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Synthesis and Evaluation of Tacrine–Huperzine A Hybrids as Acetylcholinesterase Inhibitors of Potential Interest for the Treatment of Alzheimer's Disease

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Abstract—Seventeen polycyclic compounds related to tacrine and huperzine A have been prepared as racemic mixtures and tested as acetylcholinesterase (AChE) inhibitors. The conjunctive pharmacomodulation of huperzine A (carbobicyclic substructure) and tacrine (4-aminoquinoline substructure) led to compound **7jy**, 2.5 times less active than tacrine as AChE inhibitor, but much more active than its (Z)-stereoisomer (**7iy**). Derivatives **7dy** and **7ey**, lacking the ethylidene substituent, showed to be more active than tacrine. Many other structural modifications of **7jy** led to less active compounds. Compounds **7dy** and **7ey** also showed to be much more active than tacrine in reversing the partial neuromuscular blockade induced by *d*-tubocurarine.

Introduction

Some time ago[1] we published the synthesis and evaluation of a series of tacrine-related AChE inhibitors for the treatment of Alzheimer's disease (AD), conceptually derived from tacrine by molecular duplication. Only one of these compounds showed to be of interest since although it was somewhat less active than tacrine it was also less toxic. Since then, tacrine (Cognex®) **1** and another AChE inhibitor, donepezil (Aricept®) **2**[2] have been marketed in different countries. Although donepezil is much less active than tacrine, its much lower hepatotoxicity makes it safer than tacrine. Much work has been done in the last few years for the development of new AChE inhibitors. Huperzine A, **3**, and its derivatives have been extensively studied by the groups of Kozikowski[3–8] and Bai,[9–12] some of them being tested in clinical trials. The modeling of the interaction of huperzine A[13,14] with AChE[15] led to the development of the first huperzine A derivatives more potent than the natural product. New tacrine-based compounds were also developed.[16–19] Among them, a compound containing two tacrine subunits whose amino groups are connected by an heptamethylene chain, **4**, which was designed taking into account the existence of two binding sites for tacrine in AChE, is about 1000 times more potent than tacrine,[19] although its toxicity is not known yet. Moreover, other kinds of AChE inhibitors[20–24] are also being studied for the treatment of AD. Although other approaches for the treatment of AD are under way,[25] to the best of our knowledge, all of them are still at different stages of clinical trials (Figure 1).

In continuing our interest on the development of new AChE inhibitors for the treatment of AD we planned the synthesis of compound **7jy**, designed by conjunctive pharmacomodulation of huperzine A (carbobicyclic substructure) and tacrine (4-aminoquinoline substructure), and related compounds. The protonated form of **7jy** (heterocyclic nitrogen protonated) shows

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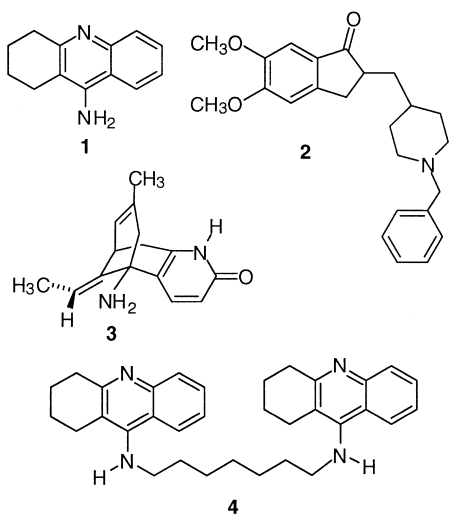


Figure 1. Acetylcholinesterase inhibitory compounds: tacrine **1**, donepezil **2**, huperzine A **3**, *N*,*N*′-heptamethylenebis(1,2,3,4-tetrahydroacridin-10-ylamine), **4**.

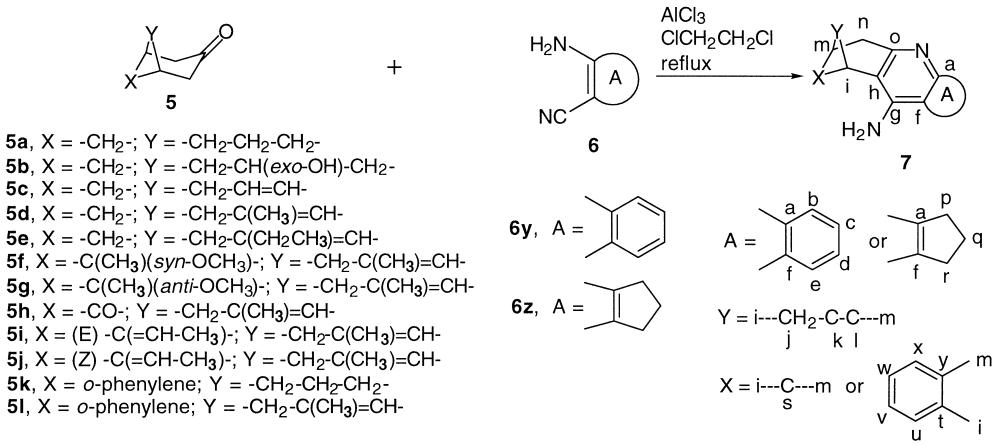
clear structural similarities with the protonated form of huperzine A (primary amine protonated). In both cases, on interaction with AChE, a hydrogen bond through the heterocyclic N–H group and an electrostatic interaction through the electron-deficient amino or ammonium group may be established.

Results

Chemistry

The synthetic route utilized for the preparation of most of these compounds is shown in Scheme 1. The Friedländer condensation of *o*-aminobenzonitrile (**6y**) or 2-aminocyclopentenecarbonitrile (**6z**) with an achiral or racemic carbonyl compound **5a–l**, under standard conditions, provided the corresponding racemic products **7**, designated with the number followed by two letters, the first one indicating the starting ketone and the second one the starting aminonitrile. These aminoquinolines were purified by column chromatography, transformed into their hydrochlorides, and crystallized from the appropriate solvent, usually methanol or mixtures methanol/water or methanol/ethyl acetate (see Experimental). The yields of chromatographed **7** derived from aminonitrile **6y** were usually in the range 48–79%, although in some cases were lower: **7hy** (25%), **7gy** (29%) and **7hy** (28%). The yield was also lower for **7dz** (10%) derived from the less stable aminonitrile **6z**. Worthy of note, compound **7hy**, derived from diketone **5h**, hydrated when transformed into their hydrochloride, as it was clearly shown by the disappearance of the ketonic absorption in the IR spectrum in passing from **7hy** to **7hy·HCl**.

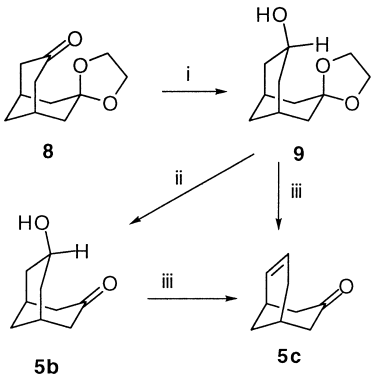
Most of the required starting ketones had been previously described: **5a**,[26] **5d**,[27,28] **5d–h**,[29] **5k,l**.[30] The known ketone **5c**[31,32] was prepared by a new and simpler procedure through the intermediacy of the new compound **5b** as shown in Scheme 2. Reduction of the known ketone acetal **8**[26] with sodium in abs. ethanol gave the thermodynamically more stable exo-alcohol **9**, which was hydrolyzed to the corresponding ketone alcohol **5b**. Dehydration of **5b** to **5c** was easily carried out by pyrolysis of its derived O-(*p*-tolyl)thiocarbonate.[33] Alternatively, **5c** was obtained by dehydration of **9** using the same procedure, followed by acid hydrolysis.



5a, X = -CH_2-; Y = -CH_2-CH_2-CH_2-
5b, X = -CH_2-; Y = -CH_2-CH(*exo*-OH)-CH_2-
5c, X = -CH_2-; Y = -CH_2-CH=CH-
5d, X = -CH_2-; Y = -CH_2-C(CH_3)=CH-
5e, X = -CH_2-; Y = -CH_2-C(CH_2CH_3)=CH-
5f, X = -C(CH_3)(*syn*-OCH_3)-; Y = -CH_2-C(CH_3)=CH-
5g, X = -C(CH_3)(*anti*-OCH_3)-; Y = -CH_2-C(CH_3)=CH-
5h, X = -CO-; Y = -CH_2-C(CH_3)=CH-
5i, X = (E) -C(=CH-CH_3)-; Y = -CH_2-C(CH_3)=CH-
5j, X = (Z) -C(=CH-CH_3)-; Y = -CH_2-C(CH_3)=CH-
5k, X = *o*-phenylene; Y = -CH_2-CH_2-CH_2-
5l, X = *o*-phenylene; Y = -CH_2-C(CH_3)=CH-

Yields (%) of chromatographed compounds **7** as bases: **7ay**, 25; **7by**, 48; **7cy**, 59; **7dy**, 60; **7dz**, 10; **7ey**, 59; **7fy**, 49, **7gy**, 29, **7hy**, 58, **7iy**, 71, **7jy**, 79, **7ky**, 28, **7dy**, 71.

