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Synthesis and antimicrobial activity of imidazole and pyridine appended cholestane-based conjugates

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ABSTRACT

A series of 3α-amino-5α-cholestane and 3α,7α-diamino-5α-cholestane derivatives containing imidazole and pyridine rings were synthesized by simple and effective reductive amination, and their in vitro activities against a range of Gram-positive and Gram-negative strains were evaluated. Most of the compound exhibited enhanced activity against MRSA pathogen. 3α,7α-Di(pyridylmethyl)amino-5α-cholestane **10** showed the highest potency in these series toward the Gram-positive bacteria, *Staphylococcus epidermidis* 887E, with the lowest MIC value of 1 μg/mL.

The rapid increase in the prevalence of multiple drug-resistance Gram-positive bacteria has highlighted the urgent need to discover novel active agents against a range of Gram-positive pathogens. One of the strategies to overcome this problem is to identify new drugs with a new molecular target.[1] Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *entercocci* (VRE) are of particular concern.[2] Initially, MRSA was observed only in hospital settings, but it is now apparent that community-acquired MRSA (CA-MRSA) infections can occur in individuals without any identifiable risk factors. Moreover, the prevalence of CA-MRSA is increasing,[3a] particularly in children.[3b,c] This is currently recognized as a growing problem worldwide.[3d] These factors make the treatment of Gram-positive infections a greater challenge than in the past.

The imidazole nucleus is an important building block in drug discovery. Recently imidazole and imidazolium derivatives have been the subject of extensive study of the antimicrobial activity for their potential as effective therapeutic agents.[4] Based on several literature surveys, imidazole derivatives show a range of pharmacological activities, such as anti-fungal and anti-bacterial,[4a,b] anti-inflammatory and analgesic,[4c] anti-tubercular,[4a,d] anti-depressant,[4e] anti-cancer,[4f] anti-viral,[4a,g] and anti-lishmanial.[4h] On the other hand, the high therapeutic properties of pyridine-related drugs have encouraged medicinal chemists to synthesize a large number of chemotherapeutic agents.[5] The medicinal properties of pyridine include a variety of pharmacological applications, such as antibacterial,[5a] antitumor,[5b] antiparacite,[5c] and analgesic activity.[5d]

A class of cationic steroid antibiotics as steroid-polyamine conjugates were designed by Savage with the intension of mimicking the antimicrobial activities of polymyxin B (PMB),[6] whereas Regen et al. described the utilization of bile acid–polyamine conjugates as synthetic ionophore leads in the process of drug discovery.[7] Over the last decade, Kim et al. actively studied 23,24-bisnorcholane-based sqaulamine analogs to determine the structure–activity relationship (SAR) of sqaulamine mimics.[8]

Resistance to membrane active antibiotics requires major changes in the membrane structure that in turn affects the permeability barrier, increasing the susceptibility to hydrophobic antibiotics. As a result, a variety of molecules that are active against Gram-positive bacteria are much less active against Gram-negative bacteria and vice versa. The general belief is that the permeability barrier of the outer membrane is formed via cross bridging between lipid molecules and divalent cations (Ca^{2+} and Mg^{2+}).[9] Therefore, metal ion chelators, such as EDTA, cationic peptides, and polyamines, which can attack the binding sites of divalent cations, can disrupt the organization of the outer membrane, increasing its permeability and therefore sensitizing bacteria to hydrophobic antibiotics.[10] Although required, the hydrophilic part is not sufficient itself to cause either permeabilization of the bacterial membranes or bacteria killing.[11] The hydrophobic moiety of amphiphilic antimicrobials is responsible for their insertion into the hydrophobic core of the bacterial lipid bilayer, leading to membrane permeabilization, and eventually to amphiphilic antimicrobials-mediated bacteria killing.[12]

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Therefore, a series of 3α-amino-5α-cholestane-7-one and 3α,7α-diamino-5α-cholestane conjugates were synthesized by reductive amination with a group containing a heterocyclic ring, such as imidazole and pyridine as shown in Figure 1, and their antimicrobial activities against a range of human pathogens were evaluated. The cholesterol backbone was chosen because it is an essential metabolite required for major biological functions such as the cell membrane structure, where the steroid combines together with phospholipids molecules, an integral part of the lipid bilayer.[13]

