Quaternary Alkylammonium Conjugates of Steroids: Synthesis, Molecular Structure, and Biological Studies

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Abstract: The methods of synthesis as well as physical, spectroscopic (1H-NMR, 13C-NMR, and FT-IR, ESI-MS), and biological properties of quaternary and dimeric quaternary alkylammonium conjugates of steroids are presented. The results were contrasted with theoretical calculations (PM5 methods) and potential pharmacological properties (PASS). Alkylammonium sterols exhibit a broad spectrum of antimicrobial activity comparable to squalamine.

Keywords: squalamine; bile acids; sterols; quaternary alkylammonium salt; conjugates; prediction of activity spectra for substances (PASS); PM5 calculations

1. Introduction

Steroids are an enormous group of very important natural products. The most significant compounds of this group are sterols (cholesterol, ergosterol, stigmasterol), bile acids (lithocholic, deoxycholic, cholic), and hormones (testosterone, estrogens, progesterone) [1–5]. Sterols are crucial constituents of the cell membrane of eukaryotes. Bile acids are amphipathic molecules with large, curved and rigid skeletons; chirality as well as the specific orientation of their chemically different polar hydroxy groups play an important role in metabolic processes. In turn, hormones determine the characteristics of sex and regulate pregnancy in animals, while plant hormones (brassinosteroids) cause elongation of stems and stimulate cell division (e.g., brassinolide) [6].

Another class of compounds that are involved in many biological processes are polyamines (spermidine, spermine, putrescine, cadaverine) [7–10]. Some of these are very important plant hormones and coenzymes.

The connection of steroids and biogenic amines give the new conjugates unusual biological properties. The best-known compound of this type is squalamine (3β-spermidine-7α-hydroxy-5α-cholestan-24R-yl sulphate) (1) (Figure 1). The steroid–polyamine conjugate was isolated from the liver tissues of the dogfish shark (Squalus acanthias) [11–14]. This aminosterol is a novel broad-spectrum antibiotic and exhibits a biocidal activity against Gram-positive and Gram-negative bacteria, fungi, protozoa, and viruses [15–23]. The antimicrobial activity of the squalamine has inspired work to design and synthesize new derivatives of steroidal–polyamine conjugates [24–32].
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Figure 1. (a) The stereochemistry and numbering of squalamine and (b) a molecular model calculated by the PM5 method.

2. Quaternary Alkylammonium Conjugates of Steroids

The basic criteria for the synthesis of biologically active conjugates of steroids and polyamines have been given by Salunke et al. [11]. Firstly, the structure must have a rigid extensive hydrophobic part and a flexible hydrophilic chain with a polar head group attached to a hydrophobic part. Secondly, the sulfate groups can be removed or replaced by a hydroxyl or carboxylate group. In turn, the structure of the polyamine is not important, and parts of steroids can be modified in various ways.

On this basis, Kim et al. described the synthesis of a squalamine analogue from bisnoralcohol (2) (Scheme 1) [16]. The structure of the product was confirmed by 1H-NMR, 13C-NMR, DEPT, COSY, HETCOR, and FT-IR, as well as low- and high-resolution mass spectra. Additionally, the biological activity of (4) has been determined. The squalamine analogue shows biocidal activity against M. luteus 9341, S. aureus 6538P, K. pneumoniae 10031, S. equi 6580C, and B. subtilis 6633. However, E. coli 25922, P. aeruginosa 27853, P. mirabilis 25933, S. marcescens 27117, and S. typhimurium 14028 are not sensitive to (4). In general, the antimicrobial activity of compound (4) is weaker in comparison to the antibacterial activity of squalamine.

Scheme 1. Synthesis of analogue of squalamine (4) from bisnoralcohol (2).
Other analogs (6–15) of MSI-1436 (5) have been synthesized from stigmasterol by Shu et al. (Figure 2) [33]. The multistep reactions gave final products with very good yields. All analogs exhibit a broad spectrum of antimicrobial activity, which strongly depend on the stereochemistry of C(7) and C(3). By contrast, the stereochemistry at the C(24) has a negligible effect on the antibacterial activity. The structure of MSI-1436 (5) and its synthesized analogs (6–15).

