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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2558–2561
Bioorganic & Medicinal Chemistry Letters

Synthesis and antimicrobial activity of 7α-amino-23,24-bisnor-5α-cholan-22-ol derivatives

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Received 20 November 2007; revised 29 February 2008; accepted 17 March 2008
Available online 20 March 2008

Abstract—A series of 7α-aminobisnorsteroids were synthesized and their in vitro antimicrobial activity was evaluated regarding Gram-positive and Gram-negative bacteria. The stereoselective reductive amination of 7-ketosteroid **3** with NH_4OAc, in the presence of $NaBH_3CN$, afforded a high yield of 7α-aminosteroid **4**. The 3,7-diaminobisnorsteroids were obtained by the reductive amination of **4** with NH_4OT_f, Boc-spermidine, and Boc-spermine. 3α,7α-Diaminobisnorsterol dihydrochloride **15** showed the highest antimicrobial activity against *Streptococcus pyogenes* 308A with a MIC value of 1.6 μg/mL. Hemolytic activities of the compounds **13**–**20** were determined. Compound **13** showed MHC value at 100 μg/mL.

Resistance against man-made antibiotics in human pathogens has increased tremendously, thus posing a constant challenge to drug designers.[1] Aminosteroids, compounds that possess an amino group in a steroid scaffold, have recently been recognized as potential antibiotics. The most important example of such an aminosteroid is squalamine (Fig. 1), a polyamine–steroid conjugate isolated from the tissue of *Squalus acanthias*. Squalamine, which displays antimicrobial activity against Gram-positive and Gram-negative bacteria, possesses antiangiogenic activity and exhibits low hemolytic activity.[2] Recently, seven new aminosterols related to squalamine were isolated and found to be more potent than squalamine.[3] The high potency and low natural abundance of squalamine has prompted efforts toward its synthetic production resulting in a multi-step total synthesis, although in low overall yield.[4] Analogues of squalamine have been prepared and some of these compounds display stronger activity against bacteria, fungi, and protozoa than squalamine.[5] Hence, focus has been diverted away from the total synthesis of squalamine to the synthesis of its analogues to eliminate the long, multi-step, low-yielding syntheses of these potential antibiotics. Toward this end, a variety of aminosteroids have been synthesized for the investigation of their structure–activity relationship (SAR). Recently, a series of cholesterol-based 3-amino and 7-aminosterols were synthesized and found to be active against microbes.[6] In one study, an amino group was attached to cholesterol through oximation and reduction or azide formation and reduction to synthesize 7α and 7β-spermidinylcholesterol. But these methods required multi-step process and overall yields were not so high.[6] In another study, amphiphilic 3α- and 3β-amino derivatives were synthesized from 5α-cholestan-3β-ol through a multi-step process involving mesylation, bromination, azide formation, and reduction which provided the final products in low overall yields.[7] In contrast to these multi-step methods, employing a reductive amination in the synthesis of aminosteroids offers advantages potentially leading to one-step syntheses of aminosterols in high yields.[8,9] As part of a research program directed toward the development of aminosteroid antibiotics, we have synthesized a series of aminosteroids from 3-keto-23,24-bisnorchola-4-en-22-ol and various amines using a reductive amination approach in order to examine the SAR against bacteria. Our study has focused on the significance of the functional group (hydroxyl, fluorine and amino) at C-7 and the amino or polyamino

Keywords: 7α-Aminobisnorsteroids; 3α,7α-Diaminosterol; Reductive amination; Antimicrobial activity.
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doi:10.1016/j.bmcl.2008.03.042

