

FUSED THIAZOLOANDROSTANES AND THEIR QUATERNARY SALTS, SYNTHESIS AND COOPERATIVE LIGAND BINDING TO MUSCARINIC ACETYLCHOLINE RECEPTOR⁺

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Steroidal thiazolium salts were prepared and their interaction with [³H]N-methylscopolamine ([³H]NMS) at M₂ muscarinic receptors using radioligand binding were studied. The results were consistent with a ternary complex theory. The compounds diminished [³H]NMS binding with K_d values in the micromolar or submicromolar range. In particular, the presence of bulky benzylic group at the thiazole nitrogen atom caused a substantial increase in affinity of allosteric ligand for receptor. Our results demonstrate that steroid thiazolium salts could be a new class of potentially useful tools for mapping the muscarinic receptors.

Key words: Steroids; Androstane; Thiazoles; Thiazolium salts; Muscarinic receptors; Allosteric ligands.

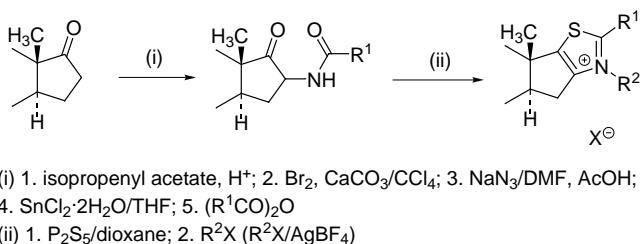
Steroids containing quaternary ammonium functions are an important group of substances in which the positively charged center mimics acetylcholine to some extent, thus affecting cholinergic transmission. For instance, pancuronium dibromide, a nicotinic acetylcholine receptor antagonist, is clinically used as a skeletal muscle relaxant and has attracted further attention because of its high affinity to cardiac muscarinic acetylcholine receptor^{2,3}. Since the thiazolium function has been exploited in the preparation of non-steroidal muscle relaxants⁴, we assumed that steroid thiazolium salts might be a potentially useful tools for cholinergic receptor studies.

Steroids containing thiazole moiety fused to the D ring of the androstane skeleton were studied in the sixties as potential antihormonal sub-

⁺ Part CDII in the series On Steroids; Part CDI see ref.¹

stances⁵⁻⁸. Reported preparations were based on condensation of 16-bromo-17-oxo steroids with thioamides or thioureas (Hantzsch reaction) affording 2'-substituted thiazolo[5',4':16,17]androstanes. We focused on the feasibility to synthesizing hitherto unknown, regioisomeric 2'-substituted thiazolo[4',5':16,17]androstanes using intramolecular cyclization of 16-acylamino-17-oxo steroids. Finding a suitable method for quaternization of fused thiazoloandrostanes was our next target.

The synthesis was based on a modification of the ring D in 17-oxo-5 α -androstane-3 β -yl acetate (Scheme 1). It implied bromination in position 16, conversion to 16-azido-17-ketone, reduction followed by acylation, and phosphorus pentasulfide mediated cyclocondensation. 2'-Unsubstituted-, 2'-methyl-, and 2'- (trifluoromethyl)thiazolo[4',5':16,17]-5 α -androstan-3 β -yl acetates were successfully prepared. Methyl-, ethyl-, and benzyl halides were used for the preparation of quaternary thiazolium salts.



SCHEME 1

Final products were examined by radioligand binding studies for their affinity for the cardiac M₂ subtype of muscarinic cholinoreceptors.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured in chloroform at 25 °C and $[\alpha]_D$ values are given in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. Infrared spectra were recorded on a Perkin-Elmer PE 580 spectrometer (wavenumbers in cm^{-1}). ¹H NMR spectra were taken on a Varian UNITY-200 (200 MHz, FT mode) at 23 °C in deuteriochloroform with tetramethylsilane as internal standard unless stated otherwise. Chemical shifts are given in ppm (δ -scale), coupling constants (J) and width of multiplets (W) in Hz. Mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer. Thin-layer chromatography was performed on silica gel G (ICN Biochemicals), with detection by spraying with concentrated sulfuric acid and followed by heating. For preparative flash column chromatography silica gel 32-63, 60A (ICN Biochemicals) or Silpearl (Kavalier) were used. The petroleum ether used was a fraction boiling at 40-62 °C. Molecular sieve 4A, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), atropine sulfate, and pancuronium dibromide were purchased from Sigma-Aldrich Co Ltd., (-)-[N-methyl-³H]scopolamine methyl chloride (³H]NMS) was obtained from Amersham, U.K.

16 α -Azido-17-oxo-5 α -androstan-3 β -yl Acetate (2a)

Sodium azide (11.0 g, 169 mmol) was added to a solution of the bromoketone **1a** (11.0 g, 27 mmol) in a mixture of dimethylformamide (100 ml) and acetic acid (7 ml). After stirring for 20 min at room temperature the reaction mixture was poured into cold water (200 ml). The resulting precipitate was filtered off, washed with water and dissolved in ethyl acetate (250 ml). The solution was washed with sodium hydrogen carbonate solution, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was applied on a column of silica gel. Elution with toluene–petroleum ether–ethyl acetate (5 : 5 : 0.3) afforded in the less polar fraction 2.86 g (29%) of **2a**, m.p. 171–172 °C dec. (methanol), $[\alpha]_D$ +264 (c 1.4). ^1H NMR: 4.68 m, 1 H (H-3 α , W = 32); 4.12 m, 1 H (H-16 β , W = 10); 2.02 s, 3 H (CH_3CO); 0.93 s, 3 H (3 \times H-18); 0.84 s, 3 H (3 \times H-19). IR (CHCl_3): 2 102, 1 311 (N_3); 1 746 (C=O); 1 725 (C=O, acetate); 1 254, 1 024 (C–O). EI MS, m/z (%): 345 (13, M – N_2); 317 (14, M – CH_2N_3); 276 (16); 257 (10, M – CH_2N_3 – CH_3COOH); 216 (71); 201 (100). For $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_3$ (373.5) calculated: 67.53% C, 8.37% H, 11.25% N; found: 67.41% C, 8.27% H, 11.38% N.

