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Synthesis and neuromuscular blocking activity of 16-(2- and 3-pyridylmethylene) dehydroepiandrosterone derivatives

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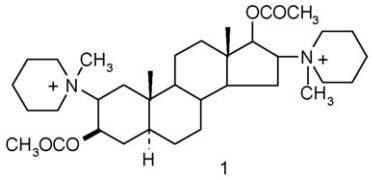
ABSTRACT

The interonium distance plays a major role in neuromuscular blocking activity of bis-quaternary ammonium compounds. In this study we tried to alter the distance between two quaternary nitrogens in some of the steroidal derivatives synthesized and evaluated them for neuromuscular blocking activity using *in vivo* (in chicks) and *in vitro* models (rectus abdominus and chick biventer cervis muscle) for their mechanism of action. All the synthesized compounds have shown to possess good depolarizing, competitive neuromuscular blocking activity, particularly the 17-acetoxy derivative and the increase in the distance between two quaternary nitrogens decreased the activity.

1. Introduction

Neuromuscular blocking agents are the drugs which cause interruption of the nerve impulse at the skeletal neuromuscular junction. They interact with a common family of receptor called nicotinic cholinergic receptor. These agents are distinguished by whether or not they cause depolarization of the muscle end plate and hence they are classified as competitive (stabilizing) agents, e.g. tubocurarine, pancuronium and depolarizing agents like succinylcholine [1–3]. Due to the complex problem of limited botanical source, it is difficult to obtain a good amount of alkaloid at once. Also the methods of purification of the curare and its identification are very complex. This increases the cost of the drug.

In addition to all above problems the currently available drug has got side effects like histamine release, ganglionic blockade and vagal blockade which may precipitate hypotension, prolonged apnea and cardiovascular collapse. Thus, a search for new molecules devoid of cardiovascular effects and histamine release has necessitated the search of novel neuromuscular blocking agents. While designing neuromuscular blockers in the bis-quaternary steroidal series the distances between two quaternary nitrogens have shown to play a major role. In the earlier studies it was reported that for good neuromuscular blocking activity the ideal interonium distance should be between 1.0 and 1.2 nm like pancuronium bromide **1**, but steroidal derivatives like pipecuronium bromide **2** and non-steroidal benzylisoquinolium derivative – atracurium and related compounds do not follow this rule [4–6]. Pipecuronium bromide is a non-depolarizing neuromuscular blocking agent with insignificant cardiovascular side effects and histamine release effect. So it was thought to synthesis certain 16-benzylidene substituted derivatives of dehydroepiandrosterone (DHEA) **3** with increased interonium distance, while keeping the other essential features taken from pipecuronium intact.



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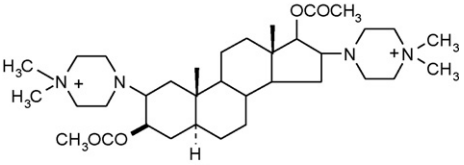

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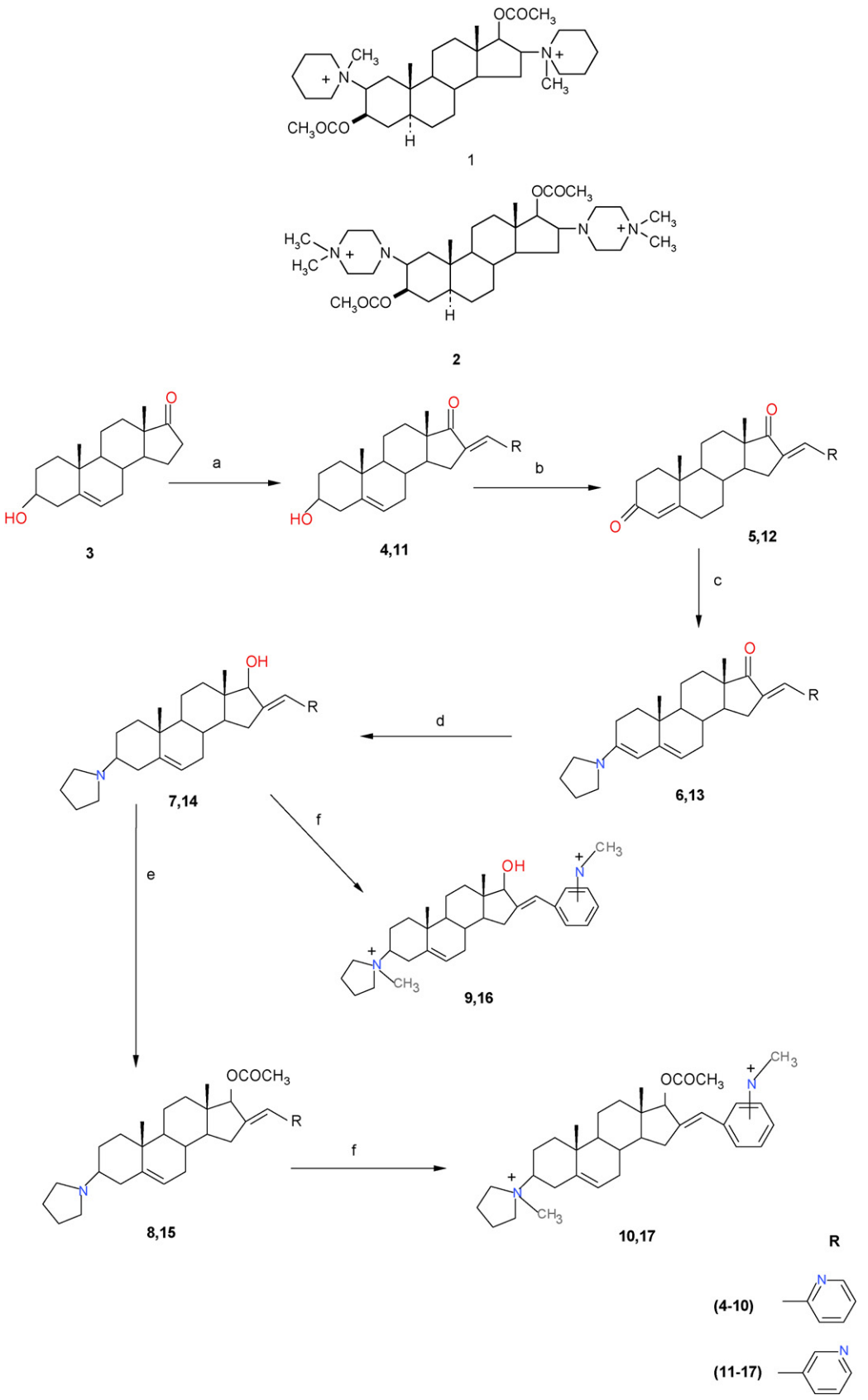