Scheme 1. Synthesis of compounds **7**. The products are denoted as a function of the starting ketone and aminonitrile (i.e. compound **7ay** is the condensation product derived from ketone **5a** and aminonitrile **6y**, and so on).

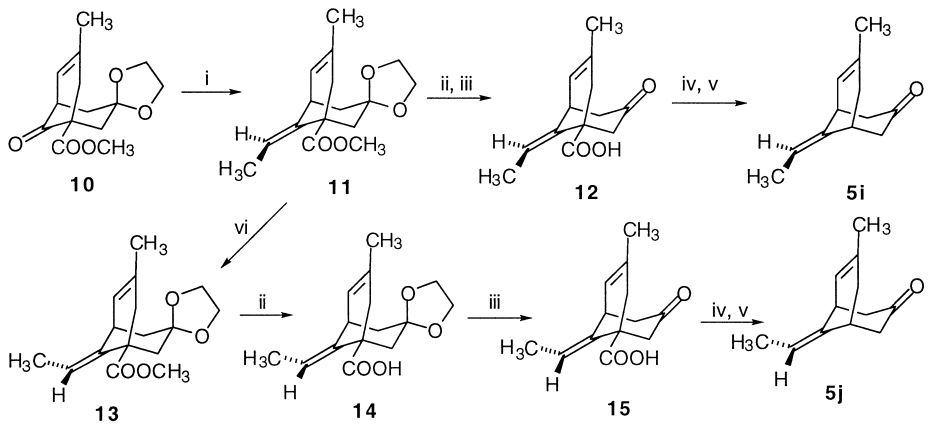


Scheme 2. Synthesis of ketones **5c**. Reagents and conditions: (i) Na, EtOH, 80%; (ii) AcOH, H_2O, Δ, 2 h, 86%; (iii) a. ClC-(S)$OC_6H_4CH_3$-*p*, pyridine, rt, 3 h, b. 5% HCl, c. 200 °C/0.5 Torr, 64% from **9** and 27% from **5b**.

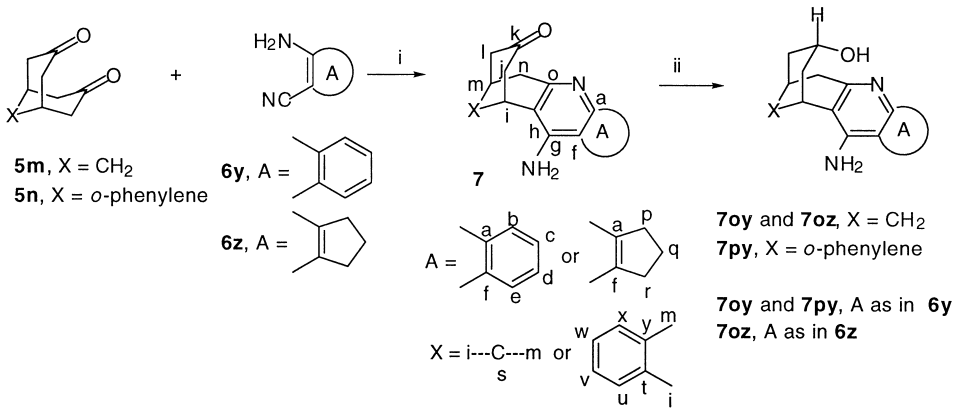
Ketones **5i** and **5j** are also new compounds whose preparation is described in Scheme 3. The starting compound **10** was first described by Kozikowski et al.[3] in connection with a synthesis of huperzine A analogues. Later, we published an improved synthesis of this compound.[34] The Wittig reaction of **10** with triphenylethylidenephosphorane to give **11** as the main diastereomer and its thiophenol-induced isomerization to a mixture in which **13** is the main component were also previously described.[3] Base hydrolysis of **11** [containing around 5% of its (*E*)-stereoisomer (**13**)] followed by acid hydrolysis of the ketal function gave in good yield pure keto acid **12**, which was decarboxylated by the Barton procedure.[35–39] The acid chloride derived from **12** was reacted with 2-mercaptopyridine N-oxide sodium salt and the thus formed ester was thermally decomposed in the presence of *t*-butylthiol as hydrogen source giving rise to ketone **5i** in 77% overall yield from **12**. Similarly, ester **13** (containing around 5% of **11**) was saponified to give acid **14** which on acid hydrolysis gave keto acid **15** in high yield. Barton's decarboxylation of **15** gave ketone **5j** in 62% yield.

Other compounds **7** were prepared from the known diketones **5m** and **5n** by condensation with aminonitriles **6y** or **6z** as previously described[1] for the cases of **7my**, **7mz** and **7ny**, and following a similar procedure for the new compound **7nz** (Scheme 4). Reduction of ketones **7my**, **7mz** and **7ny** with $NaBH_4$ in methanol solution gave the corresponding endo-alcohols which are denoted as **7oy**, **7oz** and **7py**, respectively.

All new compounds have been fully characterized through their spectroscopic data (IR, ^1H, and ^{13}C NMR spectra and elemental analysis). The ^1H and ^{13}C NMR spectra of all compounds **7**, except for **7fy**, **7gy**, **7hy**, and **7oy**, were fully assigned through the COSY $^1H/^1H$ and COSY $^1H/^{13}C$ spectra. The spectra of the rest of compounds **7** were assigned by comparison with the model compounds, while for the assignment of the quaternary carbon atoms of all compounds **7**, previous work[1] was taken into account. Tables 1 and 2 collect the ^1H NMR data of compounds **7**, while Table 3 and Table 4 collect the ^{13}C NMR data of these compounds. In order to compare these data, the different carbon atoms have been lettered (see Schemes 1 and 4) since the systematic numbering differs from one compound to another. For the rest of compounds, ketones **5b**,**c**,**i**,**j** and their precursors, the ^1H and ^{13}C NMR data are given in the experimental. Assignment of the NMR data of these compounds was straightforward.



Scheme 3. Synthesis of ketones **5i** and **5j**. Reagents and conditions: (i) $(C_6H_5)_3P{=}CHCH_3$, THF, 85%; (ii) 20% NaOH, H_2O, MeOH, THF, Δ, 48 h; (iii) 2 N HCl, dioxane, rt, 4 h, 85% global yield of **12** from **11**, 92% global yield of **15** from **13**; (iv) Cl_2SO, toluene, 80 °C, 4 h; (v) *t*-BuSH, DMAP, toluene, 2-mercaptopyridine N-oxide sodium salt, reflux, 14 h, 77% of **5i** from **12**, 62% of **5j** from **15**; (vi) Thiophenol, toluene, Δ, 90%.



Scheme 4. Synthesis of several compounds **7**, by reduction of their carbonyl precursors. The products are denoted as before, as if they were obtained from the hypothetical corresponding ketones. Reagents and conditions: (i) $AlCl_3$, $ClCH_2CH_2Cl$, reflux, 34% yield for **7nz**; (ii) $NaBH_4$, MeOH, rt, 12 h, 89, 85, and 87% yield for **7oy**, **7oz**, and **7py**, respectively, as chromatographed bases.

Pharmacology

In order to assess the potential interest of compounds **7** in AD, the AChE inhibitory activity of all compounds **7** was assayed by the method of Ellman et al.[40] These compounds were further analyzed in a cholinergic synapse, such as skeletal neuromuscular junction. In this analysis, the ability to reverse the *d*-tubocurarine-neuromuscular blockade was tested, a well-known effect of AChE inhibitors.[41] Table 5 summarizes the data comparing AChE inhibition and reversion of partial neuromuscular blockade induced by *d*-tubocurarine in the rat phrenic-hemidiaphragm preparation. As can be seen from this table, compounds **7dy** and **7ey** were approximately two- and four-times more active than tacrine, respectively, as AChE inhibitors. The rest of compounds was clearly less active. Compounds **7dy** and **7ey** were also the more potent in reversing the partial neuromuscular blockade induced by *d*-tubocurarine. In this test, several compounds **7** (**7by**, **7cy**, **7dz**, **7fy**, **7hy**, **7nz**, **7oy**, and **7oz**) were more potent than tacrine while others showed lower or no activity at 100 μM concentrations (**7ay**, **7gy**, **7iy**, **7jy**, **7ky**, **7ly**, and **7py**).