Scheme 1 illustrates the synthesis of compounds **2**–**11**. Compounds **2**–**6** were prepared by the one step reductive amination of 5α-cholestane-3,7-dione (**1**) with a variety of amines. Compounds **7**–**11** can be obtained by one or two step reductive amination as shown in Scheme 1.[14] The spectroscopic data of the obtained compounds are in agreement with those reported in publications.[15]

Compounds **2**–**11** were examined for their antimicrobial activities against a wide range of Gram-positive strains, such as eleven strains of *S. aureus* ATCC6538P, *S. aureus* ATCC25923, *S. aureus* giorgio, *S. aureus* 77, *S. aureus* 241, *Staphylococcus epidermidis* 887E, *Enterococcus faecalis* ATCC29212, *Micrococcus luteus* ATCC9341, *Bacillus cereus* ATCC27348, *Bacillus subtilis* ATCC6633, *Bacillus licheniformis* EMR and a range of Gram-negative strains, such as *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC13311, *Klebsiella pneumoniae* 2011E, *Proteus vulgaris* 6059, *Pseudomonas aeruginosa* 6065Y, and *Klebsiella aerogenes* (SHV-1) 1976E with oxacillin (OXA) and vancomycin (VAN) as a control.[8h,16] Compounds **2**–**11** exhibited excellent in vitro activity against all Gram-positive bacteria.

The structures of compounds **2**–**6** and **7**–**12** were similar in all aspects with stereochemistry at the C3, C5 and C7-positions except for the amino groups at 3 and 3,7-positions. Most of the 3-amino- and 3,7-diaminocholestane conjugates containing imidazole and pyridine groups exhibited good in vitro activity against most of the Gram-positive bacteria. Table 1 lists the minimum inhibitory concentrations (MICs).

The antimicrobial activity of 3-imdazole conjugates (**2**–**4**) showed a reasonable value toward most of the Gram-positive bacteria. 3-Ethylimidazole conjugate (**2**) and 3-propylimidazole conjugate (**3**) showed similar activity toward the majority of the tested Gram-positive strains with the lowest MIC value of 8 μg/mL. Compound **3** was four times potent against *S. aureus* 77 and *B. subtilis* ATCC6633 than compound **2**, whereas compound **2** exhibited two and four times higher potency against *S. aureus* 241 and *E. faecalis* ATCC29212 strain, respectively, than compound **3**. 3-Ethylhistidine conjugate (**4**) exhibited moderate activity toward most of the Gram-positive bacteria with a MIC value of 16 μg/mL with the exception of *S. aureus* ATCC6538P, which was 8 μg/mL.

3,7-Di(imidazole) conjugates (**7**–**9**) improved the antimicrobial potency 2- to 4-fold compare to that of 3-imdazole conjugates (**2**–**4**). 3,7-Di(ethylimidazole) conjugate (**7**) was two times more active toward *S. aureus* ATCC6538P, *S. aureus* giorgio, *M. luteus* ATCC9341 and *B. cereus* ATCC27348 Gram-positive bacterial strains (MIC values of 4–16 μg/mL) than compound **2**. Compound **8** showed 2–8 times higher activity than compound **3** with MIC values from 2 to 4 μg/mL against all Gram-positive bacteria. Although compound **4** showed moderate activity, the compound **9** showed double the potency toward most of the Gram-positive bacteria tested than compound **4**.

Among the 3,7-di(imidazole) conjugates (**7**–**9**), the best antimicrobial results were observed for compound **8** with the lowest MIC value of 2 μg/mL toward *S. aureus* ATCC6538P and *S. aureus* ATCC25923 and MIC value of 4 μg/mL toward *S. aureus* giorgio, *S. aureus* 77, *S. aureus* 241, *S. epidermidis* 887E, *E. faecalis* ATCC29212, *M. luteus* ATCC9341, *B. subtilis* ATCC6633 and *B. licheniformis* EMR Gram-positive bacteria. Compound **8** was 2–4 times more potent than compounds **7** and **9**.

