Recent Advances in Neuromuscular Blocking Agents

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Abstract: Since the introduction of (+)-tubocurarine into anaesthetic and surgical practice (1942), a number of non-depolarizing neuromuscular blocking agents (NMBs) with improved pharmacological properties have been developed during the last sixty years. However, after withdrawal of rapacuronium from clinical use, there is still a need for an ultra-short acting non-depolarizing muscle relaxant with rapid onset as substitution for the polarizing suxamethonium, which has several undesirable side-effects. In this paper, structure-activity relationships within four different series of NMBs (tetrahydroisoquinolinium, bistropinyl diester, aminosteroid, and amino peptide analogues) published in this millennium have been reviewed. The NMB properties of the most promising drug candidates from each series were discussed and compared to those of the already existing muscle relaxants.

INTRODUCTION

Neuromuscular blocking agents (NMBs) find utility in induction of muscle relaxation during anaesthesia for effective surgery. Without such drugs deeper anaesthesia, requiring more anaesthetic, would be needed to achieve the same degree of muscle relaxation; tracheal intubation would also be impossible because of strong reflex response to tube insertion. Since the introduction of the curare alkaloid (+)-tubocurarine chloride (**1**) (Intocostrin) into anaesthetic and surgical practice (1942) [1,2], a number of NMBs with improved pharmacological properties have been developed over the last sixty years. Methylation of (+)-tubocurarine provided the bisquaternary dimethylether metocurine iodide (*O,O´,N*-(+)-trimethyltubocurarine) (**1a**) (1949) with a 2-3 fold higher potency and fewer autonomic and cardiovascular effects than the parent monoquaternary compound [3]. The calabash curare alkaloid toxiferine I (**2**) served as a lead for the development of the dimeric strychnine-like agent alcuronium chloride (**3**) (Alloferin , 1963) [4]. Alcuronium is twice as potent as (+)-tubocurarine **1** having a shorter duration of action [5]. However, it occasionally gives rise to moderate increase in heart rate [6], probably due to its great affinity for muscarinic M_2 receptors [7]. Another agent with muscarinic antagonistic properties causing an undesired strong vagolytic action is the trisquaternary compound gallamine triethiodide (**4**) (Flaxedil , 1949) [8]. In 1951, suxamethonium (**5**) (succinylcholine chloride, Anectine , Sucostrin), a muscle relaxant with a different mechanism of action, was introduced [9]. While other NMBs are competitive nicotinic acetylcholine (ACh) receptor antagonists preventing the ion channel receptors from opening (non-depolarizing blocking agents), suxamethonium is an agonist which mimics the effect of ACh at the neuromuscular junction first causing muscle contractions and then paralysing (depolarizing blocking agent). This mechanism of action is responsible for a number of undesired side effects including fasciculations, muscle pain, increased intraocular pressure and increased intragatric tension, and rarely, anaphylaxis, malignant hyperthermia and

life-threatening hyperkalaemia [10]. While the action of non-depolarizing NMBs can be reversed by ACh-esterase inhibitors such as neostigmine, the effect of suxamethonium is terminated by a rapid degradation of the dimeric ACh-like molecules by plasma cholinesterases resulting in a very short duration of action under 5 minutes. Based on the structure of another arrow poison alkaloid malouetine, scientists at Organon developed the non-depolarizing aminosteroidal muscle relaxant pancuronium bromide (**6**) (Pavulon , 1968) [11] with an approximately five-fold higher potency than (+)-tubocurarine **1** and a shorter serum half-life time ($t_{1/2}$ = 107 min) due to the metabolitic inactivation by hydrolysis of both lateral ACh-like moieties [12]. Other advantages of pancuronium over (+)-tubocurarine are lack of histamine-releasing and of ganglion-blocking activity. However, pancuronium was still not an ideal NMB due to its slow onset, its cardiac vagolytic effects and its long duration of action [13]. Demethylation of the nitrogen atom in the ring A of pancuronium led to the monoquaternary vecuronium bromide (**7**) (Norcuron , 1982) [14] which was devoid of cardiac effects, indicating that the muscarinic receptor activity of pancuronium **6** is due to the *trans* ACh-like moiety incorporated in ring A, while the corresponding *cis* ACh-like structure in ring D is responsible for the activation of nicotinic ACh-receptors [11]. Vecuronium has a shorter duration of action ($t_{1/2}$ = 71 min) than pancuronium. However, as the latter, it lacks the rapid onset desirable for an intubating agent.

After it had been recognized, that the speed of onset is relatad to the potency of the aminosteridal NMBs [15], two poorly potent muscle relaxants with a short speed of onset comparable to that of suxamethonium **5** - rocuronium bromide (**8**) (Zemuron , 1994) [16,17] and rapacuronium bromide (**9**) (2000) [18,19] - were introduced by Organon. Rapacuronium **9**, which was the first non-depolarizing muscle relaxant with both rapid onset and short duration was withdrawn from clinical use shortly after its introduction due to five cases of bronchospasm resulting in death [20,21]. A mechanism by which rapacuronium may potentiate bronchoconstriction by blockade of autonomic M_2 muscarinic receptors on prejunctional parasympathetic nerves, leading to increased release of ACh and thereby