Discussion

An examination of the results of Table 5 shows that compound **7jy**, designed by modification of the pharmacophores of huperzine A (carbobicyclic substructure) and tacrine (4-aminoquinoline substructure) through a conjuctive process, exhibited an AChE inhibitory activity approximately 2.5 times lower than tacrine. The configuration of the ethylidene group is very important for the activity, since its (Z)-stereoisomer (**7iy**), showed much lower activity. Worthy of note, compound **7dy**, derived from **7jy** by elimination of the ethylidene substituent was twice more active than tacrine and **7ey**, derived from **7dy** by substituting the methyl substituent at position 9 by an ethyl group, was still more active (approximately 4 times more active than tacrine) (Figure 2).

Compounds **7** derived from **7dy** by introduction of polar substituents at position 13 (**7fy**, **7gy**, and **7hy**), by elimination of the methyl substituent at position 9 (**7cy**) or by substitution of the benzene ring by a cyclopentene (**7dz**) were clearly less active than tacrine (10 to 50 times, approximately). Curiously, **7ay**, derived from **7cy** by saturation of the C8-C9 double bond was the least active tested compound (more than 300 times less active than tacrine). However, much of the lost activity of **7ay** was recovered by introduction of an exo-9-OH group to

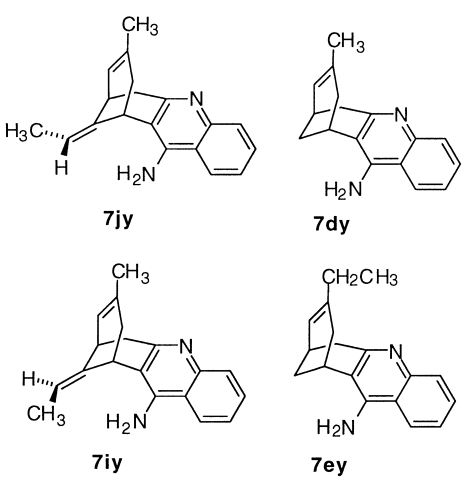


Figure 2. Selected acetylcholinesterase inhibitors **7**.

Table 1. ^{1}H NMR chemical shifts[a,b] and coupling constants of the hydrochlorides of several compounds **7**

Hydrogen	**7ay**[c]	**7by**[d]	**7dz**	**7ky**	**7ly**	**7nz**	**7oy**[e]	**7oz**[e]	**7py**[f]
δ (ppm)									
b-H	7.76	7.77		7.73	7.78		7.80		7.70
c-H	7.86	7.87		7.85	7.84		7.88		7.82
d-H	7.60	7.61		7.60	7.61		7.65		7.59
e-H	8.33	8.35		8.34	8.41		8.37		8.34
i-H	3.28	3.38	3.13	4.59	4.62	4.57	3.32	2.96	4.54
j-H_{exo}	1.77	1.55	2.47	1.82	2.61	2.82*	1.96–2.26	1.87	2.16
j-H_{endo}	1.72	2.35	1.89	2.17	2.50	2.98*	1.96–2.26	1.99	2.33
k-H_{exo}	1.53			1.82			4.21	4.04	4.25
k-H_{endo}	1.18	3.46		1.82					
l-H_{exo}	1.77	1.64	5.56	1.82	5.81	2.82*	1.96–2.26	1.87	2.23
l-H_{endo}	1.77	2.20		2.17		2.98*	1.96–2.26	1.99	2.30
m-H	2.44	2.62	2.73	3.39	3.74	3.59	2.61	2.36	3.49
n-H_{exo}	3.35	3.34	2.95	3.51	3.38*	3.31	3.40	3.02	3.44
n-H_{endo}	2.86	2.92	2.58	3.47	3.54*	3.26	3.18	2.85	3.66
NH_2	4.82	4.81	4.82	4.82	4.85	4.82	4.98	4.89	
NH^+	4.82	4.81	4.82	4.82	4.85	4.82	4.98	4.89	
OH		4.81					4.98	4.89	
k-CH_3			1.60		1.62				
p-H_2			3.06			2.82*		2.80	
q-H_2			2.27			2.23		2.07	
r-H_2			2.85			2.98*		2.70	
s-H_{syn}	1.89	1.88*	1.83				1.96–2.26	1.79	
s-H_{anti}	2.04	1.99*	1.91				1.96–2.26	1.87	
u-H				7.27	7.21*	7.41			7.27
v-H				7.15	7.21*	7.30			7.17
w-H				7.20	7.21*	7.35			7.23
x-H				7.24	7.33*	7.38			7.26
J (Hz)									
b-H/c-H	8.5	8.0		8.5	8.0		8.4		8.5
b-H/d-H	1.0	1.0		1.0	1.0		1.5		1.0
c-H/d-H	7.0	7.0		7.0	7.0		7.0		7.0
c-H/e-H	1.0	1.0		1.5	1.0		1.5		1.0
d-H/e-H	8.5	8.5		8.5	8.5		8.4		8.5
i-H/j-H_{exo}		4.0	4.5	1.5	4.0	3.0			4.0
i-H/j-H_{endo}				6.5	4.0	6.5			5.0
j-H_{exo}/j-H_{endo}	13.5	12.0	17.5		18.5				14.5
l-H_{exo}/m-H		4.0	4.5		8.5				3.5
m-H/n-H_{exo}	7.5	8.5	5.5	5.5	4.0*	5.0	7.5	7.5	5.5
m-H/n-H_{endo}				3.0	4.5*	3.5			2.5
n-H_{exo}/n-H_{endo}	19.0	19.0	18.5	19.0	18.0	18.5	18.6	17.5	18.0
s-H_{syn}/s-H_{anti}	13.0	13.0	12.5					13.0	
u-H/v-H				7.5		7.5			7.5
u-H/w-H				1.5		1.5			1.5
v-H/w-H				7.5		7.5			7.5
v-H/x-H				1.5		1.5			1.5
w-H/x-H				7.5		7.5			7.5

[a]Except where otherwise indicated, the spectra were recorded at 500 MHz in CD_3OD.

[b]The values indicated with * within a column can be interchanged.

[c]Other coupling constants: j-H_{endo}/k-H_{exo} = j-H_{endo}/k-H_{endo} = 4.0, k-H_{exo}/k-H_{endo} = 14.5.

[d]Other coupling constants: j-H_{exo}/k-H_{endo} = k-H_{endo}/l-H_{exo} = l-H_{exo}/l-H_{endo} = 12.0.

[e]Recorded at 300 MHz.

[f]Other coupling constants: j-H_{exo}/k-H = 4.0, j-H_{endo}/k-H = k-H/l-H_{endo} = 5.0, j-H_{endo}/l-H_{endo} = 1.5, k-H/l-H_{exo} = 3.5, l-H_{exo}/l-H_{endo} = 15.0, l-H_{endo}/m-H = 5.0.

give **7by** (12-times less active than tacrine). Its endo-9-OH stereoisomer, **7oy**, was somewhat less active (33 times less active than tacrine). Substitution of the benzene ring of **7oy** by a cyclopentene ring gave the less active compound **7oz**. All compounds in which the bicyclo[3.3.1]nonane substructure characteristic of huperzine A was substituted by the related benzobicyclo[3.3.2]decane substructure (**7ky**, **7ly**, **7nz** and **7py**) were much less active than tacrine.

