Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 3357–3362
Bioorganic & Medicinal Chemistry

ω-Pyridiniumalkylethers of steroidal phenols: new compounds with potent antibacterial and antiproliferative activities

C. Lange,[a] N. Holzhey,[a] B. Schönecker,[a,*] R. Beckert,[a] U. Möllmann[b] and H.-M. Dahse[b]

[a]*Institut für Organische Chemie und Makromolekulare Chemie der Friedrich-Schiller-Universität Jena, Lessingstr. 8, D-07743 Jena, Germany*
[b]*Hans-Knöll-Institut für Naturstoffforschung, Beutenbergstr. 11, D-07745 Jena, Germany*

Received 3 September 2003; accepted 19 March 2004
Available online 10 May 2004

Abstract—Novel ω-pyridiniumalkylethers of two steroidal phenols were synthesized as compounds with potential antimicrobial activity. 3-Hydroxy-estra-1,3,5(10)-triene-17-one and 1-hydroxy-4-methyl-estra-1,3,5(10)-triene-17-one were reacted with ω,ω′-dibromoalkanes to ω-bromoalkoxy-estra-1,3,5(10)-trienes followed by reaction with pyridine to obtain the desired steroidal ω-pyridiniumalkoxy compounds as bromides. Their antimicrobial activity against strains of multiresistant *Staphylococcus aureus* (MRSA), a vancomycin resistant *Enterococcus faecalis* and fast growing mycobacteria depends clearly on the length of the alkyl chain. A strong broadband activity has been found for the compounds with eight or 10 C-atoms; in some cases better than ciprofloxacin or cetylpyridinium salts. In addition, the antiproliferative and cytotoxic activity depends on the chain length, too. The differentiation between antibacterial and cytotoxic activity is better for the steroid hybrid molecules than the cetylpyridinium salts. These new compounds can serve as lead compounds for further optimization.

1. Introduction

The resistance of pathogenic organisms towards drugs has become a serious problem in the last decades. As an example, the resistance of mycobacteria to the front-line antituberculous agents is alarming, since a dramatical increase of such infections has been observed in the last few years.[1] Therefore, there is an urgent need to discover new compounds possessing potent antimicrobial activities for developing new drugs. One possibility to achieve this goal is the combination of a steroid molecule with structural elements possessing appropriate biological activities.[2–7] The advantage of employing hydrophobic steroid units is their ability to interact with cell membranes[8] and thus paves the way for biological activity of such hybrid molecules.

Long-chain aliphatic pyridinium salts, especially cetylpyridinium chloride (CPC), have potent antibacterial, fungistatic and virustatic activities. Recently, an interesting method has been described using poly(4-vinyl)-N-alkylpyridinium bromide covalently attached to amino glass slides. These surfaces are able to kill airborne gram-positive and gram-negative bacteria.[9] The *n*-hexyl compound showed the best activity.

We report here on the synthesis of compounds, which combine a phenolic steroid [hydroxy-estra-1,3,5(10)-trienes] and a N-alkylpyridinium unit across an ether bridge as well as their antibacterial, antiproliferative and cytotoxic activities. For the steroidal unit, we selected 3-hydroxy-estra-1,3,5(10)-triene-17-one (estrone, a natural estrogen) and 1-hydroxy-4-methyl-estra-1,3,5(10)-triene-17-one,[10] a synthetic product without estrogenic activity. The chain lengths of the N-alkylpyridinium unit was varied in order to obtain some insight into structure activity relationships.

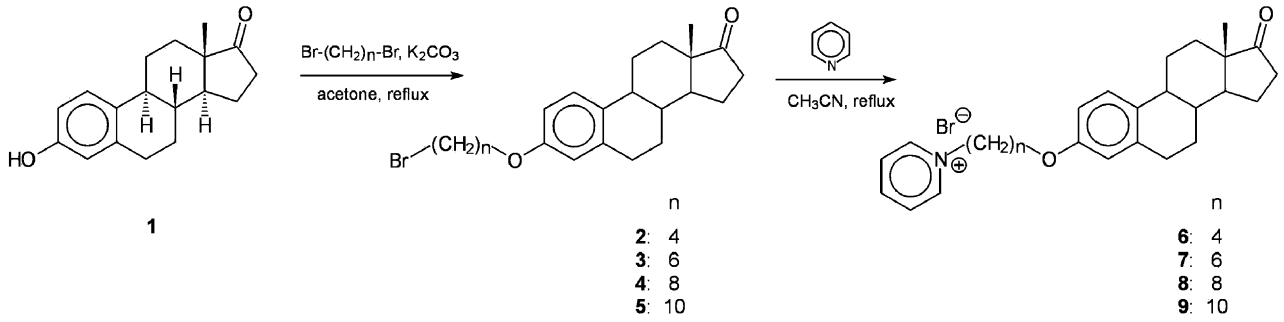
2. Results and discussion

2.1. Chemistry

Compound **1** is a pharmaceutical (estrone); compound **10** was synthesized by aromatization of androsta-1,4-diene-3,17-dione (ADD) as described in the literature.[10]