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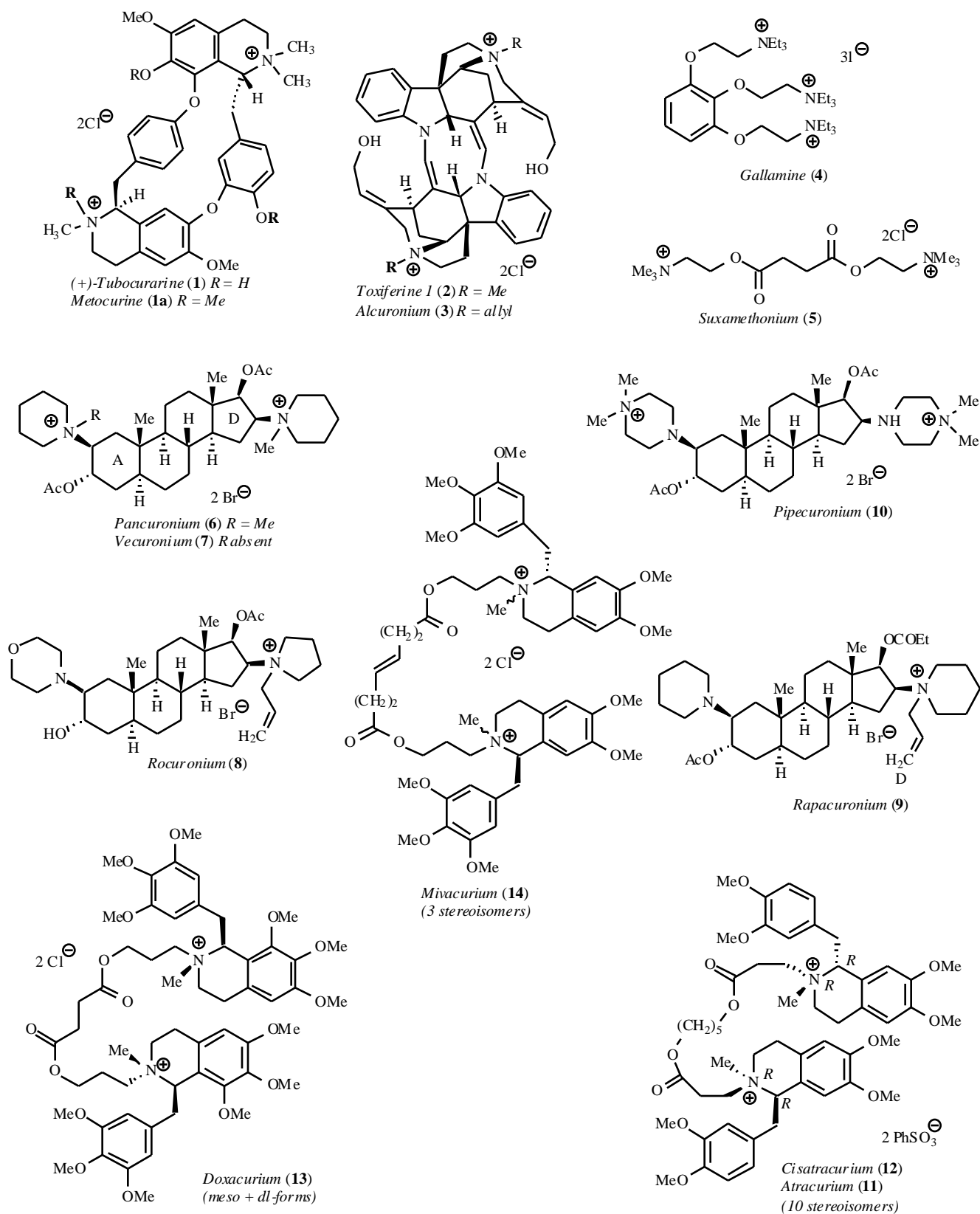
Fig. (1). Neuromuscular blocking drugs.

resulting in M_3 muscarinic receptor-mediated airway smooth muscle constriction has been proposed [22]. Scientists at Gedeon Richter Ltd. developed pipecuronium bromide (**10**) [23,24]. (Arduan, 1982), a bisquaternary aminosteroid NMB devoid of muscarinic receptor mediated cardiovascular side effects due to the absence of ACh-like structural units. Both quaternary N-atoms of pipecuronium **10** are three atom distance farther away from the steroid nucleus than in

pancuronium **6** resulting in a 5 Å greater separation of both onium heads (ca. 16 Å). Guided by the spontaneus Hofmann degradation of the alkaloid pentaline, researchers at Wellcome Laboratories and the University of Strathclyde designed a bisbenzylisoquinoline muscle relaxant atracurium besylate (**11**) (Tracrium , 1982) [25]. Atracurium, which is a mixture of 10 diastereomers, is 2.5 times more potent than (+)-tubocurarine **1** and exhibits slow onset and an intermediate duration of action ($t_{1/2}$ = 21 min) [26]. In 1996, Glaxo Wellcome launched cisatracurium besylate (**12**) (Nimbex) [27], the 1R,1′R-*cis-cis*-isomer of atracurium, which has 3-4 times the potency of atracurium and does not release histamine [28]. Like atracurium, it is mainly nonenzymatically metabolized by a Hofmann elimination to yield laudanosine and a smaller monoquaternary benzyltetrahydroisoquinoline compound, which do not have activity as neuromuscular blocker. In search for non-depolarizing NMBs with shorter and longer duration of action as atracurium, the chain lenght, ester linkage and aromatic substitution in a series of bisbenzyltetrahydro-isoquinolinium compounds were altered resulting in two novel muscle relaxants. Doxacurium chloride (**13**) (Nuromax , 1991) [29] is a long-acting NMB devoid of cardiovascular side effects. Mivacurium chloride (**14**) (Mivacron ,1992) [30] is a rapidly enzymatically metabolized, short acting drug with a fast recovery profile.

The existing NMBs can be categorized by their duration of action as long (doxacurium **13**, pipecuronium **10**, pancuronium **6**), intermediate (atracurium **11**, cisatracurium **12**, rocuronium **8**, vecuronium **7**), short-acting (rapacuronium **9**, mivacurium **14**), and ultra-short-acting (suxamethonium **5**) relaxants [31].

Due to the above-mentioned unwanted side-effects of the polarizing drug suxamethonium, the recent research efforts mainly focused on the development of ultra-short-acting non-depolarizing muscle relaxants with rapid onset and without cardiovascular side-effects for rapid sequence emergency intubations and treatment of laryngospasm. The present

paper reviews the literaure from the current millennium focusing on new structures and structure-activity relationships, as well as on the pharmacological profile of some novel NMBs being in preclinical development. For earlier literature, the reader is referred to the comprehensive reviews by Tuba *et al.* (2002) [32], Lee (2001) [33], Hill *et al.* (1994) [34], and Kharkevich (1990) [35]. A short historical overview on discovery and development of NMBs is given by McKenzie (2000) [36] and Raghavendra (2002) [37]. Comparison of the pharmacological properties of the newly approved drugs to those of the older agents was published by Moore and Hunter (2001) [38], Donati (2000) [39] Paul *et al.* (2002) [40] and Sparr *et al.* (2001) [41].