In connection with the AChE inhibitory activity, some qualitative structure–activity relationships can be derived from the above data: (a) the presence of the bicyclo[3.3.1]nonene substructure, characteristic of huperzine A, seems to be essential; (b) although the configuration of the ethylidene group at position 13 is of great importance for the activity, the absence of this substituent gives more active compounds; (c) polar substituents at position 13 give less active compounds; (d) elimination of the methyl substituent at position 9 greatly reduces the inhibitory activity, which is further reduced by saturation of the C8-C9 double bond; (e) the presence of polar groups at position 9 (exo-OH, endo-OH, = O) gives compounds more active than their saturated parent precursors but less active than the

Table 2. ^{1}H NMR chemical shifts[a] and coupling constants of the hydrochlorides of the rest of compounds **7**

Hydrogen	**7cy**[b]	**7dy**[c]	**7ey**[d]	**7fy**	**7gy**	**7hy**[e]	**7iy**	**7jy**
δ (ppm)								
b-H	7.72	7.73	7.72	7.75	7.75	7.20	7.74	7.74
c-H	7.79	7.85	7.86	7.85	7.87	7.43	7.86	7.85
d-H	7.55	7.60	7.61	7.60	7.62	7.20	7.62	7.61
e-H	8.31	8.34	8.34	8.34	8.36	7.70	8.37	8.35
i-H	3.35	3.39	3.40	3.33	3.28	3.08	4.33	3.74
j-H_{exo}	2.51	2.51	2.53	2.53	2.67	2.59	2.52	2.55
j-H_{endo}	2.08	1.98	2.02	2.07	1.82	2.01	2.20	2.19
k-H	5.64							
l-H	5.82	5.57	5.58	5.53	5.41	5.43	5.54	5.54
m-H	2.77	2.78	2.80	2.67	2.67	2.65	3.13	3.64
n-H_{exo}	3.20	3.20	3.21	3.33	3.22	3.23	3.17	3.15
n-H_{endo}	2.92	2.88	2.87	2.80	3.00	2.84	3.05	3.08
NH_2	4.95	4.82	4.87	4.82	4.82	4.64	4.83	4.83
NH^+	4.95	4.82	4.87	4.82	4.82	4.64	4.83	4.83
k-CH_3		1.57		1.57	1.56	1.50	1.56	1.56
s-H_{syn}	1.96	1.95	1.97					
s-H_{anti}	2.11	2.07	2.08					
s-CH_3				1.44	1.26			
s-OCH_3				3.17	3.33			
s = $CHCH_3$							5.52	5.54
s = $CHCH_3$							1.76	1.71
k-CH_2CH_3			1.88					
k-CH_2CH_3			0.89					
J (Hz)								
b-H/c-H	8.0	8.5	8.5	8.5	8.5	8.0	8.0	8.5
b-H/d-H	1.0	1.0	1.0	1.0	1.0		1.0	1.5
c-H/d-H	7.0	7.0	7.0	7.0	7.0		7.0	7.0
c-H/e-H	1.0	1.5	1.5	1.0	1.5		1.0	1.5
d-H/e-H	8.5	8.5	8.5	8.5	8.5	8.5	8.0	8.5
i-H/j-H_{exo}		4.5	5.5	6.0		5.5	5.5	5.5
j-H_{exo}/j-H_{endo}	18.0	18.0	18.0	18.5	17.0	18.0	17.5	17.0
l-H_{exo}/m-H		4.5	5.5		6.0	5.5	5.5	
m-H/n-H_{exo}	5.5	5.5	5.5		6.0	6.0	5.5	5.0
m-H/n-H_{endo}	1.5	2.0	2.0	1.0	1.5			2.0
n-H_{exo}/n-H_{endo}	18.0	18.0	18.0	18.0	18.5	18.0	17.0	17.5
s-H_{syn}/s-H_{anti}	13.0	12.5	13.0					

[a]See corresponding caption in Figure 1.
[b]Other coupling constants: j-H_{exo}/k-H = 5.0, k-H/l-H = 9.5, n-H_{endo}/s-H_{anti} = 1.5.
[c]Another coupling constant: n-H_{endo}/s-H_{anti} = 2.0.
[d]Another coupling constant: k-CH_2-CH_3/k-CH_2-CH_3 = 7.5 Hz.
[e]Recorded in D_2O.

Table 3. ^{13}C NMR chemical shifts[a,b] (ppm) of the hydrochlorides of several compounds **7**

Carbon	**7ay**	**7by**	**7dz**	**7ky**	**7ly**	**7nz**	**7oy**	**7oz**	**7py**
C-a	138.7	138.9	168.6*	138.8	138.8	155.8*	138.5	161.3*	138.2
C-b	120.0	120.1		119.9	119.9		120.0		119.6
C-c	134.3	134.5		134.6	134.6		133.6		134.0
C-d	126.9	127.2		127.3	127.4		126.6		127.0
C-e	124.2	124.3		124.3	124.4		124.0		124.2
C-f	116.4	116.6	122.6†	116.6	116.8	122.1	116.4	119.9	116.6
C-g	156.0	156.3	165.9*	157.4*	157.6	156.1*	154.5*	155.5*	156.3*
C-h	114.5	114.7	122.4†	113.5	114.1	117.5	114.4	118.5	115.3
C-i	28.3	29.1	29.5	43.0	42.2	39.0	26.3	26.8	40.6
C-j	29.4	38.2	36.3	31.9	39.3	49.0	35.6†	36.2	37.8
C-k	19.2	65.5	133.6	23.9	137.5	212.0	66.5	66.9	70.4
C-l	33.7	42.9	125.7	34.0	123.3	50.5	39.1	40.0	39.0
C-m	27.3	28.5	28.8	42.3	42.2	39.7	26.0	27.3	40.4
C-n	34.7	34.9	34.5	38.2	41.0	37.9	35.3†	39.2	39.5
C-o	154.4	153.5	161.5*	156.1*	154.2	147.8	153.5*	149.3*	156.2*
C-p			33.6			32.1		34.7	
C-q			24.3			23.1		23.6	
C-r			29.9			29.1		28.5	
C-s	32.1	31.6	28.6				31.9	32.4	
C-t				144.8	142.0	143.0†			143.7
C-u				130.0	128.1*	129.6			129.9
C-v				128.3	128.4*	129.3			128.4
C-w				128.9	129.0*	129.9			129.1
C-x				129.4	130.0*	130.1			129.1
C-y				145.0	145.2	143.1†			144.6
k-CH_3			23.4		26.2				

[a]Except where otherwise indicated, the spectra were taken at 50.3 MHz in CD_3OD.
[b]The values indicated with * or † within a column can be interchanged.

corresponding 9-alkyl substituted C8–C9 unsaturated analogues; (f) substitution of the benzene ring from the tacrine substructure for a cyclopentene ring also gives less active compounds.

About the reversion of the partial neuromuscular blockade induced by *d*-tubocurarine, the more active compounds were also **7ey** and **7dy** (850 and 410 times more active than tacrine, respectively). In this assay, other compounds **7** (**7by**, **7cy**, **7dz**, **7fy**, **7hy**, **7nz**, **7oy**, and **7oz**) showed greater activity than tacrine (between 6 and 19 times more active) in spite of their lower AChE inhibitory activity. This fact may be due to the simultaneous ocurrence in this assay of different mechanisms of action, such as potassium channel blocking.[42–44]

Conclusion

Conjunctive pharmacomodulation of huperzine A and tacrine has led to **7jy**, a compound with lower AChE inhibitory activity than tacrine, whose modification has given two compounds (**7dy** and **7ey**) more active than tacrine not only as AChE inhibitors but also in reversing the partial neuromuscular blockade induced by *d*-tubocurarine. These findings open the way for the preparation of more potent AChE inhibitors through the modification of the above compounds by introducing substituents at the benzene ring or by changing the substitution at position 9. Modeling of the interaction of the potential candidates with AChE will be helpful to this end.

Experimental

Chemistry

Melting points were determined on a Gallemkamp melting-point apparatus, model MFB 595010M. IR spectra were recorded on a FT/IR Perkin–Elmer, model 1600 spectrophotometer. NMR spectra were determined on Varian Gemini 200, Varian Gemini 300 and VXR 500 spectrometers; chemical shifts are given in ppm relative to TMS (δ scale); the coupling constants are expressed in Hz; standard abbreviations are used. COSY $^1H/^1H$ experiments were performed using standard procedures and COSY $^1H/^{13}C$ experiments were

Table 4. ^{13}C NMR chemical shifts[a] (ppm) of the hydrochlorides of the rest of compounds **7**