Similarly, Kim and co-workers focused on the effect of stereochemistry at the C(3) and C(5) atoms of steroids’ skeleton, as well as the types of polyamine attached to C(3) on activity against various human pathogens (Figure 3) [34–37]. The results showed that the stereochemistry of the C(3) and C(5) carbon atoms has a significant influence on the antimicrobial activity. For example, 3α-spermidine-23,24-bisnor-5α-cholane (16) was found to be more active than other spermidine analogues (16–19). However 3β-spermine-23,24-bisnor-5β-cholane (23) exhibits the highest biological activity among all the compounds (16–23). The conjugate (17), which is similar to (24–26) with the exception of the functional group at position C(7), has comparable antimicrobial activity to (25). Both compounds were much more active than the compounds (24) and (26). All synthesized conjugates (16–26) exhibited very good activity against Gram-positive bacteria.

The synthesis of a series of 7-fluoro-3-aminosteroids (36–42) is shown in Scheme 2 [37]. These compounds demonstrate a high antimicrobial activity, especially against Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, and Escherichia coli (Table 1).
Scheme 2. Synthesis of 7-fluoro-3-aminosterols (36–42).

Table 1. Minimum inhibitory concentrations (MIC, µg/mL) of 7-fluoro-3-aminosterols [37].

| Microorganisms       | Conjugate/MIC (µg/mL) |
|----------------------|-----------------------|
|                      | 25        | 36        | 37        | 38        | 39        | 40        | 41        | 42        |
| *S. pyogenes* 308A   | 6.3       | 25.0      | 12.5      | 12.5      | 12.5      | 50.0      | 25.0      | 50.0      |
| *S. pyogenes* 77A    | 6.3       | 12.5      | 6.3       | 12.5      | 12.5      | 25.0      | 6.3       | 50.0      |
| *S. ureus* 503       | 6.3       | 12.5      | 6.3       | 12.5      | 25.0      | 25.0      | 6.3       | 50.0      |
| *E. coli* DC2        | 6.3       | 50.0      | 12.5      | 12.5      | 25.0      | 50.0      | 25.0      | 25.0      |
| *P. aeruginosa* 9027 | 6.3       | 50.0      | 12.5      | 12.5      | 25.0      | 6.3       | 50.0      | 50.0      |
| *P. aeruginosa* 1771M| 3.1       | 100.0     | 25.0      | 6.3       | 50.0      | 25.0      | 50.0      | 50.0      |
| *S. typhimurium*     | 100.0     | 100.0     | 50.0      | 100.0     | 100.0     | 50.0      | 100.0     | 50.0      |
| *E. cloacae* 1321E   | 100.0     | 100.0     | 100.0     | 100.0     | 100.0     | 50.0      | 50.0      | 50.0      |

Great efforts have also been made to synthesize squalamine. Okumura et al. synthesized squalamine from a derivative of desmosterol via 12 steps with 7.4% of the total yield [38]. Moriarty and co-workers synthesized (1) from 3β-acetoxy-5-cholic acid by 17 steps [39,40]. Jones et al. described a practical synthesis of squalamine from stigmasterol in 15 steps [41,42]. An excellent review of methods for the synthesis of spermine and spermidine analogues of squalamine is made by Brunel and Letourneux [43]. They reviewed the synthesis of squalamine from cholestane and dinorholenic acid and described its biological activity and clinical perspectives.

In turn, Rao and co-workers isolated six other aminosterols (43–48) from the liver of the dogfish shark (Figure 4) [15]. The authors presented a very accurate spectral analysis based on 2D NMR (COSY, HETCOR, HMBC) as well as low- and high-resolution mass spectra (FAB, ESI, MALDI). The antimicrobial activity of aminosterols (43–48) and squalamine (1) is summarized in Table 2.

Figure 4. The structures of aminosterols (43–48) isolated from the dogfish shark.
Synthesis of 6β-hydroxy-3-α-(or β-)aminosterols (53–58) from hyodeoxycholic acid (49) has been presented by Jones et al. (Scheme 3) [44]. The modification of hyodeoxycholic acid was carried out by the esterification of the carboxyl group and oxidation of both hydroxyl groups to ketones, followed by a conversion of the A/B ring system from cis to trans by acid-catalyzed isomerization. Then various polyamines were added and the corresponding stereoconjugates were obtained.

Scheme 3. Synthesis of analogues of squalamine (53–58) from hyodeoxycholic acid (49).

The synthesized aminosterol conjugates (53–58) exhibit a broad spectrum of antimicrobial activity, similar to other aminosterols (Table 3).

