Recent Advances in the Synthesis of Spermine and Spermidine Analogs of the Shark Aminosterol Squalamine

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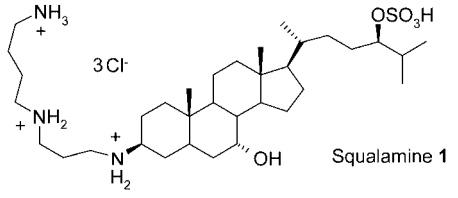
Great attention has recently been focused on the shark aminosterol squalamine (**1**) because of its important antimicrobial and antiangiogenic activities. Herein, we present the different series of spermine and spermidine analogs that have been synthesized. Many of the squalamine mimics demonstrate a broad spectrum activity against microorganisms. Squalamine is currently undergoing Phase II clinical trials in cancer patients.

Introduction

As a number of microorganisms exist in their environment, animals have developed means of controlling microbial growth. A variety of higher organisms synthesize antimicrobial substances supplementing the cellular and humoral systems. In recent years, a wide variety of low-molecular-weight antibiotics, including peptides, lipids, and alkaloids, have been isolated from diverse animal species.[1,2]

Among these substances, one of them, the shark aminosterol squalamine (**1**) (Scheme 1), has recently been the focus of a great deal of attention.[3] Moore et al. reported in 1993 the isolation, structural determination, and characterization of a water-soluble cationic steroid from the dogfish shark, *Squalus acanthias*, that exhibits potent antimicrobial activity against fungi, protozoa, and both Gram-negative and -positive bacteria.[4] Subsequent studies have shown it to exhibit also a potent antiangiogenic activity in animal systems. This natural amphiphilic steroid is a 7,24-dihydroxylated, 24-sulfated cholestane conjugated to spermidine at C-3.[5]



Squalamine **1**

Scheme 1

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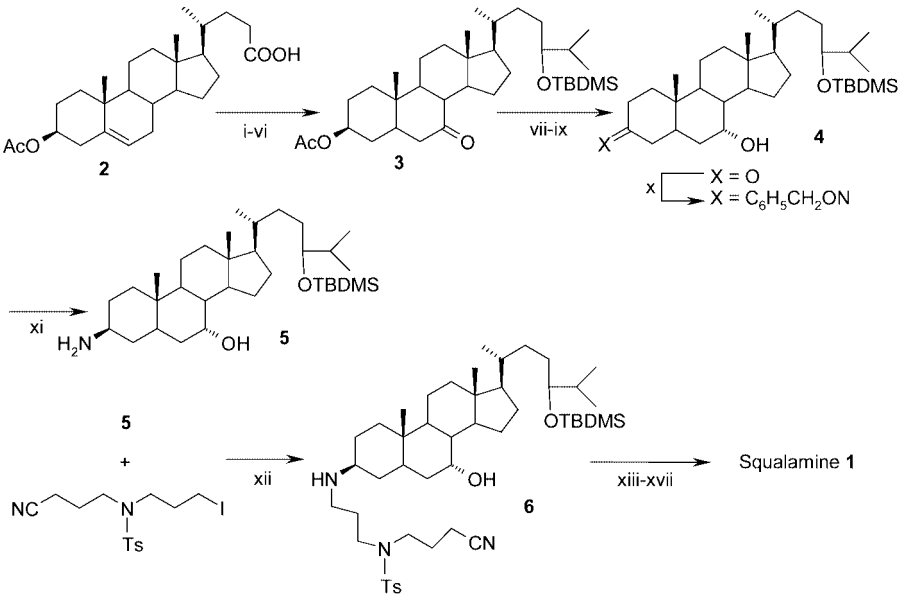
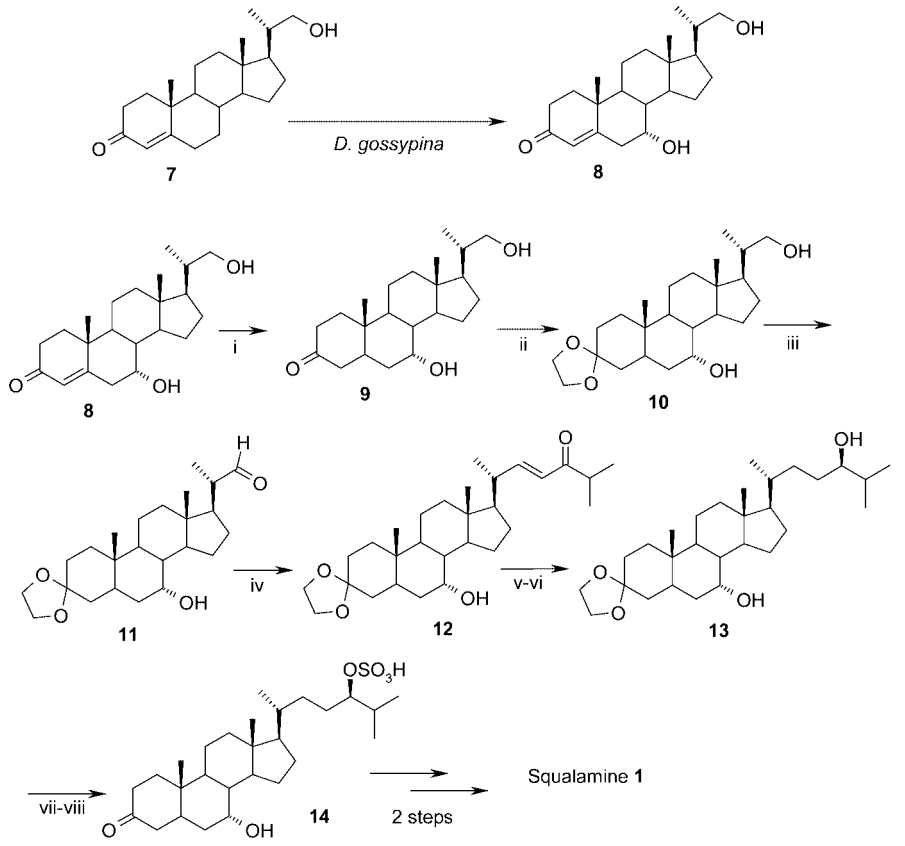
Jean Michel Brunel (left) was born in 1968 in Marseille, France. He was graduated from the School of Chemistry (Ecole Supérieure de Chimie de Marseille, ESCM) in 1991. He obtained his Ph.D. degree in 1994 from Université Aix Marseille III in the field of enantioselective synthesis and organophosphorus chemistry. In 1994, he joined the group of Professor H. B. Kagan (Université Paris Sud) as a postdoctoral fellow working on the enantioselective catalytic oxidation of sulfides (1994−1996). In 1997, he joined the CNRS as Chargé de Recherche and is now working in the Institut Méditerranéen de Recherche en Nutrition (IMRN INRA 1111). His research program is focused on the development of novel synthetic methods in asymmetric catalysis as well as the synthesis of a new class of antifungal agents.

