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Synthesis of 7α- and 7β-spermidinylcholesterol, squalamine analogues

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Abstract—Stereoselective synthesis of squalamine dessulfates analogues, 7α and 7β-*N*-[3*N*-(4-aminobutyl) aminopropyl]aminocholesterol are reported, using 7α and 7β-aminocholesterol as a key intermediate. It's the first example in which the position of spermidine is modified at the steroid ring. These molecules showed a comparable antibacteria and fungi activities to squalamine. Then, they have a cytotoxic activity on a human non-small cell bronchopulmonary carcinoma line (NSCLC-N6).

1. Introduction

Squalamine is the first aminosterol isolated from tissues of dogfish shark *Squalus acanthias*.[1] It has been shown a potent antimicrobial activity against Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeroginosa*), Gram positive bacteria (*Staphilococus aureus*, *Enterococcus faecalis*) and fungi (*Candida albicans*, *Paramecium caudatum*).[2]

Squalamine inhibits also angiogenesis and tumour growth in several animal models and is currently in phase II clinical trial for treatment of advanced non-small cell lung cancer.[3] The most significant event was the presentation of anti-angiogenic activity, which first directly led to the development of squalamine into an anticancer chemotherapeutic.[4] However, squalamine is obtained in small amounts (0.001–0.002 wt %). More recently, attempts to obtain large amounts of squalamine from the liver of the dogfish shark resulted in the discovery, isolation and characterization of seven new aminosterols related to squalamine and showed a comparable anti-bacteria and fungi properties as squalamine. These aminosterols sulfate and dessulfate compounds, have a relatively invariant steroid skeleton with a *trans* A/B ring junction, a spermidine or spermine introduced at equatorial C3 position. These other aminosterols such as squalamine were founded in low quantities (0.00005–0.00025%).[5]

Several syntheses of squalamine have been published.[6–8] However, the published methods afforded a mixture of epimers at C3 using the introduction of the polyamine side chain via a reductive amination and required an expensive starting material in some cases. Fourteen to seventeen steps were necessary to synthesize this compound.[9,10] Thus, it needed to develop analogues of squalamine. The synthesis of squalamine analogues has been reported in literature.[11–13] Different biological studies of squalamine and analogues led to the following conclusions:[14] the sterol side chain could be dessulfated. The sulfate group could be taken away. The structure and the position of polyamine on steroid could varied. The steroid can have other functions on the side chain.

However, large amounts of squalamine analogues necessary for chemotherapeutic application, hardly obtained from multistep synthesis, affording the design of simplified squalamine analogues.

In the frame of our work on aminosterol derivatives, 7α,β-aminocholesterol (77% α and 23% β) were shown a

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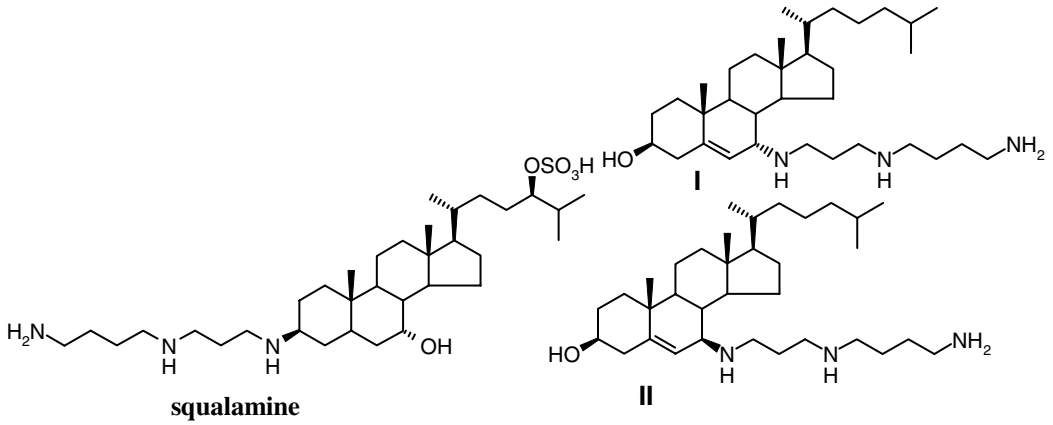


Scheme 1. Structures of squalamine and analogues dessulfates **I**, **II**.

fungicidal[15] and anti-bacteria[16] activities. Its shown also strong anti-proliferative properties on three cell lines: murine leukemia P388, KB and a contenious human non-small cell bronchopulmonary carcinoma line (NSCLC-N6).[17]

