Antimicrobial Activities of Squalamine Mimics

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We investigated the antimicrobial properties of compounds with structural features that were designed to mimic those of squalamine, an antibiotic isolated from the stomach of the dogfish shark. The mimics, like squalamine, are sterol-polyamine conjugates. Unlike squalamine, the mimics were simple to prepare, at high yield, from readily available starting materials. Several squalamine mimics showed activity against gram-negative rods, gram-positive cocci including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and fungi. Some had little or no hemolytic activity. The hydrophobicity of the sterol backbone and the length and the cationic charge of the side chains appeared to be critical determinants of activity. One of the squalamine mimics, SM-7, was bactericidal against *Escherichia coli*, *Pseudomonas aeruginosa*, and *S. aureus*; its activity was decreased by divalent or monovalent cations and by bovine serum albumin. Subinhibitory concentrations of SM-7 markedly enhanced the antimicrobial activity of rifampin against gram-negative rods. These results suggest that the compounds may disrupt an outer membrane of gram-negative rods. Squalamine mimics are a new class of broad-spectrum antimicrobial agents. The antagonism of their activity by serum and albumin and their hemolytic properties may limit their use as systemic agents. The squalamine mimics, because of their potencies, broad spectra of antimicrobial activity, and potential for systemic toxicity, appear to be good candidates for development as topical antimicrobial agents.

The emergence of multidrug-resistant microorganisms, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and drug-resistant *Mycobacterium tuberculosis*, has prompted efforts to develop new classes of antibiotics (1, 22). Squalamine, a novel antimicrobial agent isolated from the stomach of the dogfish shark, has an unusual chemical structure, consisting of a steroid backbone with a spermidine side chain (14). The antimicrobial spectrum of squalamine includes gram-negative rods, gram-positive cocci, and fungi. The compound has been postulated to play a role in the immune defense system of the dogfish shark (14). It is very difficult to obtain large amounts of squalamine from natural sources, and its synthesis requires many steps and the use of expensive starting materials (15).

Recently, we reported that a synthetic analog of squalamine had significant antimicrobial activity and reduced hemolytic potential (19). We have prepared a large number of other squalamine mimics. Our aims were to develop more active and less toxic analogs of squalamine and to study the mode of action of this class of compounds.

MATERIALS AND METHODS

Synthesis of squalamine mimics (sterol-polyamine conjugates). The following procedure, which was used for the preparation of SM-7, was typical. Deoxycholic acid (7.86 g, 20.0 mmol) was first converted into the corresponding succinimidyl ester by reaction with *N*-hydroxysuccinimide (2.38 g, 20.7 mmol), by using dicyclohexylcarbodiimide (4.8 g, 23.3 mmol) as the condensing agent and 120 ml of anhydrous tetrahydrofuran as the solvent. After stirring for 3.5 h, the ester was isolated via filtration; concentration under reduced pressure; dissolution in chloroform; sequential washing with aqueous sodium bicarbonate, water, and aqueous sodium chloride; drying over anhydrous sodium sulfate; removal of solvent