16 β -Azido-17-oxo-5 α -androstan-3 β -yl Acetate (2b)

The chromatographic separation described above yielded in the more polar fraction 6.6 g (66%) of the 16 β -azidoketone **2b**, m.p. 130–132 °C dec. (ethyl acetate–petroleum ether), $[\alpha]_D$ -63 (c 1.4). ^1H NMR: 4.68 m, 1 H (H-3 α , W = 32); 3.72 t, 1 H (H-16 α , $J_{\text{AX}} + J_{\text{BX}}$ = 17.1); 2.32 m, 1 H (H-15, W = 24); 2.02 s, 3 H (CH_3CO); 0.92 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19). IR (CHCl_3): 2 101, 1 310 (N_3); 1 749 (C=O); 1 724 (C=O, acetate); 1 256, 1 025 (C–O). EI MS, m/z (%): 345 (28, M – N_2); 317 (15, M – CH_2N_3); 276 (44); 257 (10, M – CH_2N_3 – CH_3COOH); 216 (79); 201 (100). For $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_3$ (373.5) calculated: 67.53% C, 8.37% H, 11.25% N; found: 67.61% C, 8.30% H, 11.31% N.

16 α -Formamido-17-oxo-5 α -androstan-3 β -yl Acetate (3a)

To a solution of the azidoketone **2a** (2.0 g, 5.4 mmol) in tetrahydrofuran (80 ml) tin(II) chloride dihydrate (3.6 g, 15.9 mmol) was added. The mixture was refluxed under argon atmosphere for 1 h. Mixed anhydride of formic and acetic acids (prepared from 15 ml of acetic anhydride and 30 ml of anhydrous formic acid) was added to the cooled solution and the mixture was allowed to stand at room temperature for 16 h. Water (80 ml) was added and the mixture was neutralized by portionwise addition of solid potassium carbonate. The organic material was separated from the inorganic precipitate by repeated treatment and decantation with ethyl acetate. The combined extracts were washed with brine and dried over anhydrous sodium sulfate. After evaporation of the solvent, the crude product was chromatographed on a column of silica gel (60 g) in toluene–ethyl acetate–methanol (5 : 5 : 0.3) to afford 1.77 g (88%) of the amide **3a**, m.p. 192–193.5 °C (ethyl acetate–petroleum ether), $[\alpha]_D$ -32 (c 1.3). ^1H NMR: 8.23 s, 1 H (CHO); 5.98 bd, 1 H (NH, J = 5.8); 4.68 m, 1 H (H-3 α , W = 32); 4.59 m, 1 H (H-16 α , W = 24); 2.18 m, 1 H (H-15, W = 36); 2.02 s, 3 H (CH_3CO); 1.01 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19). IR (CHCl_3): 3 410 (NH); 1 747 (C=O); 1 723 (C=O, acetate); 1 685 (amide I); 1 494 (amide II); 1 256, 1 028 (C–O). FAB MS, m/z (%): 376 (100, M + 1); 360 (16, M – CH_3); 348 (18, M – CO + 1). For $\text{C}_{22}\text{H}_{33}\text{NO}_4$ (375.5) calculated: 70.37% C, 8.86% H, 3.73% N; found: 70.54% C, 9.04% H, 3.69% N.

16 β -Formamido-17-oxo-5 α -androstan-3 β -yl Acetate (3b)

Following the same procedure described above for **3a**, 16 β -azidoketone **2b** (500 mg, 1.3 mmol) gave amide **3b** (490 mg, 97%), m.p. 185–186 °C (ethyl acetate–petroleum ether), $[\alpha]_D$ +69 (c 1.3). ^1H NMR: 8.23 s, 1 H (CHO); 5.85 bd, 1 H (NH, J = 5.8); 4.69 m, 1 H (H-3 α , W = 32); 4.13 m, 1 H (H-16 α , W = 24); 2.61 m, 1 H (H-15, W = 22); 2.02 s, 3 H (CH_3CO); 0.91 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19). IR (CHCl₃): 3 417, 3 333 (NH); 1 745 (C=O); 1 724 (C=O, acetate); 1 689 (amide I); 1 500 (amide II); 1 260, 1 255, 1 026 (C=O). EI MS, m/z (%): 375 (24, M); 360 (47, M – CH_3); 330 (15, M – HCONH₂); 315 (11, M – CH_3COOH); 276 (31); 261 (11); 216 (100); 201 (90). For C₂₂H₃₃NO₄ (375.5) calculated: 70.37% C, 8.86% H, 3.73% N; found: 70.54% C, 9.04% H, 3.69% N.

16-Acetamido-17-oxo-5 α -androstan-3 β -yl Acetate (4)

Following a similar procedure to that used for the preparation of **3a**, tin(II) chloride reduction of **2b** (500 mg, 1.3 mmol) with subsequent acetylation with acetic anhydride (7.6 ml, 80.5 mmol) gave a mixture of epimeric 16-acetylaminoketones **4a** and **4b** (497 mg, 95%). ^1H NMR: signals of the 16 α -epimer **4a**: 5.80 d, 1 H (NH, J = 6.1); 4.68 m, 1 H (H-3 α , W = 32); 5.78 m, 1 H (H-16 β , W = 19); 2.02 s, 6 H (2 \times CH₃CO); 1.00 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19); signals of the 16 β -epimer **4b**: 5.75 d, 1 H (NH, J = 5.2); 4.68 m, 1 H (H-3 α , W = 32); 4.08 m, 1 H (H-16 α , W = 24); 2.02 s, 6 H (2 \times CH₃CO); 0.90 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19). IR (CHCl₃): 3 430, 3 347 (NH); 1 743 (C=O); 1 725 (C=O, acetate); 1 674 (amide I); 1 507 (amide II); 1 254, 1 027 (C=O). EI MS, m/z (%): 389 (86, M); 374 (46, M – CH_3); 330 (25, M – CH_3CONH_2); 276 (18); 261 (11); 216 (86); 201 (100). For C₂₃H₃₅NO₄ (389.5) calculated: 70.92% C, 9.06% H, 3.60% N; found: 70.83% C, 9.16% H, 3.58% N.