Table 3. Minimum inhibitory concentrations (MIC, µg/mL) of 3α (or 3β)-aminosterols [44].

| Microorganisms      | 1  | 53 | 54 | 55 | 56 | 57 | 58 |
|---------------------|----|----|----|----|----|----|----|
| S. aureus (29213)   | 0.5–1 | 16 | 1  | 2–4| 2  | >256| 16 |
| E. coli (25922)     | 2–4 | 32–64| 8–16| 32 | 32 | >256| 16 |
| P. aeruginosa (27853) | 16 | 128 | 64 | 128| 32 | 128 | 8  |
| C. albicans (90028) | 8  | 8  | 2–4| 4  | 2  | >256| 4  |

The presented data show that the β-analogs (54, 56) are slightly more active against microorganisms than the α-analogs (53, 55). Moreover, the biocidal efficacy against S. aureus is higher for methyl esters (54, 56) in comparison to free acids (57, 58). The chain length of the polyamine has no significant effect on biocidal activity. However, for acid derivatives, a conjugate with spermine chain (58) was much more active than a conjugate with an ethylene diamine chain (57).
Maitra et al. used their own method to modify the side chain of bile acids [45,46]. The synthesis of quaternary alkylammonium conjugates of bile acids (63–75) is shown in Scheme 4.

Scheme 4. Synthesis of cationic bile salts from iodo derivatives of bile acids.

Bile acids (59, 60) were transformed to the 24-nor-23-iodo (61, 62) derivatives by a Hunsdiecker reaction followed by a reaction with secondary or tertiary amines, respectively. All conjugates (63–75) were obtained with good yields 65%–75% and were characterized by 1H-NMR, 13C-NMR, and FT-IR, as well as mass spectrometry. These quaternary ammonium conjugates were found to be good gelators. Some of the quaternary ammonium bile salts gelled water and many of them gelled aqueous salt solutions even in the presence of organic solvents such as alcohol (methanol, ethanol) as well as DMF or DMSO. These gels form fibrous networks [46].

Lopushanskii and Udovitskaya described the method to prepare cholesteryl 3β-bromoacetate and 3β-chloroacetate, which were used in the synthesis of quaternary ammonium derivatives of cholesterol and its 5α,6β-dibromo derivatives (81–91) (Scheme 5) [47].

Scheme 5. Synthesis of monoquaternary (81–83, 89) and symmetrical bisquaternary salt (84–88, 90, 91) derivatives of cholesterol.
In the first step ergosterol, cholesterol, and cholestanol were reacted with bromoacetic acid bromide (Figure 5). The conjugates were synthesized by two-step reactions. The unsymmetrical bisquaternary ammonium salts (92–105) demonstrate a bacteriostatic activity that depends on the alkyl chain length.

Brycki and co-workers obtained the series of quaternary alkylammonium conjugates of ergosterol, cholesterol, and cholestanol [48]. The conjugates were synthesized by two-step reactions. In the first step ergosterol, cholesterol, and cholestanol were reacted with bromoacetic acid bromide with TEBA and calcium hydride (or sodium hydride) in anhydrous toluene to give 3β-bromoacetates of sterols [49]. In the second step, 3β-bromoacetates have been treated with tertiary alkylamines \((\text{CH}_3-\text{CH}_2)_n-\text{N(}\text{CH}_3)_2, n = 7, 9, 11, 13\) under \(\text{Sn}_2\) reaction conditions to give conjugates of ergosterol (106–109), cholesterol (110–113), and cholestanol (114–117) (Figure 6).

The authors also obtained a series of \(N,N\)-dimethyl-3-phthalimidopropylammonium conjugates of sterols (ergosterol, cholesterol, cholestanol) (118–120) and bile acids (lithocholic, deoxycholic, cholic) (121–123) (Figure 7) [50]. The synthesis and physicochemical properties of quaternary \(N,N\)-dimethyl-3-phthalimidopropylammonium conjugates of ergosteryl 3β-bromoacetate, cholesteryl 3β-bromoacetate, and dihydrocholesteryl 3β-bromoacetate, as well as methyl litocholate 3α-bromoacetate, methyl deoxycholate 3α-bromoacetate, and methyl cholate 3α-bromoacetate with \(N,N\)-dimethyl-3-phthalimidopropylamine in acetonitrile were investigated and described.
The symmetrical dimeric quaternary alkylammonium conjugates of sterols (124–132) prepared by two-step reactions of ergosterol, cholesterol, or cholestanol with bromoacetic acid bromide, followed by bimolecular nucleophilic substitution with \(N,N',N''\)-tetramethyl-1,3-propanediamine, \(N,N',N''\)-pentamethyldiethylenetriamine, and 3,3′-iminobis-(\(N,N\)-dimethylpropylamine) have been also described by Brycki et al. (Figure 8) [51]. The final reactions were carried out in acetonitrile to favor bimolecular nucleophilic substitution and optimize the reaction yields.

