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Synthesis of quaternary ammonium salts of 16*E*-[4-(2-alkylaminoethoxy)-3-methoxybenzylidene]androstene derivatives as skeletal muscle relaxants

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ABSTRACT

Synthesis of eighteen new quaternary ammonium salts of 16*E*-arylidene androstene derivatives as skeletal muscle relaxants is reported in the present study. The effects of possibly extended interonium distances on muscle relaxant activity are discussed. All the quaternary ammonium steroids produced reduction in the twitch responses, when screened for *in vitro* neuromuscular blocking activity using isolated chick biventer cervicis muscle preparation. However, the variable interonium distance, which is believed to range from 11 to 17 Å in these quaternary compounds and is associated with the built in flexibility of these structures about the single bonds on the moieties linked to ring D of the steroid skeleton, resulted in varied degrees of muscle relaxant activity. Some of the compounds also inhibited acetylcholinesterase activity in low concentrations so that they would not be directly suitable for use as muscle relaxants.

1. Introduction

Neuromuscular junction (NMJ) blockers are clinically used as adjuncts to surgical anaesthesia to relax skeletal muscles to avoid any complications arising due to muscle contractions of the patients during surgery. Quaternary ammonium steroids represent an important class of skeletal muscle relaxants. The rigid steroid skeleton with onium centers at different positions has been an excellent target for producing highly active and selective neuromuscular blocking drugs and provides an excellent method of studying structure activity relationships within the series of these agents [1–4]. There is a general assumption that neuromuscular blocking activity is related to the optimal interonium distance of about 10–14 Å [5]. However, pipecuronium bromide (**1**) (Fig. 1) has proved to be an extremely useful neuromuscular blocking agent of medium to long duration of action without cardiovascular side effects, even though the interonium distance is much longer (16.07 Å) [6,7] as compared to pancuronium bromide (11.00 Å) (**2**) (Fig. 1) [8] with moderate cardiovascular side effects. The lack of an intact acetylcholine-like fragment in pipecuronium bromide has not compromised its potency but rather increased it, presumably due to increased interonium distance. The search for a more potent, side effect-free neuromuscular junction blocker with shorter duration of action continues. Several research groups [5,9] are involved in search for a therapeutically useful NMJ blocker and a number of reports in this regard have also been originated from our laboratory [10–12].

Recently, we have reported the synthesis of a new series of 16*E*-arylidene androstene derivatives (**3**, **4**) (Fig. 1) with significant cytotoxic activity [13]. The presence of accessible well placed bis-tertiary amino functionalities in the steroid skeleton prompted us to prepare their quaternary ammonium salts with longer interonium distance and study them as muscle relaxants.

In continuation with earlier research work on steroidal NMJ blockers reported from our laboratory, herein we present the synthesis and skeletal muscle relaxant activity of quaternary ammonium steroids with extended interonium distance in the 16-arylidene series.

2. Experimental

2.1. General

Melting points were determined on a Veego melting point apparatus and are uncorrected. UV (wavelengths in nm) were recorded on Lambda 15 and IR (wave numbers in cm^{-1}) spectra on Perkin-Elmer spectrum RX 1, FT-IR spectrophotometer models using KBr pellets. ^1H NMR spectra were recorded on Bruker

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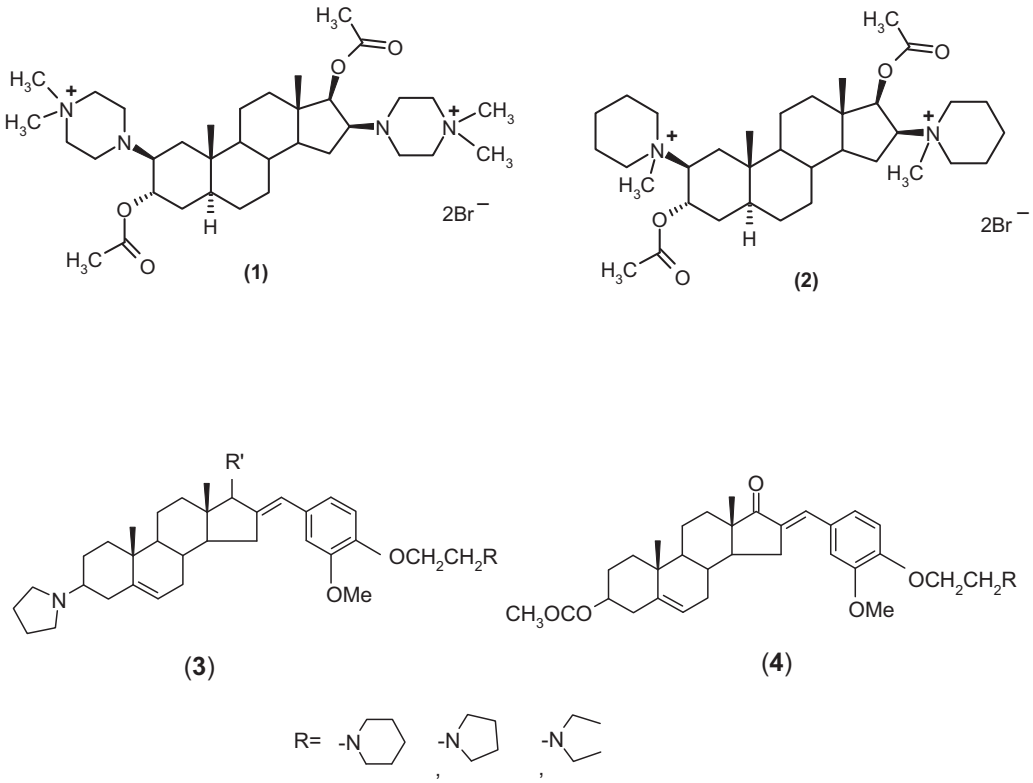


Fig. 1. Structures of pipecuronium bromide (**1**), pancuronium bromide (**2**) and 16*E*-arylidene androstene derivatives (**3**, **4**).

AC-300F, 300MHz using deuterated-chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) containing tetramethylsilane as internal standard (chemical shifts in δ, ppm). Elemental analyses were carried out on a Perkin-Elmer-2400 model CHN analyzer. Plates for TLC were prepared according to Stahl (E. Merck) using EtOAc as solvent (activated at 110 °C for 30 min) and were visualized by exposure to iodine vapors. Anhydrous sodium sulphate was used as a drying agent. All solvents were distilled prior to use according to standard procedures.