Yves Letourneux (right) is corrently Professor of Biochemistry at the Université Aix-Marseille III. He received his Doctorate in 1975 from the Université Paris XI in the field ofmultistep synthesis of sterol derivatives. As Assistant of Biochemistry at the faculté de Médecine Pitié-alpétrière (Paris), he started his research career at the Institut de Chimie des Substances Naturelles, CNRS, then he moved to the Worcester Foundation (Massachusetts (USA) under the guidance of Marcel Gut, Principal Scientist, with a grant from the National Institute of Health. After a Post-Doctoral Fellowship from the World Health Organisation, Special Programm Research in Human Reproduction, with Professor P. Crabbé at the Université J. Fourier (Grenoble), he joined the Faculté de Pharmacie de Nantes as Maître de Conférences of Organic Chemistry. His field of interest is based essentially in the design and synthesis of medified natural lipids interfering with the human and microbial metabolism.

MICROREVIEWS: This feature introduces the readers to the author's research through a concise overview of the selected topic. Reference to important work from others in the field is included.



Scheme 2. Conditions: i) $(COCl)_2$, CH_2Cl_2, reflux, 2 h (100%); ii) $(CH_3)_2CHCdBr$, C_6H_6, room temp., 1 h (60%); iii) $Ca(BH_4)_2$, THF, room temp., 5 h (80%); iv) TBDMSCl, imidazole, CH_2Cl_2, room temp., 16 h (90%), v) $Cr(CO)_6$, *t*BuOOH, CH_3CN, reflux, 12 h (46%); vi) Li, liq. NH_3, Et_2O, −78 °C, 10 min (81%); vii) K-selectride, THF, −50 °C, 5 h (80%); viii) NaCN, MeOH, reflux, 8 h (88%); ix) $(t\text{BuO})_3Al$, cyclohexanone, toluene, 110 °C, 20 h (59%): x) $C_6H_5CH_2ONH_2{\cdot}HCl$, C_5H_5N, C_2H_5OH, reflux, 16 h (97%); xi) $LiAlH_4$, Et_2O, reflux, 16 h (98%); xii) K_2CO_3, CH_3CN, reflux, 20 h; xiii) $C_6H_5CH_2OCOCl$, NaOH, THF, 0 °C to room temp., 4 h (70%); xiv) Na, liq. NH_3, THF, −78 °C to room temp., 18 h (91%); xv) $LiAlH_4$, Et_2O, reflux, 6 h (93%); xvi) HCl, EtOH, room temp., 3 h (98%); xvii) $C_5H_5N{\cdot}SO_3$, C_5H_5N, 75 °C, 2 h (10%)



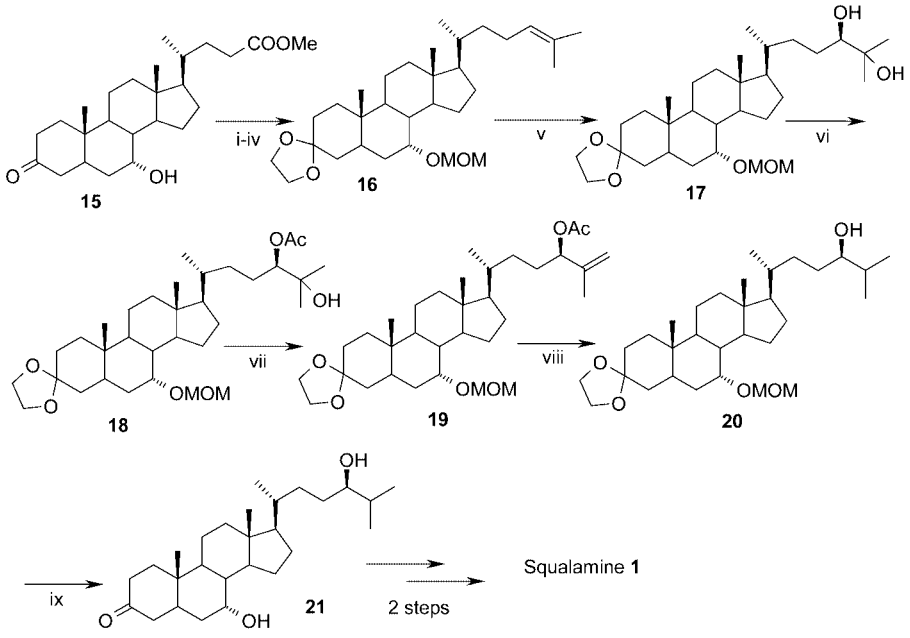
Scheme 3. Conditions: i) Li, NH_3, THF (71%); ii) TMSCl, ethylene gycol (84%); iii) NaOCl, TEMPO, NaBr, CH_2Cl_2 (98%); iv) $(EtO)_2P(O)CH_2C(O)CH(CH_3)_2$, *t*BuONa, THF (82%); v) (*R*)-MeCBS, $BH_3{\cdot}THF$, THF/toluene (80%); vi) Et_3N, toluene, 10% Pt/C, H_2 (3.5 bar) (92%); vii) *p*TsOH, water, acetone (89%); viii) $SO_3{\cdot}Py$ (1.05 equiv.), pyridine (77%)

At present the feasibility of obtaining large quantities of this steroidal antibiotic from natural sources appears questionable, since only trace amounts are present in the liver and gallbladder of the shark. Moreover, the actual synthesis requires expensive materials, making such a route impractical for large-scale preparations. Thus, numerous studies have been devoted to the synthesis of molecules mimicking not only the structure of squalamine but also its remarkable antimicrobial properties. Thus, squalamine mimics are based on the cholestane or bis(norcholenic acid) skeleton.