All structures of the conjugates were confirmed by spectral (\(^1\)H-NMR, \(^{13}\)C-NMR, and FT-IR) analysis and mass spectrometry as well as theoretical semiempirical methods (PM5). PM5 semiempirical calculations were performed using the WinMopac 2003 program [52–54]. In all cases, the heat of formation (HOF) was consistent with the expected values. The lowest values of HOF for sterols were observed for conjugates of cholestanol (118–120) and bile acids (121–123).

Figure 7. \(N,N\)-dimethyl-3-phthalimidopropylammonium conjugates of sterols (118–120) and bile acids (121–123).

Figure 8. The symmetrical bisquaternary alkylammonium conjugates of sterols (124–132).
increase the reactivity of the molecule, thereby increasing values of HOF (Figure 9). In turn, the HOF of conjugates of methyl esters of bile acids (121–123) can be explained in a similar manner. For these compounds the number of hydroxyl groups in the steroid skeleton lowers the value of HOF.

Figure 9. The representative quaternary alkylammonium conjugates of sterols calculated by the PM5 method.

The potential pharmacological activities of the synthesized compounds have been studied using a computer-aided drug discovery approach with the in silico Prediction of Activity Spectra for Substances (PASSs) program. It is based on a robust analysis of the structure–activity relationships in a heterogeneous training set currently including about 60,000 biologically active compounds from different chemical series with about 4500 types of biological activities. Since only the structural formula of the chemical compound is necessary to obtain a PASS prediction, this approach can be used at the earliest stages of investigation. There are many examples of the successful use of the PASS approach leading to new pharmacological agents [55–59]. The PASS software is useful for the study of the biological activity of secondary metabolites. The types of activities that were predicted for a potential compound with the highest probability (focal activities) have been selected. If predicted activity (PA) > 70, the substance is very likely to exhibit experimental activity and the chance of the substance being the analogue of a known pharmaceutical agent is also high. If 50 < PA < 70, the substance is unlikely to exhibit the activity in experiment, the probability is less, and the substance is unlike any known pharmaceutical agent. A research group led by Brycki selected the types of activity that were predicted for a potential compound with the highest probability (Table 4).
Table 4. Probability “to be Active” (PA) values for predicted biological activity of compounds (106–132).

| Focal Predicted Activity (PA > 80) | Conjugates |
|-----------------------------------|-------------|
|                                   | 106–109     | 110–113 | 114–117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 |
| Cholesterol antagonist            | 88           | 90       | 87       | -   | -   | -   | -   | -   | -   | -   | -   | 81   | 85   | -   | 87   | 89   | 82   | 82   | 86   | -   |
| Antihypercholesterolemic          | 91           | 87       | -        | -   | -   | -   | -   | -   | -   | -   | -   | 88   | 83   | 86   | 85   | 80   | 83   | -   | 94   | -   |
| Glyceryl-ether monooxygenase inhibitor | 89       | 92       | 95       | 87  | 91  | 93  | 93  | 94  | 95  | 89  | 89  | 88   | 92   | 92   | 91   | 95   | 95   | 94   | -   |
| Acylcarnitine hydrolase inhibitor | -            | 87       | 97       | -   | -   | 81  | 83  | 91  | 94  | -   | -   | -   | 85   | 80   | -   | 96   | 94   | -   | -   | -   |
| Alcohol O-acetyltransferase inhibitor | 91        | -        | -        | -   | -   | -   | -   | -   | -   | 91  | 90  | 90   | -   | -   | -   | -   | -   | -   | -   | -   |
| Oxidoreductase inhibitor          | 81           | -        | -        | -   | -   | -   | -   | -   | -   | 87  | 86  | 85   | -   | -   | -   | -   | -   | -   | -   | -   |
| Prostaglandin-E2 9-reductase inhibitor | -       | 86       | -        | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| Alkylacylglycerophosphatase inhibitor | -      | -        | 92       | -   | -   | 84  | 82  | 90  | 86  | -   | -   | -   | -   | -   | -   | 90   | 87   | 83   | -   |
| Alkenylglycerophosphocholine hydrolase inhibitor | -  | -        | 90       | -   | -   | -   | -   | -   | 80  | -   | -   | -   | -   | -   | -   | 88   | 82   | 80   | -   |
3. Conclusions