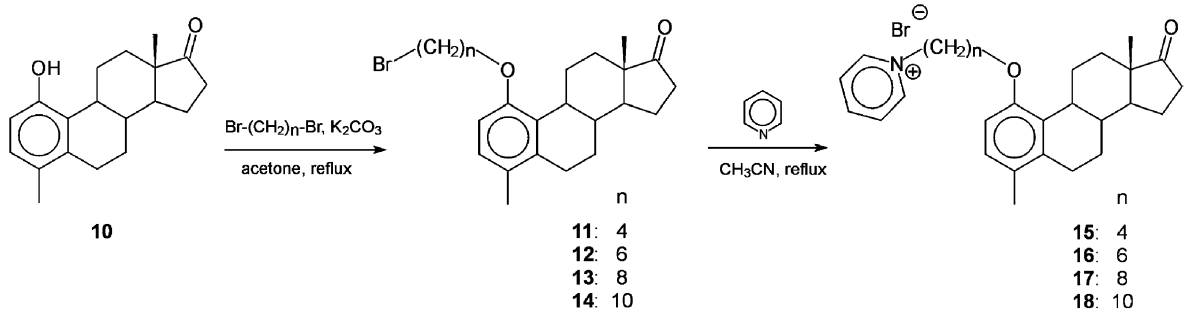
Keywords: Steroids; Pyridiniumalkyl salts; Hybrid molecules; Antimicrobial activity.
* Corresponding author. Tel.: +49-3641-948225; fax: +49-3641-9482-92; e-mail: c8scbr@uni-jena.de

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.03.046



1

Scheme 1. Syntheses of ω-bromoalkylethers and ω-pyridiniumalkylethers of 3-hydroxy-estra-1,3,5(10)-triene-17-one.



10

Scheme 2. Syntheses of ω-bromoalkylethers and ω-pyridiniumalkylethers of 1-hydroxy-4-methyl-estra-1,3,5(10)-triene-17-one.

The phenolic compounds **1** and **10** were then reacted under basic conditions with ω,ω′-dibromoalkanes in boiling acetone. The ω-bromoalkoxy-estra-1,3,5(10)-trienes **2**–**5** (Scheme 1) and **11**–**14** (Scheme 2) thus obtained were purified by chromatography and crystallization. Only the compounds **2**, **3** and **5** have been reported in the literature.[11–13] Reaction of **2**–**5** and **11**–**14** with anhydrous pyridine in boiling acetonitrile gave the desired ω-pyridinium salts of the alkylethers of the hydroxy-estra-1,3,5(10)-trienes as crystalline compounds (**6**–**9**, Scheme 1; **15**–**18**, Scheme 2). In some cases, the reaction was carried out in an autoclave at 100 °C with methanol as solvent. Interestingly, these compounds are in contrast to the most steroids soluble in water.

2.2. Biology

The steroidal pyridinium compounds **6** to **9** and **15** to **18** were screened in vitro for antimicrobial activity using strains of multiresistant *staphylococci* (MRSA), a vancomycin resistant *Enterococcus* and fast growing mycobacteria. For comparison, the steroidal phenols **1** and **10**, cetylpyridinium chloride (CPC) and bromide (CPB) as well as ciprofloxacin as a standard were included (Table 1). The minimal inhibitory concentrations (MIC) were determined by a broth dilution method according to the NCCLS-guidelines.[14]

These compounds and some additional steroid ω-bromoalkyl ethers were assayed against cell lines K-562 and

Table 1. In vitro antibacterial activities of pyridiniumalkylethers of hydroxy-estra-1,3,5(10)-trienes [minimal inhibition concentration, MIC (μg/mL)]

Compound	*Staphylococcus aureus* 994/93	*S. aureus* 134/94	*E. faecalis* 1528	*Mycobacterium smegmatis* SG 987	*Mycobacterium vaccae* 10670	*Mycobacterium aurum* SB 66	*Mycobacterium fortuitum* B
6	100	>100	50	12.5	12.5	25	12.5
7	12.5	50	12.5	3.12	1.56	12.5	0.8
8	1.56	6.25	1.56	0.8	0.4	3.12	0.4
9	1.56	6.25	1.56	1.56	0.4	3.12	0.8
15	100	>100	25	6.25	6.25	25	12.5
16	6.25	25	6.25	1.56	0.8	12.5	0.8
17	0.8	3.12	1.56	0.4	0.4	3.12	0.4
18	0.4	1.56	0.4	1.56	0.4	1.56	1.56
1	>100	>100	>50	100	>100	100	100
10	>100	>100	>50	100	>100	50	100
CPB	0.8	3.12	0.2	3.12	0.8	6.25	6.25
CPC	0.8	3.12	0.2	3.12	0.8	6.25	6.25
Ciprofloxacin	>100	12.5	0.4	0.8	0.1	<0.05	0.1

Table 2. Antiproliferative and cytotoxic effects of ω-bromoalkyl ethers and ω-pyridiniumalkyl ethers of hydroxy-estra-1,3,5(10)-trienes