Carbon	7cy	7dy	7ey	7fy	7gy	7hy[b,c]	7iy[c]	7jy[c]
C-a	139.0	138.6	138.8	139.3	139.0	143.6	136.9	137.0
C-b	120.1	119.8	120.0	120.6	120.1	125.3	120.0	120.0
C-c	134.4	134.1	134.4	134.0	134.6	139.8	134.4	134.3
C-d	127.1	127.0	127.1	126.9	127.3	132.6	127.2	127.2
C-e	124.2	124.2	124.2	124.0	124.2	128.9	124.2	124.2
C-f	116.8	116.5	116.7	116.9	116.6	121.5	116.8	116.7
C-g	157.3	156.3	156.7	156.5	157.4	162.0	156.4	156.0
C-h	114.9	114.8	114.9	113.7	114.8	119.0	114.8	115.6
C-i	27.2	27.4	27.5	35.6	36.9	44.1	30.6	38.7
C-j	31.2	35.9	34.3	35.8	33.8	40.9	38.1	39.1
C-k	127.4	134.8	140.4	134.0	—	140.6	135.0	135.6
C-l	131.0	125.0	123.3	125.2	122.9	129.2	125.7	124.7
C-m	28.0	28.0	28.1	37.8	36.1	44.7	39.4	31.6
C-n	35.6	35.8	36.0	33.3	35.2	40.6	38.4	37.5
C-o	152.3	152.1	152.3	153.1	151.1	156.6	152.4	152.1
C-s	29.2	29.2	29.5	74.5	74.1	100.4	138.8	138.8
k-CH_3		23.5		22.7	23.1	28.6	23.0	23.0
s-CH_3				19.8	20.2			
s-OCH_3				49.3	49.7			
s=$CHCH_3$							116.5	116.6
s=$CHCH_3$							12.5	12.5
k-CH_2CH_3			30.9					
k-CH_2CH_3			12.6					

[a]See corresponding caption in Table 1.
[b]Recorded at 75.4 MHz in D_2O.
[c]Recorded at 75.4 MHz.

performed using the HMQC sequence with an indirect detection probe. Thin-layer chromatography was carried out on silica gel 60 F_{254} (Alugran R sil G/UV254). Column chromatography was performed by using silica gel 60 (Merck, 230-440 mesh). Microanalyses were carried out at the Microanalysis Service of the Centro de Investigación y Desarrollo, (C.I.D.), Barcelona. Unless otherwise stated, all compounds were dried in vacuo (1 Torr) at 80 °C for 2 days (standard conditions).

Preparation of exo-7-hydroxybicyclo[3.3.1]nonan-3-one (5b)

(a) 7,7-Ethylenedioxybicyclo[3.3.1]nonan-exo-3-ol (9). To a stirred solution of the known[26] keto acetal **8** (500 mg, 2.55 mmol) in abs ethanol (30 mL) small pieces of sodium (1.0 g, 43 mmol) were slowly added keeping the reaction mixture in an argon atmosphere. After heating under reflux for 4 h, the mixture was allowed to cool to room temperature, poured into water (30 mL) and extracted with diethyl ether (5×40 mL). The combined organic extracts were dried with anhydrous Na_2SO_4 and concentrated in vacuo. The residual yellow oil was distilled in a rotary microdistillation equipment to give **9** (405 mg, 80% yield), bp 150 °C/0.5 Torr. IR ($CHCl_3$) ν: 3600 and 3450 (O–H st) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.2–2.1 (complex signal, 11 H, 2(4)-H_{exo}, 2(4)-H_{endo}, 6(8)-H_{exo}, 6(8)-H_{endo}, 9-H_{syn}, 9-H_{anti} and OH), 2.21 (m, 2 H, 1(5)-H), 3.8–4.0 (m, 4 H, O-CH_2CH_2-O), 4.78 (m, 1 H, 3-H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 28.8 [CH, C1(5)], 31.9 (CH_2, C9), 40.1 (CH_2) and 41.0 (CH_2) [C2(4) and C6(8)], 63.1 (CH_2) and 64.1 (CH_2) (O-CH_2CH_2-O), 63.2 (CH, C3), 108.0 (C, C7). Found: C, 66.57; H, 9.32; calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.16.

(b) exo-7-Hydroxybicyclo[3.3.1]nonan-3-one (5b). A mixture of **9** (450 mg, 2.27 mmol), acetic acid (15 mL) and water (35 mL) was heated under reflux for 2 h. The cold (room temperature) mixture was made basic with aqueous 5 N NaOH, and it was extrated with ethyl acetate (3×50 mL). The combined organic phases were dried with anhydrous Na_2SO_4 and concentrated in vacuo. The brown oily residue was distilled at 150 °C/0.5 Torr to give **5b** (300 mg, 86% yield) as a solid, mp 157–159 °C (sublimed). IR ($CHCl_3$) ν 3415 (O–H st), 1684 (C=O st) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.51 [dt, $J = 3.5$ Hz, $J' = 12.0$ Hz, 2 H, 6(8)-H_{exo}], 1.66–2.06 [complex signal, 5 H, 6(8)-H_{exo}, 9-H_{syn}, 9-H_{anti} and OH], 2.40–2.56 [complex signal, 6 H, 1(5)-H, 2(4)-H_{exo} and 2(4)-H_{endo}), 3.79 (m, 1 H, 7-H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 30.9 [CH, C1(5)], 32.0 (CH_2, C9), 40.3 [CH_2, C6(8)], 47.3 [CH_2, C2(4)], 63.5 (CH, C7), 211.9 (C, C3). Found: C, 70.05; H, 9.26; calcd for $C_9H_{14}O_2$: C, 70.09; H, 9.16.

Table 5. Pharmacological data of compounds **7**[a]

Compounds	IC_{50} (μM)	AI_{50}(μM)
Tacrine	0.13 ± 0.01	71.7
7ay	41.8 ± 7.6	—[b]
7by	1.61 ± 0.13	6.57
7cy	4.89 ± 0.26	5.75
7dy	0.065 ± 0.015	0.176
7dz	5.58 ± 1.22	11.7
7ey	0.0385 ± 0.004	0.084
7fy	1.34 ± 0.27	4.25
7gy	6.77 ± 1.03	—[b]
7hy	2.09 ± 0.46	6.54
7iy	1.15 ± 0.09	—[b]
7jy	0.32 ± 0.03	—[c]
7ky	2.58 ± 0.88	—[b]
7ly	2.09 ± 0.08	—[b]
7nz	2.98 ± 0.50	12.5
7oy	4.3 ± 0.8	3.81
7oz	13.4 ± 2.6	11.2
7py	15.2 ± 1.6	—[b]

[a]All values are expressed as mean ± standard error of the mean of at least 10 experiments. IC_{50}: 50% inhibitory concentration of acetylcholinesterase activity (μM); AI_{50}: drug concentration that inhibits a 50% *d*-tubocurarine blockade in neuromuscular junction. All compounds were used in the form of hydrochlorides and the values were determined taking into account the water of crystallization deduced from the elemental analysis.
[b]No reversion at 100 μM concentration.
[c]Only 20.6 ± 6.1% reversion was obtained at 3 μM.

***rac*-Bicyclo[3.3.1]non-6-en-3-one (5c)[31,32]. Method 1**. To a cold (ice-bath) solution of **9** (800 mg, 4.03 mmol) in dry pyridine (10 mL), *O*-(*p*-tolyl) chlorothionoformate (0.74 mL, 4.03 mmol) was slowly added with magnetic stirring keeping the reaction mixture in an argon atmosphere. After addition was finished the mixture was stirred at room temperature for 3 h. Then, it was poured into ice (50 g) and extracted with toluene (3×20 mL). The combined organic phases were washed with aqueous 5% HCl (3×20 mL), water (3×20 mL) and brine (3×20 mL), dried with anhydrous Na_2SO_4 and concentrated in vacuo. The oily residue (2.10 g) was pyrolized in a rotary microdistillation apparatus at 200 °C/0.5 Torr collecting three fractions, the most volatile one consisting mainly of impure oily **5c** (650 mg). This fraction was taken in CH_2Cl_2 (20 mL) and the solution was washed with aqueous 5 N NaOH (5×50 mL). Evaporation of the organic phase gave a residue (430 mg), which was chromatographed [silica gel (50 g, hexane] yielding pure **5c** (350 mg, 64% yield). **Method 2**. A similar experimental procedure to that described in method 1 was followed, except for the starting compound. From **5b** (1.00 g, 6.48 mmol) and *O*-(*p*-tolyl) chlorothionoformate (1.00 mL, 6.48 mmol), pure **5c** (510 mg, 72% yield) was obtained. IR ($CHCl_3$) ν 1703 (C=O st) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.94 (dd, J = 18.5 Hz, J' = 4.2 Hz, 1 H, 8-H_{endo}), 2.04 (broad s, 2 H, 9-H_{syn} and 9-H_{anti}), 2.20–2.65 (complex signal, 6 H, 2-H_{exo}, 2-H_{endo}, 4-H_{exo}, 4-H_{endo}, 8-H_{exo} and 1-H), 2.69 (broad s, 1 H, 5-H), 5.62 (ddd, J = 10.0 Hz, J' = 4.2 Hz, J'' 2.0 Hz, 1 H, 7-H), 5.71 (m, 1 H, 6-H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 29.9 (CH, C1), 30.2 (CH_2, C9), 31.0 (CH, C5), 32.6 (CH_2, C8), 46.3 (CH_2, C4), 49.1 (CH_2, C2), 125.7 (CH, C7), 130.5 (CH, C6), 212.4 (C, C3).