*2.2. General procedure for the synthesis of quaternary ammonio salts of 16-arylidene steroids **5–7a–d** and **8–10a,b***

Methyl iodide/allyl bromide (1 mL) was added to a solution of bistertiary/monotertiary steroids (**3**, **4**) [13] (0.4 mmol) in dry dichloromethane (50 mL). The reaction mixture was kept aside at room temperature for days as mentioned in Table 1. The crystalline material obtained was filtered, washed with dry dichloromethane, dried and recrystallized from appropriate solvent (Table 1) to afford their corresponding quaternary ammonium salts.

*2.2.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-ol dimethiodide (**5a**)*

Yield: 53.98%; m.p. 278–280 °C. UV_{max} (MeOH): 261.8 nm ($\log \varepsilon$ 4.26); IR: 3410, 2936, 1626, 1512, 1464, 1264, 1231, 1140, 1076, 1026, 938, 820 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.72 (s, 3H, 18-CH_3), 1.08 (s, 3H, 19-CH_3), 2.60 (s) and 3.02 (s) (1.2:1 area ratio, 3H, –$\overset{\oplus}{N}$–CH_3, pyrrolidine), 3.40 (s, 3H, –$\overset{\oplus}{N}$–CH_3, piperidine), 3.66–3.72 (m, 8H, 2x–$\overset{\oplus}{N}$–$(CH_2)_2$–, piperidine and pyrrolidine), 3.84 (s, 3H, –OCH_3), 4.08 (m, 3H, 17α-*H* and –$CH_2\overset{\oplus}{N}$⟨), 4.46 (t, 2H, *J* = 4.32 Hz, –OCH_2–), 5.57 (s, 1H, 6-C*H*), 6.47 (s, 1H, vinylic-*H*, 16-arylidene), 6.92–6.95 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{40}H_{62}N_2O_3I_2$: C, 55.04; H, 7.16; N, 3.21. Found: C, 55.29; H, 7.56; N, 3.58%.

*2.2.2. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-yl acetate dimethiodide (**5b**)*

Yield: 48.28%; m.p. 268–270 °C. UV_{max} (MeOH): 261.8 nm ($\log \varepsilon$ 4.31); IR: 2933, 1722, 1620, 1511, 1456, 1370, 1243, 1130, 1038, 946 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.77 (s, 3H, 18-CH_3), 1.07 (s, 3H, 19-CH_3), 2.19 (s, 3H, –$OCOCH_3$), 2.98 (s, 3H, –$\overset{\oplus}{N}$–CH_3, pyrrolidine), 3.33 (s, 3H, –$\overset{\oplus}{N}$–CH_3, piperidine), 3.55–3.77 (m, 8H, 2x–$\overset{\oplus}{N}$–$(CH_2)_2$–, piperidine and pyrrolidine), 3.84 (s, 3H,

Table 1
Quaternization time and crystallizing solvent used for various quaternary ammonium steroids.

Compound no.	Days for quaternization	Crystallizing solvent
5a	4	B
5b	3	B
5c	3	A
5d	3	A
6a	7	B
6b	7	B
6c	7	A + B
6d	7	A
7a	1	B
7b	3	B
7c	3	A + B
7d	3	B + D
8a	6	C
8b	6	C
9a	7	C
9b	7	C
10a	3	C + D
10b	7	C + D

A = dry dichloromethane, B = absolute alcohol, C = dry diethyl ether, D = dry acetone.

–OCH_3), 4.01 (t, 2H, J = 4.35 Hz, –CH_2N⟨), 4.44 (t, 2H, J = 4.37 Hz, –OCH_2–), 5.28 (s, 1H, 17α-*H*), 5.55 (d, 1H, J = 4.23 Hz, 6-C*H*), 6.10 (s, 1H, vinylic-*H*, 16-arylidene), 6.89 (m, 2H, 2-C*H* and 5-C*H*, aromatic), 6.98 (d, 1H, J_o = 8.26 Hz, 6-C*H*, aromatic); Anal. Calc. for $C_{42}H_{64}N_2O_4I_2$: C, 55.14; H, 7.05; N, 3.06. Found: C, 55.29; H, 7.16; N, 3.36%.

2.2.3. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-ol diallyl bromide (**5c**)

Yield: 56.7%; m.p. 263–266 °C. UV_{max} (MeOH): 262.0 nm (log ε 4.43); IR: 3408, 2942, 1626, 1511, 1463, 1266, 1228, 1140, 1019 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.71 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3), 3.67–3.77 (m, 8H, 2x–N$^{\oplus}$–(CH_2)$_2$–, piperidine and pyrrolidine), 3.83 (s, 3H, –OCH_3), 4.03 (brs, 3H, 17α-*H* and –CH_2N$^{\oplus}$⟨), 4.18 (d, 2H, J = 7.01 Hz, –N$^{\oplus}$–CH_2–CH=CH_2, pyrrolidine), 4.42 (d, 2H, J = 7.22 Hz, –N$^{\oplus}$–CH_2–CH=CH_2, piperidine), 4.47 (t, 2H, J = 4.8 Hz, –OCH_2–), 5.54 (d, 1H, J = 4.15 Hz, 6-C*H*), 5.72–5.90 (m, 4H, 2x–N$^{\oplus}$–CH_2–CH=CH_2), 6.04–6.13 (m, 2H, 2x–N$^{\oplus}$–CH_2–CH=CH_2), 6.46 (s, 1H, vinylic-*H*, 16-arylidene), 6.93 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{44}H_{66}N_2O_3Br_2$: C, 63.60; H, 8.01; N, 3.37. Found: C, 63.90; H, 8.09; N, 3.38%.