17-Oxo-16 α -trifluoroacetamido-5 α -androstan-3 β -yl Acetate (5a)

Azide **2b** (1.2 g, 3.2 mmol) in tetrahydrofuran (50 ml) was reduced with tin(II) chloride dihydrate (2.2 g, 9.8 mmol) at the reflux for 1 h. Trifluoroacetic anhydride (6.7 ml, 48.2 mmol) was added and the mixture allowed to stand for 16 h at room temperature. The mixture was evaporated to dryness and the solid residue applied onto a column of silica gel (50 g). Elution with petroleum ether–ethyl acetate (9 : 1) afforded amide **5a** (230 mg, 16%), m.p. 240–242 °C (ethyl acetate–petroleum ether), $[\alpha]_D$ −39 (c 1.4). ^1H NMR: 6.81 bd, 1 H (NH, J = 5.1); 4.51–4.76 m, 2 H (H-3 α , H-16 β); 2.22 m, 1 H (H-15, W = 37); 2.02 s, 3 H (CH₃CO); 1.03 s, 3 H (3 \times H-18); 0.86 s, 3 H (3 \times H-19). IR (CHCl₃): 3 399, 3 288, 3 260 (NH); 1 745 (C=O); 1 725 (C=O, acetate and amide I); 1 528, 1 540, 1 556 (amide II); 1 256, 1 029 (C=O); 1 172 (CF₃). EI MS, m/z (%): 443 (10, M); 428 (13, M – CH_3); 383 (16, M – CH_3COOH); 276 (52); 261 (10); 216 (100); 201 (90). For C₂₃H₃₂F₃NO₄ (443.5) calculated: 62.29% C, 7.27% H, 12.85% F, 3.16% N; found: 62.11% C, 7.20% H, 13.01% F, 3.12% N.

17-Oxo-16 β -trifluoroacetamido-5 α -androstan-3 β -yl Acetate (5b)

Further elution from the chromatography of the preceding preparation of compound **5a** afforded 1.02 g (71%) of the amide **5b**, m.p. 201–202.5 °C (ethyl acetate–petroleum ether), $[\alpha]_D$ +72 (c 1.7). ^1H NMR: 6.61 bd, 1 H (NH, J = 5.5); 4.69 m, 1 H (H-3 α , W = 32); 4.08 m, 1 H (H-16 α , W = 24); 2.65 m, 1 H (H-15); 2.03 s, 3 H (CH₃CO); 0.94 s, 3 H (3 \times H-18); 0.86 s, 3 H (3 \times H-19). IR (CHCl₃): 3 410, 3 319 (NH); 1 749 (C=O); 1 727 (C=O, acetate and amide I);

1 534 (amide II); 1 254, 1 026 (C–O); 1 173 (CF_3). EI MS, m/z (%): 443 (13, M); 428 (13, M – CH_3); 383 (18, M – CH_3COOH); 276 (50); 261 (11); 216 (100); 201 (87). For $\text{C}_{23}\text{H}_{32}\text{F}_3\text{NO}_4$ (443.5) calculated: 62.29% C, 7.27% H, 12.85% F, 3.16% N; found: 62.19% C, 7.22% H, 12.98% F, 3.12% N.

17-Oxo-16 α -trichloroacetamido-5 α -androstan-3 β -yl Acetate (6a)

Azide **2b** (1.0 g, 2.7 mmol) in tetrahydrofuran (40 ml) was reduced with tin(II) chloride dihydrate (1.8 g, 8.0 mmol) under reflux for 1 h. At 0 °C trichloroacetyl chloride (1.5 ml, 13.4 mmol) and triethylamine (1.87 ml, 13.4 mmol) were added. The mixture was stirred at room temperature for 0.5 h and allowed to stand for 16 h at room temperature. The mixture was evaporated to dryness and the solid residue applied onto a column of silica gel (50 g). Elution with petroleum ether–ethyl acetate (9 : 1) afforded amide **6a** (230 mg, 16%), m.p. 222.5–224.5 °C (methanol), $[\alpha]_D$ –41 (c 1.3). ^1H NMR: 7.07 bd, 1 H (NH, J = 4.9); 4.68 m, 1 H (H-3 α , W = 32); 4.50 m, 1 H (H-16 β , W = 18); 2.22 m, 1 H (H-15, W = 36); 2.02 s, 3 H (CH_3CO); 1.04 s, 3 H (3 \times H-18); 0.86 s, 3 H (3 \times H-19). IR (CHCl_3): 3 398 (NH); 1 748 (C=O); 1 715 (C=O, acetate and amide I); 1 500 (amide II); 1 256, 1 028 (C–O). EI MS, m/z (%): 491 (5, M); 476 (10, M – CH_3); 456 (11, M – Cl); 318 (8); 276 (44); 261 (13); 216 (100); 201 (76). For $\text{C}_{23}\text{H}_{32}\text{Cl}_3\text{NO}_4$ (492.9) calculated: 56.05% C, 6.54% H, 21.58% Cl, 2.84% N; found: 56.04% C, 6.61% H, 21.58% Cl, 2.78% N.

17-Oxo-16 β -trichloroacetamido-5 α -androstan-3 β -yl Acetate (6b)

Further elution from the chromatography of the preceding preparation of compound **6a** afforded 624 mg (47%) of the amide **6b**, m.p. 211–212.5 °C (methanol), $[\alpha]_D$ +35 (c 1.5). ^1H NMR: 6.96 bd, 1 H (NH, J = 5.6); 4.71 m, 1 H (H-3 α , W = 32); 4.02 m, 1 H (H-16 α , W = 24); 2.65 m, 1 H (H-15, W = 23); 2.03 s, 3 H (CH_3CO); 0.96 s, 3 H (3 \times H-18); 0.86 s, 3 H (3 \times H-19). IR (CHCl_3): 3 408 (NH); 1 747 (C=O); 1 718 (C=O, acetate and amide I); 1 509 (amide II); 1 261, 1 254, 1 026 (C–O). EI MS, m/z (%): 491 (3, M); 476 (8, M – CH_3); 456 (10, M – Cl); 318 (8); 276 (40); 261 (11); 216 (100); 201 (74). For $\text{C}_{23}\text{H}_{32}\text{Cl}_3\text{NO}_4$ (492.9) calculated: 56.05% C, 6.54% H, 21.58% Cl, 2.84% N; found: 55.83% C, 6.61% H, 21.44% Cl, 2.81% N.

16 β -Dichloroacetamido-17-oxo-5 α -androstan-3 β -yl Acetate (7)

The most polar fraction from the chromatography of the preparation of **6a** yielded 297 mg (24%) of the dehalogenated amide **7**, m.p. 203–205 °C (methanol), $[\alpha]_D$ +60 (c 1.3). ^1H NMR: 6.76 bd, 1 H (NH, J = 6.2); 5.95 s, 1 H (Cl_2CHCO); 4.70 m, 1 H (H-3 α , W = 32); 4.05 m, 1 H (H-16 α , W = 24); 2.61 m, 1 H (H-15, W = 22); 2.03 s, 3 H (CH_3CO); 0.95 s, 3 H (3 \times H-18); 0.86 s, 3 H (3 \times H-19). IR (CHCl_3): 3 412 (NH); 1 747 (C=O); 1 716 (C=O, acetate); 1 698 (amide I); 1 520 (amide II); 1 255, 1 027 (C–O). EI MS, m/z (%): 457 (5, M); 442 (10, M – CH_3); 330 (8, M – $\text{CHCl}_2\text{CONH}_2$); 276 (39); 261 (11); 216 (100); 201 (79). For $\text{C}_{23}\text{H}_{33}\text{Cl}_2\text{NO}_4$ (458.4) calculated: 60.26% C, 7.26% H, 15.47% Cl, 3.06% N; found: 60.09% C, 7.32% H, 15.47% Cl, 3.15% N.