TETRAHYDROISOQUINOLINIUM ANALOGUES

In search for the substitution of the ultra-short-acting NMB suxamethonium **5**, scientists at GlaxoSmithKline examined a new class of non-depolarizing, mivacurium-derived NMBs possessing a chlorofumarate moiety in the middle-chain spacer [42]. Unlike mivacurium **14**, which is a mixture of three diastereomers, all compounds were synthesized as single stereoisomers. The NMB properties were determined in rhesus monkeys, which provide the best predictive profile for the duration of action in humans [43]. The potency was expressed as a dose producing 95% supression of the twitch response at the *extensor digitorum* (ED_{95}), the onset was measured as the time from injection to peak inhibition of the twitch response at the ED_{95} dose, duration was determined as the time from injection to 95% recovery of the twitch response at the ED_{95} dose. The cardiovascular side-effects were assessed as a dose producing >10% of change in heart rate or arterial pressure (CV dose), The selectivity index (SI) was defined as CV dose devided by the ED_{95} dose. The biological data of all discussed compounds including suxamethonium **5** and mivacurium **14** are listed in (Table **1**). The initial lead molecule was the symmetrical bisbenzylisoquinolinium agent **15**, which is 16-fold more potent than suxamethonium having a two minutes
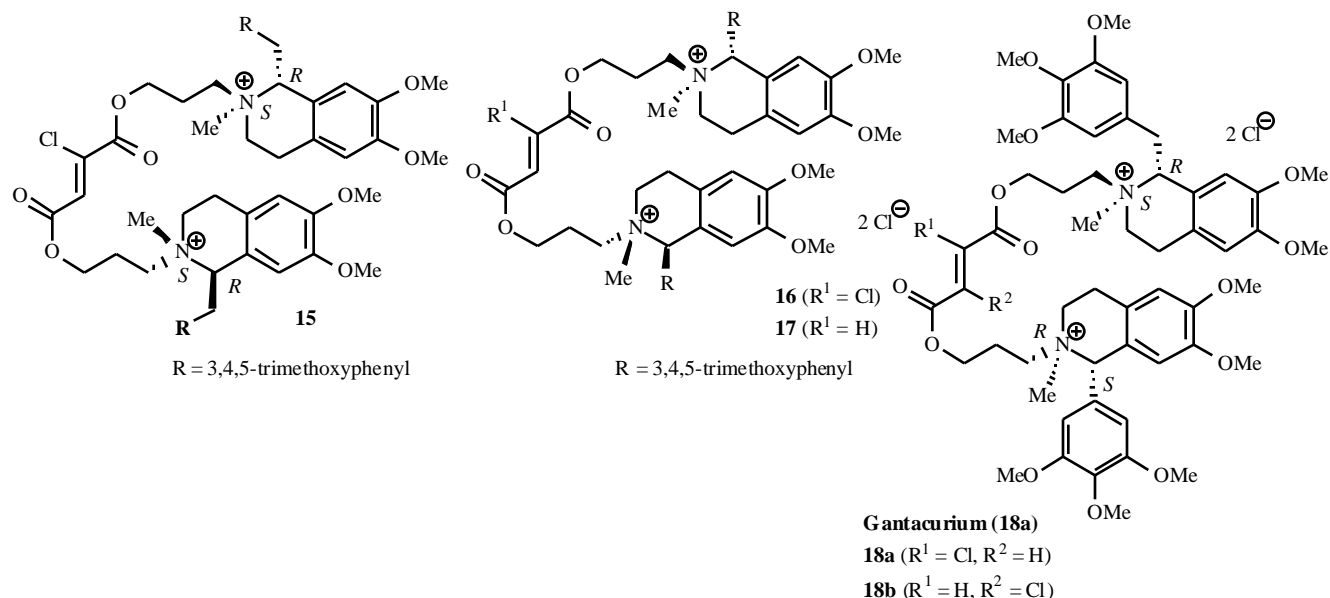

R = 3,4,5-trimethoxyphenyl

15

R = 3,4,5-trimethoxyphenyl

16 (R^1 = Cl)
17 (R^1 = H)

Gantacurium (18a)
18a (R^1 = Cl, R^2 = H)
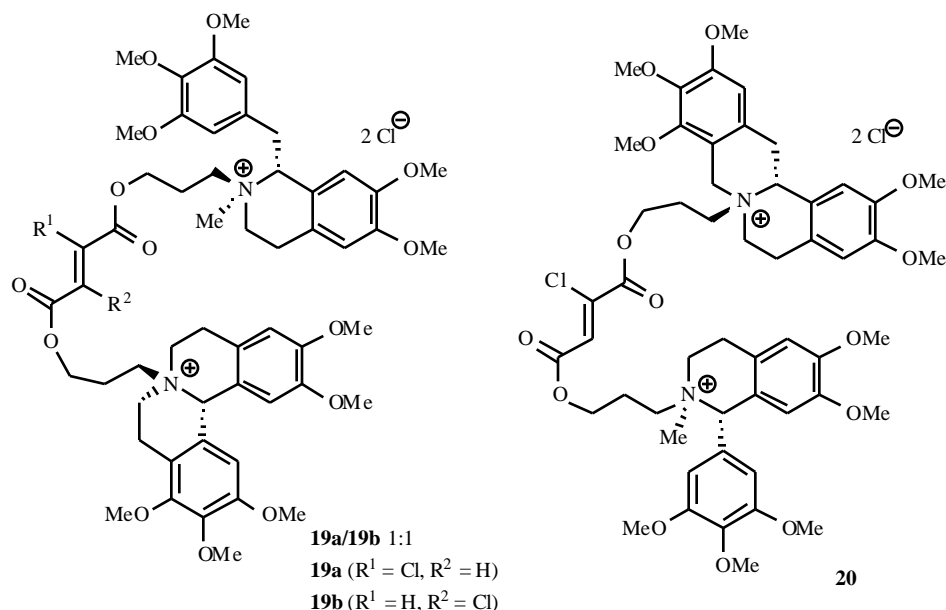**18b** (R^1 = H, R^2 = Cl)

Fig. (2).

Fig. (3).

longer duration of action (7.1 min), a twice as long onset (96 s) as the latter, and a selectivity index similar to that of mivacurium (SI = 20) [44]. Replacement of the trimethoxybenzyl groups in **15** by the equally substituted phenyl moieties to give **16** produced a ca. 20-fold decrease in neuromuscular blocking activity, reduction of onset time (46 s) and a 4-fold higher CV dose. The rapid onset time of **16** (46 s) is typicall of NMBs with low potency [45] due to the high plasma concentrations required and the resulting rapid equlibration between plasma and the receptor binding site [46]. The deschloro analogue of **16** (compound **17**) was found to be 5-fold longer acting than **16** indicating the importance of the chlorine atom in determining ultra-short duration of action.

