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Synthesis of 6-methylimidazo[2,1-*b*]thiazole derivatives acting on neuromuscular transmission

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Introduction

Since the first report on the effects of neuromuscular blocking agents in about 1510, research on curariform drugs has remained an active field [1–8]. Among the numerous structures employed for this purpose, 6-methylimidazo[2,1-*b*]thiazolium salts have also been examined [9] since they are analogs of the potent imidazo[1,2-*a*]pyridinium salts [10]. Our interest in the derivatives of 6-methylimidazo[2,1-*b*]thiazole with potential antiinflammatory [11], cardiotonic [12], diuretic [13], herbicidal [14] and antitumor [15] activity, prompted us to design new derivatives which could prove active on neuromuscular transmission (NT). Further interest in this field is inspired by recent reports about the activity of several cognition enhancers on NT [16–21]. These findings suggest that compounds active on NT may be a pool where new candidates for anti-Alzheimer tests can be selected, even though not all the drugs which are used in Alzheimer therapy are effective on NT [22].

For our first approach to this area, we planned the synthesis of two symmetrical bisammonium salts arising from 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid and containing a four carbon spacer: the amide **1a** and the ester **2a** (fig 1). Since the preliminary pharmacological results revealed an interesting dose-related NT activity which was greater for the ester **2a**, we decided to prepare the analog with a six carbon spacer only for the ester **3a**. In order to study the activity of simpler monoquaternary salts, compounds **4a** and **5a** were also prepared. Finally, the starting materials **1–5** were submitted to the pharmacological tests because, if any activity had resulted, they could have been useful orally active candidates for further Alzheimer-oriented testing.

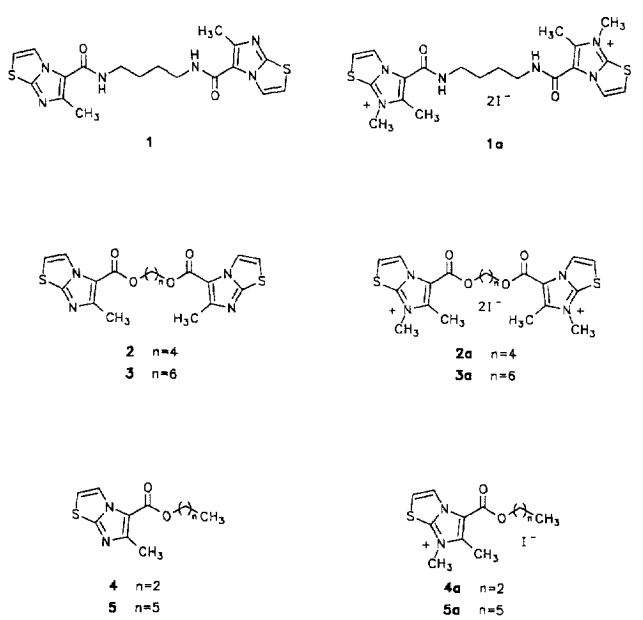

Fig 1. Compounds **1–5**.

Chemistry

A general, convenient, one-pot procedure reported for the conversion of carboxylic acids into their *t*-butyl esters [23] was used to prepare the acyl derivatives **1–5**. Accordingly, the reaction of 6-methylimidazo-[2,1-*b*]thiazole-5-carboxylic acid [24] with 1,1'-carbonyldiimidazole yielded the 1-acylimidazole which, as expected, easily gave the bisamide **1** on reaction with 1,4-diaminobutane, even in the absence of any activator. On the other hand, the reaction of the same 1-acylimidazole with 1,4-butanediol, 1,6-hexanediol, 1-propanol and 1-hexanol in the presence of 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) as activator of the alcoholic function, gave the alkyl carboxylates **2–5** in fairly good yields. The quaternary salts **1a–5a** were readily prepared by quaternization of the tertiary bases **1–5** with a large excess of refluxing iodomethane. Physical and spectral data for compounds **1–5** are given in tables I and II.

Pharmacological results

The activity of all the compounds on NT was tested on a rat sciatic nerve/gastrocnemius muscle prepara-

Table I. Compounds **1–5** and **1a–5a**.