Compound	Antiproliferative effect		Cytotoxic effect
	L-929 (mouse fibroblast cells) GI_{50} (μg/mL)[a]	K-562 (human leukemia) GI_{50} (μg/mL)[a]	HeLa (human cervical carcinoma) CC_{50} (μg/mL)[a]
3	49.3	4.9	20.7
4	>50	7.9	23.4
5	>50	7.5	22.2
11	18.8	6.6	20.3
12	>50	10.1	22.2
13	>50	37.4	>50
14	>50	>50	>50
6	>50	>50	>50
7	42.5	15.0	22.0
8	13.4	2.7	7.3
9	11.3	2.5	7.4
15	>50	38.2	37.7
16	29.6	6.8	17.6
17	6.1	1.0	6.3
18	4.7	0.9	4.1
1	>50	>50	3.7
10	>50	8.1	32.8
CPB	1.4	0.2	2.5
CPC	1.2	0.2	2.5

[a] <1 μg/mL: very strong activity; 1–10 μg/mL: strong activity; >10 μg/mL: weak activity.

L-929 for their antiproliferative effects (GI_{50}: concentration which inhibited cell growth by 50%), and against HeLa for their cytotoxic effects (CC_{50}: cytotoxic concentration which contains a specific destructive action by 50%; used particularly in referring to the lysis of cells). The cells were incubated with 10 concentrations of the target compounds. The antiproliferative and cytotoxicity assays have been previously described.[15]

As shown in Table 1, a high antibacterial activity of the new hybrid molecules can be achieved against all tested organisms. This activity clearly depends on the length of the alkyl chain.

The maximum should be reached at a length of eight or 10 C-atoms. The 1-alkylethers seem to be slightly more active than the 3-alkylethers. As expected, the starting steroidal phenols **1** and **10** are practically inactive, since cetylpyridinium bromide (CPB) and chloride (CPC) have a high activity. The best hybrid molecule **18** (1-decylpyridinium ether) seems to be slightly better than CPB and CPC (except against *Enterococcus faecalis*). The compounds **8**, **9** and **17** have a higher activity than CPB and CPC against *Mycobacterium* strains. Ciprofloxacin strongly inhibits *Mycobacterium* strains but is inactive against MRSA. In contrast the hybrid molecules are active against the MRSA strains, particularly molecule **18**.

The antiproliferative and cytotoxic effects (Table 2) of the hybrid molecules also depend on the length of the alkyl group, but a higher concentration is generally necessary as compared to the antibacterial activity. This is not the case for CPB and CPC where the antimicrobial and the cytotoxic active concentrations are in the same range. It means that the hybrid molecules show a better differentiation between antibacterial and cytotoxic activity.

Interestingly enough, the steroid phenol **1** has a remarkable cytotoxic and **10** a good antiproliferative activity. Some of the investigated ω-bromoalkylethers possess notable antiproliferative activity against K-562 (human leukemia).

3. Conclusion

It could be shown that the hitherto unknown ω-pyridiniumalkylethers of steroidal 3- and 1-hydroxy-estra-1,3,5(10)-trienes have a strong antibacterial activity against MRSA, a vancomycin resistant *E. faecalis* and against fast growing mycobacteria. A clear dependence of the antibacterial activity upon the length of the alkylchain was observed. In some cases, a better activity as ciprofloxacin and cetylpyridinium salts (CPB, CPC) and a better dissociation of antibacterial and cytotoxic activities in comparison to CPB and CPC have been found. These results demonstrate that the combination of a steroid unit, which is able to interact with cell membranes, and another functionality with potential antimicrobial activity can be successful for creating new lead structures by structure activity relationship studies. The great potential of steroidal alcohols in combination with different pyridiniumalkyl structures should be an interesting field for further investigations.

4. Experimental

4.1. General methods

Melting points were measured on a Boëtius micromelting point apparatus and are corrected values. Optical rotations were measured at room temperature in chloroform or dichloromethane with a Polamat A (Carl Zeiss Jena) polarimeter and are given in units of $g\,100^{-1}\,mL^{-1}$.

Elemental analyses were performed with a CHNS-932 (LECO) instrument. ^{1}H NMR spectra were recorded on a Bruker DRX-400 spectrometer either in $CDCl_3$ or CD_2Cl_2 (^{1}H NMR 400 MHz). Solvents were purified, dried and distilled according to conventional methods. All reactions were carried out using inert conditions. The reactions were monitored by TLC aluminium sheets, silica gel 60 F_{254} (Merck), 0.2 mm, detection by UV (254 nm) and spraying with a solution of concd sulfuric acid (80 mL) and methanol (20 mL) and heating at 170 °C. For flash chromatography silica gel 60 (Lichroprep Si 60, 40–63 μm, Merck) was used.