A novel unsymmetrical analogue of **15** was prepared by replacing one of the *trans*-(1*R*,2*S*)-benzyltetrahydroisoqui-nolinium group by the *trans*-(1*S*,2*R*)-pheny-ltetrahy-droisoquinolinium moiety. The resulting mixed-onium -chlorofumarate gantacurium chloride (GW 280430A) **(18a)** displayed good NMB potency (ED$_{95}$ = 0.063 mg/kg), ultra-short duration of action (5.6 min) and an improved selectivity index (SI = 57). The corresponding -chlorofumarate **18b** was ca. 4-fold less potent than its -chloroisomer **18a** and exhibited a shorter onset (52 s) and a shorter duration of action (3.4 min). Replacing the *trans*-(1*S*,2*R*)-phenyltetrahydroisoquinoline relative configuration in a **18a**/**18b**-mixture by the enantiotopic (1*R*,2*S*)-stereochemistry decreased both duration of action and NMB potency. Removal of the *N*-methyl substituent from the phenyltetrahydroisoquinoline group of **18a** to give the corresponding monoquaternary agent reduced NMB potency by a factor of 14 and did not affect the duration of action. Replacing methoxy groups on the exocyclic phenyl ring of **18a** with hydrogen or fluorine atoms maintained the high potency and ultra-short duration, but reduced the selectivity ratios (SI = 6-18).
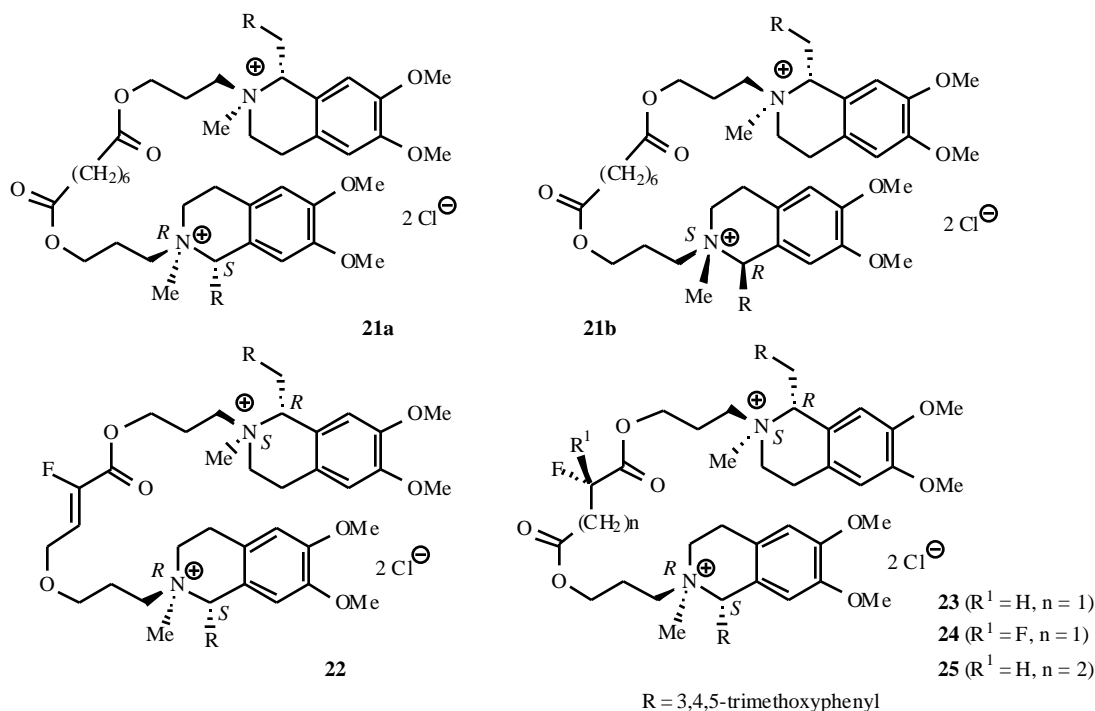
Effects of conformational restriction of the aromatic groups and alkanedioate middle-chain linkers on the NMB properties of mixed-onium NMBs were also examined. Restricting the phenyltetrahydroisoquinolinium group of the **18a/18b**-mixture in the form of a dibenzo[*a*,*h*]quinolizidine resulted in an equally high potent **19a/19b** mixture with a 11-fold lower selectivity index (SI = 5). On the other hand, constraining the benzyltetrahydro-isoquinolinium moiety of **18a** as dibenzo[*a*,*g*]quinolizidine yielded compound **20** with a 13-fold lower potency.

Table 1. **Neuromuscular Blocking Effects and Cardio-Vascular Selectivity of New Tetrahydroisoquino-linium Analogues in Rhesus Monkeys [42]. (for Details, See Text)**

compd	ED$_{95}$ [mg/kg]	onset [s]	duration [min]	SI[a]
suxamethonium **5**	1.29	50	5.0	nt
mivacurium **14**	0.062	107	12	24
15	0.079	96	7.1	20
16	1.79	46	5.1	3.6
17	1.55	114	25.5	0.65
gantacurium **18a**	0.063	88	5.6	57
18b	0.25	52	3.4	nt
19a/19b (1:1)	0.078	94	5.2	5
20	0.8	28	4.5	nt
21a	0.067	117	10.9	12
21b	0.103	75	8.6	47
22	0.137	66	4.4	37
23	0.05	80	35	nt
24	0.056	78	7.3	96
25	0.22	48	5.7	nt

[a] cardiovascular selectivity index (CV dose/ ED$_{95}$)

21a   **21b**

22

23 ($R^1 = H$, $n = 1$)
24 ($R^1 = F$, $n = 1$)
25 ($R^1 = H$, $n = 2$)

R = 3,4,5-trimethoxyphenyl

Fig. (4).

Replacement of the chlorofumarate middle chain in **18a** by an octanedioate linker (compound **21a**) prolonged the duration of action (10.9 min) and caused a worse selectivity (SI =12) without changing the high NMB activity. The (1*R*,2*S*)-stereoisomer of **21a** (**21b**) had an excellent selectivity index (SI = 47). Increasing or decreasing of the lenght of the midle chain of **21a** by one or two carbon atoms produced less potent NMBs. Substitution of the chlorine atom in **18a** by the fluorine to give compound **22** produced a 2-fold decrease in NMB potency, a 20% shorter duration of action, a 25% shorter onset, and less favourable selectivity (SI = 37). Saturation of the double bond in the middle-chain spacer of **22** yielding the monofluorosuccinate **23** increased NMB potency ba a factor 3, but prolonged remarkably the duration of action (35 min). The corresponding difluorosuccinate **24** was shorter acting than **23** (duration = 7.3 min) indicating that the number of linker fluorine atoms controls the duration of NMB effect. Difluorosuccinate **24** is an especially potent NMB (ED_{95} = 0.056 mg/kg) with an exceptionally high selectivity index value (SI = 96). Unfortunately, its extreme hydrolytic instability makes its further pharmaceutical developement difficult. The corresponding difluoroglutarate **25** was also ultra-short acting (duration 5.7 min), but four times less potent NMB agent.

Gantacurium chloride **18a** is currently in a clinical evaluation as a possible alternative to suxamethonium **5**. The results of the preliminary trials in healthy human volunteers were described by Belmont *et al*. [47]. Studies in whole human blood revealed that the ultra-short duration of action of **18a** is neither due to plasma cholinesterases nor to chemical hydrolysis of the chlorofumarate ester group. The major deactivation pathway of **18a** involves the reaction with serum cysteine to give the inactive thiazolidine **26** (Fig. **5**) [42].


26, R = 3,4,5-trimethoxyphenyl
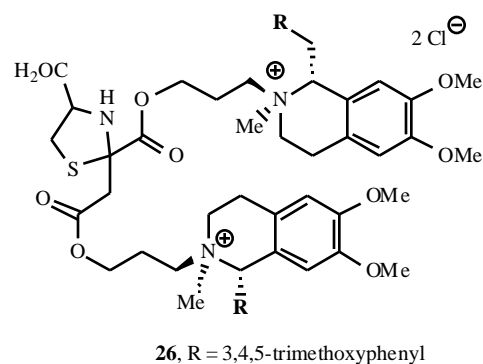
Fig. (5). Main metabolite of gantacurium in whole human blood [42].