2.2.4. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-yl acetate diallyl bromide (**5d**)

Yield: 75.17%; m.p. 271–273 °C. UV_{max} (MeOH): 262.2 nm (log ε 4.47); IR: 2937, 1731, 1626, 1514, 1461, 1237, 1142, 1036, 958 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.75 (s, 3H, 18-CH_3), 1.04 (s, 3H, 19-CH_3), 2.19 (s, 3H, –OCOCH_3), 3.73–3.81 (m, 8H, 2x–N$^{\oplus}$–(CH_2)$_2$–, piperidine and pyrrolidine), 3.83 (s, 3H, –OCH_3), 4.07 (s, 2H, –CH_2N$^{\oplus}$⟨), 4.22 (d, 2H, J = 6.57 Hz, –N$^{\oplus}$–CH_2–CH=CH_2, pyrrolidine), 4.47 (m, 4H, –OCH_2 and –N$^{\oplus}$–CH_2–CH=CH_2, piperidine), 4.49 (t, 2H,), 5.26 (s, 1H, 17α-*H*), 5.54 (d, 1H, J = 3.3 Hz, 6-C*H*), 5.63 (d, 1H, J_{cis} = 9.91 Hz, –N$^{\oplus}$–CH$_2$–CH=CH(*H*)), 5.72 (d, 1H, J_{cis} = 10.55 Hz, –N$^{\oplus}$–CH$_2$–CH=CH(*H*)), 5.79–5.91 (m, 2H, 2x–N$^{\oplus}$–CH$_2$–CH=CH(*H*)), 6.03–6.18 (m, 3H, 2x–N$^{\oplus}$–CH$_2$–CH=CH_2 and s (merged), vinylic-*H*, 16-arylidene), 6.82 (s, 1H, 2-C*H*, aromatic), 6.89 (d, 1H, J_o = 8.50 Hz, 5-C*H*, aromatic), 7.00 (d, 1H, J_o = 8.39 Hz, 6-C*H*, aromatic); Anal. Calc. for $C_{46}H_{68}N_2O_4Br_2$: C, 63.29; H, 7.85; N, 3.21. Found: C, 63.52; H, 7.41; N, 3.57%.

2.2.5. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-ol dimethiodide (**6a**)

Yield: 40.16%; m.p. 230 °C (decomp). UV_{max} (MeOH): 262.0 nm (log ε 4.28). IR: 3397, 2931, 1599, 1512, 1463, 1316, 1264, 1229, 1139, 1075, 1025, 939 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.78 (s, 3H, 18-CH_3), 1.08 (s, 3H, 19-CH_3), 2.61 (s) and 3.24 (s) (1:1 area ratio, 3H, 3–N$^{\oplus}$–CH_3), 2.97 (s, 3H, –N$^{\oplus}$–CH_3), 3.73–3.83 (m, 8H, 2x–N$^{\oplus}$–(CH_2)$_2$–, pyrrolidine), 3.84 (s, 3H, –OCH_3), 3.92 (t, 2H, J = 4.44 Hz, –CH_2N$^{\oplus}$⟨), 3.97 (s, 1H, 17α-*H*), 4.43 (t, 2H, J = 4.26 Hz, –OCH_2–), 5.54 (d, 1H, J = 4.08 Hz, 6-C*H*), 6.41 (s, 1H, vinylic-*H*, 16-arylidene), 6.91–6.99 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{39}H_{60}N_2O_3I_2$: C, 54.55; H, 7.04; N, 3.26. Found: C, 54.39; H, 7.22; N, 3.49%.

2.2.6. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-yl acetate dimethiodide (**6b**)

Yield: 51.36%; m.p. 197–200 °C. UV_{max} (MeOH): 262.6 nm (log ε 4.37); IR: 2938, 1727, 1601, 1513, 1461, 1371, 1237, 1141, 1038, 927 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.80 (s, 3H, 18-CH_3), 1.08 (s, 3H, 19-CH_3), 2.21 (s, 3H, –OCOCH_3), 2.96 (s, 3H, 3–N$^{\oplus}$–CH_3), 3.29 (s, 3H, –N$^{\oplus}$–CH_3,), 3.68 (m, 2H, 3–N$^{\oplus}$–CH_2–, pyrrolidine), 3.73 (m, 2H, 3–N$^{\oplus}$–CH_2–, pyrrolidine), 3.79 (m, 4H, –N$^{\oplus}$–(CH_2)$_2$–, pyrrolidine), 3.85 (s, 3H, –OCH_3), 3.98 (t, 2H, J = 4.44 Hz, –CH_2N$^{\oplus}$⟨), 4.44 (t, 2H, J = 4.8 Hz, –OCH_2–), 5.33 (s, 1H, 17α-*H*), 5.54 (d, 1H, J = 5.1 Hz, 6-C*H*), 6.14 (s, 1H, vinylic-*H*, 16-arylidene), 6.91–6.98 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{41}H_{62}N_2O_4I_2$: C, 54.67; H, 6.94; N, 3.11. Found: C, 54.72; H, 6.88; N, 3.49%.

2.2.7. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-ol diallyl bromide (**6c**)

Yield: 42.22%; m.p. 180–182 °C. UV_{max} (MeOH): 262.2 nm (log ε 4.37); IR: 3405, 2936, 1628, 1513, 1462, 1264, 1142, 1028 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.71 (s, 3H, 18-CH_3), 1.07 (s, 3H, 19-CH_3), 3.70 (brs, 4H, 3–N$^{\oplus}$–(CH_2)$_2$–, pyrrolidine), 3.78 (m, 4H, –N$^{\oplus}$–(CH_2)$_2$–, pyrrolidine), 3.84 (s, 3H, –OCH_3), 3.94 (t, 2H, J = 4.24 Hz, –CH_2N$^{\oplus}$⟨), 4.01 (d, 1H, J = 5.3 Hz, 17α-*H*), 4.11 (d, 2H, J = 6.46 Hz, 3–N$^{\oplus}$–CH_2–CH=CH_2), 4.24 (d, 2H, J = 7.1 Hz, –N$^{\oplus}$–CH_2–CH=CH_2), 4.45 (t, 2H, J = 4.17 Hz, –OCH_2–), 5.54 (d, 1H, J = 4.39 Hz, 6-C*H*), 5.68–5.85 (m, 4H, 2x–N$^{\oplus}$–CH$_2$–CH=CH_2), 6.00–6.17 (m, 2H, 2x–N$^{\oplus}$–CH$_2$–CH=CH_2), 6.45 (s, 1H, vinylic-*H*, 16-arylidene), 6.93 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{43}H_{64}N_2O_3Br_2$: C, 63.23; H, 7.90; N, 3.43. Found: C, 63.32; H, 7.72; N, 3.61%.