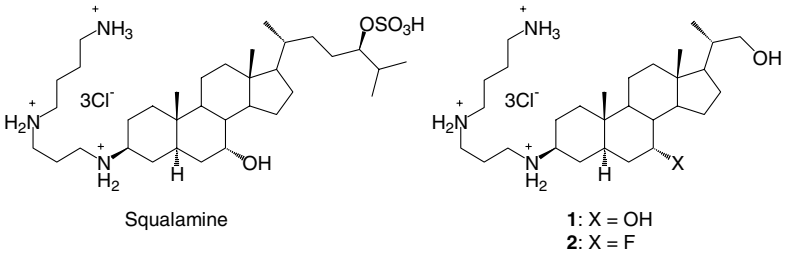
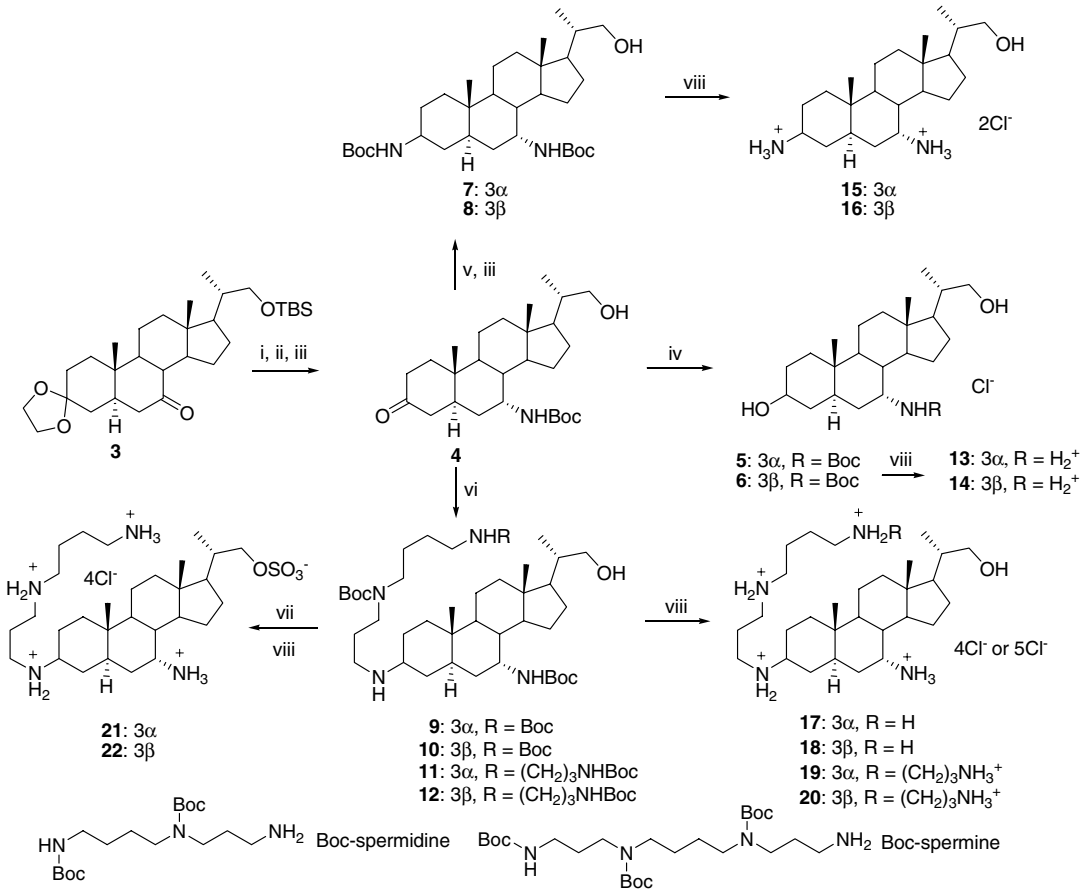


Figure 1. Squalamine and its analogues.

group at C-3, along with the relative stereochemistry at these two positions. Recently, we reported on the synthesis and antibacterial activity of 7-fluoro-3-aminosteroids.[10] In a continuation of our efforts, we now report on the synthesis and antibacterial activity of 7α-aminobisnorsteroids.