Scheme 1. Reagents and conditions: (a) 2- or 3-pyridine carboxaldehyde, NaOH, R.T. 2 h; (b) cyclohexanone, aluminium isopropoxide, toluene as solvents, reflux 4 h, steam distillation; (c) pyrrolidine in MeOh, reflux; (d) $NaBH_4$ reduction in MeOH at R.T.; (e) acetic anhydride, dried pyridine; (f) CH_3I in DCM/absolute alcohol.

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Table 1
Effect of the synthesized compounds tested in chicks using pancuronium bromide as standard.

Drug	Dose (μg/kg)	No. of animals	Flaccid paralysis	Mean onset of action (min) ± SD	Mean duration of action (min) ± SD	Difficulty in breathing	Death
9	62.5	6	–	2.59 ± 0.01	43.16 ± 0.10	√	–
	125	6	–	2.38 ± 0.02	45.32 ± 0.03	√	–
	250	6	√	2.15 ± 0.03	8.16 ± 0.05	√	√
	500	6	√	1.46 ± 0.01	5.31 ± 0.02	√	√
	1000	6	√	1.30 ± 0.03	3.36 ± 0.06	√	√
10	62.5	6	–	2.28 ± 0.09	40.58 ± 0.01	√	–
	125	6	√	1.62 ± 0.01	26.39 ± 0.02	–	–
	250	6	√	1.59 ± 0.02	6.85 ± 0.05	√	√
	500	6	√	1.49 ± 0.02	6.13 ± 0.06	√	√
	1000	6	√	1.56 ± 0.01	4.11 ± 0.02	√	√
16	62.5	6	–	3.74 ± 0.048	38.25 ± 0.01	√	–
	125	6	√	3.23 ± 0.05	41.21 ± 0.03	√	√
	250	6	√	2.68 ± 0.02	44.62 ± 0.04	√	√
	500	6	√	2.14 ± 0.01	6.11 ± 0.03	√	√
	1000	6	√	1.53 ± 0.02	4.02 ± 0.01	√	√
17	62.5	6	–	2.20 ± 0.085	42.58 ± 0.09	√	–
	125	6	√	1.58 ± 0.01	44.39 ± 0.03	-	-
	250	6	√	1.52 ± 0.03	6.89 ± 0.05	√	√
	500	6	√	1.41 ± 0.02	4.13 ± 0.02	√	√
Pancuronium bromide	125	6	√	2.58	48.25	–	–
	250	6	√	1.31	3.62	√	√
	500	6	√	1.19	2.35	√	√

At lower doses 62.5 and 125 μg/kg recovery was seen after 25–45 min.
At higher doses 250–1000 μg/kg death was observed in 3–6 min.

2. Results

2.1. Chemistry

The DHEA **3** was condensed with piconaldehyde (2-pyridinecarboxaldehyde) and niconaldehyde (3-pyridine carboxyldehyde) by aldol condensation [7] to get 16-(2- and 3-pyridylmethylene)-17-oxo-5-androsten-3β-ol (**4** and **11**). The X-ray crystallography studies have shown that the pyridyl methylene moiety has E-configuration with respect to the carbonyl at position-17 for **4** [8]. The compounds **4** and **11** on oppenauer [9] oxidation using cyclohexanone–toluene system gave 4-en-3-one **5** and **12**. The XRD studies of these structures have already been reported in our earlier studies [10,11]. The 4-en-3-one derivatives were refluxed with freshly distilled pyrrolidine in methanol to yield the enamines (**6** and **13**), which on reduction with sodium borohydride yielded 16-(2-pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-ol (**7** and **14**). On acetylation with acetic anhydride 17β-ols gave 17-acetoxy derivative (**8** and **15**). The target bis-quaternary steroidal amines were synthesised from 17-hyroxy (**7** and **14**) and 17-acetoxy (**8** and **13**) tertiary amines by using methyl iodide in dry dicholoromethane to get compounds **9** and **16** and **10** and **17**, respectively (Scheme 1). The new compounds were characterized by spectroscopic (UV, ^{1}H NMR, IR, MS) and their purity was established by elemental analyses.

2.2. Pharmacology

To evaluate the synthesized quaternary ammonium compounds for their neuromuscular blocking activity the following parameters were used: *in vivo* estimation was done by observing the paralysis of chicks and *in vitro* by using isolated frog rectus abdominis muscle preparation, and chick biventer cervicis muscle preparation – a preparation selected for its ability to elicit the potency of nicotinic antagonists and reveal indicative information on mechanism of action. The results of *in vivo* studies in terms of flaccid paralysis and death are reported in Table 1. The rectus abdominis muscle preparation results for these compounds are given in Table 2.

3. Discussion

For *in vivo* studies, after intravenous administration of compounds **9**, **10**, **16** and **17** in 1-day-old chicks, a dose-dependent

Table 2
Results of effect of compounds on rectus abdominis muscle.