2.2.8. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}benzylidene]-3β-pyrrolidino-5-androsten-17β-yl acetate diallyl bromide (**6d**)

Yield: 43.16%; m.p. 155–158 °C. UV_{max} (MeOH): 262.2 nm (log ε 4.30); IR: 2967, 1731, 1626, 1514, 1461, 1371, 1237, 1142, 1041 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.80 (s, 3H, 18-CH_3), 1.05 (s, 3H, 19-CH_3), 2.21 (s, 3H, –OCOCH_3), 3.84 (brs, 9H, –OCH_3; 3–N$^{\oplus}$–(CH_2)$_2$–, pyrrolidine and –N$^{\oplus}$–CH_2–, pyrrolidine), 4.13 (m, 4H, –CH_2N$^{\oplus}$⟨ and –N$^{\oplus}$–CH_2–, pyrrolidine), 4.24 (d, 2H, J = 7.01 Hz, 3–N$^{\oplus}$–CH_2–CH=CH_2), 4.30 (d, 2H, J = 6.7 Hz, –N$^{\oplus}$–CH_2–CH=CH_2), 4.47 (t, 2H, J = 4.31 Hz, –OCH_2–), 5.35 (s, 1H, 17α-*H*), 5.55 (d, 1H, J = 4.71 Hz, 6-C*H*), 5.68–5.86 (m, 4H, 2x–N$^{\oplus}$–CH$_2$–CH=CH_2), 5.99–6.20 (m, 3H, 2x–N$^{\oplus}$–CH$_2$–CH=CH_2 and vinylic-*H*, 16-arylidene), 6.85 (s, 1H, 2-C*H*, aromatic), 6.94 (s, 2H, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{45}H_{66}N_2O_4Br_2$: C, 62.93; H, 7.75; N, 3.26. Found: C, 62.89; H, 7.55; N, 3.33%.

2.2.9. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-3β-pyrrolidino-5-androsten-17β-ol dimethiodide (**7a**)

Yield: 50.93%; m.p. 266–270 °C. UV_{max} (MeOH): 265.8 nm (log ε 4.46); IR: 3399, 2931, 2365, 1602, 1513, 1464, 1266, 1229, 1142, 1027, 805 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.72 (s, 3H, 18-CH_3), 1.07 (s, 3H, 19-CH_3), 1.46 (t, 6H, J = 7.14 Hz, –N$^{\oplus}$(CH$_2$CH$_3$)$_2$), 3.03 (s, 3H, –N$^{\oplus}$–CH_3, pyrrolidine), 3.31 (s, 3H, H_3C–N$^{\oplus}$(CH$_2$CH$_3$)$_2$),

3.68 (m, 8H, –$\overset{\oplus}{N}(CH_2CH_3)_2$ and –$\overset{\oplus}{N}$–$(CH_2)_2$, pyrrolidine), 3.84 (s, 3H, –OCH_3), 3.97 (t, 2H, J = 4.29 Hz, –$CH_2\overset{\oplus}{N}$–), 4.04 (s, 1H, 17α-*H*), 4.44 (t, 2H, J = 4.31 Hz, –OCH_2–), 5.55 (d, 1H, J = 4.25 Hz, 6-C*H*), 6.47 (s, 1H, vinylic-*H*, 16-arylidene), 6.92–6.95 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{39}H_{62}N_2O_3I_2$: C, 54.42; H, 7.26; N, 3.26. Found: C, 54.44; H, 7.66; N, 3.22%.

2.2.10. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-3β-pyrrolidino-5-androsten-17β-yl acetate dimethiodide (**7b**)

Yield: 49.35%; m.p. 258–260 °C. UV_{max} (MeOH): 262.0 nm (log ε 4.50); IR: 2937, 1728, 1625, 1513, 1462, 1372, 1241, 1142, 1039 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.78 (s, 3H, 18-CH_3), 1.05 (s, 3H, 19-CH_3), 1.37 (t, 6H, J = 7.21 Hz, –$\overset{\oplus}{N}(CH_2CH_3)_2$), 2.21 (s, 3H, –OCOC$H_3$), 3.06 (s, 3H, –$\overset{\oplus}{N}$–C$H_3$, pyrrolidine), 3.35 (s, 3H, H_3C–$\overset{\oplus}{N}(CH_2CH_3)_2$), 3.75 (q, 8H, –$\overset{\oplus}{N}(CH_2CH_3)_2$ and –$\overset{\oplus}{N}$–$(CH_2)_2$–, pyrrolidine), 3.84 (s, 3H, –OCH_3), 4.05 (t, 2H, J = 4.36 Hz, –$CH_2\overset{\oplus}{N}$⟨), 4.47 (t, 2H, J = 4.2 Hz, –OCH_2–), 5.32 (s, 1H, 17α-*H*), 5.56 (d, 1H, J = 4.21 Hz, 6-C*H*), 6.15 (s, 1H, vinylic-*H*, 16-arylidene), 6.86 (s, 1H, 2-C*H*, aromatic), 6.93–6.99 (m, 2H, 5-C*H* and 6-C*H*, aromatic); Anal. calcd. for $C_{41}H_{64}N_2O_4I_2$: C, 54.55; H, 7.15; N, 3.10. Found: C, 54.70; H, 7.29; N, 3.14%.

2.2.11. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-3β-pyrrolidino-5-androsten-17β-ol diallyl bromide (**7c**)

Yield: 56.34%; m.p. 250–253 °C. UV_{max} (MeOH): 262.4 nm (log ε 4.55); IR: 3362, 2936, 1629, 1514, 1463, 1260, 1141, 1025, 800 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.71 (s, 3H, 18-CH_3), 1.07 (s, 3H, 19-CH_3), 1.45 (t, 6H, J = 7 Hz, –$\overset{\oplus}{N}(CH_2CH_3)_2$), 3.55 (q, 4H, J = 7.24 Hz, –$\overset{\oplus}{N}(CH_2CH_3)_2$), 3.71 (brs, 4H, –$\overset{\oplus}{N}$–$(CH_2)_2$–, pyrrolidine), 3.83 (brs, 5H, –OCH_3 and –$CH_2\overset{\oplus}{N}$⟨), 4.04 (s, 1H, 17α-*H*), 4.12 (d, 2H, J = 6.19 Hz, –$\overset{\oplus}{N}$–CH_2–CH=CH_2, pyrrolidine), 4.18 (d, 2H, J = 6.28 Hz, H_2C–HC=H_2C–$\overset{\oplus}{N}(CH_2CH_3)_2$, 4.44 (sbr, 2H, –$OCH_2$–), 5.55 (s, 1H, 6-C*H*), 5.68–5.86 (m, 4H, 2x–$\overset{\oplus}{N}$–CH_2–CH=CH_2), 6.01–6.09 (m, 2H, 2x–$\overset{\oplus}{N}$–CH_2–CH=CH_2), 6.46 (s, 1H, vinylic-*H*, 16-arylidene), 6.92–6.95 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{43}H_{66}N_2O_3Br_2$: C, 63.07; H, 8.13; N, 3.42. Found: C, 63.22; H, 8.50; N, 3.49%.