4.2. Syntheses

4.2.1. General procedure for the 3-(ω-bromoalkoxy)-estra-1,3,5(10)-triene-17-ones (2–5) and 1-(ω-bromoalkoxy)-4-methyl-estra-1,3,5(10)-triene-17-ones (11–14). To a solution of the phenolic steroid (estrone **1** or 1-hydroxy-4-methyl-estra-1,3,5(10)-triene-17-one **10**; 1 mmol) in anhydrous acetone (10 mL) containing freshly dried potassium carbonate (2 mmol) was added the corresponding ω,ω′-dibromoalkane (4 mmol). The reaction mixtures were refluxed while stirring for 10–20 h until completion, as determined by TLC. After cooling to room temperature, the potassium carbonate was filtered off and washed with acetone. Evaporation gave oily crude products, which were purified by column chromatography on silica gel with *n*-heptane–ethyl acetate (90:10).

4.2.1.1. 3-(4′-Bromobutyloxy)-estra-1,3,5(10)-triene-17-one (2). Colorless crystals (80% yield); mp 112–114 °C (MeOH), mp 97–100 °C (cyclohexane);[11] $[\alpha]_D^{24}$ +95 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 0.85 (s, 3H, 18-H), 2.83 (m, 2H, 6-H), 3.46 (t, 2H, $J = 6.7$ Hz, 4′-H), 3.91 (t, 2H, $J = 6.1$ Hz, 1′-H), 6.58 (d, 1H, $J = 2.4$ Hz, 4-H), 6.63 (dd, 1H, $J = 2.4$ Hz, $J = 8.6$ Hz, 2-H), 7.14 (d, 1H, $J = 8.6$ Hz, 1-H). Anal. Calcd for $C_{22}H_{29}O_2Br$ (405.37): C, 65.18; H, 7.21; Br, 19.71. Found: C, 64.93; H, 6.80; Br, 19.44.

4.2.1.2. 3-(6′-Bromohexyloxy)-estra-1,3,5(10)-triene-17-one (3). Colorless crystals (76% yield); mp 74–76 °C (MeOH), mp 74.5–76.5 °C (*n*-hexane);[12,13] $[\alpha]_D^{24}$ +82 (*c* 0.1, CH_2Cl_2), $[\alpha]_D^{24}$ +101 (*c* 1.04, $CHCl_3$);[12] ^{1}H NMR (CD_2Cl_2): δ 0.88 (s, 3H, 18-H), 2.87 (m, 2H, 6-H), 3.44 (t, 2H, $J = 6.9$ Hz, 6′-H), 3.91 (t, 2H, $J = 6.4$ Hz, 1′-H), 6.62 (d, 1H, $J = 2.4$ Hz, 4-H), 6.66 (dd, 1H, $J = 2.4$ Hz, $J = 8.6$ Hz, 2-H), 7.16 (d, 1H, $J = 8.6$ Hz, 1-H). Anal. Calcd for $C_{24}H_{33}O_2Br$ (433.42): C, 66.51; H, 7.67; Br, 18.44. Found: C, 66.14; H, 7.89; Br, 18.02.

4.2.1.3. 3-(8′-Bromooctyloxy)-estra-1,3,5(10)-triene-17-one (4). Colorless crystals (76% yield); mp 62–65 °C (MeOH); $[\alpha]_D^{24}$ +90 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 0.88 (s, 3H, 18-H), 2.85 (m, 2H, 6-H), 3.42 (t, 2H, $J = 6.8$ Hz, 8′-H), 3.90 (t, 2H, $J = 6.5$ Hz, 1′-H), 6.61 (d, 1H, $J = 2.6$ Hz, 4-H), 6.65 (dd, 1H, $J = 2.6$ Hz, $J = 8.4$ Hz, 2-H), 7.16 (d, 1H, $J = 8.4$ Hz, 1-H). Anal. Calcd for $C_{26}H_{37}O_2Br$ (461.48): C, 67.67; H, 8.08; Br, 17.31. Found: C, 67.36; H, 8.32; Br, 16.99.

4.2.1.4. 3-(10′-Bromodecyloxy)-estra-1,3,5(10)-triene-17-one (5). Colorless crystals (75% yield); mp 67–68 °C (MeOH), mp 66–68 °C (*n*-hexane);[12] $[\alpha]_D^{24}$ +80 (*c* 0.1, CH_2Cl_2), $[\alpha]_D^{24}$ +88 (*c* 1.02 $CHCl_3$);[12] ^{1}H NMR (CD_2Cl_2): δ 0.88 (s, 3H, 18-H), 2.85 (m, 2H, 6-H), 3.42 (t, 2H, $J = 6.9$ Hz, 10′-H), 3.90 (t, 2H, $J = 6.6$ Hz, 1′-H), 6.61 (d, 1H, $J = 2.7$ Hz, 4-H), 6.66 (dd, 1H, $J = 2.7$ Hz, $J = 8.6$ Hz, 2-H), 7.16 (d, 1H, $J = 8.6$ Hz, 1-H). Anal. Calcd for $C_{28}H_{41}O_2Br$ (489.53): C, 68.70; H, 8.44; Br, 16.32. Found: C, 68.20; H, 8.65; Br, 15.71.