under reduced pressure; and recrystallization from ethyl acetate-hexane. The resulting ester (7.08 g) was obtained as a colorless powder with a melting point of 167 to 169°C and ¹H nuclear magnetic resonance (NMR) (CDCl₃, 360 MHz) δ 0.69 (s, 3H), 0.91 (s, 3H), 0.95-1.95 (m, 29H), 2.55 (m, 1H), 2.67 (m, 1H), 2.83 (δ, *J* = 4.8 Hz, 4H), 3.61 (m, 1H), 3.98 (s, 1H). To a stirred solution of spermine (241 mg, 1.19 mmol) in 4 ml of anhydrous chloroform was added a solution of the succinimidyl ester of deoxycholic acid (351 mg, 0.71 mmol) in 10 ml of anhydrous chloroform over a 5-min period. After stirring for 14 h at room temperature, the solvents were removed under reduced pressure and the residue was dissolved in 8 ml of chloroform. Sequential washing with 0.1 M NaOH, water, and aqueous sodium chloride, followed by drying over anhydrous K₂CO₃ and concentration under reduced pressure, afforded 320 mg of solid residue. Purification by column chromatography (5 g of silica gel, CH₃OH-30% NH₄OH; 3/2 [vol/vol]) afforded 255 mg of SM-7 as a glassy solid with ¹H NMR (CD₃OD, 500 MHz) δ 0.70 (s, 3H), 0.92 (s, 3H), 1.02 (δ, *J* = 6.5 Hz, 3H), 0.97-1.91 (m, 34H), 2.11 (m, 1H), 2.23 (m, 1H), 2.57-2.65 (m, 4H), 2.65 (t, *J* = 7.3 Hz, 2H), 2.70 (t, *J* = 7.1 Hz, 2H), 3.21 (t, *J* = 6.7 Hz, 2H), 3.52 (m, 1H), 3.95 (m, 1H). ¹³C NMR (CD₃OD) 177.2, 74.0, 72.6, 50.5, 48.23, 48.16, 47.7, 47.6, 43.7, 40.6, 38.0, 37.5, 37.2, 36.9, 36.5, 35.3, 34.9, 34.2, 33.5, 32.8, 31.1, 30.2, 30.0, 28.7, 28.4, 28.2, 28.1, 27.5, 24.9, 23.7, 17.7, and 13.3. Fourier transform infrared spectroscopy (KBr): 1046, 1448, 1558, 1646, 2863, 2934, 3362 cm⁻¹. Analysis calculated for C₃₄H₆₄O₃N₄ · H₂O: C, 68.73; H, 11.18; N, 9.42. Found: C, 69.03; H, 10.91; N, 8.73.

Sulfated analogs were prepared by procedures similar to those described above, except that a sulfated form of the sterol succinimidyl ester was used. In all cases, sulfation was accomplished by reaction of the sterol succinimidyl ester with sulfur trioxide-pyridine complex in anhydrous *N,N*-dimethylformamide, in which the extent of conversion was monitored by ¹H NMR spectroscopy.

All the squalamine mimics were dissolved in dimethyl sulfoxide at 4 mg/ml and were diluted in distilled water.

Microorganisms. Reference strains were purchased from the American Type Culture Collection (Rockville, Md.). Other clinical strains were obtained from the Microbiology Laboratory at Memorial Sloan-Kettering Cancer Center. Bacterial strains were maintained on Columbia-5% sheep blood agar plates (BBL Microbiology Systems, Cockeysville, Md.), and fungi were maintained on Sabouraud dextrose agar plates (BBL).

Determination of MICs. MICs were determined by a broth microdilution method (16, 17), with a minor modification in that we used half-strength Trypticase soy broth (BBL), and the highest drug concentration was 100 µg/ml. MICs were also determined under various pH conditions with calcium chloride or magnesium chloride or with immobilized horse serum (Gibco BRL, Grand Island, N.Y.). An MIC of 12.5 µg/ml or less was considered to be evidence of significant antimicrobial activity.

Time-kill curves. Exponential-phase cells were suspended in fresh, prewarmed 0.5× TSB at 10⁵ to 10⁶ CFU/ml. After 2 h of incubation at 37°C, drugs were added. Cultures were reincubated and samples were withdrawn at various times.

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FIG. 1. Chemical structures of squalamine and squalamine mimics.

Samples were diluted in sterile saline and plated onto Trypticase soy agar in duplicate. After 24 h of incubation at 35°C aerobically, colonies were counted.

Effects of cations and bovine serum albumin on bactericidal activity. Exponential-phase *Escherichia coli* ATCC 25922 cells were suspended at 10⁶ CFU/ml in 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.4) with or without calcium chloride, magnesium chloride, sodium chloride, potassium chloride, or bovine serum albumin (BSA). SM-7 was added at a final concentration of 12.5 μ g/ml (0.02 mM), and the cultures were incubated for 1 h. Samples were withdrawn and viable colonies were counted as described above.