2.2.12. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-5-androsten-17β-yl acetate diallyl bromide (**7d**)

Yield: 77.65%; m.p. 200–203 °C. UV_{max} (MeOH): 262.0 nm (log ε 4.38); IR: 2968, 1731, 1633, 1515, 1463, 1371, 1237, 1143, 1041, 957, 801 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.80 (s, 3H, 18-CH_3), 1.07 (s, 3H, 19-CH_3), 1.42 (t, 6H, J = 7.06 Hz, –$\overset{\oplus}{N}(CH_2CH_3)_2$), 2.21 (s, 3H, –OCOC$H_3$), 3.53 (q, 4H, J = 7.13 Hz, –$\overset{\oplus}{N}(CH_2CH_3)_2$), 3.82 (t, 2H, J = 8.92 Hz, –$CH_2\overset{\oplus}{N}$⟨), 3.84 (s, 3H, –OCH_3), 3.67 (brs, 4H, –$\overset{\oplus}{N}$–$(CH_2)_2$–, pyrrolidine), 4.09 (d, 2H, J = 7.46 Hz, –$\overset{\oplus}{N}$–CH_2–CH=CH_2, pyrrolidine), 4.16 (d, 2H, J = 7.17 Hz, H_2C–HC=H_2C–$\overset{\oplus}{N}(CH_2CH_3)_2$, 4.44 (t, 2H, J = 4.06 Hz, –OCH_2–), 5.33 (s, 1H, 17α-*H*), 5.54 (s, 1H, 6-C*H*), 5.67–5.86 (m, 4H, 2x–$\overset{\oplus}{N}$–CH_2–CH=CH_2), 6.03–6.09 (m, 2H, 2x–$\overset{\oplus}{N}$–CH_2–CH=CH_2), 6.14 (s, 1H, vinylic-*H*, 16-arylidene), 6.88–6.94 (m, 3H, 2-C*H*, 5-C*H* and 6-C*H*, aromatic); Anal. Calc. for $C_{45}H_{68}N_2O_4Br_2$: C, 62.78; H, 7.96; N, 3.25. Found: C, 62.49; H, 8.11; N, 3.38%.

2.2.13. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}benzylidene]-17-oxo-5-androsten-3β-yl acetate methiodide (**8a**)

Yield: 92.94%; m.p. 250–253 °C. UV_{max} (MeOH): 332.8 nm (log ε 4.19); IR: 2938, 1724, 1621, 1596, 1511, 1462, 1371, 1247, 1140, 1098, 1029 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (s, 3H, 18-CH_3), 0.98 (s, 3H, 19-CH_3), 2.04 (s, 3H, –OCOCH_3), 3.54 (s, 3H, –$\overset{\oplus}{N}$–CH_3), 3.76–3.83 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, piperidine), 3.93 (s, 3H, –OCH_3), 3.94–4.01 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, piperidine), 4.31 (t, 2H, J = 4.25 Hz, –$CH_2\overset{\oplus}{N}$⟨), 4.56 (t, 2H, J = 4.26 Hz, –OCH_2–), 4.62 (m, 1H, 3α-*H*), 5.43 (d, 1H, J = 4.62 Hz, 6-C*H*), 7.02–7.04 (m, 2H, 2-C*H* and 5-C*H*, aromatic), 7.16 (dd, 1H, J_m = 1.29 Hz, J_o = 8.56 Hz, 6-C*H*, aromatic), 7.35 (s, 1H, vinylic-*H*, 16-arylidene); Anal. Calc. for $C_{37}H_{52}NO_5I$: C, 61.92; H, 7.30; N, 1.95. Found: C, 62.02; H, 7.38; N, 2.01%.

2.2.14. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}benzylidene]-17-oxo-5-androsten-3β-yl acetate methiodide (**9a**)

Yield: 67.85%; m.p. 157–159 °C. UV_{max} (MeOH): 327.2 nm (log ε 4.41); IR: 2940, 1720, 1624, 1513, 1462, 1371, 1253, 1141, 1098, 1030 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 2.04 (s, 3H, –OCOCH_3), 3.46 (s, 3H, –$\overset{\oplus}{N}$–CH_3), 3.86 (s, 3H, –OCH_3), 3.95 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, pyrrolidine), 4.09 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, pyrrolidine), 4.33 (s, 2H, –$CH_2\overset{\oplus}{N}$⟨), 4.54 (s, 2H, –OCH_2–), 4.58 (s, 1H, 3α-*H*), 5.43 (d, 1H, J = 4.52 Hz, 6-C*H*), 7.02 (d, 2H, J_o = 8.57 Hz, 2-C*H* and 5-C*H*, aromatic), 7.17 (dd, 1H, J_m = 1.50 Hz, J_o = 8.42 Hz, 6-C*H*, aromatic), 7.36 (s, 1H, vinylic-*H*, 16-arylidene). Anal. Calc. for $C_{36}H_{50}NO_5I$: C, 61.44; H, 7.16; N, 1.99. Found: C, 61.31; H, 7.28; N, 2.14%.

2.2.15. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-17-oxo-5-androsten-3β-yl acetate methiodide (**10a**)

Yield: 86.28%; m.p. 178–181 °C. UV_{max} (MeOH): 327.2 nm (log ε 4.19); IR: 2942, 1724, 1619, 1512, 1466, 1239, 1145, 1098, 1029, 804 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.98 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 1.49 (t, 6H, J = 7.22 Hz, –$\overset{\oplus}{N}$–$(CH_2CH_3)_2$), 2.04 (s, 3H, –OCOCH_3), 3.41 (s, 3H, –$\overset{\oplus}{N}$–CH_3), 3.78 (m, 4H, –$\overset{\oplus}{N}$–$(CH_2CH_3)_2$) 3.86 (s, 3H, –OCH_3), 4.19 (t, 2H, J = 4.4 Hz, –$CH_2\overset{\oplus}{N}$⟨), 4.55 (t, 2H, J = 4.35 Hz, –OCH_2–), 4.62 (m, 1H, 3α-*H*), 5.43 (d, 1H, J = 4.67 Hz, 6-C*H*), 7.03 (d, 2H, J_o = 8.42 Hz, 2-C*H* and 5-C*H*, aromatic), 7.17 (dd, 1H, J_m = 1.22 Hz, J_o = 8.54 Hz, 6-C*H*, aromatic), 7.36 (s, 1H, vinylic-*H*, 16-arylidene); Anal. Calc. for $C_{36}H_{52}NO_5I$: C, 61.27; H, 7.43; N, 1.98. Found: C, 62.22; H, 7.03; N, 1.77%.