A recovery mechanism of this type could provide a clinical advantage by eliminating problems of prolonged neuromuscular blockade in patients with reduced plasma cholinesterase activity. The solid state characterization [48] and preformulation studies of gantacurium chloride have been recently reported [49].
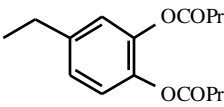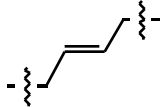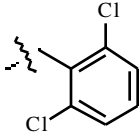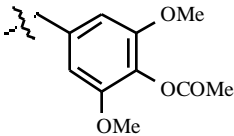
BISTROPINYL DIESTER ANALOGUES

Bisquaternary agents comprised of two tropane skeletons connected with different spacers have been known as potent NMBs for ca. 65 years. However, due to disturbing degree of autonomic ganglion block and/or cardiac vagal block/tachycardia, no muscle relaxant from this series has been introduced into the clinical practice so far. A comprehensive paper by Gyermek describing structure-activity relationships among over 250 derivatives of dicarboxylic esters of tropine has been published recently [50]. The most promising agents in terms of rapid and short neuromuscular block with negligible side-effects are

Table 2. **Neuromuscular Blocking Effects and Cardiovascular Selectivity of Bistropinyl Diester Analogues in Rats [50]**
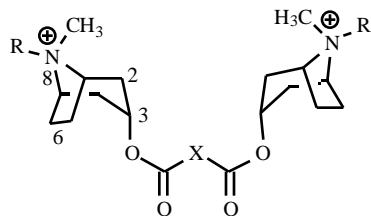
compd	R	X	ED$_{50}$ [μmol/kg]	onset [min]	recovery index ^{a}[min]	CVB^b [%]
mivacurium **14**			0.14	2.2	3.9	-
atracurium **11**			0.4	1.5	2.9	0
rapacuronium **9**			1.6	0.75	0.65	92
rocuronium **8**			0.87	0.95	1.3	60
27	-Me	-(CH$_2$)$_3$-	4.7	1.2	4	0
28	-Me	-(CH$_2$)$_8$-	0.8	1.3	2.0	-
29	-Me	-(CH$_2$)$_{10}$-	1.2	0.9	2.0	100
30	-C$_2$H$_5$	-(CH$_2$)$_3$-	3.6	1.6	2.6	-
31	-benzyl	-(CH$_2$)$_3$-	4.8	1.8	3.7	53
32	-benzyl	-(CH$_2$)$_8$-	0.2	1.5	3.2	80
TAAC3 (**33**)		-(CH$_2$)$_3$-	0.29	0.75	0.55	7
34	" "	-(CH$_2$)$_8$-	0.17	0.5	0.5	90
35	" "		0.10	0.65	0.5	32
36	" "		0.40	0.9	0.9	1
37	" "		0.27	0.7	0.45	5
38			0.19	0.6	0.5	22
39			0.08	1.0	1.9	32
40			0.45	1.4	2.0	66

(Table 2). contd.....

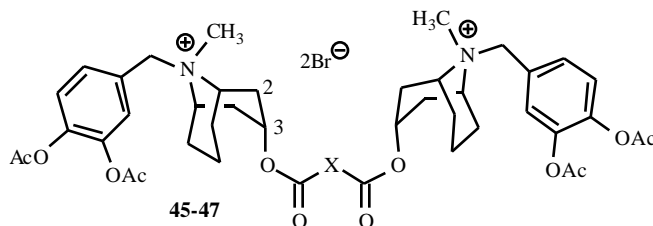| compd	R	X	ED_{50} [μmol/kg]	onset [min]	recovery index [a][min]	CVB[b] [%]
41	OCOPr / OCOPr	-(CH$_2$)$_3$-	0.36	0.7	0.5	0
42	" "		0.36	1.0	0.75	0
43 (G-1-64)	Cl / Cl	-(CH$_2$)$_3$-	1.3	1.2	3.5	90
44	OMe / OCOMe / OMe	-(CH$_2$)$_8$-	0.38	0.4	0.5	99

[a]25-76% recovery time of the evoked muscle response, [b]cardiovascular block at the 80% NMB dose

bisquaternary 3 -tropinyl diesters. Structure-activity relationships in this series of compounds are demonstrated based on NMB properties of selected agents listed in (Table **2**).

The pharmacological data were assessed using electromyography in anesthetized rats. Due to the relatively low sensitivity of the rat to most types of NMBs, the relatively large dosis needed to produce NMB also produce cardiovascular block (CVB) making the rat to a particularly valuable species for detecting the CVB side-effects in the early stage of pharmacological evaluation. Additional studies in pig, cat, and monkey were also carried out for selected compounds. Comparison of the *N*-methylated tropinyl diesters with different chain lenghts of the middle spacer exemplified by **27** (n=3), **28** (n=8), and **29** (n=10) indicated a maximal NMB potency for the C$_7$-C$_9$ compounds, while no clear correlation of the onset time and the presence of cardiovascular block with the chain lenght was observed. Substitution of the methyl groups in **27** by the ethyl substituents, yielding compound **30,** did not change the NMB properties remarkably. Further increase in the alkane chain length produced less potent NMBs. Replacement of the methyl groups in **27** and **28** by benzyl substituents yielding compounds **31** and **32**, respectively, produced compounds with cardiovascular blocking properties. Monosubstitution of the benzyl rings in **31** by chloro-, bromo-, methyl-, methoxy-, and nitro groups did not change the NMB properties remarkably leading generally to slightly more potent compounds; none of these agents were ultra-short acting. Introduction of two acetoxy groups in positions 3 and 4 of the benzene rings of **31** and **32**, yielding compounds **33** and **34**, respectively, resulted in a very favourable NMB action profile including high potency (ED$_{50}$ = 0.29 and 0.17 μmol/kg, respectively), very fast onset (0.75 and 0.5 min, respectively), and short recovery times

(both 0.5 min) caused by the hydrolyzable acetate groups. While compound **34** with the octylene middle chain exhibited a CVB of 90 %, the corresponding agent **33** with the propylene middle chain had only minimal CVB (7 %). Conformational restriction of the middle chain in the form of the *trans*-5-norbornene-2,3-*trans*-dicarboxylyl spacer (compound **35**) produced a very potent agent (ED$_{50}$ = 0.10 μmol/kg) with rapid onset (0.65 min) and short recovery index (RI = 0.5 min) exhibiting a cardiovascular blockade of 32%. Replacing of the norbornene spacer by the fumaryl diester (compound **36**) and cyclobutane-1,2-*trans*-dicarboxylyl (compound **37**) moieties reduced the CV blockade to 1% and 5%, respectively, while the NMB potency was only slightly lower. Introduction of other spacers in the 3,4-diacetoxybenzyl series such as cyclohexane-1,3-*trans*-dicarboxylyl-, hydromuconyl-, phthaloyl-, *iso*-phthaloyl-, tere-1,4-phthaloyl-, and 4,5-dichlorophthaloyl moieties produced potent agents (ED$_{50}$ = 0.15-0.64 μmol/kg) with CV blocking activity from 12% to 64%.

The effect of other benzyl substituents than acetoxy groups was also examined. Replacing of the 4-acetoxy groups in **35** by methoxy substituents (compound **38**) did not change the pharmacological profile remarkably. The 2,5-dimethoxybenzyl analogue with a norbornene spacer (compound **39**) was the most potent agent in this series (ED$_{50}$ = 0.08 μmol/kg) with a 32% CVB. Introduction of a nitro group in a 5-position of the 2-methoxybenzyl substituted agents, exemplified by compound **40**, led generally to CVB active agents. 2,5-dimethoxybenzyl-, 2,4-difluorobenzyl, and 2-chloro-6-fluoro-derivatives with the propylene middle chain also exhibited CVB activity (43-60 %). In contrast, the 3,4-dipropionyloxybenzyl analogues with the propylene and fumaryl spacers (compounds **41** and **42**, respectively) showed no CVB activity at NMB doses.