Assay for bacterial permeability-increasing activity. Synergy tests with rifampin were used to study the effects of the squalamine mimic on membrane permeability. Rifampin, a hydrophobic antibiotic, penetrates the intact outer membrane of *E. coli* very poorly unless the membrane is damaged. For the synergistic growth inhibition assay, we used a checkerboard method as described previously (7). A fractional inhibitory concentration (FIC) index was calculated by using the following formula: FIC of rifampin (the concentration of rifampin used with SM-7 to produce inhibition divided by the MIC of rifampin alone) + FIC of SM-7 (defined similarly). A FIC index of 0.5 was considered to indicate synergy.

Assay of hemolytic activity. Sheep erythrocytes were incubated for 24 h at 37°C in saline (final concentration, 0.5%) with or without respective concentrations of squalamine mimics. The minimal hemolytic concentration (MHC) was determined and was the lowest concentration of a drug that caused visible hemolysis.

RESULTS

Chemical design of squalamine mimics. The chemical structures of squalamine and the 24 mimics are presented in Fig. 1.

In SM-2, the first squalamine mimic with significant antimicrobial activity, the placement of the sulfate and polyamine groups is the reverse of that found in squalamine (19). Most of the squalamine mimics were sterol-polyamine conjugates that derived from combinations of one of seven bile acid bases and one of three polyamine side chains.

Antimicrobial and hemolytic activities of squalamine mimics. Table 1 summarizes the activities of the squalamine mimics. The MICs for 21 strains of bacteria and fungi and the MHCs for sheep erythrocytes are also presented in Table 1, which also lists the MICs of squalamine that were reported previously (16). Four of the conjugates, SM-3, SM-4, SM-14, and SM-15, were inactive; they did not inhibit the growth of any strain at concentrations of less than 100 μ g/ml (data not shown).

Many squalamine mimics exhibited significant antimicrobial activity that was similar in magnitude and spectrum to that reported for squalamine (16). We were unable to obtain an authentic sample of squalamine and so could not compare the compounds in direct tests. However, the activities of some squalamine mimics were similar to that of squalamine, and their activities even extended to vancomycin-resistant *Entero-*

TABLE 1. Antimicrobial and hemolytic activities of squalamine and squalamine mimics