General Procedure for Cyclization of 16-Acylamino-17-oxo-5 α -androstan-3 β -yl Acetates

To a solution of an acylaminoketone (1.2 mmol) in dry dioxane (45 ml) phosphorus pentasulfide (400 mg, 1.8 mmol) was added. The vigorously stirred mixture was kept at reflux under argon on an oil bath at 120 °C for 1–2 h (until no more starting material was detected by TLC). Sodium carbonate (1.3 g, 24.3 mmol) in water (10 ml) was added to the cooled mixture and stirring continued for 2 h at room temperature. The solution was poured into water (500 ml). Resulting suspension was extracted with several portions of ether. The combined extracts were washed with brine and dried over sodium sulfate. The solvent was taken down and the crude material chromatographed on a column of silica gel (\approx 30 g) in petroleum ether–ethyl acetate (9 : 1) in the case of **8** and **10**, petroleum ether–diethyl ether (9 : 1) in the case of **11**.

Thiazolo[4',5':16,17]-5 α -androstan-3 β -yl acetate (8). Acetylaminoketone **3b** (450 mg, 1.2 mmol) gave 309 mg (69%) of thiazole **8**, m.p. 187–188.5 °C (methanol), $[\alpha]_D$ -6 (c 1.4). 1 H NMR: 8.58 s, 1 H (H-2'); 4.70 m, 1 H (H-3 α , W = 32); 2.79 dd, 1 H (H-15 β , J_{vic} = 6.5, J_{gem} = 14.3); 2.46 dd, 1 H (H-15 α , J_{vic} = 11.3, J_{gem} = 14.3); 2.03 s, 3 H (CH_3CO); 0.98 s, 3 H (3 \times H-18); 0.89 s, 3 H (3 \times H-19). IR (CHCl₃): 1 724 (C=O); 1 558, 1 498, 1 444, 1 046 (thiazole ring); 1 259, 1 026 (C–O). EI MS, m/z (%): 373 (15, M); 358 (100, M – CH₃); 298 (32, M – CH₃ – CH₃COOH). For C₂₂H₃₁NO₂S (373.6) calculated: 70.74% C, 8.36% H, 3.75% N, 8.58% S; found: 70.49% C, 8.62% H, 3.72% N, 8.75% S.

2'-Methylthiazolo[4',5':16,17]-5 α -androstan-3 β -yl acetate (9). Acetylaminoketone **4** (467 mg, 1.2 mmol) gave 349 mg (74%) of thiazole **9**, m.p. 223–224 °C (methanol), $[\alpha]_D$ -13 (c 1.4). 1 H NMR: 4.70 m, 1 H (H-3 α , W = 32); 2.69 dd, 1 H (H-15 β , J_{vic} = 6.6, J_{gem} = 14.3); 2.67 s, 3 H (CH_3 -2'); 2.39 dd, 1 H (H-15 α , J_{vic} = 11.2, J_{gem} = 14.3); 2.03 s, 3 H (CH_3CO); 0.96 s, 3 H (3 \times H-18); 0.89 s, 3 H (3 \times H-19). IR (CHCl₃): 1 724 (C=O); 1 515, 1 443, 1 045 (thiazole ring); 1 259, 1 025 (C–O). EI MS, m/z (%): 387 (13, M); 372 (100, M – CH₃); 327 (6, M – CH₃COOH); 312 (18, M – CH₃ – CH₃COOH). For C₂₃H₃₃NO₂S (387.6) calculated: 71.28% C, 8.58% H, 3.61% N, 8.27% S; found: 71.09% C, 8.41% H, 3.63% N, 8.11% S.

2'-(Trifluoromethyl)thiazolo[4',5':16,17]-5 α -androstan-3 β -yl acetate (10). Acetylaminoketone **5b** (532 mg, 1.2 mmol) gave 486 mg (92%) of thiazole **10**, m.p. 215–217 °C (ethanol), $[\alpha]_D$ -11 (c 1.8). 1 H NMR: 4.70 m, 1 H (H-3 α , W = 32); 2.82 dd, 1 H (H-15 β , J_{vic} = 6.4, J_{gem} = 14.3); 2.46 dd, 1 H (H-15 α , J_{vic} = 11.1, J_{gem} = 14.3); 2.03 s, 3 H (CH_3CO); 1.04 s, 3 H (3 \times H-18); 0.90 s, 3 H (3 \times H-19). IR (CHCl₃): 1 725 (C=O); 1 505, 1 441, 1 045 (thiazole ring); 1 259, 1 254, 1 026 (C–O). EI MS, m/z (%): 441 (19, M); 426 (100, M – CH₃); 381 (10, M – CH₃COOH); 366 (87, M – CH₃ – CH₃COOH). For C₂₃H₃₀F₃NO₂S (441.6) calculated: 62.56% C, 6.85% H, 12.91% F, 3.17% N, 7.26% S; found: 62.55% C, 6.86% H, 12.50% F, 3.15% N, 7.38% S.

3 β -Acetoxy-3'-methyl-5 α -androst-16-eno[16,17-d]thiazolium Iodide (11)

A solution of thiazole **8** (207 mg, 0.6 mmol) in freshly distilled 1,2-dichloroethane (3 ml) and methyl iodide (1.7 ml, 27.3 mmol) were sealed in a glass tube. It was then kept at 100 °C for 12 h. After cooling to room temperature, the reaction mixture was diluted with chloroform (30 ml), washed successively with aqueous sodium thiosulfate solution (10%), saturated potassium iodide solution, water, and dried over anhydrous sodium sulfate. The solvents were taken down and the residue crystallized from 1,2-dichloroethane–ethyl acetate to give 199 mg (70%) of the thiazolium iodide **11**, m.p. 250–253 °C. 1 H NMR: 11.15 s, 1 H (H-2'); 4.69 m, 1 H (H-3 α , W = 32); 4.42 s, 3 H (CH₃-3'); 2.93 dd, 1 H (H-15 α , J_{vic} = 6.8, J_{gem} =

14.9); 2.74 dd, 1 H (H-15 β , $J_{\text{vic}} = 11.3$, $J_{\text{gem}} = 14.9$); 2.31 m, 1 H (H-14, $W = 29$); 2.03 s, 3 H (CH_3CO); 1.15 s, 3 H (3 \times H-18); 0.89 s, 3 H (3 \times H-19). IR (CHCl_3): 3 086 ($\text{C}_2\text{-H}$); 1 725 (C=O); 1 620, 1 565 (thiazolium); 1 254, 1 027 (C-O). FAB MS, m/z (%): 388 (100, M - I); 328 (8, M - I - CH_3COOH). For $\text{C}_{23}\text{H}_{34}\text{INO}_2\text{S}$ (515.5) calculated: 53.59% C, 6.65% H, 24.62% I, 2.72% N, 6.22% S; found: 53.29% C, 7.03% H, 24.37% I, 2.69% N, 6.30% S.