Table 3. **Neuromuscular Blocking Effects and Cardiovascular Selectivity of Granatane Diester Analogues in Rats [50]**

45-47

compd	X	ED_{50} [μmol/kg]	onset [min]	recovery index[a] [min]	CVB^b [%]
45	-(CH$_2$)$_3$-	0.2	0.6	3.2	80
46		0.38	0.55	0.45	
47		0.15	0.50	0.35	2

[a]25-76% recovery time of the evoked muscle response, [b]cardiovascular block at the 80% NMB dose.

The 2,6-dichlorobenzyl substituted analogue with a propylene spacer **G-1-64** (compound **43**) is, despite its CVB activity in the rat, one of the best explored agents with rapid onset and short duration of action in this series [51]. Pharmacological studies in anesthetized rats, rabbits, cats, ferrets, pigs and monkeys revealed for G-1-64 ED$_{50}$ values ranging between 60 and 800 µg/kg with varying degree of CVB, dependent on the species, at doses exceeding the ED$_{80}$ for NMB. G-1-64 showed a faster onset (0.9-2.1 min) and/or shorter duration of action (5-12 min) than either atracurium **11** or mivacurium **14**. Cardiovascular changes, ganglion block and histamine release were absent.

A number of trisubstituted benzyl derivatives was also examined. Notable compound is the 3,5-dimethoxy-4-acetoxy derivative (**44**) with the octylene spacer having the fastest onset (0.4 min) among the entire series.

48
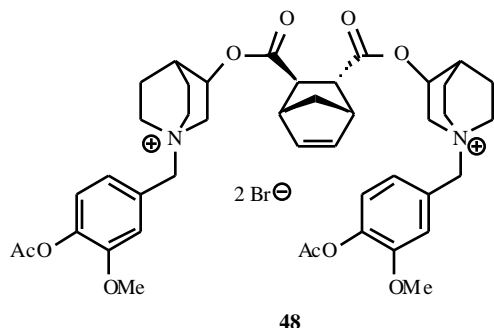
Fig. (6).

The effect of several structural changes of the tropane ring system was also explored. Changing the steric position of the 3-OR group from 3 to 3 yielded less potent agents with more CVB. Shifting the ester group from position 3 to the C2 carbon atom of the tropane ring also resulted in inferior compounds. Changing the position of the quaternary nitrogen atom from 8 to 6 produced compounds with lower

potency, although rapid onset and short recovery pattern were maintained. Reducing the tropane (8-azabicyclo[3.2.1]octane) ring system to 7-azabicyclo[2.2.1]heptane (2 -stereoisomer) yielded inferior compounds. In contrast, expanding the tropane ring to 9-azabicyclo[3.3.1]nonane ring resulted in highly potent and rapidly acting granatane 3 -ol esters, as illustrated by the pharmacological data of compounds **45-47** shown in (Table **3**). The overall NMB profile in the rat was identical or better than that of the corresponding 3,4-diacetoxybenzyl substituted tropine diester derivatives. Similar agents incorporating the quinuclidinium heterocycles, exemplified by compound **48**, were published in a patent literature [52].

Table 4. **Neuromuscular Blocking Effects and Cardiovascular Selectivity of TAAC3 (33) in Different Animal Species in Comparison to Rocuronium 8 [54]**

animal		ED_{90} [mg/kg]	%CVB at $ED0_{80}$ NMB	onset [min]	duration [min]
rat	TAAC3	0.43	10	0.75	1.8
	rocuronium	0.99	60	0.95	2.5
rabbit	TAAC3	0.11	-	0.9	2.5
	rocuronium	0.09	-	1.2	5.8
cat	TAAC3	0.34	8	1	2.4
	rocuronium	0.10	10	1.25	4.2
dog	TAAC3	0.30	-	0.9	2.5
	rocuronium	0.12	-	1.9	6.7
monkey	TAAC3	0.15	-	0.9	2.7
	rocuronium	0.07	-	1.9	5.7

Considering all aspects of the NMB and side effect profiles, as as well as the facility of synthesis, bis[*N*-(3,4-diacetoxybenzyl) tropanium-3 -yl] glutarate dibromide **TAAC3 (33)** was chosen for further pharmaceutical development of an ultra-short-acting non-depolarizing muscle relaxant. TAAC3 is a mixture of two *N*-quaternary isomers in a 9:1 ratio. Within the major isomer, the *N*-diacetoxybenzyl group is at the *equatorial* position with respect to the piperidine ring of tropane [53]. TAAC3 was evaluated for NMB actions and for autonomic and cardiovascular side-effects in seven different animal species [54]. The potency, onset, duration of action and CVB for selected animals are summarized in comparison to rocuronium **8** in (Table **4**).

TAAC3 exhibited NMB 90% effective doses ranged from 0.09 to 0.43 mg/kg, depending on the species. It had a faster onset (0.8-1.0 min), shorter recovery index (0.6-1.1 min), and shorter duration of action (1.8-3.5 min) than rocuronium. It produced a slight cumulative effect on infusion, but not on repeated single-dose administration. The CVB effect of TAAC3 in equipotent NMB doses is larger than those of atracurium **11**, mivacurium **14**, and vecuronium **7**, is similar to that of rocuronium **8**, and is of lesser degree than those of pancuronium **6** and rapacuronium **9**. Projected to humans, such a degree of CVB may be clinically acceptable. There was no demonstrable sympathetic ganglion block in the cat. The short duration of action of TAAC3 can be explained by the rapid hydrolysis of the phenol acetate groups followed by a subsequent 1,4-elimination of tropanyl glutamate as illustrated in Scheme **1** [54].
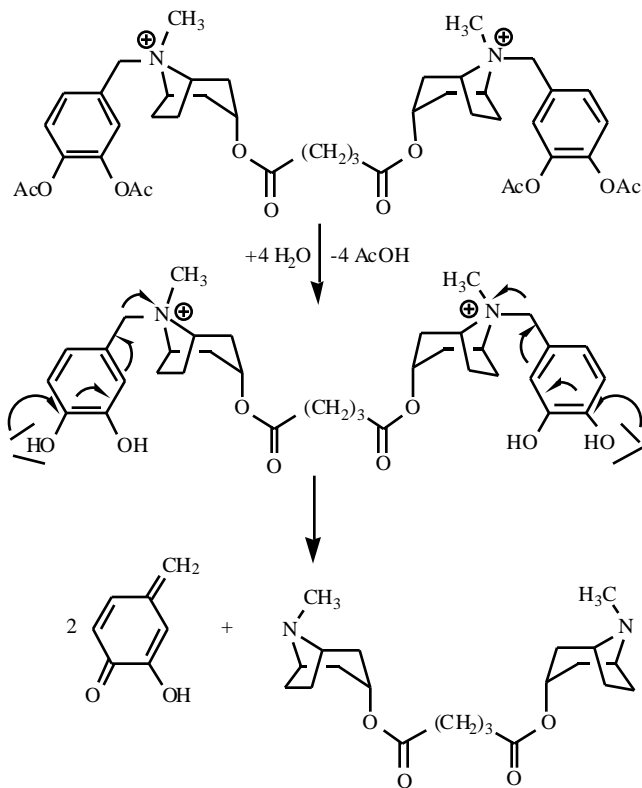
Scheme 1. Putative metabolic breakdown of **TAAC3** (**33**) [54].