A comparative study of 3-pyridine conjugates (**5**, **6**) showed that, 3-methylpyridine conjugate (**5**) exhibited the reasonable activity with a MIC value of 2 μg/mL toward *S. aureus* ATCC6538P and 4 μg/mL toward *S. aureus* ATCC25923, *S. aureus* giorgio, *S. epidermidis* 887E and *B. subtilis* ATCC6633, which was 2–4 times higher than that of the 3-ethylpyridine conjugate (**6**). Moderate antimicrobial activity was encountered with compound **6** with a MIC value in range of 8–32 μg/mL. Superior activity was observed with the 3,7-di(methylpyridine) conjugate (**10**) with the lowest MIC value of 1 μg/mL against *S. epidermidis* 887E and 2 μg/mL all against *S. aureus* strains, *B. subtilis*, *M. luteus* ATCC9341 and *B. licheniformis* EMR bacteria. The MIC values encountered for compound **10** was 2–8 times higher than compound **5**, and 4–8 times more potent than 3,7-di(ethylpyridine) conjugate (**11**). Compound **11** showed improved MIC values toward *M. luteus* ATCC9341, *B.*

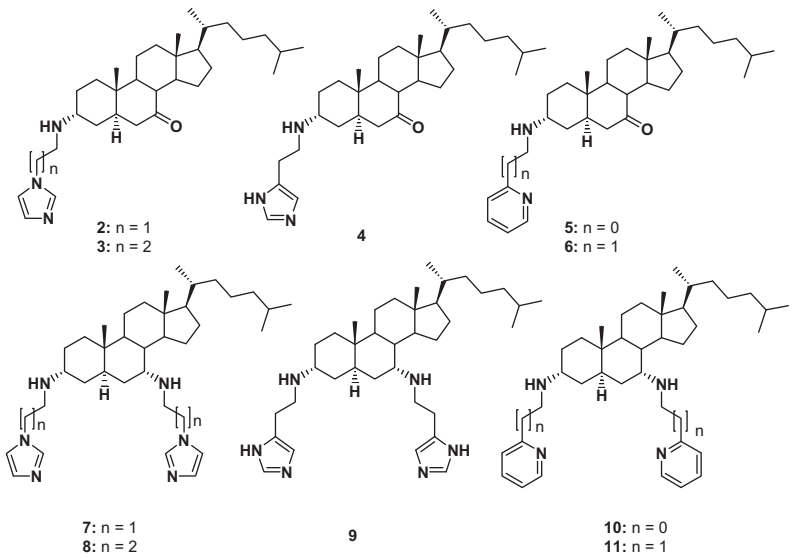
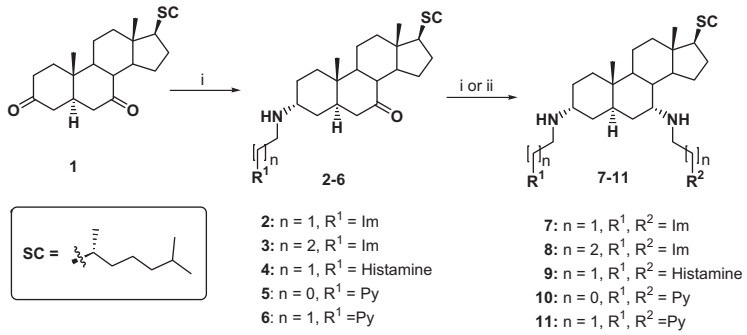


Figure 1. Structures of the imidazole and pyridine appended cholestane-based conjugates.



Scheme 1. Reagents and conditions: (i) amine, $NaBH(OEh)_3$, THF, rt; (ii) amine, $NaBH_3CN$, THF:MeOH (1:1), rt.