Drug	Conc.	Log molar concentration	log(Dr − 1)	pA_2	Mean	SD
9	1	−5.78	1.95	7.73	6.81	0.92
	5	−5.09	0.81	5.90		
	10	−4.78	0.69	5.13		
10	1	−5.86	3.66	9.35	7.22	1.24
	5	−5.25	2.65	7.19		
	10	−4.88	0.76	5.12		
16	1	−5.78	3.45	9.22	7.53	1.13
	5	−5.30	2.67	7.97		
	10	−4.78	0.62	5.39		
17	1	−5.88	0.66	6.54	6.60	0.62
	5	−5.18	2.52	7.70		
	10	−4.88	0.68	5.56		

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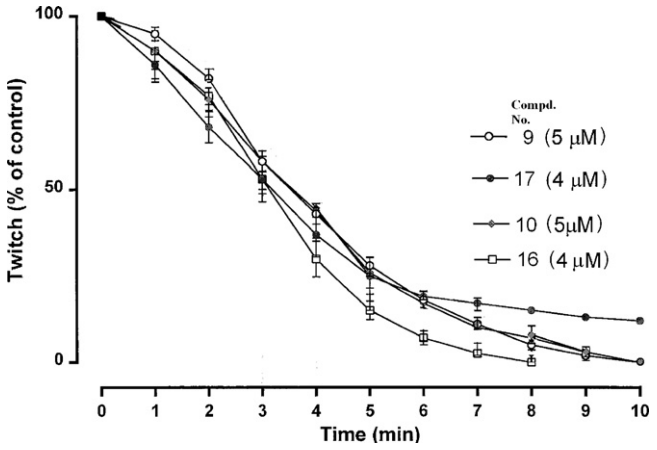

Fig. 1. Time course of the effects of **16** (4 μM), **9** and **10** (5 μM) and **17** (4 μM), on the twitch response of chick biventer cervicis preparations to nerve stimulation. Each point represents the mean + S.E.M. of 4 experiments.

response was observed. At low dose of 0.010 mg/kg none of the compound produced paralysis. Mild sedation was observed which was reversed rapidly.

At lower doses of 0.0625 and 0.125 mg/kg these molecules exhibited flaccid paralysis. The chicks recovered after about 25–45 min. At higher doses of 0.250, 0.500 and 1.0 mg/kg, the onset of flaccid paralysis was rapid but sudden death was observed. Breathing was slowed down and death occurred in about 3–6 min. Therefore the test compounds produced reversible flaccid paralysis at lower doses while irreversible flaccid paralysis and death at higher doses.

We have used acetylcholine-induced contractions of isolated rectus abdominis muscle of frog to understand the type of antagonism, i.e. competitive or non-competitive. A parallel shift of drug response curve to the right indicated the depolarizing type competitive antagonism of acetylcholine by **9**, **10**, **16** and **17**.

Further mechanistic investigations were done on these final bis-quaternary ammonium compounds by testing them on chick biventer cervicis preparations for their ability to reduce twitch responses to nerve stimulation. Both produced time- and concentration-dependent block of twitch responses at concentrations of 1 μM and above. The order of potency of bisonium compounds to reduce the twitch response to nerve stimulation is **16** > **9** = **10** > **17** by reversible binding to acetylcholine receptors. Compound **16** was about two times more potent than compounds **9** and **10**; 4 μM of **16** caused complete twitch blockade in 8 min, whereas 5 μM of **9** and **10** and 4 μM of **17** completely blocked twitches in 10 min (Fig. 1). The block of twitches was completely reversed by washout of the compounds. Addition of the anticholinesterase neostigmine (1 μM) before complete twitch blockade led to a rapid recovery of twitch height in the presence of these compounds.

Thus based on these results it can be concluded that these compounds are competitive antagonist of acetylcholine and change in the twitch response was observed with the change from 17-acetoxy **16** to 17-hydroxy **9** and **10**. This indicates that the introduction of 17-acetoxy in place of 17-OH in quaternary ammonium compounds led to a marked increase in the potency, which may be attributed to the increased bulkiness and increased lipophilicity of the molecule. These results are in agreement with our previous studies on similar types of quaternary ammonium compounds [12,13]. But as suggested by the results the increase in the distance between two quaternary nitrogen group, from 11.1 to 11.6 Å in **16** and **17**, respectively, led to a marked decrease in the neuromuscular blocking activity. This observation further strengthens the need for an optimal distance between two quaternary nitrogens for good neuromuscular blocking activity.

4. Experimental protocol

4.1. Chemistry

Melting points reported are uncorrected ^{1}H and ^{13}C NMR spectra were recorded on AC-300F, 300 MHz NMR instruments using tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are expressed in ppm. IR spectra (using KBr pellets) and UV spectra were recorded on PerkinElmer 882 and Lamda 15 spectrophotometer models, respectively. The purity of the compounds was established by thin layer chromatography and by elemental analyses (CHN). Elemental analyses were carried out on a PerkinElmer 2400. Mass spectra were recorded on a V6-11-250J70S. Anhydrous sodium sulphate was used as a drying agent and iodine vapors as developing agent.

4.1.1. General procedure for the synthesis of *4* and *11*

A mixture of dehydroepiandrosterone (**3**, 1.0 g, 3.47 mmol), pyridine carboxaldehyde (0.8 g, 7.47 mmol) and sodium hydroxide (1.75 g) in methanol (20 mL) was shaken continuously for 1 h at room temperature. The completion of reaction was monitored by TLC. The reaction mixture was poured into ice-cold water. The resulting precipitate was filtered, washed with water, dried and crystallized from methanol to obtain the compounds **4** and **11**.