4.2.1.5. 1-(4′-Bromobutyloxy)-4-methyl-estra-1,3,5-(10)-triene-17-one (11). Colorless crystals (98% yield); mp 99–102 °C (*n*-heptane); $[\alpha]_D^{24}$ +228 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (s, 3H, 18-H), 2.16 (s, 3H, 4-CH_3), 3.45 (t, 2H, $J = 6.8$ Hz, 4′-H), 3.93 (m, 2H, 1′-H), 6.60 and 6.93 (2×d, 2×1H, $J = 8.6$ Hz, 2-H and 3-H). Anal. Calcd for $C_{23}H_{31}O_2Br$ (419.38): C, 65.87; H, 7.45; Br, 19.05. Found: C, 66.12; H, 7.98; Br, 19.67.

4.2.1.6. 1-(6′-Bromohexyloxy)-4-methyl-estra-1,3,5-(10)-triene-17-one (12). Colorless oil (91% yield); $[\alpha]_D^{24}$ +203 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.93 (s, 3H, 18-H), 2.15 (s, 3H, 4-CH_3), 3.40 (t, 2H, $J = 6.8$ Hz, 6′-H), 3.89 (m, 2H, 1′-H), 6.59 and 6.92 (2×d, 2×1H, $J = 8.6$ Hz, 2-H and 3-H). Anal. Calcd for $C_{25}H_{35}O_2Br$ (447.45): C, 67.11; H, 7.88; Br, 17.86. Found: C, 66.97; H, 8.14; Br, 17.68.

4.2.1.7. 1-(8′-Bromooctyloxy)-4-methyl-estra-1,3,5-(10)-triene-17-one (13). Colorless crystals (88% yield); mp 52–54 °C (*n*-heptane); $[\alpha]_D^{24}$ +191 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (s, 3H, 18-H), 2.15 (s, 3H, 4-CH_3), 3.39 (t, 2H, $J = 6.8$ Hz, 8′-H), 3.88 (m, 2H, 1′-H), 6.60 and 6.92 (2×d, 2×1H, $J = 8.4$ Hz, 2-H and 3-H). Anal. Calcd for $C_{27}H_{39}O_2Br$ (475.50): C, 68.20; H, 8.27; Br, 16.80. Found: C, 68.25; H, 8.58; Br, 17.03.

4.2.1.8. 1-(10′-Bromodecyloxy)-4-methyl-estra-1,3,5-(10)-triene-17-one (14). Colorless oil (94% yield); $[\alpha]_D^{24}$ +192 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (s, 3H, 18-H), 2.15 (s, 3H, 4-CH_3), 3.39 (t, 2H, $J = 6.8$ Hz, 10′-H), 3.86 (m, 2H, 1′-H), 6.60 and 6.91 (2×d, 2×1H, $J = 8.6$ Hz, 2-H and 3-H). Anal. Calcd for $C_{29}H_{43}O_2Br$ (503.55): C, 69.17; H, 8.61; Br, 15.87. Found: C, 68.94; H, 8.91; Br, 16.05.

4.2.2. General procedure for the reaction of pyridine with 1-(ω-bromoalkoxy)-4-methyl-estra-1,3,5(10)-triene-17-ones (15–18). A solution of the corresponding 1-(ω-bromoalkoxy)-4-methyl-estra-1,3,5(10)-triene-17-one (**11–14**) (1 mmol) and pyridine (3 mmol) in anhydrous acetonitrile (10 mL) was refluxed under an argon atmosphere for 20–30 h until a complete conversion was achieved (TLC). The mixture was concentrated to the half. The precipitate that separated out was filtered off, washed twice with cold *n*-heptane and dried in vacuo.

4.2.2.1. N-1′[(4-Methyl-17-oxo-estra-1,3,5(10)-trien-1-yloxy)-butyl]-pyridinium bromide (15). Light yellow solid (84% yield); mp 131–134 °C; $[\alpha]_D^{24}$ +154 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.87 (s, 3H, 18-H), 2.11 (s, 3H, 4-

CH_3), 3.92 (m, 2H, –CH_2–O–), 5.10 (t, 2H, $J = 7.4$ Hz, –CH_2–N), 6.58 and 6.88 (2×d, 2×1H, $J = 8.6$ Hz, 2-H and 3-H), 8.10 (t, 2H, $J = 7.5$ Hz, H_{Py}), 8.48 (t, 1H, $J = 7.8$ Hz, H_{Py}), 9.48 (d, 2H, $J = 5.5$ Hz, H_{Py}). Anal. Calcd for $C_{28}H_{36}O_2NBr$ (498.50): C, 67.46; H, 7.28; N, 2.81; Br, 16.03. Found: C, 66.90; H, 7.52; N, 2.88; Br, 14.83.