Table 1
Antimicrobial activities of compounds **2**–**11** against wide ranges of bacteria[a]

Entry	Strains	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	OXA[b]	VAN[c]
1	*S. aureus* ATCC6538P	8	8	8	2	8	4	2	4	2	8	0.25	2
2	*S. aureus* ATCC25923	8	8	16	4	8	8	2	8	2	8	0.25	2
3	*S. aureus* giorgio	8	8	16	4	8	4	4	8	2	8	0.5	2
4	*S. aureus* 77	>32	8	16	32	32	4	4	8	2	16	4	2
5	*S. aureus* 241	16	32	32	32	>32	16	4	8	2	8	>32	2
6	*S. epidermidis* 887E	16	16	16	4	16	16	4	8	1	8	>32	2
7	*E. faecalis* ATCC29212	8	32	16	8	16	16	4	8	4	16	32	2
8	*M. luteus* ATCC9341	8	8	16	8	32	4	4	16	2	8	0.03	1
9	*B. cereus* ATCC27348	32	16	32	16	>32	16	8	32	8	32	>32	>32
10	*B. subtilis* ATCC6633	32	8	32	4	32	8	4	>32	2	8	16	2
11	*B. lichenifomis* EMR	32	32	32	32	32	32	4	>32	2	8	>32	>32
12	*E. coli* ATCC25922	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
13	*S. typhimurium* ATCC13311	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
14	*K. pneumoniae* 2011E	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
15	*P. vulgaris* 6059	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
16	*P. aeruginosa* 6065Y	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
17	*K. aerogenes* (SHV-1) 1976E	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32

[a] MIC values are reported in μg/mL.
[b] Oxacillin.
[c] Vancomycin.

subtilis and *B. licheniformis* EMR bacteria, which was four times better than compound **6**.

The relative study in between the single strand and double strand imidazole and pyridine appended 5α-cholestane conjugates showed that cholestane bearing a double strand moiety was more potent than a single strand toward the majority of Gram-positive bacteria. The antimicrobial activity was also varied by the chain length between the amino and hetero aromatic ring attached to cholestane through a N–H linkage. 3,7-Di(propylimidazole) conjugate **8** showed superior activity to 3-propylimidazole conjugate (**3**) and 3,7-di(ethylimidazole) conjugates (**7**, **9**). In the case of pyridine conjugates, 3,7-di(methylpyridine) conjugate (**10**) showed the higher activity than 3-methylpyridine conjugate (**5**), 3-ethylpyridine conjugate (**6**) and 3,7-di(ethylpyridine) conjugate (**11**). The conjugate **10** showed similar potency compare to vancomycin, but less potent than oxacillin against *S. aureus* ATCC6538P, *S. aureus* ATCC25923, *S. aureus* giorgio and *M. luteus* ATCC9341. In the case of *S. epidermidis* 887E, conjugate **10** exhibited very strong activity compare to oxacillin and vancomycin having a MIC value of 1 μg/mL.

The mechanism of the action for Gram-negative bacteria can be explained as follows: the positively charged amino groups of aminosteroid derivatives interact with the negatively charged bacterial LPSs (lipopolysaccharide) resulting in a disruption of the outer membrane and subsequent cell death.[17,18] The mechanism of action of the same compound against Gram-positive bacteria cannot be explained by this fact because the membrane of Gram-positive bacteria are essentially constituted of peptidoglycans, which are less negatively charged. Therefore, the precise mode of action of antimicrobial agents on Gram-positive bacteria is unknown. On the other hand, it has been proposed and widely believed that the compounds interact with and disrupt the cytoplasmic membrane, leading to a dissolution of the proton motive force and a leakage of essential molecules, resulting in cell death.[19]

Compounds **2**–**11** showed moderate to excellent activity towards a range of Gram-positive bacteria. In contrast, none of the Gram-negative bacteria were resistant to the compounds, due to a different mechanism by which Gram-negative bacteria produce their cell walls and various factors relating to the outer membrane of Gram-negative organisms.[17,18]

In summary, a series of 3α-amino- and 3α,7α-diamino-5α-cholestane conjugates appended with imidazolyl and pyridyl moieties at the 3- and 7-positions of cholestane were synthesized and their antimicrobial activity against selected human pathogenic bacterias were evaluated. Most of the compounds showed excellent antimicrobial activity over a wide range of Gram-positive bacteria. The presence of an alkyamino moiety along with a heterocyclic unit is essential for the antimicrobial activity. The compounds **8** and **10** can be developed further as antibiotic drugs because they exhibit better antibacterial activity against most of the tested MRSA and CA-MRSA pathogens with the lowest MIC value of 1 μg/mL.