4.1.1.1. 16-(2-Pyridylmethylene)-17-oxo-5-androsten-3β-ol ***4***. Yield: 1.0 g (72.9%), θ_{mp} 205–210 °C; UV_{max} (MeOH): 295.6 nm ($\log\varepsilon$ 4.30) and 270.8 nm ($\log\varepsilon$ 4.12); $IR_{\nu max}$ (KBr): 3200, 2980, 1718, 1630, 1580 and 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$): 1.08 (s, 3H, 18-C*H*$_3$), 1.11 (s, 3*H*, 19-C*H*$_3$), 3.52 (m, 1H, 3α-*H*), 5.40 (d, 1H, 6-C*H*), 7.21 (m, 1H, 5-C*H* aromatic proton), 7.40 (m, 1H, 3-C*H* aromatic proton), 7.44 (s) and 7.47 (s) [0.9:1 area ratio, integrating for 1H, vinyl-*H* of 16-(2-pyridylmethylene)], 7.72 (m, 1H, 4-C*H* aromatic proton) and 8.70 (s, 1H, 6-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$):37.2 (C-1), 31.7 (C-2), 76.8 (C-3), 41.8 (C-4), 140.8 (C-5), 121.8 (C-6), 31.8 (C-7), 27.7 (C-8), 50.6 (C-9), 37.7 (C-10), 20.3 (C-11), 34.1 (C-12), 52.8 (C-13), 57.4 (C-14), 24.8 (C-15), 149.6 (C-16), 208.8 (C-17), 19.3 (C-18), 19.0 (C-19), 128.4 (C-20), 122.7, 124.3,137, 148.8 and 154.7 (C-aromatic)

4.1.1.2. 16-(3-Pyridylmethylene)-17-oxo-5-androsten-3β-ol ***11***. Yield: 0.8 g (58.36%), θ_{mp} 260–264 °C; UV_{max} (MeOH): 281.6 nm ($\log\varepsilon$ 4.32); $IR_{\nu max}$ (KBr): 3350, 2920, 1715, 1630 and 900 cm^{-1}; ^{1}H NMR ($CDCl_3$): 0.99 (s, 3H, 18-C*H*$_3$), 1.08 (s, 3H, 19-C*H*$_3$), 3.54 (t, 1H, 3α-*H*), 5.4 (d, 1H, 6-C*H*), 7.35–7.40 [m, 2H, one vinyl-*H* of 16-(3-pyridylmethylene) and 5-C*H* aromatic proton], 7.83 (d, 1H, J_o = 7.8, 4-C*H* aromatic proton), 8.57 (d, 1H, 6-C*H* aromatic proton) and 8.80 (s, 1H, 2-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 37.2 (C-1), 31.7 (C-2), 76.8 (C-3), 41.8 (C-4), 140.8 (C-5), 121.8 (C-6), 31.8 (C-7), 27.7 (C-8), 50.6 (C-9), 37.7 (C-10), 20.3 (C-11), 34.1 (C-12), 52.8 (C-13), 57.4 (C-14), 24.8 (C-15), 149.6 (C-16), 208.8 (C-17), 19.3 (C-18), 19.0 (C-19), 135.6 (C-20), 123.8, 132.5, 132.6, 148.1, 149.5 (C-aromatic); Calcd. for $C_{25}H_{31}NO_2$: C, 79.54; H, 8.28; N, 3.71. Found: C, 79.35; H, 8.15; N, 4.00.

4.1.2. General procedure for the synthesis of *5* and *12*

The 16-(substituted-pyridylmethylene)-17-oxo-5-androsten-3β-ol (1.0 g, 2.65 mmol) was dissolved in dry toluene (150 mL) by refluxing and then cyclohexanone (10 mL) was added. Traces of moisture were removed by azeotropic distillation. The distillation was continued at a slow rate while adding a solution of aluminium isopropoxide (1.0 g) in dry toluene (15 mL) drop wise. The reaction mixture was refluxed for 4 h. It was allowed to stand overnight at

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room temperature. The slurry was filtered and residue was washed thoroughly with dry toluene. The combined filtrate and washing were steam distilled until complete removal of organic solvent was affected. The solid residue was allowed to stand overnight and then filtered, washed, dried and crystallized from methanol to obtain **5** and **12**.

*4.1.2.1. 16-(2-Pyridylmethylene)-4-androstene-3,17-dione **5**.* Yield: 1.8 g (90.48%), θ_{mp} 188–190 °C; UV_{max} (MeOH): 296.0 nm ($\log \varepsilon$ 4.18) and 243.8 nm ($\log \varepsilon$ 4.18); $IR_{\nu max}$ (KBr): 2980, 1720, 1670, 1590, 1430 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): 1.01 (s, 3H, 18-C*H*$_3$), 1.25 (s, 3H, 19-C*H*$_3$), 5.76 (d, 1H, 4-C*H*), 7.21 (m, 1H, 5-C*H* aromatic proton), 7.39 (m, 1H, 3-C*H* aromatic proton), 7.43 (s) and 7.45 (s) [0.7:1 area ratio, integrating for 1H, vinyl-*H* of 16-(2-pyridyl methylene)], 7.71 (m, 1H, 4-C*H* aromatic proton) and 8.71 (d, 1H, 6-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 35.2 (C-1), 34.2 (C-2), 198.9 (C-3), 123.0 (C-4), 162.7(C-5), 127.2 (C-6), 140.6(C-7), 32.0 (C-8), 50.4 (C-9), 36.1 (C-10), 20.2 (C-11), 34.2 (C-12), 52.8 (C-13), 58.8 (C-14), 24.8 (C-15), 149.6 (C-16), 208.8 (C-17), 16.3(C-18), 19.1 (C-19), 128.4 (C-20), 122.7, 124.3, 137.0, 148.8, 154.7(C-aromatic); MS Calcd. for $C_{25}H_{29}NO_2$: *m*/*z*: 375 [M^+].