2.2.16. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}benzylidene]-17-oxo-5-androsten-3β-yl acetate allyl bromide (**8b**)

Yield: 92.55%; m.p. 216–220 °C. UV_{max} (MeOH): 312.0 nm (log ε 4.26); IR: 2937, 1716, 1623, 1513, 1461, 1370, 1327, 1256, 1143, 1098, 1026 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 2.04 (s, 3H, –OCOCH_3), 3.70 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, piperidine), 3.84 (s, 3H, –OCH_3), 3.97 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, piperidine), 4.20 (t, 2H, J = 3.6 Hz, –$CH_2\overset{\oplus}{N}$ <), 4.46 (d, 2H, J = 7.18 Hz, –$\overset{\oplus}{N}$–CH_2–CH=CH_2), 4.58 (t, 2H, J = 3.93 Hz, –OCH_2–), 4.61 (m, 1H, 3α-*H*), 5.42 (d, 1H, J = 4.36 Hz, 6-C*H*), 5.77 (d, 1H, J_{cis} = 9.91 Hz, –$\overset{\oplus}{N}$–CH_2–CH=CH(*H*)), 5.91 (d, 1H, J_{trans} = 16.65 Hz, –$\overset{\oplus}{N}$–CH_2–CH=CH(*H*)), 6.09–6.17 (m, 1H, –$\overset{\oplus}{N}$–CH_2–CH=CH_2), 7.03 (s, 1H, 2-C*H*, aromatic), 7.06 (d, 1H, J_o = 8.47 Hz, 5-C*H*, aromatic),

7.17 (dd, 1H, J_m = 1.09 Hz, J_o = 8.56 Hz, 6-C*H*, aromatic), 7.35 (s, 1H, vinylic-*H*, 16-arylidene); Anal. Calc. for $C_{39}H_{54}NO_5Br$: C, 67.23; H, 7.81; N, 2.01. Found: C, 67.01; H, 7.88; N, 2.39%.

*2.2.17. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}benzylidene]-17-oxo-5-androsten-3β-yl acetate allyl bromide (**9b**)*

Yield: 61.71%; m.p. 83–85 °C. UV_{max} (MeOH): 312.4 nm (log ε 4.35); IR: 2936, 1712, 1624, 1596, 1514, 1462, 1328, 1261, 1143, 1060, 1027, 945, 875 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 2.00 (s, 3H, –OCOCH_3), 3.85 (s, 3H, –OCH_3), 3.80 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, pyrrolidine), 4.20 (m, 2H, –$\overset{\oplus}{N}$–CH_2–, pyrrolidine), 4.24–4.30 (m, 4H, –C$H_2\overset{\oplus}{N}$ < and –$\overset{\oplus}{N}$–CH_2–CH=CH_2), 4.56–4.62 (m, 3H, –OCH_2– and 3α-*H*), 5.43 (d, 1H, *J* = 4.47 Hz, 6-C*H*), 5.76 (d, 1H, J_{cis} = 9.96 Hz, –$\overset{\oplus}{N}$–CH$_2$–CH=CH(*H*)), 5.85 (d, 1H, J_{trans} = 16.85 Hz, –$\overset{\oplus}{N}$–CH$_2$–CH=CH(*H*)), 6.16 (m, 1H, –$\overset{\oplus}{N}$–CH_2–CH=CH_2), 7.02 (m, 2H, 2-C*H* and 5-C*H*, aromatic), 7.17 (dd, 1H, J_m = 1.10 Hz, J_o = 8.18 Hz, 6-C*H*, aromatic), 7.36 (s, 1H, vinylic-*H*, 16-arylidene); Anal. Calc. for $C_{38}H_{52}NO_5Br$: C, 66.85; H, 7.68; N, 2.05. Found: C, 66.98; H, 7.52; N, 2.22%.

*2.2.18. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-17-oxo-5-androsten-3β-yl acetate allyl bromide (**10b**)*

Yield: 98.8%; m.p. 175–177 °C. UV_{max} (MeOH): 327.4 nm (log ε 4.21); IR: 2936, 1712, 1624, 1514, 1463, 1371, 1329, 1260, 1143, 1098, 1059, 1028, 940, 813 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 0.98 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 1.52 (t, 6H, *J* = 7.18 Hz, –$\overset{\oplus}{N}$(CH$_2$CH_3)$_2$), 2.04 (s, 3H, –OCOCH_3), 3.63 (m, 4H, –$\overset{\oplus}{N}$(CH_2CH$_3$)$_2$), 3.85 (s, 3H, –OCH_3), 4.20 (t, 2H, *J* = 4.57 Hz, –C$H_2\overset{\oplus}{N}$⟨), 4.28 (d, 4H, *J* = 7.11 Hz, –$\overset{\oplus}{N}$–CH_2–CH=CH_2), 4.60 (m, 3H, –OCH_2– and 3α-*H*), 5.43 (d, 1H, *J* = 4.36 Hz, 6-C*H*), 5.78 (d, 1H, J_{cis} = 10.38 Hz, –$\overset{\oplus}{N}$–CH$_2$–CH=CH(*H*)), 5.87 (d, 1H, J_{trans} = 16.53 Hz, –$\overset{\oplus}{N}$–CH$_2$–CH=CH(*H*)), 6.07 (m, 1H, –$\overset{\oplus}{N}$–CH_2–CH=CH_2), 7.01 (d, 1H, J_m = 1.20 Hz, 2-C*H*, aromatic), 7.09 (d, 1H, J_o = 8.48 Hz, 5-C*H*, aromatic), 7.19 (d, 1H, J_o = 8.55 Hz, 6-C*H*, aromatic), 7.36 (s, 1H, vinylic-*H*, 16-arylidene); Anal. Calc. for $C_{38}H_{54}NO_5Br$: C, 66.65; H, 7.95; N, 2.06. Found: C, 66.66; H, 8.02; N, 2.13%.