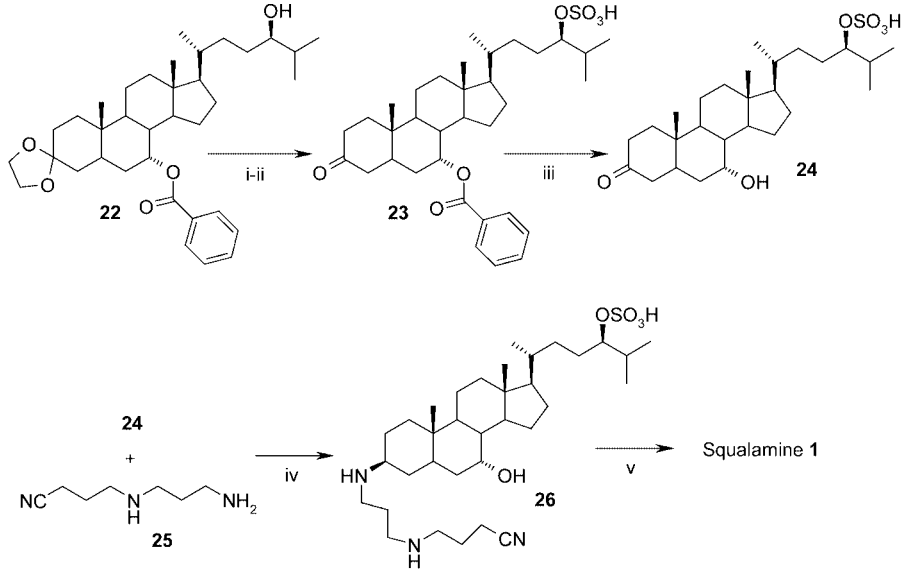
Synthesis of Spermine and Spermidine Analogs of Squalamine

a) Cholestane Analogs

Confirmation of the proposed structure of squalamine (**1**) was obtained by comparison with the synthetic compound, epimeric in C-24, synthesized for the first time in 1994 by Moriarty et al. in a 17-step sequence from 3β-acetoxy-5-cholenic acid (**2**) with an overall yield of 0.3% (Scheme 2).[6]



Scheme 4. Conditions: i) $CH_3OCH_2OCH_3$, P_2O_5, $CHCl_3$, room temp. (94%); ii) ethylene glycol, PTSA, benzene, reflux (96%); iii) $LiAlH_4$, THF, room temp. (96%); iv) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; BuLi, $Ph_3P^+CH(CH_3)_2I^-$, THF, room temp. (93%); v) $(DHQD)_2PHAL$, $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, *tert*-butyl alcohol/*tert*-butyl methyl ether/H_2O (2.5:3:2.5), room temp. (83%); vi) Ac_2O, pyridine, room temp. (92%); vii) CH_3SO_2Cl, DMAP, Et_3N, CH_2Cl_2, 0−20 °C (64%); viii) NH_2OH, EtOAc, DMF, 95 °C, then KOH, MeOH, reflux (86%); ix) PPTS, *t*BuOH, reflux (92%)



Scheme 5. Conditions: i) Amberlyst 15, acetone, room temp.; ii) SO_3·Py (2 equiv.), pyridine, 80 °C (77%); iii) KOH (6 equiv.), MeOH, reflux (90%); iv) trimethyl orthoformate (10 equiv.), $NaBH_4$ (1.5 equiv.), MeOH, −78 °C; v) EtOH, PtO_2 (2.8 bar), TFA to pH = 2 (60%)

In 2000, Kinney et al. reported an eleven-step preparation of squalamine (**1**) from a microbial metabolite **8** which is available in one step from 3-oxo-23,24-dinorchol-4-en-22-ol (Scheme 3).[7]

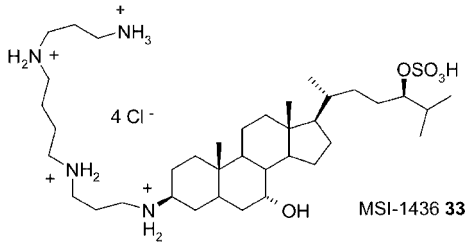
More recently, Zhou et al. have described a stereoselective construction of the squalamine side chain by using methyl 3-oxo-5α-chenodeoxycholanate (**15**) as the starting material and an improved Sharpless catalytic asymmetric dihydroxylation as the key step (Scheme 4).[8]

The short route to the precursor of squalamine was achieved in nine steps with an overall yield of 31% and 99% *de*.

In 1998, a straightforward five-step synthesis of squalamine (**1**) was presented from **22** and the easily prepared reagent **25**. This reagent was shown to be the most appropriate one since it is stable to reductive amination conditions and is converted into spermidine under weakly acidic conditions (Scheme 5).[9]

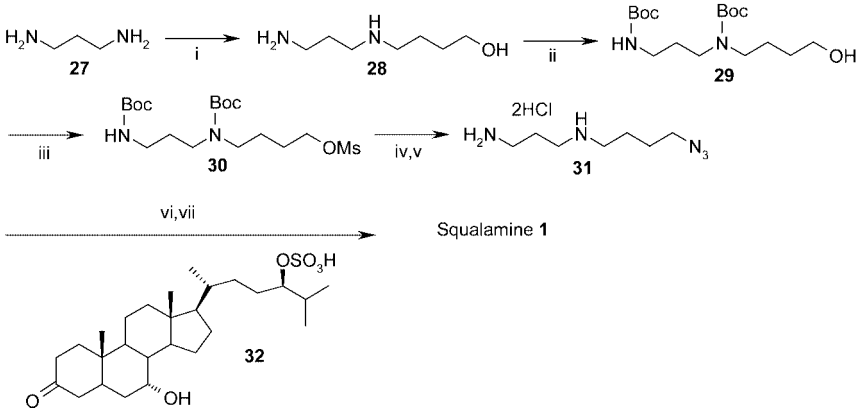
An efficient synthesis and conversion of an azidospermidine equivalent for squalamine synthesis has also been reported. Thus, the reagent **31** is stable to sodium borohydride reduction, is converted into spermidine under mild conditions and is not prone to internal cyclization (Scheme 6).[10]

In the same area, Shu et al. have investigated the synthesis of a new class of aminosterols, since squalamine is only one of a number of aminosterols isolated from the dogfish shark. Among these, MSI-1436 {[(24*R*)-3β-{[3-({4-[(3-aminopropyl)amino]butyl}amino)propyl]amino}-7α-hydroxy-5α-chlorestan-24-yl] sulfate} (**33**) is structurally identical to squalamine except for the polyamine at C-3 (Scheme 7).[11]