Organism	MIC (μg/ml)																			Squal- amin ^e	
	SM-1	SM-2	SM-5	SM-7	SM-7H	SM-8	SM-9	SM-9H	SM-11	SM-13	SM-17	SM-19	SM-21	SM-22	SM-25	SM-27	SM-28	SM-32	SM-34		SM-38
Gram-positive cocci																					
<i>Enterococcus faecalis</i> ATCC 29212	ND ^b	>100	>100	3.13	6.25	>100	25			50	50	6.25	3.13	>100	3.13	12.5	50	100	3.13	6.25	1-2
<i>Enterococcus faecium</i> T64809 (vanco- mycin resistant)	ND	>100	>100	6.25	6.25	100	50			50	50	3.13	1.56	>100	3.13	12.5	50	100	6.25	NA ^c	
<i>Staphylococcus aureus</i> ATCC 25923	3.13	12.5	50	1.56	0.78	25	25			6.25	25	3.13	3.13	1.56	0.78	6.25	6.25	25	0.78	NA	
<i>Staphylococcus aureus</i> ATCC 29213	6.25	>100	100	1.56	0.78	25	25			12.5	25	3.13	3.13	1.56	0.78	6.25	6.25	25	1.56	1-2	
<i>Staphylococcus aureus</i> H20809 (methi- cillin resistant)	ND	ND	>100	1.56	0.78	25	25			6.25	50	3.13	3.13	3.13	0.39	6.25	6.25	25	1.56	NA	
<i>Staphylococcus epidermidis</i> ATCC 12228	1.56	3.13	25	0.78	0.39	25	25			3.13	25	1.56	1.56	1.56	0.39	3.13	3.13	25	0.78	NA	
<i>Streptococcus agalactiae</i> ATCC 13813	ND	>100	>100	3.13	0.78	50	12.5			12.5	12.5	3.13	1.56	>100	0.78	3.13	12.5	25	1.56	NA	
<i>Streptococcus pneumoniae</i> ATCC 6305	ND	>100	>100	3.13	1.56	50	25			25	25	0.78	1.56	25	3.13	3.13	50	25	3.13	NA	
<i>Streptococcus pyogenes</i> ATCC 19615	ND	6.25	6.25	0.78	0.39	6.25	6.25			3.13	12.5	3.13	3.13	1.56	0.78	1.56	1.56	12.5	0.78	3.13	
Gram-negative rods																					
<i>Enterobacter cloacae</i> ATCC 13047	ND	>100	>100	12.5	12.5	>100	50			50	>100	>100	>100	>100	25	>100	>100	12.5	12.5	NA	
<i>Escherichia coli</i> ATCC 25922	12.5	6.25	100	3.13	6.25	>100	50			25	12.5	25	50	>100	1.56	3.13	50	25	3.13	6.25	1-2
<i>Klebsiella pneumoniae</i> ATCC 13883	ND	12.5	>100	3.13	12.5	50	50			50	50	6.25	>100	>100	>100	6.25	>100	50	6.25	12.5	NA
<i>Proteus vulgaris</i> ATCC 13315	25	>100	>100	3.13	6.25	50	50			12.5	25	>100	50	>100	12.5	12.5	50	50	12.5	25	4-8
<i>Pseudomonas aeruginosa</i> ATCC 10145	ND	12.5	25	6.25	3.13	100	100			25	100	>100	25	>100	3.13	25	25	50	25	NA	NA
<i>Pseudomonas aeruginosa</i> ATCC 27853	1.56	3.13	25	3.13	3.13	25	100			25	50	12.5	25	>100	0.78	12.5	25	25	12.5	12.5	4-8
<i>Serratia marcescens</i> ATCC 13880	>100	>100	>100	25	12.5	>100	>100			>100	>100	>100	>100	>100	>100	12.5	>100	>100	12.5	>100	>125 ^d
Fungi																					
<i>Candida albicans</i> ATCC 90028	100	12.5	>100	3.13	6.25	100	25			12.5	25	3.13	6.25	>100	3.13	25	6.25	25	3.13	25	4-8 ^e
<i>Cryptococcus neoformans</i> M68979	6.25	3.13	>100	1.56	0.39	50	25			3.13	12.5	0.78	1.56	25	0.39	3.13	3.13	25	0.78	12.5	NA
<i>Cryptococcus neoformans</i> ATCC 90012	ND	ND	>100	0.78	0.39	50	25			ND	12.5	6.25	1.56	25	0.39	3.13	3.13	25	0.78	12.5	NA
<i>Aspergillus fumigatus</i> H1120	ND	12.5	>100	12.5	12.5	>100	50			25	25	12.5	6.25	>100	12.5	100	25	50	12.5	25	NA
<i>Aspergillus fumigatus</i> H29709	ND	ND	>100	12.5	12.5	>100	50			ND	25	3.13	3.13	>100	12.5	100	25	50	12.5	25	NA
MHC	100	>100	>100	12.5	50	>100	50			>100	>100	6.25	3.13	>100	50	50	>100	>100	25	6.25	NA

^a Data taken from reference 14.^b ND, not done.^c NA, not applicable.^d *Serratia marcescens* ATCC 8100.^e *Candida albicans* ATCC 14053.