3 β -Acetoxy-2',3'-dimethyl-5 α -androst-16-eno[16,17-d]thiazolium Iodide (12)

Following the same procedure described above for **11**, the thiazole **9** (130 mg, 0.3 mmol) gave thiazolium iodide **12**, (132 mg, 74%), m.p. 261–264 °C (1,2-dichloroethane–ethyl acetate). ^1H NMR: 4.69 m, 1 H (H-3 α , $W = 32$); 4.18 s, 3 H ($\text{CH}_3\text{-}3'$); 3.14 s, 3 H ($\text{CH}_3\text{-}2'$); 2.93 dd, 1 H (H-15 α , $J_{\text{vic}} = 7.2$, $J_{\text{gem}} = 14.8$); 2.79 dd, 1 H (H-15 β , $J_{\text{vic}} = 11.0$, $J_{\text{gem}} = 14.7$); 2.24 m, 1 H (H-14, $W = 29$); 2.03 s, 3 H (CH_3CO); 1.15 s, 3 H (3 \times H-18); 0.88 s, 3 H (3 \times H-19). IR (CHCl_3): 1 725 (C=O); 1 613, 1 572 (thiazolium); 1 253, 1 027 (C-O). FAB MS, m/z (%): 402 (100, M - I); 342 (7, M - I - CH_3COOH). For $\text{C}_{24}\text{H}_{36}\text{INO}_2\text{S}$ (529.5) calculated: 54.44% C, 6.85% H, 23.97% I, 2.65% N, 6.06% S; found: 54.03% C, 6.82% H, 23.12% I, 2.40% N, 6.29% S.

3 β -Acetoxy-3'-ethyl-5 α -androst-16-eno[16,17-d]thiazolium Bromide (13)

Thiazole **8** (100 mg, 0.3 mmol) and ethyl bromide (3.0 ml, 40.2 mmol) were sealed in a glass tube and kept at 100 °C for 10 h. After cooling to room temperature, the crystalline product was filtered off and washed with ether to yield thiazolium bromide **13** (122 mg, 94%). An analytical sample was recrystallized from chloroform–ether, m.p. 258–260 °C. ^1H NMR ($\text{DMSO}-d_6$): 4.58 m, 1 H (H-3 α , $W = 32$); 4.45 q, 2 H (CH_3CH_2 , $J = 7.3$); 3.43 s, 1 H (H-2'); 1.97 s, 3 H (CH_3CO); 1.45 t, 3 H (CH_3CH_2 , $J = 7.3$); 1.04 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19). IR (CHCl_3): 3 089 ($\text{C}_2\text{-H}$); 1 725 (C=O); 1 620, 1 558 (thiazolium); 1 253, 1 027 (C-O). FAB MS, m/z (%): 402 (100, M - Br); 342 (7, M - Br - CH_3COOH). For $\text{C}_{24}\text{H}_{37}\text{BrNO}_2\text{S}$ (483.5) calculated: 59.62% C, 7.71% H, 16.53% Br, 2.90% N, 6.63% S; found: 59.35% C, 7.76% H, 16.37% Br, 2.81% N, 6.48% S.

3 β -Acetoxy-3'-ethyl-2'-methyl-5 α -androst-16-eno[16,17-d]thiazolium Bromide (14)

Following the same procedure described above for **13**, the thiazole **9** (100 mg, 0.3 mmol) gave thiazolium bromide **14** (114 mg, 89%), m.p. 213–215 °C (chloroform–ether). ^1H NMR ($\text{DMSO}-d_6$): 4.59 m, 1 H (H-3 α , $W = 32$); 4.36 m, 2 H (CH_3CH_2 , $W = 40$); 2.97 s, 3 H ($\text{CH}_3\text{-}2'$); 1.97 s, 3 H (CH_3CO); 1.37 t, 3 H (CH_3CH_2 , $J = 7.5$); 1.02 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19). IR (CHCl_3): 1 725 (C=O); 1 620, 1 568 (thiazolium ring); 1 253, 1 027 (C-O). FAB MS, m/z (%): 416 (100, M - Br); 356 (8, M - Br - CH_3COOH). For $\text{C}_{25}\text{H}_{38}\text{BrNO}_2\text{S}$ (496.6) calculated: 60.47% C, 7.71% H, 16.09% Br, 2.82% N, 6.46% S; found: 60.31% C, 7.66% H, 15.87% Br, 2.76% N, 6.34% S.

3 β -Acetoxy-3'-benzyl-5 α -androst-16-eno[16,17-d]thiazolium Bromide (15)

A solution of thiazole **8** (150 mg, 0.4 mmol) in 1,2-dichloroethane (3 ml) and benzyl bromide (0.47 ml, 4.0 mmol) were sealed in a glass tube and kept at 100 °C for 12 h. After cooling to room temperature, the volatiles were removed *in vacuo* and the residue crystallized from 1,2-dichloroethane–ethyl acetate giving thiazolium bromide **15** (149 mg, 68%), m.p.