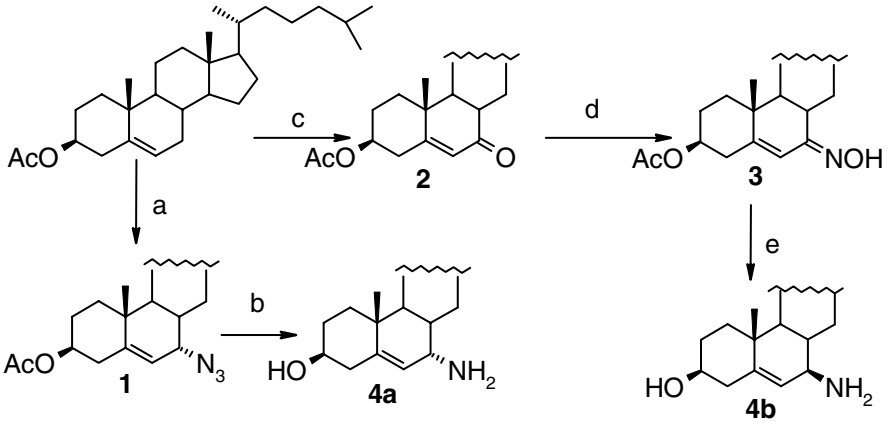
Our aim was to develop new analogues of squalamine dessulfates. We envisioned the spermidine chain at C7 position of cholesterol. Two polyaminosterols epimers **I** and **II** were selectively prepared from 7α- and 7β-aminocholesterol, the crucial intermediate selectively prepared from cholesterol. This approach preserved the hydroxyl group at C-3 position of steroid and so minimized the synthesis steps of these analogues (Scheme 1).

2. Results and discussion

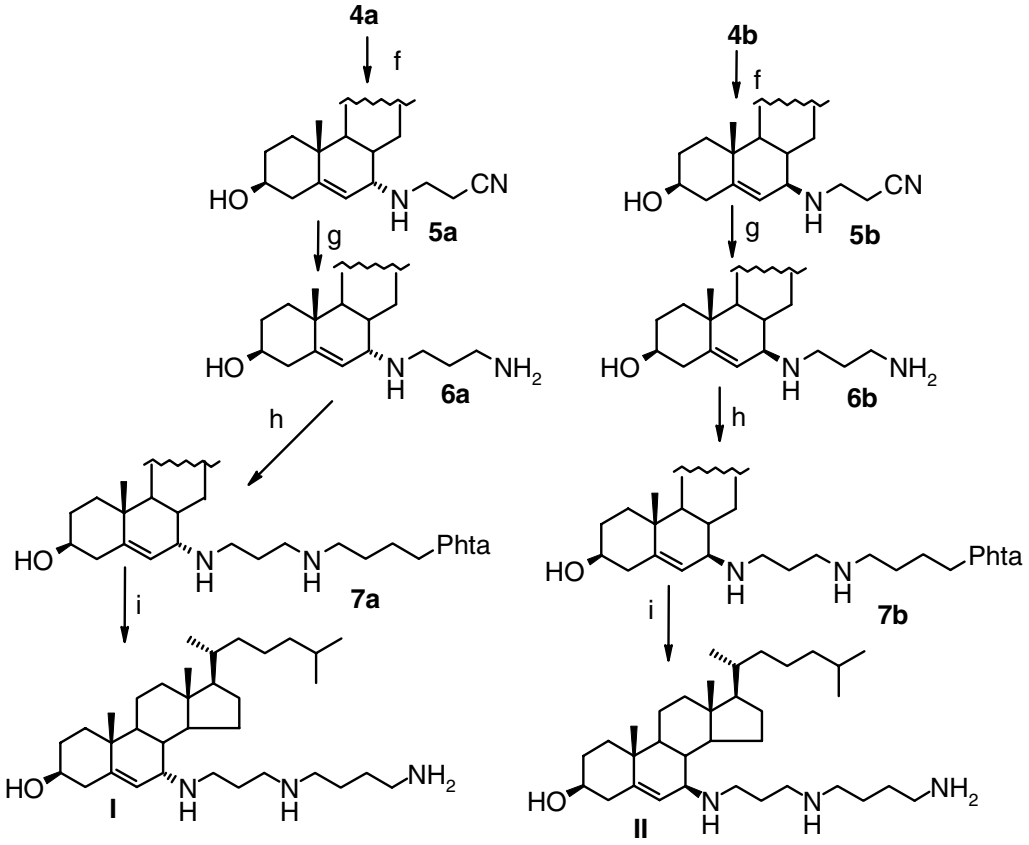
7α- and 7β-Aminocholesterol were selectively prepared by the following procedure:[18] the azido group was directly introduced in the axial allylic position C7 of cholesteryl acetate by trimethylsilyl azide action in presence of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). The mechanism of this reaction is an oxidative nucleophilic substitution (S_NOx) using oxidizing reagent (DDQ) and followed by nucleophilic attack. The azide derivative **1** was then reduced with lithium aluminium hydride to give 7α-aminocholesterol **4a**. Allylic oxidation of cholesteryl acetate was accomplished using pyridium chromium complex (compound **2**). Oxime derivative **3** was obtained by treatment of ketone with hydroxylamine hydrochloride in pyridine and reduced by di-*iso*-butylaluminiumhydride (DIBAH) in dichloromethane, to 7β-aminocholesterol (**4b**). Probably due to steric hindrance, only β epimer **4b** was observed (Scheme 2).

