konno, K.; Niwa, M.; Kallimopoulos, T.; Bukownik, R.; Lenares, D.; Nakanishi, K. *Tetrahedron* **1990**, *45*, 3267.
21. Tal, D. M.; Frisch, D.; Elliott, W. H. *Tetrahedron* **1984**, *40*, 851.
22. Geall, A. J.; Blagbrough, I. S. *Tetrahedron* **2000**, *56*, 2449.
23. Klenke, B.; Gilbert, I. H. *J. Org. Chem.* **2001**, *66*, 2480.