TABLE 2. Influence of divalent cations on the activity of SM-7 against four strains

Cation (concn [mM])	MIC ($\mu\text{g/ml}$)			
	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. marcescens</i> ATCC 13880	<i>S. aureus</i> ATCC 25923
CaCl ₂				
0.1	6.25	3.13	25	1.56
1	6.25	12.5	50	1.56
10	25	25	>100	6.25
100	>100	>100	>100	12.5
MgCl ₂				
0.1	6.25	3.13	25	1.56
1	6.25	6.25	50	1.56
10	>100	>100	>100	3.13
100	>100	>100	>100	12.5
Control (only TSB ^a)	6.25	3.13	25	1.56

^a TSB, trypticase soy broth.

coccus faecium and methicillin-resistant *S. aureus*. For example, the MICs of SM-7H and SM-25 for *S. aureus* ATCC 29213 were 0.78 $\mu\text{g/ml}$, and those of SM-1 and SM-25 for *Pseudomonas aeruginosa* ATCC 27853 were 1.56 and 0.78 $\mu\text{g/ml}$, respectively; these values are similar to those reported for squalamine. Some of the squalamine mimics, such as SM-7, SM-7H, SM-11, SM-27, and SM-34, had activity against *S. marcescens*, while squalamine has no activity against this species. Of all the compounds tested, SM-7 was the most active against gram-negative rods and SM-25 was the most active against gram-positive cocci and fungi.

Generally, among conjugates with the same sterol backbone, the compound with spermine as the side chain was the most active. The most active sterol-spermine conjugates, SM-7, SM-19, SM-25, and SM-34, incorporated deoxycholic acid,ursocholic acid, lithocholic acid, and chenodeoxycholic acid sterol backbones, respectively. The spermine-sterol conjugates that incorporated cholic acid, SM-13, and hyodeoxycholic acid, SM-28, were less active.

In contrast to squalamine, the presence of a sulfate group appeared to reduce the activity of the squalamine mimics. With the spermine-sterol conjugates SM-7 and SM-13, activity was greatly reduced with the presence of a sulfate group at position C-3 (R_1 in Fig. 1) (see structures of SM-8 and SM-14, respectively, in Fig. 1); SM-14 was inactive against all the strains tested (data not shown). There was a less marked reduction in the activity of the spermine-sterol conjugate SM-25 when a sulfate was added at C-3 (structure SM-22); in this case, the spectrum was narrowed, but potency against some species was preserved or enhanced.

Two compounds, SM-7H and SM-9H, were reduced forms of SM-7 and SM-9, respectively, in which the amide group was reduced to the amine (R_2 in Fig. 1). The activity of SM-9H was 2 to 16 times greater than that of SM-9 against gram-positive cocci, and it was 4 to 32 times greater than that of SM-9 against fungi. Only slight enhancement was seen when SM-7H and SM-7 were compared.

Some squalamine mimics, for example, SM-19, SM-21, and SM-38, had significant antimicrobial activities and caused hemolysis at low concentrations. There was, however, not an absolute correlation between the MICs and the MHCs of individual compounds; SM-7H was one of the most active antimicrobial agents and one of the least active hemolysins.

Effects of various factors on MIC determinations. Tables 2 and 3 describe the effects of divalent cations and serum, respectively, on the MICs of SM-7. Calcium or magnesium chloride decreased the antimicrobial activity of SM-7 in a dose-dependent manner. Horse serum also inhibited the antimicrobial activities of the seven squalamine mimics that were tested. The effects of pH were variable. For example, over a range from pH 5.0 to 8.5, the MICs of SM-7 for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 varied only 2-fold, while the MIC for *S. aureus* ATCC 25923 varied 32-fold. With the gram-negative organisms, the activity of SM-7 was greatest at neutral pH; with *S. aureus*, the activity increased as the pH was raised.

Bactericidal activity. Figure 2 indicates the bactericidal activity over time when SM-7 was tested against *E. coli*, *P. aeruginosa*, and *S. aureus*. SM-7 exhibited bactericidal activity in a dose-dependent manner.

Figure 3 indicates the effects of various cations and BSA on the bactericidal activity of SM-7 against *E. coli*. Monovalent and divalent cations inhibited the bactericidal activity in a dose-dependent manner. The highest concentration of BSA, equivalent to the concentration of albumin in normal human serum, completely inhibited the bactericidal activity of 12.5 μg of SM-7 per ml.