The design and preparation of new steroid conjugates allow us to develop the fields of supramolecular chemistry, material chemistry, and nanotechnology. In this paper we described the synthesis and physicochemical properties of quaternary alkylammonium conjugates of steroids. Most of the described compounds are characterized by high biological activity with a broad spectrum of antimicrobial and antifungal activity. Moreover, these compounds can actively participate in transport across biological membranes, which offers tremendous possibilities in biochemistry, pharmacology, and medicine. The spectroscopic data, semiempirical calculations, and potential pharmacological properties (PASS) obtained in this work significantly extend the library of new steroid conjugates.

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References

1. Dewick, P.M. Medicinal Natural Products A Biosynthetic Approach, 3rd ed.; John Wiley & Sons, Ltd.: Chichester, UK, 2009; pp. 275–277.
2. Nicolaou, K.C.; Montagnon, T. Molecules that Changed the World; John Wiley & Sons, Ltd.: Weinheim, UK, 2008; pp. 79–90.
3. Fieser, L.F.; Fieser, M. Steroids; Reinhold Publishing Corporation: New York, NY, USA, 1959; pp. 341–364.
4. Templeton, W. An Introduction to the Chemistry of Terpenoids and Steroids; Butterworths: London, UK, 1969; pp. 158–190.
5. Lednicer, D. Steroid Chemistry at a Glance; John Wiley & Sons, Ltd.: Chichester, UK, 2011.
6. Hayat, S.; Ahmad, A. Braassinsteroids: A Class of Plant Hormone; Springer: New York, NY, USA, 2011.
7. Lawrence, S.A. Amines: Synthesis, Properties and Applications; Cambridge University Press: Cambridge, UK, 2004.
8. Rato, C.; Amirova, S.R.; Bates, D.G.; Stansfield, I.; Wallace, H.M. Translational recoding as a feedback controller: Systems approaches reveal polyamine-specific effects on the antizyme ribosomal frameshift. Nucleic Acid Res. 2011, 39, 4587–4597. [CrossRef] [PubMed]
9. Zhang, L.; Lee, H.K.; Pruess, T.H.; White, H.S.; Bulaj, G.J. Synthesis and applications of polyamine amino acid residues: Improving the bioactivity of an analgesic neuropeptide, neurotensin. Med. Chem. 2009, 52, 1514–1517. [CrossRef] [PubMed]
10. Pandey, S.; Ranade, S.A.; Nagar, P.K.; Kumar, N. Role of polyamines and ethylene as modulators of plant senescence. J. Biosci. 2000, 25, 291–299. [CrossRef] [PubMed]
11. Salunke, D.B.; Hazra, B.G.; Pore, V.S. Steroidal conjugates and their pharmacological applications. Curr. Med. Chem. 2006, 13, 813–847. [CrossRef] [PubMed]
12. Moore, K.S.; Wehrli, S.; Roder, H.; Rogers, M., Jr; Forrest, J.N., Jr.; McCrimmon, D.; Zasloff, M. Squalamine: An aminosterol antibiotic from the shark. Proc. Natl. Acad. Sci. USA 1993, 90, 1354–1358. [CrossRef] [PubMed]
13. Wehrli, S.; Moore, K.S.; Roder, H.S.; Durell, S.; Zasloff, M. Structure of the novel steroidal antibiotic squalamine determined by two-dimensional NMR spectroscopy. Steroids 1993, 58, 370–378. [CrossRef]
14. Sadownik, A.; Deng, G.; Janout, V.; Regen, S.L.; Bernard, E.M.; Kikuchi, K.; Armstrong, D. Rapid Construction of a Squalamine Mimic. J. Am. Chem. Soc. 1995, 117, 6138–6139. [CrossRef]
15. Rao, M.N.; Shinnar, A.E.; Noecker, L.A.; Chao, T.L.; Feibush, B.; Snyder, B.; Sharkansky, I.; Sarkahan, A.; Zhang, X.; Jones, S.R.; et al. Aminosterols from the Dogfish Shark Squalus acanthias. J. Nat. Prod. 2000, 63, 631–635. [CrossRef] [PubMed]
16. Kim, H.S.; Choi, B.S.; Kwon, K.C.; Lee, S.O.; Kwak, H.J.; Lee, C.H. Synthesis and Antimicrobial Activity of Squalamine Analogue. Bioorg. Med. Chem. 2000, 8, 2059–2065. [CrossRef]
17. Bhargava, P.; Marshall, J.L.; Dahut, W.; Rizvi, N.; Trocky, H.; Hait, H.; Song, S.; Holroyd, K.; Hawkins, M.J. A phase I and pharmacokinetic study of squalamine, a novel antiangiogenic agent, in patients with advanced cancers. *Clin. Cancer Res.* 2001, 7, 3912–3919.