Preparation of *rac*-(*E*)-9-ethylidene-7-methylbicyclo[3.3.1]non-6-en-3-one (5i)

(a) *rac*-(*Z*)-9-Ethylidene-3-methyl-7-oxobicyclo[3.3.1]non-3-ene-1-carboxylic acid (12). A mixture of *rac*-(*Z*)-3,3-ethylenedioxy-9-ethylidene-7-methylbicyclo[3.3.1]non-6-ene-1-carboxylic acid methyl ester (**11**)[3] [4.33 g, 15.6 mmol, containing around 5% of the corresponding (*E*)-stereoisomer (**13**)], aqueous 20% NaOH (325 mL, 1.63 mol), THF (325 mL) and methanol (325 mL) was heated at the reflux temperature under argon for 48 h. The organic solvent was evaporated at reduced pressure and the resulting aqueous solution was washed with CH_2Cl_2 (2×50 mL), acidified with concentrated HCl and extracted with CH_2Cl_2 (3×100 mL). The combined organic extracts were washed with brine (100 mL), dried with anhydrous Na_2SO_4 and evaporated at reduced pressure to give a yellow gelatinous residue (3.38 g), which was taken in dioxane (40 mL) and treated with 2 N HCl (40 mL) at room temperature for 4 h. The resulting mixture was concentrated in vacuo, diluted with water (50 mL) and extracted with CH_2Cl_2 (3×30 mL). The combined organic extracts were washed with brine (50 mL), dried with anhydrous Na_2SO_4 and evaporated at reduced pressure affording acid **12** (2.92 g, 85% yield) as a light-yellow solid. An analytical sample was obtained by crystallization from ethanol, mp 134–136 °C; IR ($CHCl_3$) ν 3450–2400 (O–H st), 1725, 1686 (C=O st) cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 1.62 (s, 3 H, 3-CH_3), 1.65 (d, J = 7.0 Hz, 3 H, 9=CH-CH_3), 2.08 (d, J = 17.5 Hz, 1 H, 2-H_{endo}), 2.39 (dd, J = 16.0 Hz, J' = 2.5 Hz, 1 H, 8-H_{endo}), 2.40 (dt, J = 14.5 Hz, J' = 2.5 Hz, 1 H, 6-H_{endo}), 2.59 (dd, J = 14.0 Hz, J' = 4.0 Hz, 1 H, 6-H_{exo}), 3.03 (broad d, J = 17.5 Hz, 1 H, 2-H_{exo}), 3.05 (broad s, 1 H, 5-H), 3.15 (dd, J = 16.0 Hz, J' = 1.5 Hz, 1 H, 8-H_{exo}), 5.38 (dm, J = 5.0 Hz, 1 H, 4-H), 5.64 (q, J = 7.0 Hz, 1 H, 9=C*H*-CH_3), 9–12 (broad signal, 1 H, COOH). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 12.3 (CH_3, 9=CH-*C*H_3), 22.4 (CH_3, 3-CH_3), 42.3 (CH_2, C2), 44.8 (CH, C5), 47.2 (CH_2, C6), 48.9 (C, C1), 51.7 (CH_2, C8), 118.6 (CH, 9=*C*H-CH_3), 124.7 (CH, C4), 132.1 (C, C3), 134.9 (C, C9), 181.3 (C, COOH), 209.3 (C, C7). Found: C, 70.90; H, 7.37; calcd for $C_{13}H_{16}O_3$: C, 70.88; H, 7.33.

(b) *rac*-(*E*)-9-Ethylidene-7-methylbicyclo[3.3.1]non-6-en-3-one (5i). A suspension of acid **12** [1.25 g, 5.68 mmol, containing around 5% of the (*E*)-stereoisomer (**15**)] and thionyl chloride (1.65 mL, 22.7 mmol) in anhydrous toluene (185 mL) was heated at 80 °C for 4 h. The solvent and excess thionyl chloride were eliminated in vacuo and the resulting residue was twice taken in anhydrous toluene (15 mL) and evaporated at reduced pressure to completely remove the thionyl chloride, obtaining a yellow oily residue (1.35 g) of the corresponding acid chloride.

To a suspension of 2-mercaptopyridine N-oxide sodium salt (1.13 g, 7.58 mmol), 4-dimethylaminopyridine (75.5 mg, 0.62 mmol) and *t*-butylthiol (3.44 mL, 30.6 mmol) in anhydrous toluene (60 mL), heated under reflux, a solution of the previously prepared acid chloride in anhydrous toluene (30 mL) was added dropwise for 15 min and the reaction mixture was heated under reflux for 14 h. The cold mixture was washed with water (2×30 mL) and brine (30 mL), dried with anhydrous Na_2SO_4, and concentrated in vacuo affording a brown oily residue (2.22 g) consisting mainly of **12** and *t*-butyl 2-pyridyl disulfide (approximate molar ratio 58:42 by ^{1}H NMR). This mixture was taken in hexane (15 mL), washed with 3 N HCl (3×2.5 mL) and dried with anhydrous Na_2SO_4. The organic solution was evaporated at reduced pressure affording an orange oil (1.15 g), which was submitted to column chromatography [silica gel (75 g), hexane/ethyl acetate 99/1], to give **5i** [770 mg, 77% yield, containing around 5% of the corresponding (*Z*)-stereoisomer (**5j**)] as a colorless oil, bp 40–45 °C/0.5 Torr; IR ($CHCl_3$) ν 1706 (C=O st) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (s, 3 H, 7-CH_3), 1.71 (d, J=7.0 Hz, 3 H, 9=CH-CH_3), 2.00 (d, J=18.0 Hz, 1 H, 8-H_{endo}), 2.36 (dt, J=15.5 Hz, J'=1.0 Hz, 1 H, 2-H_{endo}), 2.39–2.44 (complex signal, 2 H, 4-H_{endo} and 8-H_{exo}), 2.48–2.52 (complex signal, 2 H, 2-H_{exo} and 4-H_{exo}), 2.99 (broad s, 1 H, 5-H), 3.36 (dt, J=1.0 Hz, J'=6.5 Hz, 1 H, 1-H), 5.42 (m, 1 H, 6-H), 5.49 (q, J=7.0 Hz, 1 H, 9=CH-CH_3). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 12.4 (CH_3, 9=CH-CH_3), 22.7 (CH_3, 7-CH_3), 31.6 (CH, C1), 39.6 (CH_2, C8), 41.3 (CH, C5), 48.2 (CH_2, C4), 49.5 (CH_2, C2), 115.4 (CH, 9=CH-CH_3), 125.3 (CH, C6), 132.8 (C, C7), 138.5 (C, C9), 211.7 (C, C3). Found: C, 81.67; H, 9.20; calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.16.

***rac*-(*Z*)-9-Ethylidene-7-methylbicyclo[3.3.1]non-6-en-3-one (5j).** This reaction was carried out as described for the preparation of **5i**. Starting from *rac*-(*E*)-9-ethylidene-3-methyl-7-oxobicyclo[3.3.1]non-3-ene-1-carboxylic acid (**14**)[3] [1.00 g, 4.54 mmol, containing around 5% of the corresponding (*Z*)-stereoisomer (**12**)], **5j** [492 mg, 62% yield, containing around 5% of the corresponding (*E*)-stereoisomer (**5i**)] was obtained as an oily product, bp 40–45 °C/0.5 Torr; IR ($CHCl_3$) ν 1706 (C=O st) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60 (broad s, 3 H, 7-CH_3), 1.69 (d, J=6.5 Hz, 3 H, 9=CH-CH_3), 1.97 (d, J=17.5 Hz, 1 H, 8-H_{endo}), 2.33 (dd, J=15.5 Hz, J'=1.0 Hz, 1 H, 2-H_{endo}), 2.39–2.45 (complex signal, 3 H, 4-H_{endo}, 4-H_{exo} and 8-H_{exo}), 2.53 (ddd, J=15.5 Hz, J'=6.5 Hz, J''=1.0 Hz, 1 H, 2-H_{exo}), 2.87 (dt, J=1.0 Hz, J'=6.5 Hz, 1 H, 1-H), 3.42 (m, 1 H, 5-H), 5.40 (m, 1 H, 6-H), 5.42 (q, J=6.5 Hz, 1 H, 9=CH-CH_3). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 12.8 (CH_3, 9=CH-CH_3), 22.8 (CH_3, 7-CH_3), 32.8 (CH, C5), 40.2 (CH, C1), 40.5 (CH_2, C8), 47.5 (CH_2, C4), 50.6 (CH_2, C2), 115.3 (CH, 9=CH-CH_3), 124.4 (CH, C6), 133.6 (C, C7), 138.8 (C, C9), 211.8 (C, C3). Found: C, 81.74; H, 9.19; calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.16.