4.2.2.2. N-1′[(4-Methyl-17-oxo-estra-1,3,5(10)-trien-1-yloxy)-hexyl]-pyridinium bromide (16). Light yellow solid (81% yield); mp 86–89 °C; $[\alpha]_D^{24}$ +151 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.90 (s, 3H, 18-H), 2.13 (s, 3H, 4-CH_3), 3.85 (m, 2H, –CH_2–O–), 5.04 (t, 2H, $J = 7.5$ Hz, –CH_2–N), 6.56 and 6.90 (2×d, 2×1H, $J = 8.6$ Hz, 2-H and 3-H), 8.11 (t, 2H, $J = 6.8$ Hz, H_{Py}), 8.48 (t, 1H, $J = 7.8$ Hz, H_{Py}), 9.51 (d, 2H, $J = 5.7$ Hz, H_{Py}). Anal. Calcd for $C_{30}H_{40}O_2NBr$ (526.55): C, 68.43; H, 7.66; N, 2.66; Br, 15.18. Found: C, 67.83; H, 7.73; N, 2.65; Br, 14.93.

4.2.2.3. N-1′[(4-Methyl-17-oxo-estra-1,3,5(10)-trien-1-yloxy)-octyl]-pyridinium bromide (17). Light yellow solid (89% yield); mp 143–145 °C; $[\alpha]_D^{24}$ +142 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.92 (s, 3H, 18-H), 2.14 (s, 3H, 4-CH_3), 3.85 (m, 2H, –CH_2–O–), 4.98 (t, 2H, $J = 7.5$ Hz, –CH_2–N), 6.57 and 6.90 (2×d, 2×1H, $J = 8.6$ Hz, 2-H and 3-H), 8.11 (t, 2H, $J = 7.0$ Hz, H_{Py}), 8.48 (t, 1H, $J = 7.8$ Hz, H_{Py}), 9.51 (d, 2H, $J = 5.8$ Hz, H_{Py}). Anal. Calcd for $C_{32}H_{44}O_2NBr$ (554.60): C, 69.30; H, 8.00; N, 2.53; Br, 14.41. Found: C, 68.94; H, 8.25; N, 2.36; Br, 14.08.

4.2.2.4. N-1′[(4-Methyl-17-oxo-estra-1,3,5(10)-trien-1-yloxy)-decyl]-pyridinium bromide (18). Light yellow hygroscopic solid (85% yield); $[\alpha]_D^{24}$ +135 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.90 (s, 3H, 18-H), 2.13 (s, 3H, 4-CH_3), 3.86 (m, 2H, –CH_2–O–), 4.95 (t, 2H, $J = 6.7$ Hz, –CH_2–N), 6.57 and 6.90 (2×d, 2×1H, $J = 8.6$ Hz, 2-H and 3-H), 8.11 (t, 2H, $J = 7.0$ Hz, H_{Py}), 8.47 (t, 1H, $J = 7.8$ Hz, H_{Py}), 9.47 (d, 2H, $J = 5.5$ Hz, H_{Py}). Anal. Calcd for $C_{34}H_{48}O_2NBr$ (582.66): C, 70.09; H, 8.30; N, 2.40; Br, 13.71. Found: C, 69.97; H, 8.63; N, 2.39; Br, 14.01.

4.2.2.5. N-1′[(17-Oxo-estra-1,3,5(10)-triene-3-yloxy)-butyl]-pyridinium bromide (6). 3-(4′-Bromobutyloxy)-estra-1,3,5(10)-triene-17-one **2** was reacted with pyridine in acetonitrile under reflux for 5 h as described for the reaction of 1-(ω-bromoalkoxy)-4-methyl-estra-1,3,5(10)-triene-17-ones (**15**–**18**). The crude product was recrystallized from methanol/MTBE. White solid (70% yield); mp 213–215 °C; $[\alpha]_D^{24}$ +80 (*c* 0.1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-H), 2.84 (m, 2H, 6-H), 4.00 (t, 2H, $J = 5.9$ Hz, –CH_2–O–), 4.74 (t, 2H, $J = 7.5$ Hz, –CH_2–N), 6.62 (d, 1H, $J = 2.6$ Hz, 4-H), 6.66 (dd, 1H, $J = 2.6$ Hz, $J = 8.5$ Hz, 2-H), 7.15 (d, 1H, $J = 8.5$ Hz, 1-H), 8.13 (t, 2H, $J = 7.0$ Hz, H_{Py}), 8.60 (t, 1H, $J = 7.8$ Hz, H_{Py}), 9.05 (d, 2H, $J = 5.7$ Hz, H_{Py}). Anal. Calcd for $C_{27}H_{34}O_2NBr$ (484.47): C, 66.94; H, 7.07; N, 2.89; Br, 16.49. Found: C, 66.12; H, 6.92; N, 2.76; Br, 17.04.