18. Teicher, B.A.; Williams, J.I.; Takeuchi, H.; Ara, G.; Herbst, R.S.; Buxton, D. Potential of the aminosterol, squalamine in combination therapy in the rat 13,762 mammary carcinoma and the murine Lewis lung carcinoma. *Anticancer Res.* 1998, 18, 2567–2573. [PubMed]

19. Schiller, J.H.; Bittner, G. Potentiation of platinum antitumor effects in human lung tumor xenografts by the angiogenesis inhibitor squalamine: Effects on tumor neovascularisation. *Clin. Cancer Res.* 1999, 5, 4287–4294.

20. Williams, J.I.; Weitman, S.; Gonzalez, C.M.; Jundt, C.H.; Marty, J.; Stringer, S.D.; Holroyd, K.; McLane, M.P.; Chen, Q.; Zasloff, M.; et al. Squalamine treatment of human tumors in nu/nu mice enhances platinum-based chemotherapies. *Clin. Cancer Res.* 2001, 7, 724–733.

21. Li, D.; Williams, J.I.; Pietras, R.J. Squalamine and cisplatin block angiogenesis and growth of human ovarian cancer cells with or without HER-2 gene overexpression. *Oncogene* 2002, 21, 2805–2814. [CrossRef] [PubMed]

22. Walker, B.T.; Houston, T.A. Squalamine and its derivatives as potential antitubercular compounds. *Tuberculosis* 2013, 93, 102–103. [CrossRef] [PubMed]

23. Sills, A.K., Jr.; Williams, J.I.; Tyler, B.M.; Epstein, D.S.; Sipos, E.P.; Davis, J.D.; McLane, M.P.; Pitchford, S.; Cheshire, K.; Gannon, F.H.; et al. Squalamine inhibits angiogenesis and solid tumor growth in vivo and perturbs embryonic vasculature. *Cancer Res.* 1998, 58, 2784–2792. [PubMed]

24. Novotná, E.; Waisser, K.; Kuneš, J.; Palát, K.; Buchta, V.; Stolaríková, J.; Beckert, R.; Wsól, V. Synthesis and Biological Activity of Quaternary Ammonium Salt-Type Agents Containing Cholesterol and Terpenes. *Arch. Pharm. Chem. Life Sci.* 2014, 347, 381–386. [CrossRef] [PubMed]

25. Zasloff, M.; Adams, A.P.; Beckerman, B.; Campbell, A.; Han, Z.; Luijten, E.; Meza, I.; Julander, J.; Mishra, A.; Qu, W.; et al. Squalamine as a broad-spectrum systemic antiviral agent with therapeutic potential. *Proc. Natl. Acad. Sci. USA* 2011, 108, 15978–15983. [CrossRef] [PubMed]

26. Kinney, W.A.; Zhang, X.; Williams, J.I.; Johnston, S.; Michalak, R.S.; Deshpande, M.; Dostal, L.; Rosazza, J.P. A short formal synthesis of squalamine from a microbial metabolite. *Org. Lett.* 2000, 2, 2921–2922. [CrossRef]

27. Zhou, X.D.; Cai, F.; Zhou, W.S. A new highly stereoselective construction of the side chain of squalamine through improved Sharpless catalytic asymmetric dihydroxylation. *Tetrahedron Lett.* 2001, 42, 2537–2539. [CrossRef]

28. Zhou, X.-D.; Cai, F.; Zhou, W.-S. A stereoselective synthesis of squalamine. *Tetrahedron* 2002, 58, 10293–10299. [CrossRef]