Acrylonitrile was reacted in methanol with each aminosterol (**4a** and **4b**) required the 7α- and 7β-*N*(2-cyanoethyl)aminocholesterol (**5a** and **5b**).

The nitrile function was reduced by lithium aluminium hydride and alkylated by 4-*N*-bromobutyrophthalimide to give compounds **7a** and **7b**. *N*-Phthalimido group was deprotected by methylamine in ethanol afforded squalamine analogues **I** $[\alpha]_D^{20}$ +41 (*c* 0.2, methanol) and **II** $[\alpha]_D^{20}$ −41 (*c* 0.2, methanol)[19] (Scheme 3).



Scheme 2. Reagents and conditions: (a) DDQ, $(CH_3)_3SiN_3$, CH_2Cl_2, reflux 51%; (b) $LiAlH_4$, THF reflux, 70%; (c) CrO_3, 2py, CH_2Cl_2, 64%; (d) $H_3N^+OHCl^-$, py; (e) DIBAH, THF, reflux, 42%.



Scheme 3. Reagents and conditions: (f) CH_2=CH–CN, MeOH, 50–66%; (g) $LiAlH_4$, THF, reflux; (h) Br–$(CH_2)_4$-*N*-phthalimide, DMF, 70 °C, 72 h, 68–70%; (i) $MeNH_2$ (33% in EtOH), absolute EtOH, 6 h, 39–42%.

Table 1. Determination of MIC (μg/mL) values after 48 h incubation

Strains	*E. coli* 54127	*S. aureus* CIP 54127	*E. hirae* CIP 5855	*C. albicans* CIP1180-79	*S. cerevisiae* ATCC28383
Compound **I**	6.25	3.12	3.12	6.25	3.12
Compound **II**	3.12	6.25	6.25	3.12	3.12
Squalamine	>100	1.56	—	4–8	—

Minimum inhibitory concentrations (MIC) of compounds **I** et **II** were tested against Gram negative bacteria (*E. coli*), Gram positive bacteria (*S. aureus*, *E. hirae*) and fungi (*C. albicans*, *S. cerevisiae*)[20] (Table 1).

Then, the anti-proliferative effect of these analogues on a human non-small cell bronchopulmonary carcinoma line (NSCLC-N6) was evaluated.[21] The cytotoxicity determinations showed clearly strong anti-proliferative properties on NSCLC-N6 cell line ($IC_{50} = 3$ μg/mL; significative activity when $IC_{50} < 10$ μg/mL).

From these results, we can conclude that analogues of squalamine were synthesized without a sulfate group in five steps of analogue **I** and seven steps of analogue **II**. These analogues with spermidine at C7 position of steroid were able to exhibit comparable anti-microbial and cytotoxic activities. Thus, the position of spermidine moiety at the steroid ring is a key target to develop the most active polyaminosterols. Further work is in progress to synthesize other squalamine analogues.