***rac*-12-Amino-6,7,8,9,10,11-hexahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (7ay·HCl).** To an ice-bath cooled suspension of $AlCl_3$ (964 mg, 7.23 mmol) and 2-aminobenzonitrile (**6y**) (856 mg, 7.25 mmol) in 1,2-dichloroethane (120 mL), a solution of ketone **5a** (1.00 g, 7.24 mmol) in 1,2-dichloroethane (20 mL) was added dropwise under argon, and the mixture was heated under reflux with stirring for 12 h. The resulting suspension was cooled to 0 °C, dropwise treated with a mixture of THF (120 mL) and water (60 mL), and made alkaline by stirring with aqueous 2 N NaOH for 30 min. The organic solvent was evaporated at reduced pressure and the aqueous suspension was filtered. The yellow solid residue (2.40 g) was submitted to column chromatography [silica gel (50 g), hexane/ethyl acetate/methanol mixtures of increasing polarity]. On elution with ethyl acetate/methanol 90/10, **7ay** (430 mg, 25% yield) was isolated. To a solution of **7ay** (430 mg) in methanol (50 mL), concentrated HCl (10 mL) was added and the mixture was heated under reflux for 20 min. The solution was evaporated to dryness and the residue (445 mg) was crystallized from ethyl acetate/methanol 1/1 (10 mL) to give, after drying under standard conditions, **7ay·HCl·H_2O** (310 mg, 15% overall yield) as a white solid, mp 254–256 °C (dec); IR (KBr) ν 3450, 3165, 2815 (N–H and O–H st), 1664, 1632, 1585 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 65.33; H, 6.92; N, 9.66; Cl, 12.34; calcd for $C_{16}H_{18}N_2$·HCl·H_2O: C, 65.63; H, 7.23; N, 9.57; Cl, 12.11.

***rac*-12-Amino-6,7,8,9,10,11-hexahydro-7,11-methanocycloocta[*b*]quinolin-exo-9-ol hydrochloride (7by·HCl).** This reaction was carried out as described before for **7ay**. From ketone **5b** (500 mg, 3.24 mmol), **7by** (400 mg, 48% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was decolorized with active charcoal and crystallized from ethyl acetate/methanol 1/9 (10 mL) to give, after drying under standard conditions, **7by·HCl·1.5H_2O** (200 mg, 19% overall yield) as a light-yellow solid, mp

260 °C (dec); IR (KBr) ν 3700–2000 [max at 3342, 3200 (N–H and O–H st)], 1654, 1640, 1587 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 60.21; H, 6.80; N, 8.59; Cl, 11.00; calcd for $C_{16}H_{18}N_2O{\cdot}HCl{\cdot}1.5H_2O$: C, 60.46; H, 6.98; N, 8.81; Cl, 11.15.

***rac*-12-Amino-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (7cy·HCl).** This reaction was carried out as described before for **7ay**. From ketone **5c** (500 mg, 3.67 mmol), **7cy** (510 mg, 59% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (15 mL) to give, after drying under standard conditions, **7cy·HCl·1.75H_2O** (310 mg, 28% overall yield) as a white solid, mp 177–179 °C (dec); IR (KBr) ν 3700–2000 [max at 3335, 3176 (N–H and O–H st)], 1652, 1586 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 63.14; H, 6.50; N, 8.98; Cl, 11.57; calcd for $C_{16}H_{16}N_2{\cdot}HCl{\cdot}1.75H_2O$: C, 63.15; H, 6.79; N, 9.21; Cl, 11.65.

***rac*-12-Amino-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline hydrochloride (7dy·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5d** (1.50 g, 9.79 mmol), **7dy** (1,48 g, 60% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was decolorized with active charcoal and crystallized from ethyl acetate/methanol 1/1 (20 mL) to give, after drying under standard conditions, **7dy·HCl·H_2O** (980 mg, 33% overall yield) as a white solid, mp 265–268 °C (dec); IR (KBr) ν 3700–2000 [max at 3354, 3202 (N–H and O–H st)], 1640, 1588 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 67.02; H, 7.12; N, 8.89; Cl, 11.97; calcd for $C_{17}H_{18}N_2{\cdot}HCl{\cdot}H_2O$: C, 66.98; H, 6.95; N, 9.19; Cl, 11.63.

***rac*-11-Amino-2,3,5,6,9,10-hexahydro-8-methyl-6,10-methano-1*H*-cycloocta[*e*]cyclopenta[*b*]pyridine hydrochloride (7dz·HCl).** This reaction was carried out in a similar way to that described before for **7ay**. Starting from 2-aminocyclopent-1-enecarbonitrile (**6z**) (3.57 g, 33.0 mmol), and ketone **5d** (4.90 g, 33.0 mmol), **7dz** (760 mg, 10% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (30 mL) to give, after drying under standard conditions, **7dz·HCl·3H_2O** (410 mg, 4% overall yield) as a white solid, mp 247–250 °C (dec). IR (KBr) ν 3700–2000 [max at 3341, 3187 (N–H and O–H st)], 1655, 1620 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 58.30; H, 8.24; N, 8.52; Cl, 10.85; calcd for $C_{16}H_{20}N_2{\cdot}HCl{\cdot}3H_2O$: C, 58.08; H, 8.23; N, 8.47; Cl, 10.71.

***rac*-12-Amino-9-ethyl-6,7,10,11-tetrahydro-7,11-methanocycloocta[*b*]quinoline hydrochloride (7ey·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5e** (800 mg, 4.87 mmol), **7ey** (750 mg, 59% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (25 mL) to give, after drying under standard conditions, **7ey·HCl·1.25H_2O** (330 mg, 21% overall yield) as a white solid, mp 260–263 °C (dec); IR (KBr) ν 3700–2000 [max at 3325 and 3150 (N–H and O–H st)], 1660, 1587 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 66.98; H, 6.90; N, 8.41; Cl, 11.26; calcd for $C_{18}H_{20}N_2{\cdot}HCl{\cdot}1.25H_2O$: C, 66.86; H, 7.23; N, 8.66; Cl, 10.96.

***rac*-12-Amino-6,7,10,11-tetrahydro-syn-13-methoxy-9,13-dimethyl-7,11-methanocycloocta[*b*]quinoline hydrochloride (7fy·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5f** (631 mg, 3.25 mmol), **7fy** (470 mg, 49% yield) was isolated. It was transformed into the corresponding hydrochloride by reaction with excess of an ethereal solution of HCl, and then, it was crystallized from ethyl acetate/methanol 10/1 (22 mL) to give, after drying under standard conditions, **7fy·HCl** (380 mg, 35% overall yield) as a white solid, mp 265–270 °C (dec); IR (KBr) ν 3700–2000 [max at 3331, 3144 (N–H and O–H st)], 1659, 1588 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 68.99; H, 7.06; N, 8.32; Cl, 10.68; calcd for $C_{19}H_{22}N_2O{\cdot}HCl$: C, 68.97; H, 7.01; N, 8.47; Cl, 10.71.

***rac*-12-Amino-6,7,10,11-tetrahydro-anti-13-methoxy-9,13-dimethyl-7,11-methanocycloocta[*b*]quinoline hydrochloride (7gy·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5g** (200 mg, 1.03 mmol), **7gy** (87 mg, 29% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7fy·HCl** and crystallized from ethyl acetate/methanol 10/1 (22 mL) to give, after drying under standard conditions, **7gy·HCl·1.25H_2O** (60 mg, 16% overall yield) as a white solid, mp 220 °C (dec); IR (KBr) ν 3500–2000 [max at 3338, 3179 (N–H and O–H st)], 1658, 1587 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 64.68; H, 6.98; N, 7.91; Cl, 10.38; calcd for $C_{19}H_{22}N_2O{\cdot}HCl{\cdot}1.25H_2O$: C, 64.58; H, 7.28; N, 7.93; Cl, 10.03.