*4.1.2.2. 16-(3-Pyridylmethylene)-4-androstene-3,17-dione **12**.* Yield: 0.6 g (59.68%), θ_{mp} 202–208 °C; UV_{max} (MeOH): 281.0 nm ($\log \varepsilon$ 4.34) and 242.0 nm ($\log \varepsilon$ 4.30); $IR_{\nu max}$ (KBr): 2980, 1715, 1670 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): 1.03 (s, 3H, 18-C*H*$_3$), 1.26 (s, 3H, 19-C*H*$_3$), 5.77 (s, 1H, 4-C*H*), 7.35–7.41 [m, 2H, one vinyl-*H* of 16-(3-pyridylmethylene) and 5-C*H* aromatic proton], 7.82 (d, 1H, J_o = 7.9, 4-C*H* aromatic proton), 8.58 (m, 1H, 6-C*H* aromatic proton) and 8.79 (d, 1H, J_p = 1.0, 2-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 35.2 (C-1), 34.2 (C-2), 198.9 (C-3), 123.0 (C-4), 162.7(C-5), 127.2 (C-6), 140.6(C-7), 32.0 (C-8), 50.4 (C-9), 36.1 (C-10), 20.2 (C-11), 34.2 (C-12), 52.8 (C-13), 58.8 (C-14), 24.8 (C-15), 149.9 (C-16), 208.8 (C-17), 16.3(C-18), 19.1 (C-19), 135.6 (C-20), 123.8, 132.5, 132.6, 148.0 148.1(C-aromatic); MS *m*/*z*: 375 [M^+]; Calcd. for $C_{25}H_{29}NO_2$: C, 79.96; H, 7.78; N, 3.73. Found: C, 79.30; H, 7.77; N, 3.65

*4.1.3. General procedure for the synthesis of **6** and **13***

Freshly distilled pyrrolidine (1.0 mL, 14.06 mmol) was added to a refluxing solution of androstene-3,17-dione (0.5 g, 1.34 mmol) in methanol (15 mL). Refluxing was continued for 15 min and then the reaction mixture was cooled. The precipitate obtained was filtered, washed with methanol and dried to afford **6** and **13**. Due to lesser stability of this compound the next step was followed immediately.

*4.1.3.1. 16-(2-Pyridylmethylene)-3-pyrrolidino-3,5-androsta-dien-17-one **6**.* Yield: 0.4 g (69.92%); θ_{mp} 185–190 °C; UV_{max} (MeOH): 289.6 nm; $IR_{\nu max}$ (KBr): 2975, 1715, 1595, 1420 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): 1.00 (s, 3H, 18-C*H*$_3$), 1.10 (s, 3H, 19-C*H*$_3$), 3.17 (t, 4H, *N*-methylenes of pyrrolidine function), 4.80 (s, 1H, 4-C*H*), 5.10 (m, 1H, 6-C*H*), 7.19 (m, 1H, 5-C*H* aromatic proton), 7.39 (t, 1H, 3-C*H* aromatic proton), 7.43 (s) and 7.46 (s) [0.9:1 area ratio, integrating for 1H, vinyl-*H* of 16-(2-pyridylmethylene)], 7.71 (m, 1H, 4-C*H* aromatic proton) and 8.70 (d, 1H, 6-C*H* aromatic proton).

*4.1.3.2. 16-(3-Pyridylmethylene)-3-pyrrolidino-3,5-androsta-dien-17-one **13**.* Yield: 0.4 g (69.92%); θ_{mp} 225–228 °C; UV_{max} (MeOH): 281.0 nm; $IR_{\nu max}$ (KBr): 2900, 1705, 1630, 1600 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): 1.00 (s, 3H, 18-C*H*$_3$), 1.20 (s, 3H, 19-C*H*$_3$), 3.20 (t, 4H, *N*-methylenes of pyrrolidine function), 4.80 (s, 1H, 4-C*H*), 5.10 (s, 1H, 6-C*H*), 7.33–7.55 [m, 2H, one vinyl-*H* of 16-(3-pyridylmethylene) and 5-C*H* aromatic proton], 7.78–8.0 (m, 1H, 4-C*H* aromatic proton), 8.60–8.75 (dd, 1H, 6-C*H* aromatic proton) and 8.90 (s, 1H, 2-C*H* aromatic proton).

*4.1.4. General procedure for the synthesis of **7** and **14***

To a stirred suspension of substituted-3-pyrrolidino-3,5-androstadien-17-one (0.4 g, 0.933 mmol) in methanol (75 mL) at room temperature, sodium borohydride (1.0 g) was added in small portion over a period of 2 h. Stirring was continued for next 4 h. The solvent was recovered under reduced pressure and the residue was poured into ice-cold water. The white precipitate was filtered, washed with water, dried and crystallized from methanol to give **7** and **14**.

*4.1.4.1. 16-(2-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-ol **7**.* Yield: 0.35 g (86.68%); θ_{mp} 215–218 °C; UV_{max} (MeOH): 285.8 nm ($\log \varepsilon$ 4.02) and 250.4 nm ($\log \varepsilon$ 4.24); $IR_{\nu max}$ (KBr): 3220, 2980, 1595, 1440, 1425 and 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 0.73 (s, 3H, 18-C*H*$_3$), 1.03 (s, 3H, 19-C*H*$_3$), 2.59 (br, 4H, *N*-methylenes of pyrrolidine function), 4.08 (s, 1H, 17α-*H*), 5.39 (t, 1H, 6-C*H*), 6.5 (s) and 6.6 (s) [1:0.8 area ratio, integrating for 1H, vinyl-*H* of 16-(2-pyridylmethylene)], 7.06 (m, 1H, 5-C*H* aromatic proton), 7.28 (d, 1H, J_o = 8.3, 3-C*H* aromatic proton), 7.62 (t, 1H, 4-C*H* aromatic proton) and 8.58 (d, 1H, 6-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$) δ: 37.1(C-1), 25.2 (C-2), 68.8 (C-3), 35.8 (C-4), 141.9 (C-5), 121.8 (C-6), 32.1 (C-7), 28.8 (C-8), 50.9 (C-9), 37.4 (C-10), 21.2 (C-11), 34.6 (C-12), 59.3 (C-13), 63.3 (C-14), 28.1 (C-15), 152.6 (C-16), 86.7 (C-17), 19.3 (C-18), 25.3 (C-19), 120.7 (C-20), 122.7, 124.3, 137,148.8,157.6 (C-aromatic), 23.9, 55.8 (C-pyrrolidine); MS Calcd. for $C_{29}H_{40}N_2O$: *m*/*z*: 432 [M^+].