This rapid breakdown occurs spontaneously *in vitro* und is probably catalyzed *in vivo* by nonspecific plasma esterases,

that seem to preferentially speed up the degradation of the phenol acetate groups and not the ester moieties of the dicarboxylic acid ester linker (many other quaternary ammonium derivatives of tropinyl diesters, as well as of some tetrahydroisoquinoline diesters, e.g. mivacurium **14** and doxacurium **13**, lacking acyloxy benzyl groups, show a considerably longer duration of action than compounds **33**-**37**. TAAC3 (**33**) and some of its analogues are currently undergoing intensive preclinical development by Organon-Teknika, NV, and Organon Laboratories.

AMINOSTEROID ANALOGUES

Since the introduction of pipecuronium **10** into anaesthetic and surgical practice as a NMB agent of a medium duration of action without cardiovascular side-effects in 1982, scientists at Gedeon Richter Ltd. synthesized a great number of aminosteroid NMB agents in search for a rapid acting ultra-short non-depolarizing muscle relaxant to replace suxamethonium [32]. From ca. 120 newly prepared compounds **SZ-1677** (**49**) seems to have the desired pharmacological profile. SZ-1677 can be derived from rocuronium by a formal substitution of the O-atom in the morpholine ring by the ethylene glycol acetal moiety. The action of **49** on neuromuscular transmission, muscarinic M_2 and M_3 receptors and cardiovascular reactions has been recently examined and its properties compared to those of other aminosteroid muscle relaxants (see Table **5**) [55].

Table 5.   NMB Effects of SZ-1677 and SZ-1823 in Comparison to Other Muscle Relaxants [55]

49 R = Ac
50 R = H

compd	species	ED_{90} [µg/kg]	onset [s]	recovery rate[a] [min]
SZ-1677 (**49**)	rat	186	41	3.2
	guinea pig	24	73	3.1
SZ-1823 (**50**)	guinea pig	662	66	5.9
rocuronium **8**	rat	1125	30	1.9
	guinea pig	98	51	2.8
rapacuronium **9**	rat	5953	27.5.	1.2
	guinea pig	187	32.5	3.1
pancuronium **6**	guinea pig	18	66	14.8
vecuronium **7**	guinea pig	15	72	9.0
pipecuronium **10**	guinea pig	6	88	24.0

[a] time needed to recover from 75% to 25% inhibition.

SZ-1677 is a non-depolarizing NMB with a relatively short duration of action in the rat and in the guinea pig. In *in vivo* experiments in guinea pigs, SZ-1677 was 32-fold more potent than rapacuronium **9**, 6-fold more potent than rocuronium **8**, 12-fold less potent than vecuronium **7**, 10-fold less potent than pancuronium **6**, and 30-fold less potent than pipecuronium **10**. According to the inversed potency-onset relationship observed in other NMB series, the onset time of SZ-1677 in the guinea pig (73 s) was longer than those of the less potent rapacuronium **9** (32.5 s) and rocuronium **8** (51 s), and shorter than that of pipecuronium **10** (88 s). SZ-1677 is devoid of cumulative effects, and fails to produce tachycardia. Cardiac vagal blocking effect was not observed even at concentrations 8-20 times of neuromuscular ED$_{90}$. Unlike pancuronium **6**, rocuronium **8** and rapacuronium **9**, SZ-1677 does not have an atropine-like effect on human atrial tissue and it does not increase the release of noradrenaline from the sympathetic innervation of the heart. The findings indicate that the safety margin between the doses required to produce NMB and those likely to lead to cardiovascular side-effects observed for SZ-1677 is greater than for others clinically used NMB agents.

The main metabolite of SZ-1677 is the desacetyl 17-OH derivative **SZ 1823 (50)**. Similar to the other 17-desacetyl steroidal muscle relaxants, SZ-1823 has a very week NMB activity in guinea pig (see Table **5**). In contrast to the 3-OH derivatives of other steroidal NMB agents, acetylation of the 3-OH group of SZ-1677, led to a two-fold decrease in NMB potency [32].

Further contributions to novel steroidal muscle relaxants were made by the scientists of the Panjab University, Chandigarh, India, who already developed chandonium (**51**), a potent, short-acting azasteroidal agent with rapid onset. Combination of the structural features responsible for the NMB activity of pancuronium **6** (ring D) and of chandonium **51** (ring A), led to the new steroidal muscle relaxants **52-53** (Fig. **7**) [56].

The NMB activity was assessed on the isolated chick biventer cervicis muscle preparation in comparison with (+)-tubocurarine **1**. While compound **53** was 50-fold more potent than (+)-tubocurarine **1**, the corresponding 17-desacetoxy derivative **52** exhibited only 43 % the potency of **1**, showing once again the importance of the 17-acetoxy group in combination with the 16-quaternary nitrogen for high potency. Removal of the methyl group from the pyrrolidinium nitrogen atom produced only a modest drop in potency confirming the importance of the D-ring substitution similar to pancuronium **6** and vecuronium **7**. Reducing of the size of the piperidine ring of **53** by one

carbon atom resulting in the pyrrolidine moiety caused a 15-fold decrease in NMB potency [57]. A subsequent substitution of the 2-pyrrolidinium moiety by a hydroxy group produced a 6-fold less potent agent.

The time course of action of **53** was investigated in anaesthesized cat [56]. The onset of action (2.9 min) was in the rapid category, beeing the same as that of rocuronium **8** and shorter than that of the medium-onset vecuronium **7** (5.0 min). Both the recovery and duration times of the block were long (recovery time from 25 to 75% of initial twitch = 9.9 min, time from injection to 90% recovery = 26 min). **53** had no effect on the concentration of the pre-ganglionically-stimulated nictitating membrane, indicating a lack of ganglion-blocking activity. There was no effect on general arterial blood presure, but **53** produced a moderate reduction of the bradycardial response of the heart rate to vagal stimulation, indicating a block of cardiac muscarinic receptors.

Another two related muscle relaxants **54-55** were obtained by combination of the structural features responsible for the NMB activity of pipecuronium **10** (ring D) and chandonium **51** (ring A) (Fig. **7**) [58]. Investigations on chick biventer cervicis muscle preparations revealed the 17-acetoxy derivative **55** to be 50-fold more potent than (+)-tubocurarine **1**; the corresponding 17-OH analogue **54** had only twice the potency of **1**. Interestingly, while investigated in anaesthesized cat, both compounds were equipotent beeing ca. 2-3 times more potent than rocuronium **8** and ca. 4 times less potent than vecuronium **7**. Compound **55** was considerably slower in onset (3.9 min) than compound **54** (1.6 min) and had a long duration (**55**: 38.8 min; **54**: 8.4 min) beeing twice that of vecuronium (17.4 min). Conversly, the time course of action of **54** was unexpectedly short (recovery time from 25 to 75% of initial twitch = 3.0 min) compared to that of **55** (16.9 min) and of rocuronium (5.2 min). Similarly to **53**, compound **55** exhibited no ganglion-blocking activity, and slightly blocked cardiac muscarinic receptors. In contrast, the 17-desacetoxy derivative **54** produced severe effect on the cardiovascular system, including a lasting tachycardia and profound inhibition of the vagally induced bradycardia.