For the synthesis of 7α-aminobisnorsteroids, the requisite starting material, 3-dioxolane-22-*tert*-butyldimethylsilyloxy-23,24-bisnor-5α-cholan-7-one (**3**), was prepared from commercially-available 3-keto-23,24-bisnorchola-4-en-22-ol by the previously reported procedure.[11] The stereoselective reductive amination of 3-keto and 7-ketosteroids in order to synthesize 3α, 3β and 7α-isomers, respectively, has been previously reported by us.[8] The versatility of this reductive amination protocol was extended to 23,24-bisnor-5α-cholane **3** to introduce amine functionality at C-7 by reacting compound **3** with NH_4OAc in the presence of $NaBH_3CN$ to give the 7α-aminobisnorsteroid. After the groups at C-3 and C-22 were deprotected with *p*TSA, the free amino group at C-7 was reacted with di-*tert*-butyl dicarbonate to give 3-keto-7α-(*tert*-butyloxycarbonyl)amino-23,24-bisnor-5α-cholan-22-ol **4** in 72% yield. The 3-keto group of **4** was reduced with K-selectride to yield 3α-hydroxy compound **5** in 85% yield, or with $NaBH_4$ which afforded the 3β-hydroxy compound **6** in 78% yield. The stereoselective introduction of an amino group at C-3, with the configurations 3α and 3β was dependent on the selection of a reducing reagent. Smaller reagents like $NaBH_3CN$ provided the 3β isomer and bulky reagents like sodium triacetoxyborohydride [$NaBH(OAc)_3$] and sodium tris[2-(ethylhexanoic)]borohydride [$NaBH(OEh)_3$][8a] afforded 3α isomer predominantly.[8,12] Thus, amine precursors such as NH_4OT_f, Boc-spermidine, and Boc-spermine could be reacted with **4** in the presence of $NaBH(OEh)_3$ to afford **7** (76%), **9** (93%), and **11** (91%) while the reaction of **4** with the same amine precursors using $NaBH_3CN$ produced **8** (62%), **10** (60%), and **12** (61%) as shown in Scheme 1. The structures of the obtained compounds were characterized by NMR spectroscopy and elemental analysis.[13] The ^1H NMR of **9** showed 3α-NH and 7α-NH protons at δ 4.61 and 4.80, respectively, whereas ^1H NMR of **10** revealed 3β-NH and 7α-NH proton at δ 4.60 and 4.73, respectively.[13] The chemical shifts observed were similar to those found previously.[8b] Deprotection of the Boc group in compounds **5**–**12** with hydrochloric acid in situ generated from the reaction of thionyl chloride with methanol proceed smoothly in dichloromethane to provide the corresponding hydrochloride salts **13**–**20** quantitatively. Recrystallization of the resulting hydrochloride salts in acetone–methanol afforded pure **13**–**20**. The sulfation of the hydroxyl group at C-22 in **9** and **10** with SO_3–pyridine complex in pyridine at room temperature, followed by treatment with hydrochloric acid yielded **21** and **22** in 94% and 92% yield, respectively.

Ten synthesized 7α-aminobisnorsteroids **13**–**22** were evaluated along with compounds **1** and **2** for antimicrobial activity against the strains of three Gram-positive and five Gram-negative bacteria as previously described.[10,11] and the MIC (minimum inhibitory concentration) values are summarized in Table 1. The structures of **1** and **18** are similar in all respects including stereochemistry, except for the functional group at C-7. Previously, we examined the effect of substituents by introducing the fluorine atom at C-7 in **2**, which showed more potency than the hydroxyl analogue **1**.[10] Although the 3-amino-7α-hydroxy-23,24-bisnor-5α-cholane was inactive (with a MIC of 100.0 μg/mL) against the tested strains,[12] the 3-hydroxy-7α-amino-23,24-bisnor-5α-cholanes **13** and **14** showed antimicrobial activity against *Streptococcus pyogenes* 308A, *S. pyogenes* 77A, *Staphylococcus aureus* 503, and *Escherichia coli* DC2. The facial amphiphilic 3α,7α-diaminobisnorsteroid **15** was the most potent in this series with a MIC as low as 1.6 μg/mL against *S. pyogenes* 308A. The 3β isomer **16** was less potent than the 3α isomer **15** with a MIC of 6.3 μg/mL against Gram-positive strains. Moreover, it was observed that the 3α-hydroxy isomer **13** was more potent than the 3β-isomer **14**, and in the same manner the 3α-amino isomer **15** was more active than the 3β-isomer **16**. The introduction of a spermidine moiety at C-3 in a bisnorsteroid did not further enhance activity against any of the tested bacterial strains. Compounds **1**, **2**, and **18** have a similar structure except for the functional group at C-7, and a comparison of the activity of these compounds shows that an amino substituent only marginally improves activity while a fluorine dramatically enhances potency.[10] The sulfation at C-22 in **17** gave analogue **21** which showed twice the activity as **17** against *S. pyogenes* 308A, *S. pyogenes* 77A, *S. aureus* 503, and *Pseudomonas aeruginosa* 9027. The 3β isomer **22**, however, was less active. The spermine analogues **19** and **20** showed enhanced activity against *S. pyogenes* 308A, *S. aureus* 503, and *P. aeruginosa* 9027 compared to the spermidine analogues **17** and **18**. The 3β-spermine isomer **20** was more active than the 3α-spermine isomer against *S. pyogenes* 308A, *S. aureus* 503, and *P. aeruginosa* 9027. Compound **15** was moderately active against *Salmonella typhimurium* and *Enterobacter cloacae* 1321E as