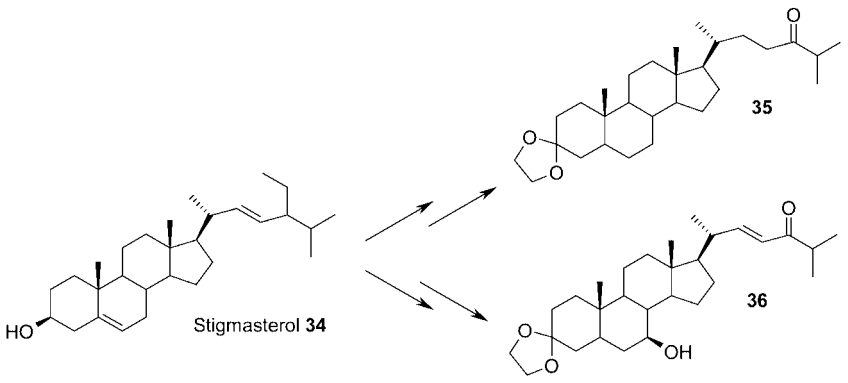


Scheme 7

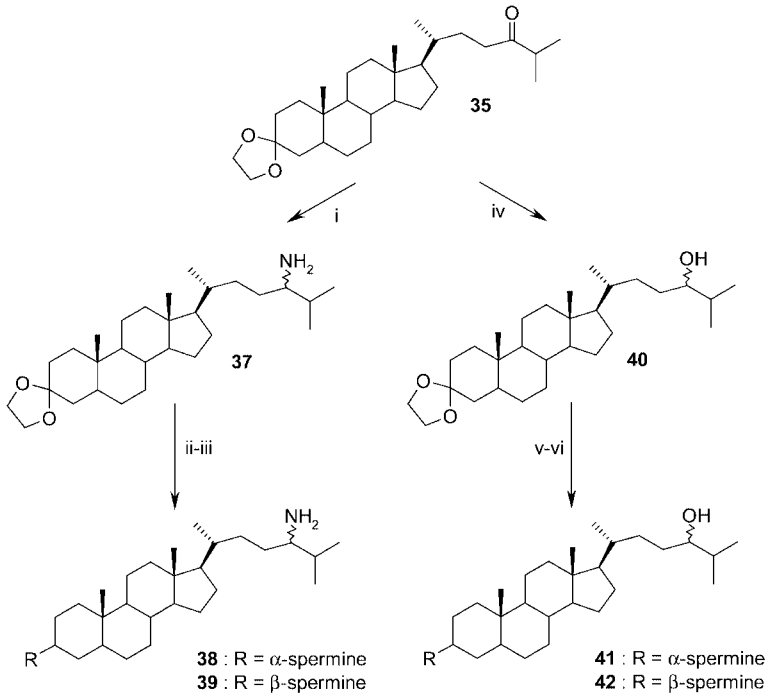
Thus, stigmasterol (**34**) was chosen as the starting material for the preparation of precursors **35** and **36** used in the synthesis of MSI-1436 analogs both with and without a 7-hydroxy group (Scheme 8).



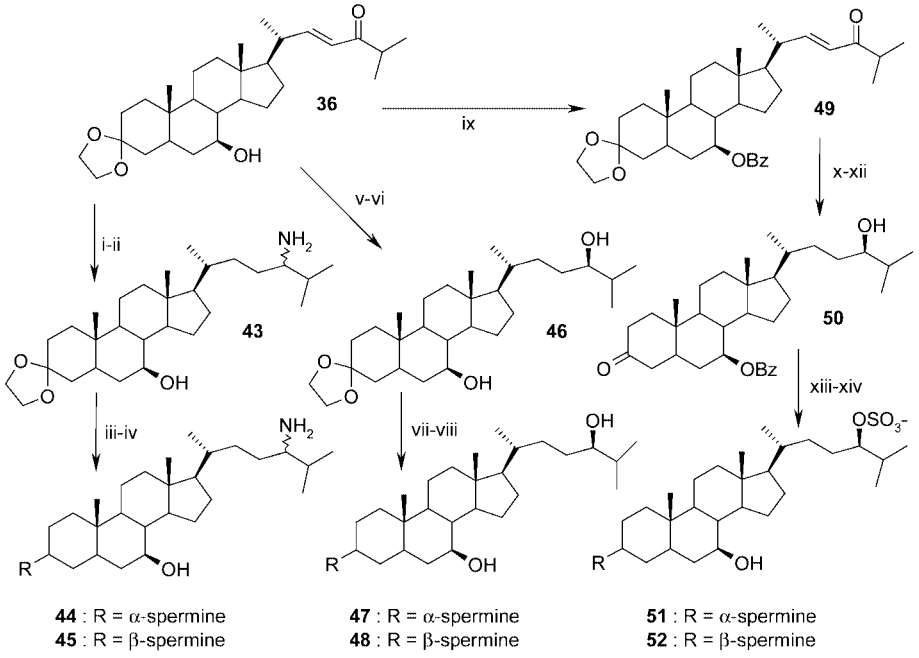
Scheme 6. Conditions: i) 0.37 equiv. 4-chlorobutanol, H_2O, 140−150 °C (48%); ii) Boc_2O, EtOH, room temp., 48 h; iii) Et_3N, MsCl, CH_2Cl_2, room temp., 16 h; iv) NaN_3, DMF, room temp., 48 h (80%); v) HCl, dioxane, room temp., 16 h; vi) 2 equiv. NaOMe, 0.5 equiv. **32**, molecular sieves (3 Å), MeOH, room temp., 24 h then $NaBH_4$, −78 °C; vii) H_2, Raney Ni (69%)



Scheme 8



Scheme 9. Conditions: i) NH_4OAc, $NaCNBH_3$ (69%); ii) HCl, acetone (95%); iii) spermine, $NaCNBH_3$; iv) $NaBH_4$ (96%); v) HCl, acetone (98%); vi) spermine



Scheme 10. Conditions: i) H_2, Pd/C (94%); ii) NH_4OAc, $NaCNBH_3$ (71%); iii) HCl, acetone (93%); iv) spermine, $NaCNBH_3$; v) chiral reduction (95%); vi) H_2, Pt (85%); vii) HCl, acetone (92%); viii) spermine, $NaCNBH_3$; ix) benzoyl chloride, DMAP (85%); x) chiral reduction (93%); xi) H_2, Pt (93%); xii) HCl, acetone (94%); xiii) SO_3, pyridine (85%); xiv) spermine, $NaCNBH_3$, LiOH

The protected 3,24-dione **34** was used as the precursor of the four different aminosterols **38**, **39**, **41**, and **42** synthesized according to the pathway outlined in Scheme 9.