Membrane-permeabilizing activity. Table 4 presents the MICs of rifampin in the presence of SM-7. Subinhibitory concentrations of SM-7 enhanced the activity of rifampin for all the strains tested, especially *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumoniae* ATCC 13883, and *Serratia marcescens* ATCC 13880.

DISCUSSION

The squalamine mimics are sterol-polyamine conjugates (19). The squalamine mimics in this series were produced in high yield with readily available starting materials and a simple two- or three-step procedure. Several of these simple conjugates not only share structural similarities with squalamine but also have similar spectra and degrees of antimicrobial activity.

The free polyamines have little antimicrobial activity. Growth inhibition of *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *Proteus vulgaris*, or *Candida albicans* is only seen with concentrations of spermidine or spermine that exceed 100 $\mu\text{g/ml}$ (14, 21). The bile acids can inhibit some gram-positive bacteria and, to a lesser extent, some gram-negative organisms (10). None of the bile salts used to prepare these squalamine mimics inhibited any of the test strains at concentrations of $\leq 100 \mu\text{g/ml}$ (data not shown). The results of studies with the squalamine mimics confirm and extend the observations made with squalamine (13, 19); they demonstrate that sterol-polyamine conjugates can have significant and broad-spectrum antimicrobial activities that exceed the activities of their individual components.

TABLE 3. Influence of horse serum on the activities of squalamine mimics against *S. epidermidis* ATCC 12228

Compound	MIC ($\mu\text{g/ml}$)		
	No horse serum	10% horse serum	50% horse serum
SM-1	3.13	25	>100
SM-2	3.13	25	>100
SM-5	12.5	25	>100
SM-7	0.78	1.56	6.25
SM-11	6.25	12.5	50
SM-13	3.13	6.25	12.5
SM-17	12.5	50	>100

FIG. 2. (A) Time-kill studies of *E. coli* ATCC 25922 (SM-7 MIC, 3.13 $\mu\text{g/ml}$). (B) Time-kill studies of *P. aeruginosa* ATCC 27853 (SM-7 MIC, 3.13 $\mu\text{g/ml}$). (C) Time-kill studies of *S. aureus* ATCC 25923 (SM-7 MIC, 1.56 $\mu\text{g/ml}$). ●, control; ○, one-half the MIC; ■, the MIC; □, two times the MIC; ▲, four times the MIC.

The antimicrobial activities of conjugates with the same sterol and different polyamines varied markedly. In general, the spermine conjugate was more active than the putrescine or triethylenetetramine conjugates. Spermine and triethylenetetramine have four positive charges, while putrescine has two positive charges. The activities of the squalamine mimics varied with the cationic charge and with the length of the pendant polyamine.

Others have reported that cholane derivatives with basic side chains at the C-24 position show antimicrobial activity, mainly against gram-positive bacteria and less so against gram-negative bacteria (3–5). Lithocholic acid, chenodeoxycholic acid, and deoxycholic acid were very active steroid backbones, of which the “ β ” face is quite free of hydrophilic contributions, while hyodeoxycholic acid and cholic acid were weakly active structures, and ursodeoxycholic acid and urocholic acid were inactive forms. It has been postulated that the antimicrobial activity of cholane-24-benzylamines is dependent on both their hydrophobicity and the basicity of their side chains (3, 9).

The steroid backbone appeared to play a key role in the antimicrobial action of the squalamine mimics. This series suggests that the hydrophobicity of the steroid backbones and the basicity of side chains at the R_2 position were critical determinants of activity.

The mechanism of antimicrobial action of squalamine is unknown. It has been postulated that it may act like melittin, a basic antimicrobial peptide, by perforating membranes (14). Others have hypothesized that the mechanism may resemble that of polyene antibiotics such as amphotericin B, which con-

FIG. 3. Effects of various factors on bactericidal activity of SM-7 (12.5 $\mu\text{g/ml}$) against *E. coli* ATCC 25922. (A) Divalent cations. (B) Monovalent cations. (C) BSA.

sist of a lipophilic unit and a polar unit in a ring. Membrane lysis could occur through pore formation involving several polyene molecules in a circular array (half pore-hydrophilic channel) or barrel stave channel formation (15).