Compound	*Formula (mw)*	*Mp (°C)*	*Recrystallization solvent*	*Yield (%)*	ν_{max} *(cm^{-1})*
1	$C_{18}H_{20}N_6O_2S_2$ (416.5)	183–185	EtOH/H_2O	63	3420, 3200, 1690, 1620, 1550, 1255
1a	$C_{20}H_{26}I_2N_6O_2S_2$ (700.4)	271–272 dec	EtOH/Et_2O[a]	86	3310, 1640, 1505, 1240, 1150, 800
2	$C_{18}H_{18}N_4O_4S_2$ (418.5)	133–135	EtOH	49	1685, 1510, 1230, 1145, 1095, 990
2a	$C_{20}H_{24}I_2N_4O_4S_2$ (702.4)	241–242 dec	EtOH/H_2O	98	1690, 1590, 1510, 1225, 1150, 925
3	$C_{20}H_{22}N_4O_4S_2$ (446.5)	172–174	EtOH	72	1675, 1510, 1235, 1145, 1100, 985
3a	$C_{22}H_{28}I_2N_4O_4S_2$ (730.4)	215–216 dec	EtOH/Et_2O[a]	98	1695, 1590, 1505, 1230, 1150, 965
4	$C_{10}H_{12}N_2O_2S$ (224.3)	58–60	Hexane	56	1680, 1540, 1520, 1240, 1140, 1105
4a	$C_{11}H_{15}IN_2O_2S$ (366.2)	167–168	EtOH/Et_2O[a]	97	1690, 1590, 1505, 1225, 1155, 1130
5	$C_{13}H_{18}N_2O_2S$ (266.4)	60–62	Hexane	51	1680, 1540, 1520, 1240, 1135, 1100
5a	$C_{14}H_{21}IN_2O_2S$ (408.3)	135–137	EtOH/Et_2O[a]	98	1710, 1595, 1510, 1230, 1140, 1105

Table II. ^{1}H-NMR of compounds **1–5** and **1a–5a**.

Compound	*δ (ppm), J (Hz) in DMSO-d_6 (th = thiazole)*
1	1.59 (4H, br s, CH_2), 2.47 (6H, s, CH_3), 3.29 (4H, m, CH_2), 7.30 (2H, d, th, J = 4.4), 7.62 (2H, t, NH), 8.04 (2H, d, th, J = 4.4)
1a	1.64 (4H, br s, CH_2), 2.60 (6H, s, CH_3-6), 3.36 (4H, br s, CH_2), 3.91 (6H, s, CH_3-7), 7.79 (2H, d, th, J = 4.1), 8.32 (2H, d, th, J = 4.1), 8.55 (2H, t, NH)
2	1.89 (4H, br s, CH_2), 2.47 (6H, s, CH_3), 4.35 (4H, br s, CH_2), 7.41 (2H, d, th, J = 4.4), 8.03 (2H, d, th, J = 4.4)
2a	1.92 (4H, br s, CH_2), 2.70 (6H, s, CH_3-6), 3.93 (6H, s, CH_3-7), 4.47 (4H, br. s, CH_2), 7.87 (2H, d, th, J = 4.2), 8.43 (2H, d, th, J = 4.2)
3	1.46 (4H, br s, CH_2), 1.74 (4H, br s, CH_2), 2.47 (6H, s, CH_3), 4.28 (4H, t, CH_2), 7.42 (2H, d, th, J = 4.4), 8.04 (2H, d, th, J = 4.4)
3a	1.47 (4H, br s, CH_2), 1.80 (4H, br s, CH_2), 2.71 (6H, s, CH_3-6), 3.95 (6H, s, CH_3-7), 4.42 (4H, t, CH_2), 7.88 (2H, d, th, J = 4.2), 8.42 (2H, d, th, J = 4.2)
4	0.97 (3H, t, CH_2CH_3, J = 7), 1.73 (2H, sex, CH_2CH_3, J = 7), 2.51 (3H, s, CH_3-6), 4.24 (2H, t, $COOCH_2$, J = 7), 7.44 (1H, d, th, J = 4.4), 8.06 (1H, d, th, J = 4.4)
4a	0.98 (3H, t, CH_2CH_3, J = 7), 1.78 (2H, sex, CH_2CH_3, J = 7), 2.71 (3H, s, CH_3-6), 3.94 (3H, s, CH_3-7), 4.37 (2H, t, $COOCH_2$, J = 7), 7.88 (1H, d, th, J = 4.3), 8.42 (1H, d, th, J = 4.3)
5	0.86 (3H, t, CH_2CH_3, J = 7), 1.32 (6H, m, CH_2), 1.71 (2H, qui, CH_2, J = 7), 2.50 (3H, s, CH_3-6), 4.27 (2H, t, $COOCH_2$, J = 7), 7.45 (1H, d, th, J = 4.4), 8.07 (1H, d, th, J = 4.4)
5a	0.88 (3H, t, CH_2CH_3, J = 7), 1.34 (6H, m, CH_2), 1.76 (2H, qui, CH_2, J = 7), 2.71 (3H, s, CH_3-6), 3.94 (3H, s, CH_3-7), 4.40 (2H, t, $COOCH_2$, J = 7), 7.88 (1H, d, th, J = 4.2), 8.42 (1H, d, th, J = 4.2)

tion, according to the procedure described in the *Experimental protocols*. Compounds **1–4**, **4a** and **5a** were inactive, whereas compound **5** produced complete blockade at 20 mg/kg.