157–159 °C. ^1H NMR: 7.46–7.38 m, 5 H (arom. H); 6.04 m, 2 H (PhCH_2 , $W = 52$); 4.68 m, 1 H (H-3 α , $W = 32$); 2.79 dd, 1 H (H-15 α , $J_{\text{vic}} = 6.3$, $J_{\text{gem}} = 14.8$); 2.46 dd, 1 H (H-15 β , $J_{\text{vic}} = 11.6$, $J_{\text{gem}} = 14.8$); 2.02 s, 3 H (CH_3CO); 1.04 s, 3 H (3 \times H-18); 0.85 s, 3 H (3 \times H-19). IR (CHCl_3): 1 725 (C=O); 1 617, 1 556 (thiazolium); 1 606, 1 497, 1 455, 912, 703 (benzene ring); 1 254, 1 027 (C–O). FAB MS, m/z (%): 464 (98, M – Br); 404 (4, M – Br – CH_3COOH); 374 (4, M – Br – C_7H_6); 150 (5, $\text{C}_8\text{H}_8\text{NS}$); 105 (5, $\text{C}_7\text{H}_7\text{N}$); 91 (100, C_7H_7). For $\text{C}_{29}\text{H}_{38}\text{BrNO}_2\text{S}$ (544.6) calculated: 63.96% C, 7.03% H, 14.67% Br, 2.57% N, 5.89% S; found: 63.75% C, 7.01% H, 14.51% Br, 2.48% N, 5.59% S.

3 β -Acetoxy-3'-benzyl-2'-methyl-5 α -androst-16-eno[16,17-*d*]thiazolium Bromide (16)

Following the same procedure described above for 15, the thiazole 9 (100 mg, 0.3 mmol) gave thiazolium bromide 16 (55 mg, 66%), m.p. 213–216 °C (1,2-dichloroethane–ethyl acetate). ^1H NMR: 7.41–7.38 m, 2 H (arom. H); 7.23–7.12 m, 3 H (arom. H); 5.94 m, 2 H (PhCH_2 , $W = 39$); 4.68 m, 1 H (H-3 α , $W = 32$); 3.17 s, 3 H (CH_3 -2'); 2.83 dd, 1 H (H-15 α , $J_{\text{vic}} = 7.1$, $J_{\text{gem}} = 14.8$); 2.69 dd, 1 H (H-15 β , $J_{\text{vic}} = 11.0$, $J_{\text{gem}} = 14.7$); 2.03 s, 3 H (CH_3CO); 1.14 s, 3 H (3 \times H-18); 0.87 s, 3 H (3 \times H-19). IR (CHCl_3): 1 725 (C=O); 1 619, 1 566 (thiazolium); 1 606, 1 498, 1 454, 910, 701 (benzene ring); 1 253, 1 027 (C–O). FAB MS, m/z (%): 478 (100, M – Br); 418 (3, M – Br – CH_3COOH); 388 (3, M – Br – C_7H_6); 164 (3); 105 (3); 91 (61). For $\text{C}_{30}\text{H}_{40}\text{BrNO}_2\text{S}$ (558.6) calculated: 64.5% C, 7.22% H, 14.30% Br, 2.51% N, 5.74% S; found: 64.40% C, 7.25% H, 14.31% Br, 2.33% N, 5.65% S.

3 β -Acetoxy-3'-methyl-2'-(trifluoromethyl)-5 α -androst-16-eno[16,17-*d*]thiazolium Tetrafluoroborate (17)

A solution of thiazole 10 (150 mg, 0.3 mmol) in 1,2-dichloroethane (3 ml) was added at 0 °C under argon to a stirred solution of silver tetrafluoroborate (130 mg, 0.67 mmol) in 1,2-dichloroethane (2 ml). Methyl iodide (0.05 ml, 0.80 mmol) was added, the mixture stirred for 30 min at 0 °C and stirring continued at room temperature for additional 5 h. Molecular sieve A4 (powder) was added in portions until the reaction mixture achieved pH \approx 7. Filtration through celite followed by evaporation of the solvent gave white solid which upon recrystallization from 1,2-dichloroethane–ethyl acetate yielded 129 mg (70%) of the thiazolium tetrafluoroborate 17, m.p. 248–249 °C. ^1H NMR: 4.69 m, 1 H (H-3 α , $W = 32$); 4.24 s, 3 H (CH_3 -3'); 2.93 m, 2 H (2 \times H-15, $W = 46$); 2.37 m, 1 H (H-14, $W = 28$); 2.03 s, 3 H (CH_3CO); 1.21 s, 3 H (3 \times H-18); 0.89 s, 3 H (3 \times H-19). IR (CHCl_3): 1 725 (C=O); 1 653, 1 560 (thiazolium); 1 324, 1 173 (CF_3); 1 255 (C–O); 1 099, 1 085, 1 057, 1 035 (BF_4). FAB MS, m/z (%): 456 (100, M – BF_4); 396 (37, M – BF_4 – CH_3COOH); 246 (7); 232 (18); 220 (19); 181 (11); 110 (42). For $\text{C}_{24}\text{H}_{33}\text{BF}_7\text{NO}_2\text{S}$ (543.4) calculated: 53.05% C, 6.12% H, 2.58% N, 5.90% S; found: 53.53% C, 6.13% H, 2.47% N, 5.83% S.

3 β -Acetoxy-3'-ethyl-2'-(trifluoromethyl)-5 α -androst-16-eno[16,17-*d*]thiazolium Tetrafluoroborate (18)

Following a procedure analogous to that described for 17, the thiazole 10 (180 mg, 0.41 mmol) was quaternized using silver tetrafluoroborate (80 mg, 0.41 mmol) and ethyl bromide (0.33 ml, 4.1 mmol). The reaction mixture was filtered through celite and the solvent evaporated. The oily residue was treated with toluene and the resulting suspension centrifuged. The supernatant contained 118 mg of starting material 10. The sediments were

washed with petroleum ether and dried under reduced pressure yielding thiazolium tetrafluoroborate **18** (62 mg, 27%), m.p. 247–248 °C (1,2-dichloroethane–toluene). ¹H NMR: 4.77–4.48 m, 3 H (H-3 α , CH₃CH₂); 2.99 m, 2 H (2 \times H-15, *W* = 45); 2.40 m, 1 H (H-14, *W* = 29); 2.03 s, 3 H (CH₃CO); 1.59 t, 3 H (CH₃CH₂, *J* = 7.3); 1.21 s, 3 H (3 \times H-18); 0.89 s, 3 H (3 \times H-19). IR (CHCl₃): 1 725 (C=O); 1 629, 1 554 (thiazolium); 1 315, 1 171 (CF₃); 1 254 (C–O); 1 104, 1 057, 1 034 (BF₄). FAB MS, *m/z* (%): 470 (100, M – BF₄); 410 (18, M – BF₄ – CH₃COOH); 260 (4); 246 (6); 234 (9); 195 (3); 124 (5). For C₂₅H₃₅BF₇NO₂S (557.4) calculated: 53.87% C, 6.33% H, 2.51% N, 5.75% S; found: 53.75% C, 6.13% H, 2.29% N, 5.54% S.