Scheme 1. Synthesis of 7α-aminosteroids. Reagents: (i) NH_4OAc, $NaBH_3CN$, THF-MeOH; (ii) *p*TSA, acetone; (iii) Boc_2O, MeOH; (iv) K-selectride, THF, −60 °C or $NaBH_4$, EtOH; (v) NH_4OTf, $NaBH(OEh)_3$, THF; (vi) amine, $NaBH_3CN$, THF-MeOH, or amine, $NaBH(OEh)_3$, THF; (vii) SO_3.py, C_5H_5N; (viii) $SOCl_2$, MeOH, CH_2Cl_2.

Table 1. In vitro antimicrobial activity (MIC: μg/mL) of 7α-aminobisnorsteroids

Strains	1	2	13	14	15	16	17	18	19	20	21	22
S. pyogenes 308A	25.0	6.3	3.1	6.3	1.6	6.3	12.5	12.5	6.3	3.1	6.3	25.0
S. pyogenes 77A	25.0	6.3	6.3	12.5	3.1	6.3	25.0	25.0	25.0	25.0	12.5	50.0
S. aureus 503	12.5	6.3	3.1	12.5	3.1	6.3	12.5	12.5	12.5	6.3	6.3	25.0
E. coli DC 2	25.0	6.3	12.5	25.0	12.5	25.0	>100	50.0	50.0	50.0	25.0	>100
P. aeruginosa 9027	12.5	6.3	>100	>100	50.0	>100	25.0	25.0	12.5	6.3	12.5	>100
P. aeruginosa 1771M	25.0	3.1	>100	>100	25.0	50.0	>100	>100	25.0	50.0	50.0	>100
S. typhimurium	50.0	>100	>100	>100	25.0	50.0	>100	>100	>100	>100	>100	>100
E. cloacae 1321E	50.0	>100	>100	>100	50.0	50.0	>100	>100	>100	>100	>100	>100

shown in Table 1. Minimal hemolytic concentrations (MHC) of **13**–**20** were determined in triplicate by the literature method.[5d] Most of the 7α-aminobisnor derivatives tested had no significant hemolytic activity up to 100 μg/mL. Compound **13** showed MHC at 100 μg/mL.

In conclusion, we have synthesized a series of 7α-aminobisnorsteroids by reductive amination in high yield. Our results suggest that the nature and stereochemistry of functional groups exerted a major impact on antimicrobial activity. The 3α-hydroxybisnorsteroid **13** and 3α-aminobisnorsteroid **15** were more active than their 3β-hydroxy and 3β-amino counterparts **14** and **16**. This seemed to be a general trend except for 3α-sperminylbisnorsteroid **19** which showed lower activity than its 3β analogue. 3α,7α-Diaminobisnorsterol dihydrochloride **15** was determined to be the most potent among the tested 7α-aminobisnorsteroids. The results obtained suggest that the stereochemistry and substituent at C-3 and a 7α-amino group are the crucial determinants of activity. Most of the 7α-aminobisnorsteorids exhibited no hemolytic activity. There was no correlation between MICs and MHCs of individual compounds. The compounds are being checked for antiangiogenic activity and a detailed study will be released in future communication.

Acknowledgments

This work was supported by the Korean Research Foundation Grant funded by the Korean Government

(MOEHRD) (KRF-2007-521-C00155) and the Kyungpook National University Research Fund, 2007.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.042.

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13. Refer to supplementary information.