On one hand, a synthetic route (Scheme 10) has described the preparation of MSI-1436 analogs **44**, **45**, **47**, **48**, **51**, and **52** with a 7β-hydroxy substituent from the key intermediate 3,3-(ethylenedioxy)-7β-hydroxy-5α-cholest-22-en-24-one (**36**), which is easily obtained in a 6-step sequence from stigmasterol (**34**).

On the other hand, Moriarty et al. have investigated the synthesis of squalamine dessulfate (24*R*) and the unnatural epimer (24*S*) from stigmasterol (**34**).[12] The key step, establishing the C-24 stereochemistry, is the attachment of the side chain at C-22, using either (2*R*)- or (2*S*)-1,2-epoxy-3-

methylbutane (**55** or **56**), to afford the corresponding squalamine dessulfate stereoisomer (Scheme 11).

Nevertheless, in this case, no results have been reported concerning the antimicrobial activities of the synthesized products.

b) Dinorcholenic Analogs

In 1995, Regen et al. reported the design and three-step synthesis of efficient squalamine mimics possessing similar antimicrobial activities. Synthesis of these analogs was realized from 3β-hydroxy-23,24-dinor-5-cholenic acid (**61**) as outlined in Scheme 12.[13]

In 2000, Kim et al. reported the synthesis of squalamine analog **74** having a shorter side chain, which they prepared from the inexpensive 22-hydroxy-23,24-dinorchol-4-en-3-one (**66**) (Scheme 13).[14]

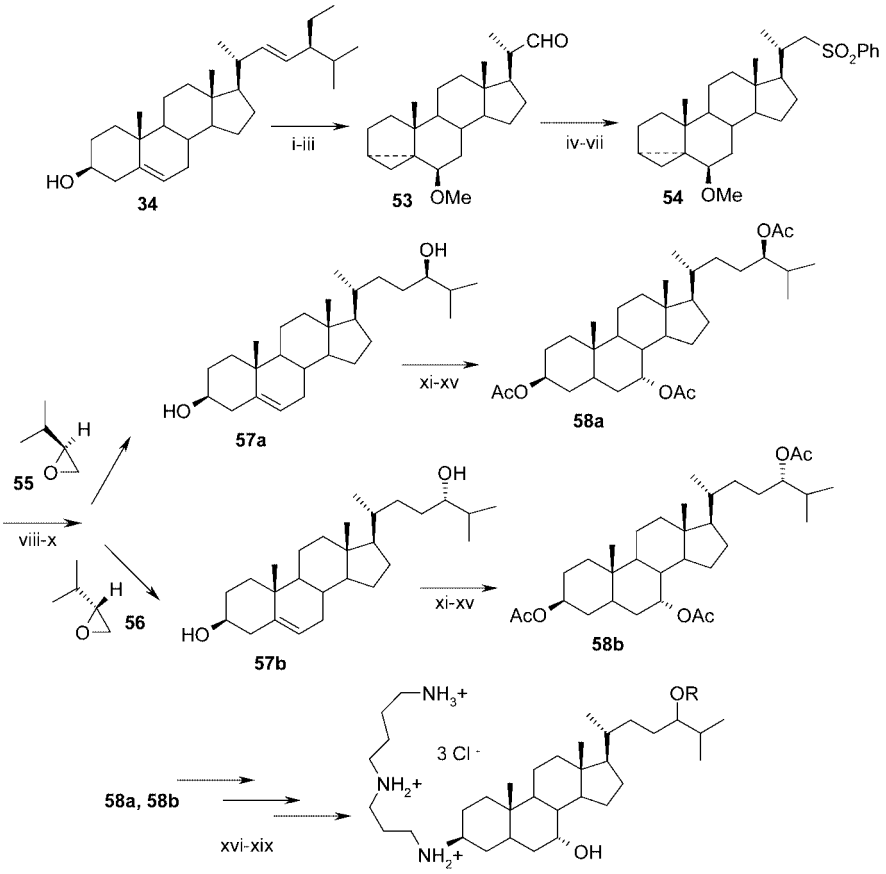
Furthermore, Gilbert et al. have investigated the role of the sulfate group of the squalamine (**1**) by preparing numerous compounds with and without this group. Their strategy developed 3β-acetoxydinor-5-cholenic acid (**75**) as the starting steroid for the synthesis of squalamine analogs **79** and **80** (Scheme 14).[15]

Moreover, in 1996, Jones et al. described the syntheses, characterization, and antimicrobial activities of squalamine analogs **85**−**90**, namely the 6β-hydroxy-3-aminosterols synthesized from hyodeoxycholic acid.[16] The synthesis of such analogs was performed as outlined in Scheme 15.

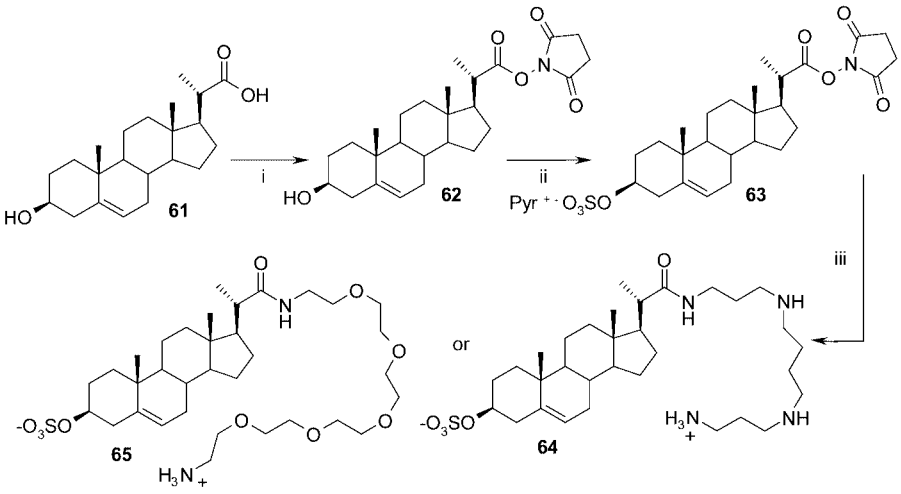
In this case, the efforts of the authors have been concentrated on bile acid analogs that do not contain the 24-sulfate unit or the squalamine side chain, but instead possess different polyamine substituents, stereochemical modifications, and substitutions on rings A and B (Scheme 16).