4.2.2.6. N-1′[(17-Oxo-estra-1,3,5(10)-triene-3-yloxy)-decyl]-pyridinium bromide (9). 3-(10′-Bromodecyloxy)-estra-1,3,5(10)-triene-17-one **5** was reacted with pyridine as described for the reaction of 3-(4′-bromobutyloxy)-estra-1,3,5(10)-triene-17-one **2** (reaction time: 20 h). White solid (81% yield); mp 103–105 °C; $[\alpha]_D^{24}$ +67 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 0.87 (s, 3H, 18-H), 2.84 (m, 2H, 6-H), 3.88 (t, 2H, $J = 6.5$ Hz, –CH_2–O–), 4.88 (t, 2H, $J = 7.5$ Hz, –CH_2–N), 6.60 (d, 1H, $J = 2.6$ Hz, 4-H), 6.64 (dd, 1H, $J = 2.6$ Hz, $J = 8.5$ Hz, 2-H), 7.15 (d, 1H, $J = 8.5$ Hz, 1-H), 8.12 (t, 2H, $J = 7.0$ Hz, H_{Py}), 8.50 (t, 1H, $J = 7.8$ Hz, H_{Py}), 9.26 (d, 2H, $J = 5.7$ Hz, H_{Py}). Anal. Calcd for $C_{33}H_{46}O_2NBr$ (568.63): C, 69.70; H, 8.15; N, 2.46; Br, 14.05. Found: C, 69.08; H, 8.19; N, 2.39; Br, 14.48.

4.2.2.7. N-1′[(17-Oxo-estra-1,3,5(10)-triene-3-yloxy)-hexyl]-pyridinium bromide (7). A solution of 3-(6′-bromohexyloxy)-estra-1,3,5(10)-triene-17-one **3** (1 mmol) and pyridine (3 mmol) in methanol (10 mL) was reacted in an autoclave at 100 °C and 3 atm for 20 h. The reaction mixture was evaporated to dryness and the oily residue obtained was dissolved in methanol (5 mL). Addition of MTBE (10 mL) gave a precipitate, which was filtered off and dried in vacuo. White solid (31% yield); mp 163–165 °C; $[\alpha]_D^{24}$ +74 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 0.87 (s, 3H, 18-H), 2.84 (m, 2H, 6-H), 3.89 (t, 2H, $J = 6.4$ Hz, –CH_2–O–), 4.94 (t, 2H, $J = 7.5$ Hz, –CH_2–N), 6.59 (d, 1H, $J = 2.6$ Hz, 4-H), 6.64 (dd, 1H, $J = 2.6$ Hz, $J = 8.6$ Hz, 2-H), 7.15 (d, 1H, $J = 8.6$ Hz, 1-H), 8.11 (t, 2H, $J = 7.0$ Hz, H_{Py}), 8.49 (t, 1H, $J = 9.0$ Hz, H_{Py}), 9.36 (d, 2H, $J = 5.6$ Hz, H_{Py}). Anal. Calcd for $C_{29}H_{38}O_2NBr$ (512.52): C, 67.96; H, 7.47; N, 2.73; Br, 15.59. Found: C, 67.19; H, 7.55; N, 2.77; Br, 15.81.

4.2.2.8. N-1′[(17-Oxo-estra-1,3,5(10)-triene-3-yloxy)-octyl]-pyridinium bromide (8). 3-(8′-Bromooctyloxy)-estra-1,3,5(10)-triene-17-one **4** was reacted with pyridine as described for the reaction of 3-(6′-bromohexyloxy)-estra-1,3,5(10)-triene-17-one **3**. White solid (35% yield); mp 113–115 °C; $[\alpha]_D^{24}$ +67 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (CD_2Cl_2): δ 0.87 (s, 3H, 18-H), 2.85 (m, 2H, 6-H), 3.88 (t, 2H, $J = 6.5$ Hz, –CH_2–O–), 4.94 (t, 2H, $J = 7.5$ Hz, –CH_2–N), 6.60 (d, 1H, $J = 2.6$ Hz, 4-H), 6.64 (dd, 1H, $J = 2.6$ Hz, $J = 8.6$ Hz, 2-H), 7.15 (d, 1H, $J = 8.6$ Hz, 1-H), 7.93 (t, 2H, $J = 7.0$ Hz, H_{Py}), 8.48 (t, 1H, $J = 7.9$ Hz, H_{Py}), 9.40 (d, 2H, $J = 5.6$ Hz, H_{Py}). Anal. Calcd for $C_{31}H_{42}O_2NBr$ (540.58): C, 68.88; H, 7.83; N, 2.59; Br, 14.78. Found: C, 68.19; H, 7.77; N, 2.57; Br, 14.97.

4.3. Biological assays

4.3.1. In vitro antibacterial activity. Antibacterial activity of the compounds was studied by determination of minimal inhibitory concentrations (MIC) according to the NCCLS guidelines[14] using the microbroth dilution method. The cells were grown overnight at 37 °C in Mueller–Hinton broth (MHB) (Difco). 50 μL of a compound solution of 400 μL/mL were serially diluted by factor two with MHB. The wells were then inoculated

with 50 μL of bacteria to give a final concentration of 5×10^5 CFU/mL. After the microtiter plates were incubated at 37 °C for 24 h, the MIC values were read with a Nepheloscan Ascent 1.4 automatic plate reader (Labsystems, Vantaa, Finland) as the lowest dilution of antibiotic allowing no visible growth. Test organisms were taken from the stock of the Hans Knöll Institute (mycobacteria); epidemic multiresistant *S. aureus* strains and a vancomycin resistant *Enterococcus* strain were kindly provided by Prof. W. Witte, Wernigerode, Germany.