BISQUATERNARY AMINO DI- AND TRIPEPTIDE ANALOGUES

Most non-depolarizing NMB agents possess two alkylated amino nitrogens separated by an approximate distance between 10 and 14 Å with at least one of these quaternized, leading to a permanent positive charge.
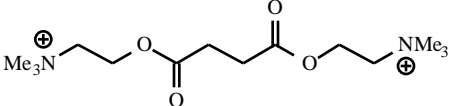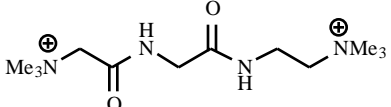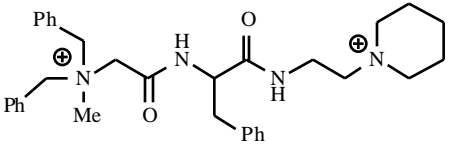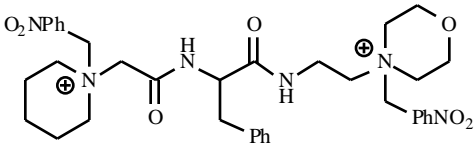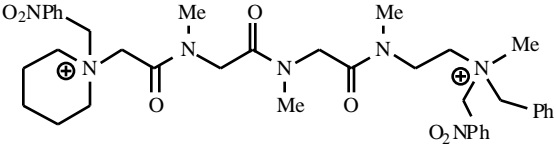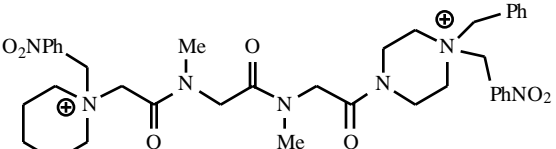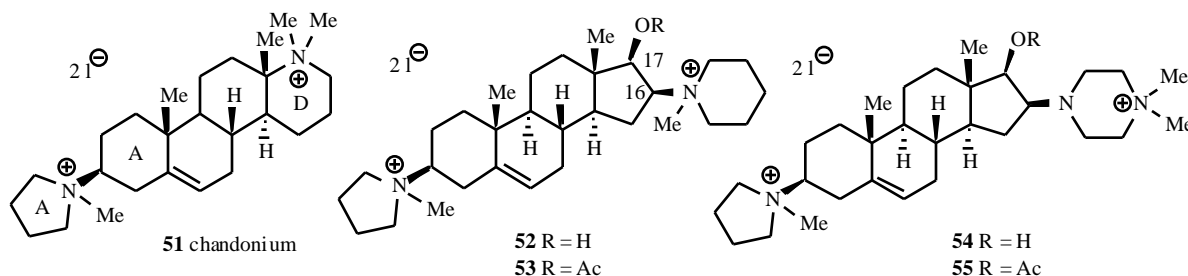

Fig. (7).

Table 6.   **Structures, *in vitro* Activity and Calculated Lipophilicity (clog*P*) of Bisquaternary Dipeptide Derivatives Measured in Chick Biventer [59]**

compd.	structure	$ED_{50}{}^{a}$ [μmol]	clog*P*
suxamethonium		0.54	
pancuronium **6**		0.2	1.21
rocuronium **8**		0.76	0.6
atracurium **11**		0.41	2.36
56		>390	-
57		134	-2.93
58		19.8	2.01
59		0.4	0.31
60		0.7	1.57

[a]dose causing a 50% block of muscle twitch.

Researchers at Organon Laboratories investigated whether compounds with a short peptide fragment between two ammonium cation heads could serve as non-depolarizing NMBs and explored the structure-activity relationship of such a series [59]. A peptide scaffold could confer a number of desirable properties including rapid *in vivo* degradation to nontoxic metabolits, low propensity to cross the blood-brain barrier, and straightforward synthesis from inexpensive starting materials. Conformational analysis suggested that the distance between the quaternary nitrogens with a tripeptide spacer is likely to be 11.5 – 14.9 Å, while the corresponding distance in the dipeptide derivatives will be 9.1 – 11.3 Å. Thus, both the di- and tripeptide analogues were investigated. The initial biological screen was *in vitro* isolated chick biventer muscle preparation. The dose causing 50% block of electrically stimulated muscle twitch was used

to assess the NMB potency of the new compounds in comparison to the corresponding data for suxamethonium **5**, pancuronium **6**, rocuronium **8** and atracurium **11** (see (Table **6**).

The first dipeptide derivative investigated, the Gly-Gly analogue **56** possessing an ethylenediamine moiety, was inactive. Replacing of the trimethylammonium group at the one side of **56** by cyclic quaternary groups present in known NMBs: i.e. the morpholine, the pyrrolidine, and the tetrahydroisoquinoline failed to increase NMB activity. However, when both of the quaternary groups of **56** were changed, as in **57**, the NMB potency was modestly increased. Increasing the lipophilicity and conformational restraint within the dipeptide scaffold by incorporating a phenyl residue and introducing cyclic quaternary groups at

both nitrogen atoms combined with *p*-nitrobenzyl substitution led to the most active compound among the dipeptides **58**. In the tripeptide series, the most active agents, **59** and **60** reached the potency of atracurium **11** and rocuronium **8**, respectively. In both compounds the amide nitrogen atoms are methylated and the quaternary nitrogen atoms are substituted with *p*-nitrobenzyl groups. The chick biventer ED_{50} values of the investigated peptides correlated well with the lipophilicity (calculated log*P* values) i.e., increasing the overall lipophilicity resulted in increased activity *in vitro*. **59** and **60** maintained their high NMB activity in anaesthetized cats. The NMB properties in rhesus monkeys are displayed in (Table **7**).

60 has a similar potency (ca. 0.2 μmol/kg) and onset time (ca. 3 min) to rocuronium. **59** has a similarly rapid onset NMB as suxamethonium **5** (ca. 1.5 min) beeing 7-fold more potent than the latter. Unfortunately, both peptides have considerably longer duration and slower recovery than rocuronium **8** and suxamethonium **5** which is an undesired profile for short duration surgery.

Table 7.   NMB Properties of the Tripeptide Agents 59 and 60 in Anaesthetized Rhesus Monkeys [59]

compd.	ED_{90} [μmol/kg]	onset [min]	duration 90% [min]	recovery 25-75% [min]
suxamethonium **5**	5.04	1.5	9.7	2.9
rocuronium **8**	0.16	3.1	5.0	13.6
59	0.68	1.7	27.7	17.6
60	0.23	2.9	42.5	15.6

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