Biological Results

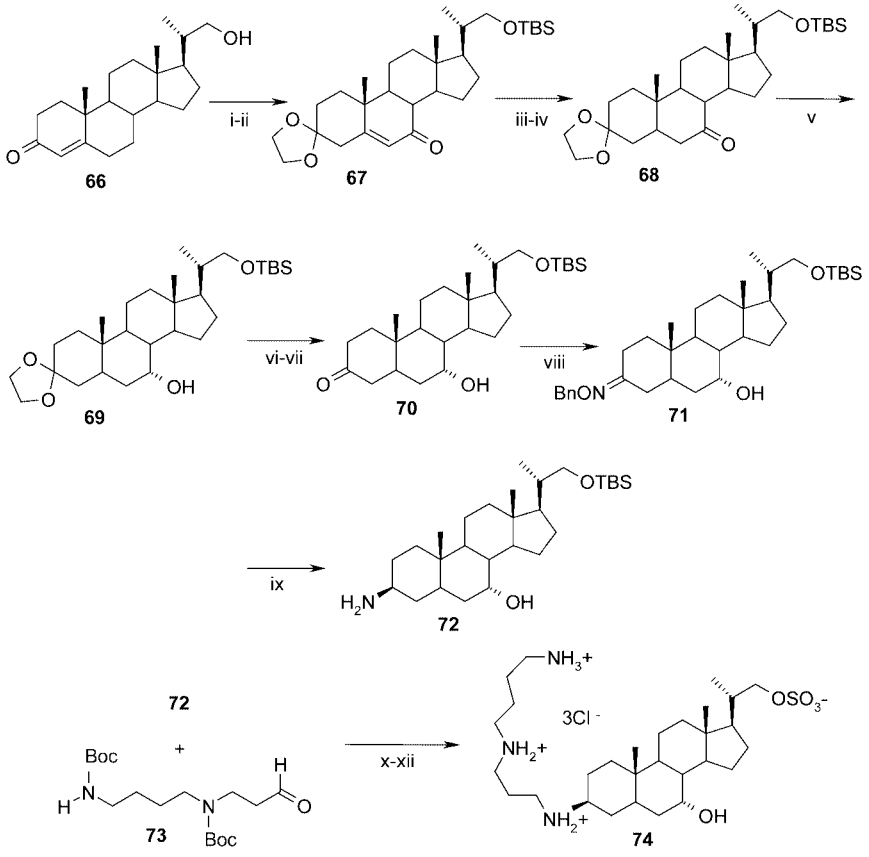
Squalamine (**1**) and many squalamine mimics display a broad-spectrum activity against bacteria and fungi. Since squalamine does interact preferentially with anionic lipids, the selectivity of squalamine for prokaryotic over eukary-



Scheme 11. Conditions: i) TsCl, pyridine, 25 °C, 14 h (100%); ii) MeOH, KOAc, reflux, 4 h (80%); iii) O_3, MeOH, −78 °C (92%); iv) $NaBH_4$, MeOH, 0−25 °C (85%); v) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C, 2 h; vi) NaI, Me_2CO, reflux, 17 h (90%, 2 steps); vii) $PhSO_2Na$, DMF, 25 °C, 32 h (91%); viii) *n*BuLi, epoxide **55** or **56**, −78 °C, 2 h (90%); ix) Li, NH_3, −78 °C, 30 min (80%); x) TsOH, dioxane/H_2O (7:3), 80 °C, 1 h (95%); xi) Ac_2O, pyridine, 25 °C, 14 h (95%); xii) CrO_3, DMP, CH_2Cl_2, −20 °C, 24 h (75%); xiii) Li, NH_3, −78 °C, 10 min (80%); xiv) $KB[CH(CH_3)C_2H_5]_3H$, THF, −50 °C, 6 h (75%); xv) Ac_2O, DMAP, CH_2Cl_2, 25 °C, 14 h (85%); xvi) NaCN, MeOH, 25 °C, 48 h (85%); xvii) CrO_3, H_2SO_4, H_2O (75%); xviii) *t*BocNH$(CH_2)_4$N(*t*Boc)$(CH_2)_3NH_2$, $NaBH_3CN$, 25 °C, 14 h (80%); xix) HCl, MeOH, 14 h (50%)

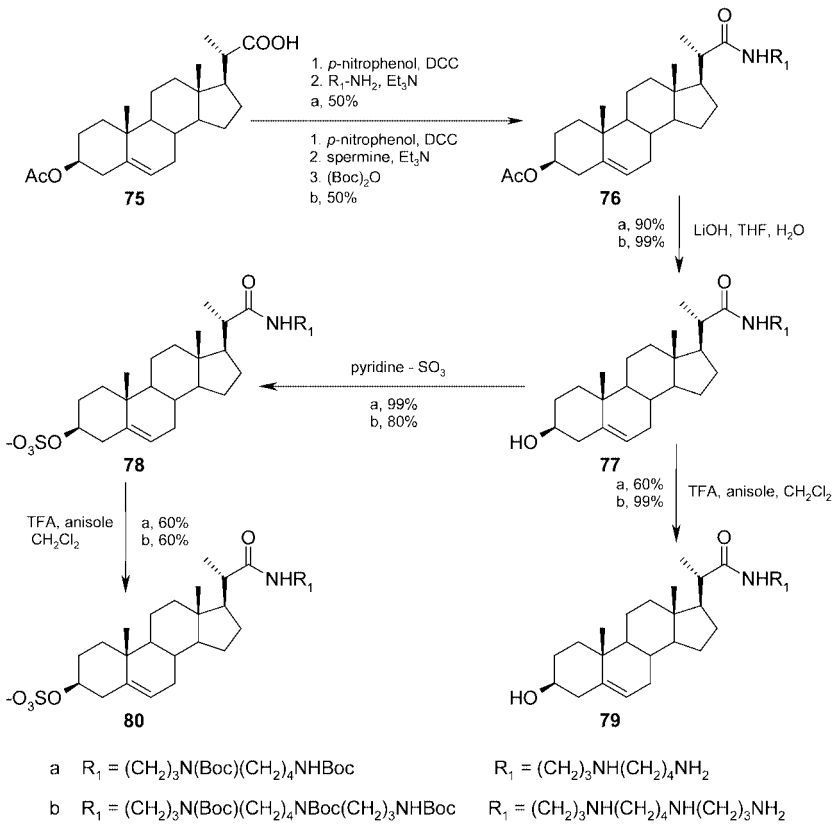


Scheme 12. Conditions: i) *N*-hydroxysuccinimide, DCC, THF, 50 °C, 3 h (58%); ii) Pyr·SO_3 (3 equiv.), $CHCl_3$, 14 h, room temp. (84%); iii) spermine (1.6 equiv.) or 1,17-diamino-3,6,9,12,15-pentaoxaheptadecane, DMF, 1.5 h, room temp. (20% for **64** and 12% for **65**)