The experiments performed on compounds **1a–3a** are reported in table III, in comparison to the activity of tetraethylammonium (TEA). It is interesting to point out the particular behaviour of these three compounds which, at low doses, showed a stimulant effect by releasing acetylcholine at a presynaptic level; this effect cannot be related to activity on the muscle. In fact on directly stimulated preparations, the compounds when injected iv at 2 mg/kg or 16 mg/kg did not modify the contractile force of the gastrocnemius muscle on rats pretreated with *d*-tubocurarine (45 μg/kg). Moreover the effect cannot depend on stimulation of the nicotinic receptor at postsynaptic level since compounds **1a–3a** were inactive (at 2 and 16 μg/mL) on frog rectus abdominalis. Additional evidence in support of this hypothesis was provided by the antagonism to *d*-tubocurarine, analogous to that of TEA. In contrast, at high doses compounds **1a–3a** produced a blockade by acting at the postsynaptic level.

Experimental protocols

Chemistry

Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses (C, H, N) were within ±0.4% of the theoretical values. The IR spectra were recorded in Nujol on a Perkin-Elmer 683. The ^{1}H-NMR spectra were recorded on a Varian Gemini (300 MHz) using TMS as the internal standard. TLC was performed on Merck Kieselgel 60 F_{254} plates using $CHCl_3/CH_3OH$ (80:20) as eluent and exposure to a UV lamp or iodine vapour as the visualization method.

Bisamide 1

1-1'-Carbonyldiimidazole (3.57 g, 22 mmol) was added to a suspension of 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid (4 g, 22 mmol) in anhydrous $CHCl_3$ (50 mL) and held at reflux under stirring until effervescence ceased. After cooling, 1,4-diaminobutane (0.97 g, 11 mmol) was added. Stirring at reflux was continued for 2 h, after which the reaction mixture was partitioned between $CHCl_3$ and 0.5 N HCl (100 mL). The aqueous layer was washed with $CHCl_3$ and kept at 50 °C under reduced pressure in order to remove residual organic solvent. Excess powdered $NaHCO_3$ was added under stirring and the crude precipitated product was collected and washed with water. Crystallization from dilute EtOH gave the bisamide **1** (tables I, II).

Alkyl carboxylates 2–5

The cooled 1-acylimidazole prepared as above was treated with the appropriate alcohol (22 mmol) or diol (11 mmol) and DBU (3.35 g, 22 mmol). The reaction mixture was stirred under reflux for 4 h, cooled, and partitioned between $CHCl_3$ and water. The organic layer was washed with water to neutrality, dried and concentrated under reduced pressure to afford a crude residue which, upon recrystallization, gave the esters **2–5** (tables I, II).

Quaternary salts 1a–5a

Tertiary bases **1–5** (7–10 mmol) and CH_3I (60 mL) were stirred under reflux using a coil condenser cooled at –2 °C. The reactions were allowed to proceed until a TLC analysis of the crude mixture (methanol solution) indicated both the lack of the tertiary base and the presence of a single adduct. The time required ranged from 20 h (**4a**, **5a**) to 100, 120 and 160 h (**2a**, **3a** and **1a** respectively). The reaction mixtures were then cooled and the suspended solid filtered and washed with Et_2O in order to remove the excess CH_3I. Additional purification from traces of unreacted tertiary base or monoquaternary salt was achieved by treating a saturated ethanol solution with Et_2O (**1a**, **3a–5a**) or by recrystallization (**2a**) (see tables I, II).

Pharmacology

Male rats weighing 350–500 g were anesthetized with urethane (1.3–1.5 g/kg ip) and kept under artificial respiration (Basile Rodent Ventilator mod 7025). Indirectly elicited muscle

Table III. Activity of compounds **1a–3a** on NT in comparison to tetraethylammonium (TEA)[a].

Compound	*Infusion*[b]			*Injection (iv)*[c]				*> 50% antagonism to d-tubocurarine*[d] *(mg/kg iv)*
	Contractile force increment (%)	*Appearance after (min)*	*50% blockade after (min)*	*Dose (mg/kg)*	*Contractile force increment (%)*	*Dose (mg/kg)*	*Blockade (%)*	
1a	12	7	16	1	50	40	50	2
2a	12	1	5	0.50	37	16	100	2
3a	20	1	5	0.25	70	16	100	2
TEA	16	10	50	–	–	–	–	2

[a]Mean of three experiments. [b]2.76 mg/kg/min. [c]Arterial blood pressure was not modified. [d]100% blockade was induced with 75 μg/kg of *d*-tubocurarine.

contractions were obtained by using a sciatic nerve/gastrocnemius muscle preparation with the following parameters of nerve stimulation: frequency = 0.2 Hz, duration = 1 ms at supramaximal voltage (HSE Stimulator type 215/S). In some experiments the muscle was stimulated directly. The preparation was stimulated for 30-40 min before the main experiment in order to allow it to become stabilized. Contractions were detected with a strain gauge transducer (Basile mod DY3) and recorded with an electronic microdynamometer (Basile Unirecord mod 7050). Drugs were administered in the jugular vein by a single injection lasting 10–20 s or by a continuous infusion (Braun Perfusor VI).

Isolated rectus abdominis muscles of frog were mounted in a 20 mL bath and dose–response curves recorded.

Drugs were dissolved in DMSO; at the concentrations used for the solubilization, DMSO does not produce appreciable effects on NT.

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