29. Weis, A.L.; Bakos, T.; Alferiev, I.; Zhang, X.; Shao, B.; Kinney, W.A. Synthesis of an azido spermidine equivalent. *Tetrahedron Lett.* 1999, 40, 4863–4864. [CrossRef]

30. Khabnadideh, S.; Tan, C.L.; Croft, S.L.; Kendrick, H.; Yardley, V.; Gilbert, I.H. Squalamine analogues as potential anti-trypanosomal and anti-leishmanial compounds. *Bioorg. Med. Chem. Lett.* 2000, 10, 1237–1239. [CrossRef]

31. Choquair, B.; Dherbomez, M.; Roussakis, C.; Khiel, L.E. Synthesis of spermidinylcholesterol and spermidinylcholesterol, squalamine analogues. *Tetrahedron* 2004, 60, 11477–11486. [CrossRef]

32. Hussey, S.L.; He, E.; Peterson, B.R. Synthesis of chimeric 7 alpha-substituted estradiol derivatives linked to cholesterol and cholesterylamine. *Org. Lett.* 2002, 4, 415–418. [CrossRef] [PubMed]

33. Shu, Y.; Jones, R.S.; Kinney, W.A.; Selinsky, B.S. The synthesis of spermine analogs of the shark aminosterol squalamine. *Steroids* 2002, 67, 291–304. [CrossRef]

34. Kim, H.S.; Khan, S.N.; Jadhav, J.R.; Jeong, J.W.; Kwak, J.H. A concise synthesis and antimicrobial activities of 3-polyamino-23,24-bisnorcholanes as steroid–polyamine conjugates. *Bioorg. Med. Chem. Lett.* 2011, 21, 3861–3865. [CrossRef] [PubMed]

35. Kim, H.S.; Kwon, K.C.; Kim, K.S.; Lee, C.H. Synthesis and antimicrobial activity of new 3α-hydroxy-23,24-bisnorcholane polyamine carbamates. *Bioorg. Med. Chem. Lett.* 2001, 11, 3065–3068. [CrossRef]
36. Kim, B.-K.; Doh, K.-O.; Bae, Y.-U.; Seu, Y.-B. Synthesis and Optimization of Cholesterol-Based Diquaternary Ammonium Gemini Surfactant (Chol-GS) as a New Gene Delivery Vector. *J. Microbiol. Biotechnol.* **2011**, *21*, 93–99. [CrossRef] [PubMed]

37. Khan, S.N.; Kim, B.J.; Kim, H.-S. Synthesis and antimicrobial activity of 7-fluoro-3-aminosteroids. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5139–5142. [CrossRef] [PubMed]

38. Okumura, K.; Nakamura, Y.; Takeuchi, S.; Kato, I.; Fujimoto, Y.; Ikekawa, N. Formal Synthesis of Squalamine from Desmosterol. *Chem. Pharm. Bull.* **2003**, *51*, 1177–1182. [CrossRef] [PubMed]

39. Moriarty, R.M.; Tuladhar, S.M.; Guo, L.; Wehril, S. Synthesis of squalamine. A steroidal antibiotic from the shark. *Tetrahedron Lett.* **1994**, *36*, 8103–8106. [CrossRef]

40. Moriarty, R.M.; Enache, L.A.; Kinney, W.A.; Allenc, C.S.; Canary, J.W.; Tuladhar, S.M.; Guo, L. Stereoselective synthesis of squalamine dessulfate. *Tetrahedron Lett.* **1995**, *36*, 5139–5142. [CrossRef]

41. Jones, S.R.; Selinsky, B.S.; Rao, M.N.; Zhang, X.H.; Kinney, W.A.; Tham, F.S. Efficient Route to 7α-(Benzoyloxy)-3-dioxolane Cholestan-24(β)-ol, a Key Intermediate in the Synthesis of Squalamine. *J. Org. Chem.* **1998**, *63*, 3786–3789. [CrossRef]

42. Zhang, X.H.; Rao, M.N.; Jones, S.R.; Shao, B.; Feibush, P.; McGuigan, M.; Tzodikov, N.; Feibubush, B.; Sharkansky, I.; Snyder, B.; et al. Synthesis of Squalamine Utilizing a Readily Accessible Spermidine Equivalent. *J. Org. Chem.* **1998**, *63*, 8599–8603. [CrossRef]