2.3. Neuromuscular blocking activity

The chick biventer cervicis nerve–muscle preparations were set up as described previously [14]. Muscles were removed from 4 to 10 day-old chicks killed by exposure to CO_2. Preparations were mounted in pairs with a resting tension of approximately 1.0 g in 10 mL tissue baths containing Krebs-Henseleit solution. The solution was maintained at 34 °C and bubbled with 95% O_2 and 5% CO_2. The muscles were stimulated via their motor nerves at 0.1 Hz with pulses of 0.2 ms duration and a voltage greater than that required for maximal contractions. Responses to acetylcholine, carbachol and KCl were obtained in the absence of nerve stimulation.

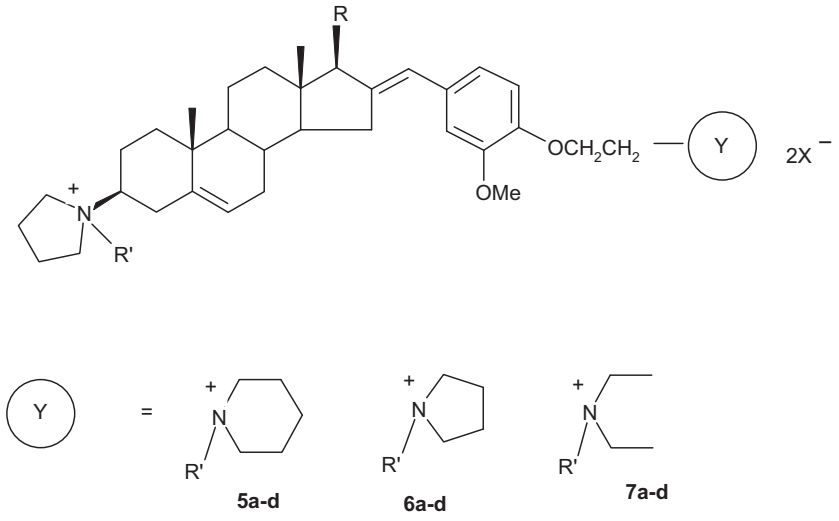
2.4. Anticholinesterase activity

The effect of compounds on the activity of acetylcholinesterase from electric eel was monitored at room temperature by the method of Ellman et al. [15] adapted to 96-well plates. Each assay was repeated three times.

3. Results and discussion

3.1. Chemistry

The bisquaternary ammonium salts **5**–**7a**–**d** (Scheme 1) were prepared from their respective bistertiary amines (**3**) [13] by treatment with alkyl halides such as methyl iodide or allyl bromide in



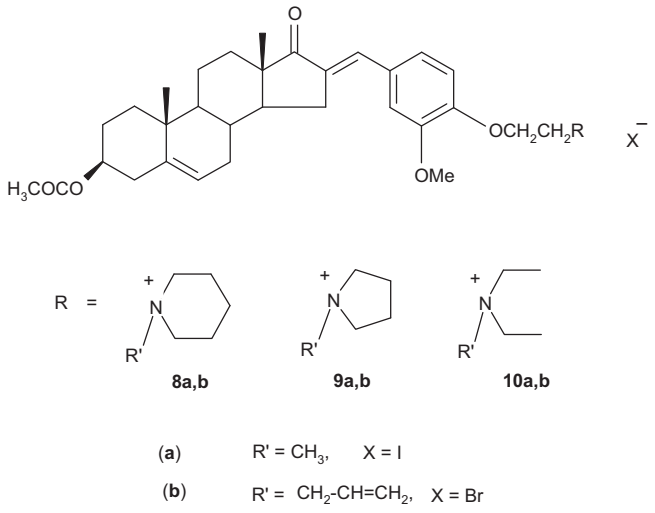
(**a**) R = OH, R' = CH_3, X = I

(**b**) R = $OCOCH_3$, R' = CH_3, X = I

(**c**) R = OH, R' = CH_2-CH=CH_2 X = Br

(**d**) R = $OCOCH_3$, R' = CH_2-CH=CH_2, X = Br

Scheme 1. Structures of bisquaternary steroidal derivatives **5**–**7a**–**d**.



Scheme 2. Structures of monoquaternary steroidal derivatives **8**–**10a**–**b**.

dry dichloromethane at room temperature for a variable period of time (Table 1). The structures of these quaternary compounds were established using various spectral and elemental analyses. In the ^{1}H NMR spectra of **5**–**7a**, protons of methyl group attached to quaternary nitrogen of 3-pyrrolidino functionality appeared as split singlets for compounds **5** and **6a** while only one singlet was observed for protons of the methyl group attached to quaternary nitrogen of other heterocyclic ring at 16-position. However, in case of **7a** the two methyl groups attached to quaternary heads appeared as single singlets. The singlets for $-\overset{\oplus}{N}-CH_3$ of two heterocyclic rings in compounds **5**–**7b** also appeared downfield at $\delta \sim 3$ and 3.33 ppm. The presence of a singlet at $\delta \sim 2.2$ ppm in **5**–**7b,d** confirmed the acetylation at 17-position. Terminal methylenes and methine proton of both the allyl groups attached to the quaternary nitrogens of pyrrolidine and piperidine rings appeared as multiplets at about δ 5–6 ppm in the proton NMR of the diallyl quaternary ammonium salts **5**–**7c** and **5**–**7d**. The triplets of $-CH_2\overset{\oplus}{N}($ and $-OCH_2-$ protons of all these quaternary ammonium derivatives **5**–**7a**–**d** appeared at $\delta \sim 4$ and 4.4 ppm, respectively. *N*-Methylenes of pyrrolidino and piperidino functionalities were also observed at downfield values due to quaternization of nitrogen.

Monoquaternary salts (Scheme 2) of the monotertiary counterparts (**4**) [13] of 16-arylidenosteroids were also prepared to observe the difference in muscle relaxant activity of the compounds among this class and to study the structure-activity relationships. Monoquaternary ammonium salts **8**–**10a,b** were prepared by quaternizing their respective monotertiary amines with methyl iodide and allyl bromide in dry dichloromethane at room temperature for a variable time period as shown in Table 1. These quaternary compounds exhibited IR absorption bands near 1720 cm^{-1} and ^{1}H NMR signal at δ 2.04 ppm (3β-OCOCH_3). The proton resonance singlets for the $-CH_3$ attached to quaternary nitrogen head of **8**–**10a** appeared at δ 3.54, 3.46 and 3.41 ppm, respectively.