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14. *Representative synthesis:* 3α-[(5-Imidazolyl)ethyl]amino-5α-cholestane-7-one (**4**). A mixture of 5α-cholestane-3,7-dione (**1**, 100 mg, 0.45 mmol), histamine hydrochloride salt (38 mg, 1.1 mmol) and triethylamine (30 mg, 1.1 mmol) in THF (15 mL) was reacted with sodium tris(2-ethylhexanoxy)borohydride [$NaBH(OEh)_3$] (2 mL) at room temperature for 8 h. After work up, the residue was chromatographed on silica gel with the elution of (CH_2Cl_2–MeOH-NH_4OH 20:1:0.2) to give **4**; 62% yield; waxy solid; TLC R_f 0.54 (CH_2Cl_2–MeOH–NH_4OH 20:1:0.5); ^1H NMR ($CDCl_3$) δ 0.58 (s, 3H, 18-CH_3), 0.78 (d, J = 6.6 Hz, 26-CH_3), 0.79 (d, J = 6.6 Hz, 27-CH_3), 0.83 (d, J = 7.3 Hz, 21-CH_3), 0.98 (s, 3H, 19-CH_3), 2.17–2.33 (m, 2H), 2.70 (t, J = 7.0 Hz, 2H, –CH_2), 2.77 (t, J = 7.3 Hz, 2H, –CH_2), 2.86 (br s, 1H, 3β-H), 6.72 (s, 1H, Im-H4), 7.46 (s, 1H, Im-H2); ^{13}C NMR ($CDCl_3$) δ 11.5, 12.5, 19.2, 21.6, 22.9, 23.2, 24.2, 25.4, 26.3, 27.0, 28.4, 28.8, 32.8, 33.3, 36.0, 36.5, 37.1, 39.1, 39.9, 42.6, 42.9, 46.5, 47.4, 49.3, 50.7, 52.6, 55.4, 56.4, 118.6, 134.9, 135.2, 213.1; HR mass Calcd for $C_{32}H_{53}ON_3$: 495.4189. Found: m/z 496.4268 $(M+H)^+$.
*3α,7α-Bis[2-(5-imidazolyl)ethyl]amino-5α-cholestane (**9**).* A mixture of compound **4** (100 mg, 0.21 mmol) and the histamine hydrochloride salt (90 mg, 3 equiv) in THF–MeOH (1:1) (15 mL) was reacted with $NaBH_3CN$ (38 mg, 3 equiv) at room temperature for 12 h. After work up, the residue was chromatographed with the elution of (CH_2Cl_2–MeOH–NH_4OH 20:1.0:0.2) on silica gel to give compound **9** as an waxy solid; yield (62%); TLC R_f 0.52 (CH_2Cl_2–MeOH–NH_4OH 20:1:0.5); ^1H NMR ($CDCl_3$) δ 0.63 (s, 3H, 18-CH_3), 0.79 (s, 3H, 19-CH_3), 0.84 (d, J = 6.6 Hz, 26-CH_3), 0.86 (d, J = 6.6 Hz, 27-CH_3), 0.89 (d, J = 7.2 Hz, 21-CH_3), 2.64 (br s, 2H), 2.67–2.83 (m, 4H, –CH_2), 2.85–2.98 (m, 4H, –CH_2), 6.75 (s, 2H, Im-H4), 7.43 (s, 1H, Im-H2), 7.49 (s, 1H, Im-H2); ^{13}C NMR ($CDCl_3$) δ 11.4, 12.2, 19.1, 21.1, 22.9, 23.2, 24.0, 24.4, 25.6, 26.1, 26.9, 28.4, 28.5, 31.9, 32.2, 32.3, 32.8, 36.2, 36.6, 36.8, 39.3, 39.7, 39.9, 43.2, 46.7, 47.4, 48.5, 51.1, 53.7, 56.2, 56.6, 117.9, 118.8, 134.8, 134.9, 135.1, 135.2; HR mass Calcd for $C_{37}H_{62}N_6$: 590.5036. Found: m/z 591.5112 $(M+H)^+$.
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