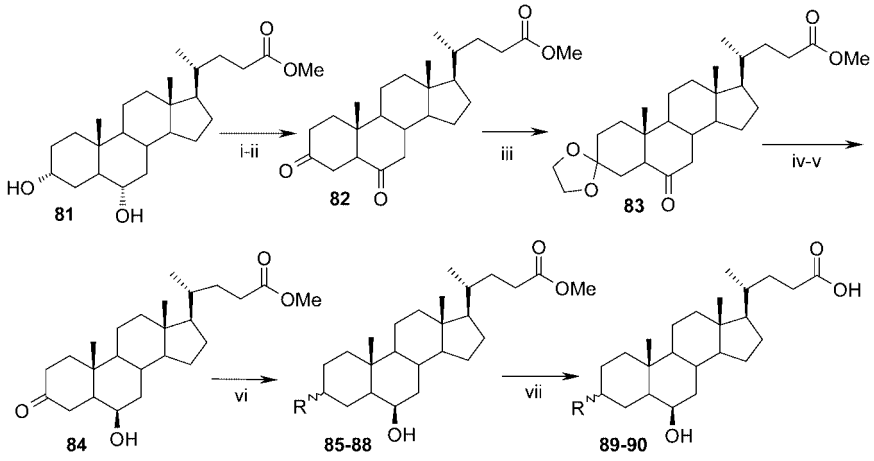


Scheme 13. Conditions: i) $HOCH_2CH_2OH$, PTSA, benzene (85%); ii) TBSCl, imidazole, DMAP, CH_2Cl_2 (95%); iii) $RuCl_3$, TBHP, cyclohexane (68%); iv) H_2, 5% Pt/C, EtOAc (80%); v) K-Selectride, THF (98%); vi) 1 N HCl, THF (97%); vii) TBSCl imidazole, DMAP, CH_2Cl_2 (95%); viii) $BnONH_2$·HCl, pyridine, EtOH (93%); ix) $LiAlH_4$, Et_2O (95%); x) $NaBH(OAc)_3$, CH_2Cl_2 (56%); xi) 10% HCl, MeOH (90%); xii) SO_3, pyridine, MeOH (15%)

otic cells, and the low hemolytic activity of squalamine, indicates that squalamine selects cellular surfaces based upon electrostatic interactions.[8] Squalamine (**1**) exhibits some hemolytic activity occurring at higher concentrations than observed for nonselective membrane disruptive amphipathic molecules, such as mellitin. Moreover, the activity of squalamine has been compared with that of several molecules and the data, summarized in Table 1, suggest that the biological activity of squalamine results from the synergistic combination of an anionic bile salt with spermidine, each of which independently exhibits considerably less antibiotic activity than squalamine.



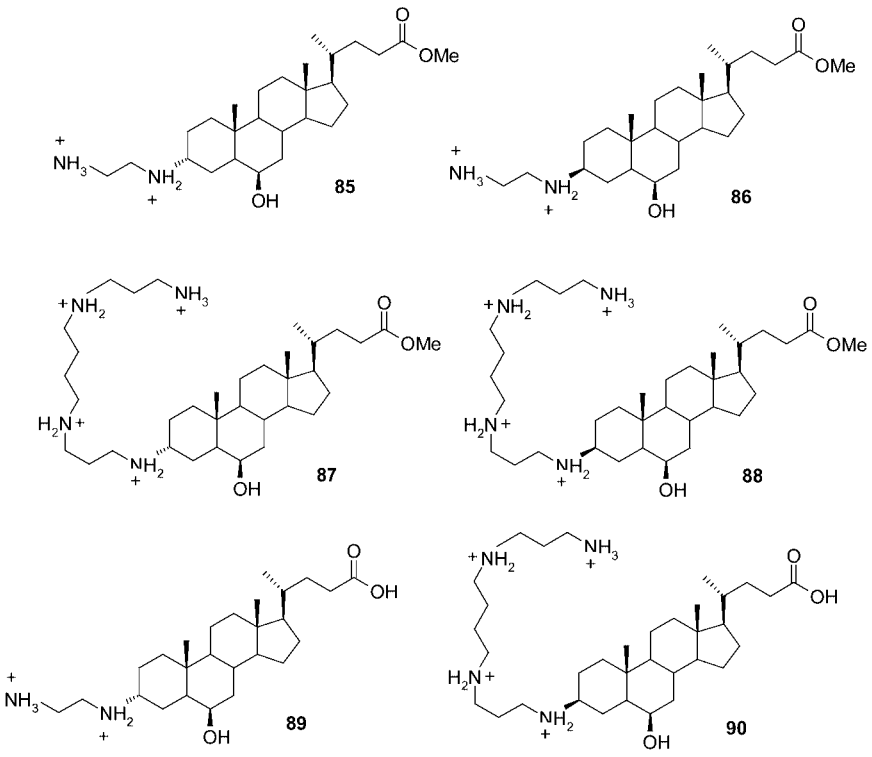
Scheme 14



Scheme 15. Conditions: i) PCC (3 equiv.), CH_2Cl_2 (96%); ii) HCl, MeOH (100%); iii) ethylene glycol, TsOH, C_6H_6, Dowex (89%); iv) $NaBH_4$, MeOH (87%); v) H^+, acetone (100%); vi) $NaBH_3CN$, ethylenediamine or spermine, THF/MeOH, pH = 6; vii) NaOH, THF

Table 1 presents a summary of the biological activities of the different synthesized spermine and spermidine analogs against representative bacteria and fungi.