*4.1.4.2. 6-(3-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-ol **14**.* Yield: 0.3 g (74.3%); θ_{mp} 238–244 °C; UV_{max} (MeOH): 254.4 nm ($\log \varepsilon$ 4.24); $IR_{\nu max}$ (KBr): 3220, 2970, 1640 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): 0.73 (s, 3H, 18-C*H*$_3$), 1.03 (s, 3H, 19-C*H*$_3$), 2.63 (br, 4H, N-methylenes of pyrrolidine function), 4.07 (s, 1H, 17α-*H*), 5.36 (d, 1H, 6-C*H*), 6.51 (s) and 6.52 (s) [1:1 area ratio, integrating for 1H, vinyl-*H* of 16-(3-pyridylmethylene)], 7.23–7.27 (m, 1H, 5-C*H* aromatic proton), 7.67 (d, 1H, J_o = 8, 4-C*H* aromatic proton), 8.40 (t, 1H, 6-CH aromatic proton) and 8.63 (d, 1H, J_p = 1.8, 2-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 37.1(C-1), 25.2 (C-2), 68.8 (C-3), 35.8 (C-4), 141.9 (C-5), 121.8 (C-6), 32.1 (C-7), 28.8 (C-8), 50.9 (C-9), 37.4 (C-10), 21.2 (C-11), 34.6 (C-12), 59.3 (C-13), 63.3 (C-14), 28.1 (C-15), 152.9 (C-16), 86.7 (C-17), 19.3 (C-18), 25.3 (C-19), 122.8 (C-20), 123.8, 132.5, 132.6, 148.1, 149.5, (C-aromatic), 23.9, 55.8 (C-pyrrolidine); MS *m*/*z*: 432 [M^+]; Calcd. for $C_{29}H_{40}N_2O$: C, 80.51; H, 9.32; N, 6.47. Found: C, 80.62; H, 9.21; N, 6.38.

*4.1.5. General procedure for the synthesis of **8** and **15***

A mixture of androsten-17β-ol (0.5 g, 1.16 mmol) in acetic anhydride (2.0 mL) and dry pyridine (0.5 mL) was heated on a steam bath for 2 h. The reaction mixture was cooled and poured into ice-cold water and then it was filtered, washed thoroughly with water, dried and crystallized from acetone-methanol to get **8** and **15**.

*4.1.5.1. 16-(2-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-yl acetate **8**.* Yield: 0.28 g (51.05%); θ_{mp} 206–210 °C; UV_{max} (MeOH): 284.0 nm ($\log \varepsilon$ 3.98) and 247.2 nm ($\log \varepsilon$ 4.22); $IR_{\nu max}$ (KBr): 2970, 1720, 1580, 1440, 1420, 1240 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): 0.79 (br, 3H, 18-C*H*$_3$), 1.02 (s, 3H, 19-C*H*$_3$), 2.20 (s, 3H, 17-OCOC*H*$_3$), 2.89 (br, 4H, *N*-methylenes of pyrrolidine function), 5.36 (d, 1*H*, 6-C*H*), 5.40 (s, 1H, 17α-*H*), 6.33 [1:0.9 area ratio, integrating for 1H, vinyl-*H* of 16-(2-pyridylmethylene)], 7.06 (m, 1H, 5-C*H* aromatic proton), 7.26 (d, 1H, J_o = 8.2, 3-C*H* aromatic proton), 7.61 (m, 1H, 4-C*H* aromatic proton) and 8.58 (d, 1H, 6-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 37.1(C-1), 25.2 (C-2), 68.8 (C-3), 35.8 (C-4), 141.9 (C-5), 121.8 (C-6), 32.1 (C-7), 28.5 (C-8), 50.9 (C-9), 37.4 (C-10), 20.9 (C-11), 34.8 (C-12), 56.0(C-13), 63.5 (C-14), 28.3 (C-15), 152.6 (C-16), 86.0 (C-17), 19.3 (C-18), 25.3 (C-19), 120.7 (C-20), 122.7, 124.3, 137,148.8,157.6, (C-aromatic),

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23.9, 55.8 (C-pyrrolidine), 170.8 (C=O), 21.1 (CH_3); MS Calcd. for $C_{31}H_{42}N_2O_2$: *m/z*: 474 [M^+].

4.1.5.2. 16-(3-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-yl acetate ***15***. Yield: 0.4 g (72.93%); θ_{mp} 170–174 °C; UV_{max} (MeOH): 251.6 nm ($\log \varepsilon$ 4.28); $IR_{\nu max}$ (KBr): 2980, 1715, 1240 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): 0.79 (s, 3H, 18-C*H*$_3$), 1.02 (s, 3H, 19-C*H*$_3$), 2.22 (s, 3H, 17-OCOC*H*$_3$), 2.56 (br, 4H, *N*-methylenes of pyrrolidine function), 5.37 (s, 1H, 17α-*H*), 5.38 (s, 1H, 6-C*H*), 6.18 (s) and 6.19 (s) [1:1 area ratio, integrating for 1H, vinyl-*H* of 16-(3-pyridyl-methylene)], 7.23–7.28 (m, 1H, 5-C*H* aromatic proton), 7.63–7.67 (m, 1H, 4-C*H*, aromatic proton), 8.41–8.43 (q, 1H, 6-C*H* aromatic proton) and 8.60 (d, 1H J_p = 1.9, 2-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 37.1(C-1), 25.2 (C-2), 68.8 (C-3), 35.8 (C-4), 141.9 (C-5), 121.8 (C-6), 32.1 (C-7), 28.8 (C-8), 50.9 (C-9), 37.4 (C-10), 21.2 (C-11), 34.6 (C-12), 59.3 (C-13), 63.5 (C-14), 28.3 (C-15), 152.9 (C-16), 86.0 (C-17), 19.3 (C-18), 25.3 (C-19), 122.8 (C-20), 123.8, 132.5, 132.6, 148.1, 149.5, (C-aromatic), 23.9, 55.8 (C-pyrrolidine), 170.2 (C=O),25.5 (CH_3); MS *m/z*: 475 [M^+]; Calcd. for $C_{31}H_{42}N_2O_2$: C, 78.44; H, 8.92, N, 5.90. Found: C, 77.99; H, 8.85; N, 5.93 ppm.