The proton resonance doublets of terminal methylenes of the allyl groups in the mono allyl quaternary ammonio salts **8**–**10b** were found in the region $\delta \sim 5.77$ (J_{cis} = 9.95 Hz) and 5.9 ppm (J_{trans} = 16.65 Hz). A multiplet at $\delta \sim 6.1$ ppm for the methine proton of the allyl group was also seen in the ^{1}H NMR spectra of all of these mono allylated quaternary ammonio derivatives.

The interonium distances of these quaternary compounds were estimated from drieding models, which indicated a wide range of values of 11–17 Å. This may be associated with the built in flexibility of these structures about the single bonds on the moieties linked to ring D of the steroid skeleton (Fig. 1).

Table 2
Skeletal muscle relaxant activity of quaternary ammonium steroids.

Compound no.	Concentration (μM)	t_{50} (min)
5a	2	49
5b	10	8–10
5c	11	6–8
5d	1	2
6a	5	10
6b	5	8
6c	5	7
6d	5	6.5
7a	10	7–9
7b	5	3–4
7c	0.4	16–17
7d	5	4
8a	>150	No block
8b	200	5–7
9a	10	t_{20} = 23 min
9b	10	t_{20} = 23 min
10a	>300	No block
10b	35	45–50
Pancuronium	0.011[a]	7[a]
Pipecuronium	0.01[a]	6[a]

All compounds were tested on the twitch responses of chick biventer cervicis preparations to stimulation of the motor nerve. t_{50} = time in which 50% block is achieved; each compound was tested against 2 preparations: the range of times to reach t_{50} is given; where a single value appears, both preparations blocked at the same rate.
[a] Ref. [16].

3.2. Pharmacological activity

3.2.1. Neuromuscular blocking activity

Quaternary ammonium steroids **5**–**7a**–**d** and **8**–**10a,b** were screened for *in vitro* neuromuscular blocking activity using an isolated nerve–muscle preparation, the chick biventer cervicis [14]. Muscles were stimulated indirectly at 0.1 Hz to monitor effects of the compounds on twitch height.

All the bisquaternary ammonium compounds (**5**–**7a**–**d**) reduced twitch responses to stimulation of the motor nerve, although there were marked differences in potency (Table 2). The most active compound was allyl salt **5d**, which reduced twitch responses to 50% of control height in 2 min when added at 1 μM. In contrast, 10 μM of methiodide **5b** took 8–10 min to give a similar reduction in twitch height. The monoquaternary compounds (**8a**–**10b**) were virtually inactive on the chick biventer cervicis muscle preparation (Table 2). When compounds had reduced responses to nerve stimulation, they also had reduced the sensitivity of the muscle preparations to the cholinoceptor agonists acetylcholine and carbachol. However, muscle contractility (as judged by responses to depolarization induced by KCl) was not affected by the compounds. The twitch blocking effects were reversed on washout of the compounds.

Although there is no marked difference in the muscle relaxant properties of the three series i.e. pyrrolidinyl, piperidinyl and diethylamino groups as the quaternary nitrogen heads on side chain at 16-position of steroid skeleton, the bisquaternary 17-acetoxy piperidinyl derivative **5d** exhibited the maximum potency by blocking 50% of the twitch response in 2 min at 1 μM. In general the 17-acetoxy derivatives **5**–**7b,d** exhibited higher potency in comparison to their 17-hydroxy counterparts **5**–**7a,c**. Out of dimethiodides or diallyl salts, the later looked more promising in agreement with earlier reports [11]. The greatest loss of potency was on changing from bisquaternary **5**–**7a–d** to monoquaternary mononitrogen compounds **8**–**10a,b**. Lack of an intact acetylcholine-like fragment in these compounds has not compromised their potency, presumably due to increased interonium distance, which was found to be ranging from 11 to 17 Å due to free rotation of side chain.

The compounds are acting by a non-depolarizing mechanism because they do not themselves cause contracture of the chick

Table 3
Anticholinesterase activity of the compounds.

Compound no.	K_i (μM)[a]
5a	6.0 (4.1–8.6)
5b	2.7 (1.2–5.8)
5c	7.5 (3.9–14.5)
5d	0.35 (0.1–3.0)
6a	0.64 (0.06–6.5)
6b	4.3 (0.07–27)
6c	1.3 (0.3–5.0)
6d	2.0 (0.6–6.1)
7a	1.0 (0.4–2.7)
7b	6.7 (2.8–16.2)
7c	0.46 (0.03–7.0)
7d	0.2 (0.1–0.4)
8a	4.2 (2.9–6.2)
8b	0.21 (0.12–0.38)
9a	3.6 (0.9–2.8)
9b	2.2 (0.9–4.9)
10a	2.6 (1.7–3.8)
10b	0.34 (0.23–0.5)
Pancuronium	7.4[b]
Pipecuronium	5.9[b]

[a] Values are mean ± 95% confidence limits ($n = 4$).
[b] Determined against acetylcholinesterase from human erythrocytes [17].

biventer cervicis preparations, as would suxamethonium, and because they also decreased responses to the cholinoreceptor agonist carbachol (data not shown). However, the compounds also displayed nonparallel shifts of dose response curve to carbachol. This implies that the compounds may also be acting by some other mechanism, such as block of receptor ion channel.

3.2.2. *Anticholinesterase activity*

The compounds were also tested for their ability to inhibit the activity of electric eel acetylcholinesterase [15]. All compounds, mono- and bis-quaternary, inhibited the enzyme and K_i values were found between 0.2 (for **8b**) and 7.5 (for **5c**) μM (Table 3).

The compounds are not only acting as antagonists at acetylcholine receptors (Table 2) but are also inhibiting acetylcholinesterase activity at low concentrations (Table 3). Thus, there are two functionally opposite effects: reduction of neuromuscular transmission because of the block of acetylcholine receptors and augmentation of transmission because of the reduced metabolism of the released acetylcholine.

Overall, the tested compounds displayed varied degrees of muscle relaxant activity with the monoquaternary salts exhibiting less potency as compared to bisquaternary ones. The present series represents a new class of steroidal neuromuscular blockers with variable interonium distance, but the compounds ability to inhibit the activity of acetylcholinesterase means that they would not themselves be candidates for potential development.

Acknowledgements

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