Muscarinic Receptor Binding Studies

Tissue preparation. Experiments were performed on homogenates of heart atria taken from male Wistar rats (200–230 g body weight). Tissue was homogenized with Polytron homogenizer in 10-fold volume of ice-cold medium. The homogenization and incubation medium consisted of 100 mM NaCl and 20 mM HEPES, pH 7.4. The homogenates were diluted with the homogenization medium to 2-fold volume and centrifuged at –4 °C for 15 min at 700 g. The pellets were discarded, supernatants were divided into the Eppendorf tubes, and kept frozen at –80 °C until the day of experiment.

Incubations. Incubations were performed at 25 °C in a total volume of 1.6 ml for 3 h. The concentration of [³H]NMS was 200 pm, close to half of the apparent dissociation constant *K*_{RL} for complex [³H]NMS–receptor (*K*_{RL} = 436 pm, under the condition used). The tissue concentration was 2 mg (original wet weight) per tube. Non-specific radioligand binding was defined using atropine (5 μ M). Incubations were terminated by the addition of 3 ml of ice-cold water and subsequent vacuum filtration on Whatman GF/B glass fiber filters (presoaked in water for 3 h), with two washes of filters with 3 ml of water. Radioactivity was determined by scintillation counting.

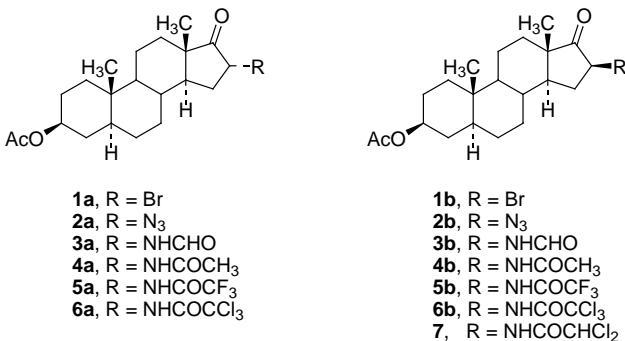
Data analysis. Data treatment was performed as described in refs^{9,10}. Data were analyzed with nonlinear regression analysis using SigmaPlot 4.0 (SPSS, Erkrath, Germany) and are presented as mean \pm standard error of the mean (S.E.M.). Symbols R, L, and A denote the receptor, the orthosteric ligand [³H]NMS, and the allosteric ligand, respectively. *K*_{RL}, *K*_{AR} denote the apparent dissociation constants of the corresponding complexes. The cooperativity coefficient, α , expresses the mutual potency of the allosteric and orthosteric ligands to bind simultaneously to the receptor¹¹. Values of α < 1 suggest positive cooperativity, values of α > 1 suggest negative cooperativity.

RESULTS AND DISCUSSION

Starting material, the bromoketone **1a**, was prepared¹² by direct bromination of 5 α -androsta-5,16-diene-3 β ,17-diyl acetate. In a large-scale preparation, a small amount of the epimeric 16 β -bromo derivative **1b** was isolated as well. Reaction of **1a** with sodium azide in dimethylformamide in the presence of acetic acid according to von Schönecker¹³ gave 16 β -azido-ketone **2b** and a minor, epimerized 16 α -derivative **2a**. Assignment of the configuration on C-16 is based on ¹H NMR data. The H-16 α of compound **2b** appears as a triplet at δ 3.72 (δ 3.67 reported for known 16 β -azido-

17-oxo-5 α -androstan-3 β -ol¹⁴). The H-16 β signal of **2a** appears as a multiplet shifted towards a lower field (δ 4.12).

For the conversion of azidoketone **2b** to acyclic intermediates **3–6**, we developed a convenient one pot reduction–acylation procedure. Tin(II) chloride in methanol has been shown to be a mild reagent for azide reduction¹⁵. Isolation of the amine formed requires alkaline workup. α -Aminoketones are, however, sensitive to basic conditions due to possible oxidative dimerization to pyrazines¹⁶. We found that reduction of **2b** with tin(II) chloride dihydrate in boiling tetrahydrofuran is completed within one hour and the intermediate inorganic complexes of α -aminoketone can be directly acylated. Stable α -acylaminoketones are then easy to separate from



the inorganic material. With an exception of the formamido derivative, the acylation process is accompanied by epimerization in position 16; presumably *via* 17-enolate. Since steroid 16-acylamino-17-oxo derivatives were not known from the literature, we examined the stereochemistry in details even though this was not an important issue for the cyclization to thiazolo derivatives. Thus, reduction of **2b** followed by reaction with mixed anhydride of formic and acetic acids gave 16 β -formamidoketone **3b** as a single product in 90% yield. The ¹H NMR exhibited an N-H signal as a doublet at δ 5.85, H-16 α appeared at δ 4.14, and the C-18 protons resonate at δ 0.91. For comparison, 16 α -formamidoketone **3a** was prepared from azide **2a** in the same way. All the characteristic signals of **3a** exhibited relative downfield shifts: δ 5.98 for N-H, 4.59 for H-16 β , and 1.01 for H-18. The reaction of reduced **2b** with trifluoroacetic anhydride yielded 71% of 16 β -trifluoroacetamidoketone **5b** together with 16% of its epimer **5a**. Analogously to the epimeric formyl derivatives, ¹H NMR signals of **5b** resonated at a relatively lower field when compared with those of **5a** (δ 6.61 vs 6.81

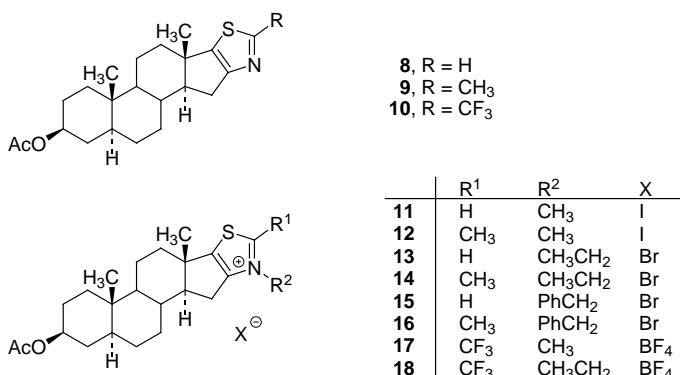
for N-H, 4.08 vs 4.58 for H-16, 0.94 vs 1.03 for H-18). Acylation with acetic anhydride resulted in a chromatographically inseparable mixture of acetamidoketones in total yield of 95%. In the ^1H NMR, particular signals were assigned for both epimers **4a** and **4b**. Integral curves revealed that the ratio of 16β - to 16α -acetamidoketone was approximately 4 : 1. Finally, the reduced **2b** was acylated with trichloroacetyl chloride in the presence of triethylamine. Besides 47% of 16β -trichloroacetamido derivative **6b** and 25% of 16α -epimer **6a**, a significant amount of a third product was chromatographically isolated from the most polar fraction. Its ^1H NMR was similar to that of compound **6b**. In addition, it showed a sharp singlet at δ 5.95. Confirmed by elemental analysis and EI mass spectrum (457, M; 442, M - CH_3), its structure was assigned to 16β -dichloroacetylamino derivative **7**. Tests of tin(II) chloride action on pure **6a** or **6b** at various temperatures in the presence or absence of triethylamine revealed total stability of trichloroacetyl derivatives under these conditions. We judge therefore, the divalent tin species present in the reaction mixture caused dehalogeno-hydrogenation of trichloroacetyl chloride, thus revealing limitations of the procedure which was used.