4.1.6. Procedure for the synthesis of ***9***

4.1.6.1. 16-(2-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-ol dimethiodide ***9***. Methyl iodide (2.0 mL) was added to a refluxing solution of 16-(2-pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-ol (**7**, 0.5 g, 1.16 mmol) in aldehyde free absolute alcohol (75 mL). The refluxing was further continued for 7 h and then solvent was removed under vacuum. The solid residue was treated with dry solvent ether and crystallized from absolute ethanol–dry acetone to afford **9** (0.4 g, 48.30%); θ_{mp} 244 °C; UV_{max} (MeOH): 297.4 nm ($\log \varepsilon$ 4.02) and 219.4 nm ($\log \varepsilon$ 4.51); $IR_{\nu max}$ (KBr): 3350, 2980, 1600 and 1440 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): 0.69 (s, 3H, 18-C*H*$_3$), 1.08 (s, 3H, 19-C*H*$_3$), 3.01 (s, 3H, $\overset{\oplus}{N}$-C*H*$_3$ of pyrrolidine function), 3.35 (m, 4H, $\overset{\oplus}{N}$-methylenes of pyrrolidine function), 4.15 (s) and 4.35 (s) (1:2.7 area ratio, integrating for 3H, -$\overset{\oplus}{N}$-C*H*$_3$ of pyridine function), 5.51–5.56 (m, 2H, 17α-*H* and 6-C*H*), 6.82 (s) and 6.86 (s) [0.83:1 area ratio, integrating for 1H, vinyl-*H* of 16-(2-pyridylmethylene)], 7.83 (m, 1H, 5-C*H* aromatic proton), 8.12 (m, 1H, 3-C*H* aromatic proton), 8.50 (m, 1H, 4-CH aromatic proton), 8.70 (s) and 9.09 (s) (1.8:1 area ratio, integrating for 1H, 6-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 32.5 (C-1),25.0 (C-2), 72.4 (C-3), 33.3(C-4), 140.8 (C-5), 121.8 (C-6), 32.1 (C-7), 28.8 (C-8), 50.9 (C-9), 37.4 (C-10), 21.2 (C-11), 34.6 (C-12), 59.3 (C-13), 63.3 (C-14), 28.1 (C-15), 152.9 (C-16), 86.7 (C-17), 19.3 (C-18), 25.3 (C-19), (C-20), 118.0 (C-21), 121.5, 127.1, 145.8, 147.1, 148.4 (C-aromatic), 22.4, 68.0 (C-pyrrolidine), 45.5, 47.7 (2× CH_3); Calcd. for $C_{31}H_{46}N_2OI_2$: C, 51.96; H, 6.47; N, 3.91. Found: C, 51.76; H, 6.31; N, 3.89.

4.1.7. General procedure for the synthesis of ***16***, ***10*** *and* ***17***

Methyl iodide (2.0 mL) was added to the solution of substituted 5-androsten-17β-ol (**14**, 0.5 g, 1.16 mmol) and substituted 5-androsten-17β-yl acetate (**8** and **15**, 0.5 g, 1.05 mmol) in dry dichloromethane (50 mL). The reaction mixture was kept at room temperature for 7 days. Solvent was removed under reduced pressure. The residue was treated with dry solvent ether and dry acetone and crystallized from dry acetone to yield **16**, **15** and **17**, respectively.

4.1.7.1. 16-(3-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-ol dimethiodide ***16***. The solid material obtained was crystallized from absolute alcohol to obtain **19** (0.5 g, 60.44%); θ_{mp} 295–298 °C; UV_{max} (MeOH): 270.4 nm ($\log \varepsilon$ 4.17) and 220.8 nm ($\log \varepsilon$ 4.51); $IR_{\nu max}$ (KBr): 3280, 2980, 1590 and 900 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-$_6$): 0.70 (s, 3H, 18-C*H*$_3$), 1.08 (s, 3H, 19-C*H*$_3$), 3.01 (s, 3H-$\overset{\oplus}{N}$ C*H*$_3$ of pyrrolidine function), 3.65 (m, 4H, -$\overset{\oplus}{N}$-methylenes of pyrrolidine function), 4.08 (d, 1H, 3α-*H*), 4.53 (s, 3H, -$\overset{\oplus}{N}$-C*H*$_3$ of pyridine function), 5.33 (d, 1H, 17α-*H*), 5.55 (d, 1H, 6-C*H*), 6.64 [s, 1H, vinyl-*H* of 16-(3-pyridylmethylene)], 8.02–8.06 (m, 1H, 5-C*H* aromatic proton), 8.45 (d, 1H, J_o = 8.3, 4-C*H* aromatic proton), 8.88 (d, 1H, J_o = 6.0-C*H* aromatic proton) and 8.99 (s, 1H, 2-C*H* aromatic proton). ^{13}C NMR ($CDCl_3$): 32.5 (C-1), 25.0 (C-2), 72.4 (C-3), 33.3(C-4), 140.8 (C-5), 121.8 (C-6), 32.1 (C-7), 28.8 (C-8), 50.9 (C-9), 37.4 (C-10), 21.2 (C-11), 34.6 (C-12), 59.3 (C-13), 63.3 (C-14), 28.1 (C-15), 152.9 (C-16), 86.7 (C-17), 19.3 (C-18), 25.3 (C-19), (C-20), 129.8, 137.0, 141.1, 143.0 143.6 (C-aromatic), 22.4, 68.0 (C-pyrrolidine), 45.5, 49.4 (2× CH_3); Calcd. for $C_{31}H_{46}N_2OI_2$: C, 51.96, H, 6.47, N, 3.91. Found: C, 51.90; H, 6.49; N, 3.90.