4.3.2. In vitro antiproliferative and cytotoxicity tests. Cells of established suspended cell lines K-562 (DSM ACC 10) and adherent L-929 (DSM ACC 2) were cultured in RPMI medium.[15] The adherent cells of L-929 and HeLa were harvested at the logarithmic growth phase after trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA (Biochrom KG Kat.-Nr. L2163). The target compounds were assayed against cell lines K-562 (human chronic myeloid leukemia), and L-929 (mouse fibroblast) for their antiproliferative effects. The adherent cells of L-929 were harvested at the logarithmic growth phase after soft trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA (Biochrom KG L2163). For each experiment with K-562, L-929 and HeLa approximately 10,000 cells were seeded with 0.1 mL RPMI 1640 (GIBCO BRL 21875-034), containing 25 μg/mL gentamicin sulfate (BioWhittaker 17-528Z), but without HEPES, per well of the 96-well microplates (K-562: NUNC 163320, L-929, HeLa: NUNC 167008). For the cytotoxic assay, the HeLa cells were preincubated for 48 h without the test substances. The dilutions of the compounds were carefully carried out on the monolayers of HeLa cells after the preincubation time.

Cells of L-929, K-562 and HeLa were incubated for 72 h at 37 °C in a humidified atmosphere and 5% CO_2. Suspension cultures of K-562 in microplates were analyzed by an electronic cell analyzer system CASY 1 (SCHÄRFE, Reutlingen, Germany) using an aperture of 150 μm. The 0.2 mL content of each well in the microplate was diluted 1:50 with CASYTON (SCHÄRFE). Every count/mL was automatically calculated from the arithmetic mean of three successive counts of 0.4 mL each. From the dose response curves the GI_{50} values were calculated with CASYSTAT. The GI_{50} value was defined as the 50% intersection line of the concentration–response curve, determined by the cell counts/mL as compared to the control. The essential parameters for the estimation of growth inhibition and for changes in diameter distribution curves are expressed as diagrams. The monolayer of the adherent L-929 and HeLa cells were fixed by glutaraldehyde and stained with a solution of methylene blue. After gently washing, the stain was eluted by 0.2 mL of 0.33 N HCl in the wells. The optical densities were measured at 630 nm in a microplate reader.

Acknowledgements

We gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 436) and the Fond der Chemischen Industrie, the Schering AG and the Jenapharm GmbH & Co. KG, Jena for the gift of steroids.

References and notes

1. Blum, B. R.; Murray, J. L. *Science (Washington DC)* **1992**, *257*, 1055.
2. Krieg, R.; Wyrwa, R.; Möllmann, U.; Görls, H.; Schönecker, B. *Steroids* **1998**, *63*, 531.
3. Tietze, L. F.; Schneider, Gy; Wölfling, J.; Nöbel, T.; Wulf, C.; Schubert, I.; Rübeling, A. *Angew. Chem.* **1998**, *110*, 2644; *Angew. Chem., Int. Ed.* **1998**, *37*, 2469.
4. De Riccardis, F.; Meo, D.; Izzo, I.; Di Filippo, M.; Casapullo, A. *Eur. J. Org. Chem.* **1998**, *9*, 1965.
5. Scherlitz-Hofmann, I.; Dubs, M.; Krieg, R.; Schönecker, B.; Kluge, M.; Sicker, D. *Helv. Chim. Acta* **1997**, *80*, 2345.
6. Ishiki, N.; Onishi, H.; Machida, Y. *Chem. Pharm. Bull.* **1997**, *45*, 1345.
7. Siddiqui, A. U.; Satyanarayana, Y.; Siddiqui, A. H. *Collect. Czech. Chem. Commun.* **1995**, *60*, 1186.
8. Voet, D.; Voet, J. G. In *Biochemie*; VCH: Weinheim, New York, Basel, Cambridge, 1990; p 271.
9. Tiller, J. C.; Liao, Ch.-J.; Lewis, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5981.
10. Wolff, T.; Dannenberg, A. *Chem. Ber.* **1970**, *103*, 917.
11. Shipchander, M. T. U.S. Patent 4,051,128, 1977; *Chem. Abstr*. **1977**, *88*, 7207.
12. Evans, D. D.; Evans, D. E.; Lewis, G. S.; Palmer, P. J.; Weyell, D. J. *J. Pharm. Pharmacol.* **1964**, *16*, 717.
13. Evans, D. D.; Evans, D. E.; Palmer, P. J. U.S. Patent 3,187,024, 1965.
14. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow Aerobically, Approved Standard. NCCLS Document M7-A4*, 4th ed.; National Committee for Clinical Laboratory Standards: Villanova, PA, 1997.
15. Dahse, H.-M.; Schlegel, B.; Gräfe, U. *Pharmazie* **2001**, *56*, 489.