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19. Compound **I**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ: 0.68 (s, 3H, Me 18); 0.86 (d, 6H, $J = 6.5$, Me 27 and Me 26); 0.91 (d, 3H, $J = 6.5$, Me 21); 0.99 (s, 3H, Me 19); 2.66 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–NH–); 2.74 (dd, 1H, $J_{7\beta-8} = 4.3$, $J_{7\beta-6} = 5.0$, H7β of α epimer); 2.80 (t, 2H, $J = 5.4$, –HN–$(CH_2)_3$–CH_2–NH_2); 2.92 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–NH–); 3.00–3.48 (m, 4H, St–NH–$(CH_2)_2$–CH_2–NH– et NH–CH_2–$(CH_2)_3$–NH_2); 3.63 (m, 1H, H3α); 5.66 (dd, 1H, $J_{6-7\beta} = 1.8$, $J_{6-4} < 1.0$, H6 of α epimer); 7.23 (br s, 2H, –NH_2, D_2O exchange); 8.15 (br s, 1H, –NH–, D_2O exchange); 8.44 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$, 100 MHz): δ: 12.1; 18.6; 18.8; 21.7; 22.7; 23.9; 25.4; 25.6; 26.3; 28.1; 28.2; 29.5; 31.7; 35.9; 36.3; 37.0; 37.4; 39.6; 39.7; 40.0; 40.7; 40.8; 43.0; 46.1; 46.36; 46.8; 47.6; 54.2; 56.3; 65.4; 71.5; 122.2; 139. IE-SM: m/z (%) = 471 (3); 457 (2); 442 (2); 414 (2); 401 (35); 400 (100); 385 (24); 384 (27); 369 (3); 351 (24); 275 (10). $[\alpha]_D^{20}$ −41 (c 0.2 M, MeOH). Anal. Calcd for $C_{34}H_{61}N_3O$: C 77.35; H 11.66; N 7.96. Found: C 77.33; H 11.69; N 7.95. Compound **II**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ: 0.69 (s, 3H, Me 18); 0.86 (d, 6H, $J = 6.5$, Me 27 and Me 26); 0.90 (d, 3H, $J = 6.5$, Me 21); 0.93 (s, 3H, Me 19); 2.37 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–); 2.42–2.47 (m, 2H, –HN–$(CH_2)_3$–CH_2–NH_2 et –HN-CH_2–$(CH_2)_3$–NH_2); 2.70 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–); 2.65 (dd, 1H, $J_{7\alpha-8} = 6.8$, $J_{7\alpha-6} = 1.3$, H7α of β epimer); 2.79 (t, 2H, $J = 8.3$, St–NH–$(CH_2)_2$–CH_2–NH–); 3.5 (m, 1H, H3α); 5.30 (d, 1H, $J = 3.0$, H6 of β epimer); 7.19 (br s, 2H, –NH_2, D_2O exchange); 8.13 (br s, 1H, –NH–, D_2O exchange); 8.39 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$, 100 MHz): δ: 12.1; 18.6; 18.8; 21.7; 22.7; 23.9; 25.4; 25.6; 26.3; 28.1; 28.2; 29.5; 31.7; 35.9; 36.3; 37.0; 37.4; 39.6; 39.7; 40.0; 40.7; 40.8; 43.0; 46.1; 46.6; 46.8; 47.6; 54.2; 56.3; 59.0; 71.5; 122.8; 139.0. IE-SM: m/z (%) = 471(1); 414 (1); 401 (19); 400 (33); 385 (50); 384 (100); 369 (11); 351 (26). $[\alpha]_D^{20}$ +41 (c 0.2 M, MeOH). Anal. Calcd for $C_{34}H_{61}N_3O$: C 77.35; H 11.66; N 7.96. Found: C 77.31; H 11.69; N 7.95.
20. The anti-microbial tests were measured in vitro using a liquid-phase turbidimetric system (Bioscreen® from Labsystem, France) and automatically evaluated every 30 min for 48 h. The tested molecules were dissolved in dimethylsulfoxide and added in liquid broth to prepare various concentrations of drugs by successives dilutions (DMSO in liquid broth did not exceed 2%). The inocula (2%) was prepared from overnight culture and diluted to obtain 0.5 value of optical density (600 nm). Microorganisms were incubate in shaker system and the growth curves were obtained by optical density readings with wide broad filter (420–580 nm) of Bioscreen system Dei-Cas, E.; Dujardin, L.; Ribiero Pinto, M. E.; Ajana, F.; Fruit, J.; Poulain, D.; Camus, D.; Vernes, A. *Mycoses* **1991**, *34*, 167.
21. Cell line and cell culture: the NSCLC-N6 cell line, derived from a human non-small cell bronchopulmonary carcinoma (moderately differentiated, rarely keratinizing, classified as T2NOMO) was used for all experiments. The cells were cultured in RPMI 1640 medium with 5% foetal calf serum, to which were added 100 IU penicillin mL^{-1}, 100 μg streptomycin mL^{-1} and 2 mM glutamine, at 37 °C in a air/carbon dioxide (95:5, v/v) atmosphere. In these conditions, cell doubling time was 48 h. Cells used in all experiments never exceeded 35 passages. Cytotoxicity determinations: continuous drug exposure. Experiments were performed in 96 wells microliter plates (2×10^5 cells mL^{-1}). Cell growth was estimated by a colorimetric assay based on the conservation of tetrazolium dye (MTT) to a blue formazan product by live mitochondria. Eight repeats were performed for each concentration. Control growth was estimated from 16 determinations. Optical density at 570 nm corresponding to solubilized formazan was read for each well on a Titertek Multiskan MKII Mossmann, T. *J. Immunol. Methods* **1983**, *65*, 55.