4.1.7.2. 16-(2-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-yl acetate dimethiodide ***10***. Yield: 0.25 g (31.29%); θ_{mp} 230–236 °C; UV_{max} (MeOH): 299.6 nm ($\log \varepsilon$ 4.07) and 219.2 nm ($\log \varepsilon$ 4.5); $IR_{\nu max}$ (KBr): 2970, 1725, 1580, 1425 and 1225 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ: 0.84 (s, 3H, 18-C*H*$_3$), 1.07 (s, 3H, 19-C*H*$_3$), 2.25 (s, 3H, 17-OCOC*H*$_3$), 3.03 (s, 3H, -$\overset{\oplus}{N}$-C*H*$_3$ of pyrrolidine function), 3.74 (br, 4H, $\overset{\oplus}{N}$-methylenes of pyrrolidine function), 4.39 (s, 3H, -$\overset{\oplus}{N}$-C*H*$_3$ of 2-pyridyl function), 5.43 (s, 1H, 17α-*H*), 5.51 (d, 1H, 6-C*H*), 6.51 (s) and 6.52 (s) [1:1 area ratio, integrating for 1H, vinyl-*H* of 16-(2-pyridylmethylene)], 7.92 (t, 1H, 5-C*H* aromatic proton), 8.13 (d, 1H, J_o = 8.0, 3-C*H* aromatic proton), 8.53 (t, 1H, 4-C*H* aromatic proton) and 9.22 (d, 1H, J_o = 6, 6-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 32.5 (C-1),25.0 (C-2), 72.4 (C-3), 33.3(C-4), 140.8 (C-5), 121.8 (C-6), 32.1 (C-7), 28.8 (C-8), 50.9 (C-9), 37.4 (C-10), 21.2 (C-11), 34.6 (C-12), 56.0 (C-13), 63.3 (C-14), 28.1 (C-15), 152.9 (C-16), 86.7 (C-17), 19.3 (C-18), 25.3 (C-19), (C-20), 118.0 (C-21), 121.5, 127.1, 145.8, 147.1, 148.4 (C-aromatic), 22.4, 68.0 (C-pyrrolidine), 45.5, 47.7,21.0 (3× CH_3), 170.2 (C=O); Calcd. for $C_{33}H_{48}N_2O_2I_2$: C, 52.25; H, 6.38; N, 3.69. Found: C, 52.13; H, 6.27; N, 3.68.

4.1.7.3. 16-(3-Pyridylmethylene)-3β-pyrrolidino-5-androsten-17β-yl acetate dimethiodide ***17***. Yield: 0.5 g (62.63%); θ_{mp} 260 °C (decomp.); UV_{max} (MeOH): 265.2 nm ($\log \varepsilon$ 4.18) and 221.2 nm ($\log \varepsilon$ 4.54); $IR_{\nu max}$ (KBr): 2980, 1725, 1240 and 900 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): 0.80 (s, 3H, 18-C*H*$_3$), 1.07 (s, 3H, 19-C*H*$_3$), 2.23 (s, 3H, 17-OCOC*H*$_3$), 3.04 (s, 3H, -$\overset{\oplus}{N}$-C*H*$_3$ of pyrrolidine function), 3.75 (br, 4H, -$\overset{\oplus}{N}$-methylenes of pyrrolidine function), 4.65 (s, 3H, -$\overset{\oplus}{N}$-C*H*$_3$ of pyridine function), 5.38 (s, 1H, 17α-*H*), 5.55 (s, 1H, 6-C*H*), 6.37 [s, 1H, vinyl-*H* of 16-(3-pyridylmethylene)], 8.04–8.09 (m, 1H, 5-C*H* aromatic proton), 8.43 (d, 1H, J_o = 6, 4-C*H* aromatic proton), 9.0 (d, 1H, J_o = 8.4, 6-C*H* aromatic proton) and 9.23 (s, 1H, 2-C*H* aromatic proton); ^{13}C NMR ($CDCl_3$): 32.5 (C-1), 25.0 (C-2), 72.4 (C-3), 33.3(C-4), 140.8 (C-5), 121.8 (C-6), 32.1 (C-7), 28.8 (C-8), 50.9 (C-9), 37.4 (C-10), 21.2 (C-11), 34.6 (C-12), 59.3 (C-13), 63.3 (C-14), 28.1 (C-15), 152.9 (C-16), 86.0 (C-17), 19.3 (C-18), 25.3 (C-19), (C-20), 129.8, 137.0, 141.1, 143.0 (C-aromatic), 22.4, 68.0 (C-pyrrolidine), 45.5, 49.4, 21.0 (3× CH_3); Calcd. for $C_{33}H_{48}N_2O_2I_2$: C, 52.25; H, 6.38; N, 3.69. Found: C, 52.22; H, 6.18; N, 3.63.

4.2. Pharmacological methods

4.2.1. Paralysis in chick

The test compounds and the standard pancuronium bromide (B.P.) were administered intravenously at doses as shown in Table 1. The animals were observed individually for development of paral-

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ysis. The initial symptom was inability of chick to hold neck due to relaxation of neck muscles. The characteristic features of flaccid paralysis observed were: bending of neck, pulling of legs near abdomen, inability to stand, characteristic semicircular shape of body. The onset and duration of action was noted. End point was determined when chick was unable to stand on its legs and showed a typical contracture with flaccid paralysis.

4.2.2. Frog rectus abdominis muscle preparation

The frogs were stunned, decapitated and spinal cord was destroyed. Rectus abdominis muscle was dissected out and kept in aerated frog ringer. The preparation was stretched for at least 30 min. Suitable cycle of operation followed when acetylcholine was added to the organ bath. Response of acetylcholine at various doses was obtained. Frog ringer solution was replaced by modified frog ringer solution containing antagonist and then original concentration of acetylcholine is tested for response. Concentration of acetylcholine was increased 4 times to obtain steady response. The animals were divided into three major groups control, test (low, medium and high dose) and standard (medium and high dose).

4.2.3. Chick biventer cervicis nerve-muscle preparation

The chick biventer cervicis nerve-muscle preparations were set up as described previously [14]. Muscles were removed from 4–10-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 carbachol were obtained in the absence of nerve stimulation; test compounds were replaced in the tissue bath after each washout of carbachol.

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