In the next step, a method developed for synthesis of steroidal thiazoles with the thiazole ring in a side chain has been followed¹⁷. Reaction of amidoketone **3b** with an excess of phosphorus pentasulfide in boiling dioxane afforded fused thiazoloderivative **8**. A singlet at δ 8.58 due to the thiazole 2'-hydrogen and signals of both protons in position 15 of the adjacent steroid skeleton (H- 15β at δ 2.79 as a doublet of doublets, $J_{\text{vic}} = 6.5$, $J_{\text{gem}} = 14.3$ Hz; H- 15α at δ 2.46 as a doublet of doublets, $J_{\text{vic}} = 11.3$, $J_{\text{gem}} = 14.3$ Hz) were clearly distinguishable in its ^1H NMR. Bands corresponding to the thiazole ring vibrations (1 558, 1 498, 1 444, and 1 046 cm^{-1}) were found in the IR. In the EI mass spectrum, besides molecular ion (373), fragments arising by loss of methyl group (358, base peak) and loss of methyl group and a molecule of acetic acid (298) are present.

2'-Methylthiazole **9** and 2'-(trifluoromethyl)thiazole **10** were prepared from acylaminoketones **4a** + **4b** and **5b**, respectively, and characterized in a similar manner. All attempts to synthesize 2'-(trichloromethyl)thiazolo derivative from the trichloroacetamidoketone **6b** failed. Since formation of strongly polar reaction products only was observed even at lower temperatures, phosphorus pentasulfide in the reaction with **6b** seems to be acting rather as a reducing agent causing cleavage of the trichloroacetamido group.

In general, thiazoles with alkyl halides or tosylates undergo $S_{\text{N}}2$ quaternization reaction, the rate of which depends mainly on steric and

electronic influence of substituents in positions 2 and 4. In our case, methyl iodide reacted readily with 2'-unsubstituted thiazolo derivative **8** and 2'-methylthiazole **9**. Complete conversion was achieved within 12 h at 100 °C using 1,2-dichloroethane solution of the substrates with fifty molar equivalents of methyl iodide. Unfortunately, elemental iodine, a product of partial radical decomposition of the reagent, formed stable complexes with the thiazolium iodides. Successive treatment of this dark brown material with sodium thiosulfate and potassium iodide followed by recrystallization gave colorless products **11** and **12** in 70 and 75% yields, respectively.



Ethylthiazolium bromides **13** and **14** were obtained in very good yields as crystalline products directly from a neat mixture of corresponding thiazole and ethyl bromide after 10 h heating. Benzylthiazolium bromides **15** and **16** were prepared in yields of nearly 70% using ten equivalents of benzyl bromide. None of the reagents, even after prolonged reaction time caused observable conversion of thiazole **10** in which the trifluoromethyl group in position 2' causes significant inductive retardation of the reaction rate. Alkyl halides in the presence of silver ions are known as an excellent system for alkylation of poor nucleophiles. Silver tetrafluoroborate was used in preparation of sterically hindered sulfonium salts¹⁸ or for S-alkylation of thiophenes. Hence, testing this method, we found that treatment of a 1,2-dichloroethane solution of thiazole **10** with two equivalents of silver tetrafluoroborate and approximately 2.5 equivalents of methyl iodide led to its quantitative conversion at room temperature within 5 h. It was necessary to use excess of both reagents because of a competitive coupling of the alkyl halide as indicated by increasing acidity of the reaction mixture. The acid together with unreacted silver ions was successfully removed with molecular sieve 4A. Pure methylthiazolium tetrafluoroborate **17** was obtained

in a 70% overall yield. Supposedly due to an additional elimination side reaction of ethyl bromide in the presence of silver ions, the analogously prepared ethylthiazolium tetrafluoroborate **18** was isolated only in moderate yield of 27%. Preferred coupling of benzyl bromide to diphenylethane in the presence of silver tetrafluoroborate was the probable cause of failure of all our attempts to complete the series by synthesis of the corresponding benzylthiazolium derivative. The structures of prepared steroidal thiazolium salts were confirmed by elemental analyses, ¹H NMR, IR, and FAB mass spectra.

All of the compounds **11–18** exhibited sufficient solubility in aqueous media to be convenient for examination in radioligand binding experiments. Their effects on the specific binding of [³H]N-methylscopolamine to M₂ subtype of muscarinic acetylcholine receptors (homogenates of rat atrial membranes) were examined. Test compounds diminished [³H]NMS binding with K_d values in the micromolar or submicromolar region (Table I). Their binding parameters are comparable with those of known negative allosteric modulator, pancuronium. Surprisingly, the presence of bulky benzyl substituent in **15** caused a substantial increase in affinity. These results confirmed our assumption that combination of lipophilic steroid skeleton with conveniently substituted ionium moiety could afford promising structures for studying the allosteric phenomena.

TABLE I

Quantitative parameters of the binding and action of thiazolium salts on M₂ muscarinic receptors (n is number of experiments, $pK_{AR} = -\log K_{AR}$, α is cooperativity factor, S.E.M. is standard error of the mean)

Compound	$pK_{AR} \pm \text{S.E.M.}$ [M] (n)	α
Pancuronium	7.105 ± 0.081 (5)	11.28
11	6.166 ± 0.103 (4)	27.14
12	6.089 ± 0.092 (4)	7.70
13	6.126 ± 0.070 (4)	10.20
14	6.056 ± 0.087 (4)	29.23
15	7.016 ± 0.135 (4)	17.21
16	6.166 ± 0.094 (4)	8.12
17	4.632 ± 0.106 (4)	25.41
18	4.876 ± 0.090 (4)	11.35

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