Squalamine possesses a spermidine moiety, while analog MSI-1436 (**33**) is linked to the symmetrical polyamine, spermine. All of the aminosterols isolated from the shark exhibit antimicrobial activity, but compound **33** displays the greatest efficiency against a variety of microorganisms. Thus, changing the functionality on C-24 at the side chain has little effect upon antimicrobial activity. A 7-hydroxy



Scheme 16

Table 1. Antimicrobial activities of spermine and spermidine analogs of squalamine

Sample	Antimicrobial activity (MIC) [μg/mL]				
	E. Coli (25922)	*Pseudomonas aeruginosa* (27853)	*Staphylococcus aureus* (29213)	*Proteus vulgaris* (13315)	*Candida albicans* (14053)
Squalamine (**1**)	1−2	4−8	1−2	4−8	4−8
Spermidine	> 500	> 500	> 500	> 500	> 500
CHAPS	> 500	> 500	> 500	> 500	> 500
Melittin	8−16	16−31	8−16	16−31	16−31
64	6.25	3.13	> 100	> 100	12.5
65	> 100	> 100	> 100	> 100	> 100
74	> 100	> 100	−	> 100	−
85	32−64	128	16	−	8
86	8−16	64	1	−	2−4
87	32	128	2−4	−	4
88	32	32	2	−	2
89	> 256	128	> 256	−	> 256
90	16	8	16	−	4
MSI-1436 (**33**)	1	4	1	−	4
38	8	8	1−2	−	2
39	16	4	2−4	−	4
40	8	32	4−8	−	4
41	64	32	4	−	4
44	16	256	2−4	−	64
45	8	128	4	−	16
47	64−128	32	4−8	−	4
48	32	32	4−8	−	16
51	64	128	16	−	64
52	8−16	8	2−4	−	16

substituent does not seem essential to the activity of squalamine. The stereochemistry at C-7, however, does affect the activity with a simultaneous change of the stereochemistry in C-3.

Of particular significance is the fact that compound **64** mimics the squalamine antibiotic properties by exhibiting potent activity against a broad spectrum of microorganisms. The fact that compound **65** exhibits negligible antimi-

crobial activity suggests that the pendant polyamine chain plays an important role with respect to the biological activity of **64**.

Furthermore, these authors have demonstrated that **64** favors the transport of ions across negatively charged bilayers (egg phosphatidylglycerol, egg PG) over ones that are electrically neutral (egg phosphatidylcholine, egg PC), suggesting that **64** is a synthetic ionophore that exhibits membrane selectivity as well as ion selectivity.[17]

Moreover, compound **74**, possessing a shorter side chain, shows weaker antimicrobial activity than squalamine under identical conditions.

Several conclusions can be drawn about the antimicrobial activities of the analogs **85**−**90** from structure/activity relationships. Thus, the identity of the polyamine chain has only limited importance in the antimicrobial activity in the case of esters, but the spermine carboxylic acid **90** is dramatically more active than the ethylenediamine carboxylic acid **89**. Furthermore, analogs possessing a spermidine moiety on squalamine attached with a β-stereochemistry at the sterol ring have been selected. Moreover, the 6β-hydroxy-substituted compounds exhibit antimicrobial activities similar to that of squalamine, suggesting that the absolute positioning of the hydroxy group is not important.

Several of compounds **76**−**80** showed significant in vitro activity against parasites like *T. brucei and L. donovani* and little against *T. cruzi*. Nevertheless, at this stage it is unclear why there is such a marked difference in activity between the species (Table 2).

Table 2. Values of ED_{50} activity [μM] for compounds **76**−**80** against trypanosomes and leishmania

Compound	*L. donovani*	*T. cruzi*	*T. brucei*	Toxicity (KB cells)
Spermidine series:				
76a	17.3	> 42	2.6	111
77a	25.7	> 15	4.7	65
78a	4.9	> 36	> 36	> 360
79a	44.7	> 63	1.9	3.3
80a	39.9	13.5	> 53	> 526
Spermine series:				
76b	4.7	> 34	1.1	31
77b	12.6	> 36	0.6	27
78b	>30	> 30	5.4	1.5
79b	5.0	> 57	0.56	15
80b	20.5	> 48	> 48	< 0.47

Clinical Perspectives

Squalamine (**1**) causes the leakage of the fluorescent calcium-selective dye from lipid vesicles or either anionic or zwitterionic phospholipids. In the same study, it was demonstrated that squalamine does not act as a proton ionophore when incorporated into large, unilamelar phospholipid vesicles, nor when added externally to a preformed vesicle preparation.[4,18,19] The antimicrobial squalamine has been shown to be an angiogenesis inhibitor and an antitumor compound. This natural aminosterol inhibits mitogen-induced proliferation and migration of endothelial cells in vitro and causes significant in vivo inhibition of angiogenesis.[20]

Mechanistic studies have revealed that squalamine inhibits the sodium/hydrogen exchanger (isoform NHE 3) causing changes in intracellular pH that lead to alterations in the shape and volume of endothelial cells.[21]

Another aspect of the mechanism of squalamine action involves the specific entry of squalamine into endothelial cells and intracellular redistribution of calmodulin (CaM), which depletes CaM in certain cellular compartments and enriches CaM in others, but without necessarily inhibiting CaM function.[19] Squalamine, therefore, represents the first of a new class of biological modifiers that act as CaM chaperones, altering cellular responses to stimuli that increase intracellular calcium concentrations.

In various xenograft models, squalamine strongly potentiates the antitumor activity of cytotoxic agents against primary lung tumors,[22,23] chemoresistant human non-small-cell lung carcinoma,[24] breast,[22] brain,[20] and ovarian[25] tumors.

It is, therefore, not surprising that the most remarkable effect seen with squalamine in preclinical studies has been in combination with the most commonly used chemotherapeutic agents: cisplatin, carboplatin, cyclophosphamide, and 5-fluorouracil.[20,23,26]

Squalamine is presently undergoing Phase II clinical trials in cancer patients.

Conclusion

Squalamine (**1**) and its spermine or spermidine analogs exhibit high antimicrobial and antiangiogenic activities. Moreover, its antimicrobial activity for immuno-compromised patients under chemotherapy seems to be of great interest. More generally, the pharmacological properties of aminosterols have been partially explored despite their high pharmacological potentials.[16,27−29]

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