43. Brunel, J.M.; Letourneux, Y. Recent Advances in the Synthesis of Spermine and Spermidine Analogs of the Shark Aminosterol Squalamine. *Eur. J. Org. Chem.* **2003**, *20*, 3897–3907. [CrossRef]

44. Jones, S.R.; Kinney, W.A.; Zhang, W.; Jones, L.M.; Selinsky, B.S. The synthesis and characterization of analogs of the antimicrobial compound squalamine: 6β-hydroxy-3-aminosterols synthesized from hyodeoxycholic acid. *Steroids* **1996**, *61*, 565–571. [CrossRef]

45. Sangeetha, N.M.; Balasubramanian, R.; Maitra, U.; Ghosh, S.; Raju, A.R. Novel Cationic and Neutral Analogues of Bile Acids: Synthesis and Preliminary Study of Their Aggregation Properties. *Langmuir* **2002**, *18*, 7154–7157. [CrossRef]

46. Bhat, S.; Maitra, U. Low molecular mass cationic gelators derived from deoxycholic acid: Remarkable gelation of aqueous solvents. *Tetrahedron* **2007**, *63*, 7309–7320. [CrossRef]

47. Lopushanskii, A.I.; Udovitskaya, V.V. Quaternary ammonium derivatives of cholesterol. *Pharm. Chem. J.* **1970**, *4*, 425–429. [CrossRef]

48. Brycki, B.; Koenig, H.; Kowalczyk, I.; Pospieszny, T. Synthesis, Spectroscopic and Semiempirical Studies of New Quaternary Alkylammonium Conjugates of Sterols. *Molecules* **2013**, *18*, 14961–14976. [CrossRef] [PubMed]

49. Aher, N.G.; Pore, V.S.; Patil, S.P. Design, synthesis, and micellar properties of bile acid dimers and oligomers linked with a 1,2,3-triazole ring. *Tetrahedron* **2007**, *63*, 12927–12934. [CrossRef]

50. Brycki, B.; Koenig, H.; Kowalczyk, I.; Pospieszny, T. Synthesis, Spectroscopic and Theoretical Studies of New Dimeric Quaternary Alkylammonium Conjugates of Sterols. *Molecules* **2014**, *19*, 9419–9434. [CrossRef] [PubMed]

51. Brycki, B.; Koenig, H.; Kowalczyk, I.; Pospieszny, T. Synthesis, Spectroscopic and Theoretical Studies of New Quaternary Alkylammonium Conjugates of Sterols. *Molecules* **2014**, *19*, 4212–4233. [CrossRef] [PubMed]

52. Fujitsu. *CAChe 5.04 User Guide*; Fujitsu: Chiba, Japan, 2003.

53. Stewart, J.J.P. Optimization of parameters for semiempirical methods. III Extension of PM3 to Be, Mg, Zn, Ga, Ge, As, Se, Cd, In, Sn, Sb, Te, Hg, Tl, Pb, and Bi. *J. Comput. Chem.* **1991**, *12*, 320–341. [CrossRef]

54. Stewart, J.J.P. Optimization of parameters for semiempirical methods I. Method. *J. Comput. Chem.* **1989**, *10*, 209–220. [CrossRef]

55. Pharma Expert Predictive Services © 2011–2013, Version 2.0. Available online: http://www.pharmaexpert.ru/PASSOnline/ (accessed on 18 November 2013).

56. Poroikov, V.V.; Filimonov, D.A.; Borodina, Y.V.; Lagunin, A.A.; Kos, A. Robustness of biological activity spectra predicting by computer program PASS for noncongeneric sets of chemical compounds. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 1349–1355. [CrossRef] [PubMed]

57. Poroikov, V.V.; Filimonov, D.A. How to acquire new biological activities in old compounds by computer prediction. *J. Comput. Aided. Mol. Des.* **2002**, *16*, 819–824. [CrossRef] [PubMed]
58. Poroikov, V.V.; Filimonov, D.A. Predictive Toxicology; Helma, C., Ed.; Taylor and Francis: Boca Raton, FL, USA, 2005; pp. 459–478.

59. Stepanchikova, A.V.; Lagunin, A.A.; Filimonov, D.A.; Poroikov, V.V. Prediction of biological activity spectra for substances: Evaluation on the diverse sets of drug-like structures. Curr. Med. Chem. 2003, 10, 225–233. [CrossRef] [PubMed]

Sample Availability: Samples of the compounds 106–132 are available from the authors.

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