It is possible that squalamine, with its lipophilic sterol backbone, may form an intramolecular salt bridge between the cationic terminus of spermidine and the negatively charged sulfate residue. However, the ability to form such an intramolecular salt bridge does not appear to be essential for antimicrobial activity, because the squalamine mimics with no sulfate residue at the C-3 position were more active than the corresponding sulfated analogs.

The inhibitory activity of SM-7 against *E. coli* was diminished by various cationic agents such as Ca^{2+} and Mg^{2+} and by

TABLE 4. MICs of rifampin in the presence of SM-7 against various organisms

Organism	MIC ($\mu\text{g/ml}$) of rifampin with SM-7 at the following MIC:			FIC index
	0	1/16	1/4	
<i>E. cloacae</i> ATCC 13047	25	0.2	0.05	0.046
<i>E. coli</i> ATCC 25922	6.25	3.13	0.05	0.156
<i>E. coli</i> ATCC 35310	6.25	0.2	≤ 0.013	0.094
<i>K. pneumoniae</i> ATCC 13883	12.5	0.39	≤ 0.013	0.063
<i>P. vulgaris</i> ATCC 13315	12.5	0.78	0.1	0.094
<i>P. aeruginosa</i> ATCC 10145	25	25	6.25	0.5
<i>P. aeruginosa</i> ATCC 27853	25	25	0.2	0.29
<i>S. marcescens</i> ATCC 13880	12.5	0.2	0.05	0.063
<i>E. faecium</i> T 64809	12.5	6.25	1.56	0.38

high concentrations of Na^+ or K^+ . The antimicrobial activities of some basic antimicrobial peptides are inhibited by various cations, apparently because they reduce the binding of the peptides to the cell surface (2, 8, 12, 23, 24). The target sites of these peptides are thought to be lipopolysaccharides in gram-negative bacteria and lipoteichoic acid or teichoic acid in gram-positive bacteria, which are negatively charged (6, 8, 18, 20, 23). The activities of other cationic agents such as aminoglycosides are inhibited by divalent cations because they compete with initial binding to lipopolysaccharides or teichoic acid (11, 23). Our results suggest that the polycationic sites of the polyamine moiety may be essential to the activities of the squalamine mimics.

Enhancement of the activity of rifampin by subinhibitory concentrations of SM-7 was observed with all the strains of gram-negative rods that were tested. Rifampin is a very hydrophobic antibiotic with low levels of activity against gram-negative rods; it can be used as a marker to detect perturbations of membrane permeability in gram-negative bacteria because agents that increase permeability enhance the action of rifampin (23).

The antimicrobial activities of the squalamine mimics decreased in serum, possibly because of binding to albumin. The bactericidal action of 12.5 μg of SM-7 per ml was completely inhibited by 40 mg of BSA per ml; this concentration of albumin is similar to that of normal human serum.

Like squalamine, most of the active squalamine mimics, such as SM-7, SM-7H, SM-25, and SM-34, had significant hemolytic activities. There was an imperfect correlation between antimicrobial and hemolytic activities; for example, reduction of the amide in SM-7 to yield SM-7H resulted in a two- to fourfold enhancement of activity against many microbial strains and a fourfold reduction in hemolytic activity. This suggests that it might be possible to improve the target selectivity of squalamine mimics.

The squalamine mimics are a new class of antimicrobial agents that are simple and inexpensive to prepare. Some of these compounds have a broad spectrum of antimicrobial activity and appear to act as membrane-disrupting agents. This study has explored the relationship between the structure and biological function of a series of these sterol-polyamine conjugates. The antagonism of their activities by serum and albumin and their hemolytic potential may limit their utility as systemic agents. As has been suggested for squalamine, the squalamine mimics may be good candidates for development as topical antimicrobial agents (13).

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