***rac*-12-Amino-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinolin-13-one (7hy) and *rac*-12-amino-6,7,10,11-tetrahydro-13,13-dihydroxy-9-methyl-7,11-methanocycloocta[*b*]quinoline hydrochloride (7hy·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5h** (490 mg, 2.98 mmol), slightly impure **7hy** (460 mg, 58% yield) was isolated. IR (KBr) ν 3357, 3257, 2926 (N–H st), 1723 (C=O st), 1650, 1589 (ar–C–C and ar–C–N st) cm^{-1}. This product was transformed into the corresponding hydrochloride as described for **7fy·HCl**, which was crystallized from ethyl acetate/methanol 10/1 (22 mL) to give, after drying under standard conditions, **7hy·HCl·0.1H_2O** (230 mg, 24% overall

yield) as a white solid, mp 225 °C (dec), which showed to be the hydrochloride of the corresponding *gem*-diol, for which the same notation as for the ketone has been retained. IR (KBr) ν 3355, 3215 (N–H and O–H st), 1651, 1588 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 63.50; H, 5.67; N, 8.80; Cl, 11.43; calcd for $C_{17}H_{18}N_2O_2 \cdot HCl \cdot 0.1H_2O$: C, 63.69; H, 6.04; N, 8.74; Cl, 11.06.

***rac*-(*Z*)-12-Amino-13-ethylidene-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline hydrochloride (7iy·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5i** [473 g, 2.69 mmol, containing around 5% molar (by ^1H NMR) of its (*Z*)-stereoisomer (**5j**)], **7iy** (527 mg, 71% yield) was isolated. This product was transformed into the corresponding hydrochloride as described for **7fy·HCl** and crystallized from methanol (2.3 mL) to give, after drying under standard conditions, **7iy·HCl·0.75H$_2$O** [263 mg, 30% overall yield, still containing around 5% molar of its stereoisomer (**7jy·HCl**)] as a white solid, mp 320 °C (dec). IR (KBr) ν 3600–2400 [max at 3345, 3189, 2906 (N–H and O–H st)], 1640, 1585 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 69.70; H, 6.93; N, 8.54; Cl, 11.26; calcd for $C_{19}H_{21}N_2Cl \cdot HCl \cdot 0.75H_2O$: C, 69.92; H, 6.95; N, 8.58; Cl, 10.86.

***rac*-(*E*)-12-Amino-13-ethylidene-6,7,10,11-tetrahydro-9-methyl-7,11-methanocycloocta[*b*]quinoline hydrochloride (7jy·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5j** [600 mg, 3.41 mmol, containing around 5% molar (by ^1H NMR) of its (*E*)-stereoisomer (**5i**)], **7jy** (740 mg, 79% yield) was isolated. This product was transformed into the corresponding hydrochloride as described for **7fy·HCl** and crystallized from methanol (2.5 mL) to give, after drying under standard conditions, **7jy·HCl** [420 mg, 39% overall yield, still containing around 5% molar of its stereoisomer (**7iy·HCl**)] as a white solid, mp 250 °C (dec). IR (KBr) ν 3650–2400 [max at 3334, 3160, 2905 (N–H st)], 1652, 1627, 1586 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 72.79; H, 6.72; N, 8.93; Cl, 11.43; calcd for $C_{19}H_{20}N_2 \cdot HCl$: C, 72.52; H, 6.77; N, 8.95; Cl, 11.33.

***rac*-12-Amino-6,7,8,9,10,11-hexahydro-7,11-*o*-benzenocycloocta[*b*]quinoline hydrochloride (7ky·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5k** (3.00 g, 15.0 mmol), **7ky** (1.25 g, 28% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (25 mL) to give, after drying under standard conditions, **7ky·HCl·2H$_2$O** (560 mg, 10% overall yield) as a light yellow solid, mp 120–122 °C (dec). IR (KBr) ν 3700–2000 [max at 3450, 3365, 3250 (N–H and O–H st)], 1642, 1570 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 67.79; H, 6.68; N, 7.33; Cl, 9.71; calcd for $C_{21}H_{20}N_2 \cdot HCl \cdot 2H_2O$: C, 67.64; H, 6.76; N, 7.20; Cl, 9.51.

***rac*-12-Amino-6,7,10,11-tetrahydro-9-methyl-7,11-*o*-benzenocycloocta[*b*]quinoline hydrochloride (7ly·HCl).** This reaction was carried out as described before for **7ay**. Starting from **5l** (200 mg, 0.94 mmol), **7ly** (210 mg, 71% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (10 mL) to give, after drying under standard conditions, **7ly·HCl·2.25H$_2$O** (160 mg, 44% overall yield) as a white solid, mp 263–265 °C (dec). IR (KBr) ν 3700–2000 [max at 3362, 3218 (N–H and O–H st)], 1655, 1635, 1583 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 67.60; H, 6.99; N, 7.04; Cl, 9.00; calcd for $C_{22}H_{20}N_2 \cdot HCl \cdot 2.25H_2O$: C, 67.85; H, 6.60; N, 7.19; Cl, 9.10.

***rac*-11-Amino-1,2,3,5,6,7,9,10-octahydro-6,10-o-benzenocycloocta[*e*]cyclopenta[*b*]pyridin-8-one, 7nz[1].** This reaction was carried out in a similar manner to that described for **7ay**. Starting from 2-aminocyclopent-1-enecarbonitrile (**6z**) (750 mg, 6.94 mmol), and diketone **5n** (1.00 g, 4.67 mmol), **7nz** (480 mg, 34% yield) was isolated. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was decolorized with active charcoal and crystallized from ethyl acetate/methanol 1/1 (10 mL) to give, after drying under standard conditions, **7nz·HCl·H$_2$O** (380 mg, 23% overall yield) as a white solid, mp 216–218 °C (dec). IR (KBr) ν 3700–2000 [max at 3425, 3335, 3167 (N–H and O–H st)], 1692 (C=O st), 1654, 1625 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 66.98; H, 6.21; N, 7.89; Cl, 9.92; calcd for $C_{20}H_{20}N_2O \cdot HCl \cdot H_2O$: C, 66.94; H, 6.46; N, 7.81; Cl, 9.88.

***rac*-12-Amino-6,7,8,9,10,11-hexahydro-7,11-methanocycloocta[*b*]quinolin-endo-9-ol hydrochloride (7oy·HCl).** To a solution of 12-amino-7,8,10,11-tetrahydro-7,11-methano-6*H*-cycloocta[*b*]quinolin-9-one[1] (200 mg, 0.79 mmol) in methanol (20 mL), $NaBH_4$ (60 mg, 1.6 mmol) was added portionwise. The reaction mixture was stirred at room temperature for 12 h and then, the solvent was evaporated at reduced pressure. The resulting residue was suspended in aqueous 2 N NaOH (30 mL), heated under reflux for 30 min, and filtered. The solid material was washed with water (10 mL) and dried in vacuo, yielding impure **7oy** (180 mg). It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (12 mL) to give, after drying under standard conditions, **7oy·HCl·0.75H$_2$O** (145 mg, 68% overall yield), mp 197–198 °C (dec). IR (KBr) ν 3700–2000 [max at 3515, 3463, 3338, 3251, 3080 (N–H and O–H st)], 1659, 1575, 1565 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 63.22; H, 6.88; N, 9.33; Cl, 12.00; calcd for

$C_{16}H_{18}N_2O{\cdot}HCl{\cdot}0.75H_2O$: C, 63.15; H, 6.79; N, 9.21; Cl, 11.65.

***rac*-11-Amino-2,3,5,6,7,8,9,10-octahydro-6,10-methano-1*H*-cycloocta[*e*]cyclopenta[*b*]pyridin-endo-8-ol hydrochloride (7oz·HCl).** This reaction was carried out as described before for **7oy**. Starting from *rac*-11-amino-1,2,3,5,6,7,9,10-octahydro-6,10-methanocycloocta[*e*]cyclopenta[*b*]pyridin-8-one[1] (500 mg, 2.06 mmol), **7oz** (420 mg, 85% yield) was obtained. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (20 mL) to give, after drying under standard conditions, **7oz·HCl·2.5H$_2$O** (330 mg, 50% overall yield) as a white solid, mp 162–164 °C (dec). IR (KBr) ν 3700–2000 [max at 3500, 3417 (N–H and O–H st)], 1640 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 55.04; H, 7.78; N, 8.46; Cl, 10.84; calcd for $C_{15}H_{20}N_2O{\cdot}HCl{\cdot}2.5H_2O$: C, 55.29; H, 8.05; N, 8.60; Cl, 10.88.

***rac*-12-Amino-6,7,8,9,10,11-hexahydro-7,11-*o*-benzenocycloocta[*b*]quinolin-endo-9-ol hydrochloride (7py·HCl).** This reaction was carried out as described before for **7oy**. Starting from 12-amino-7,8,10,11-tetrahydro-6*H*-7,11-*o*-benzenocycloocta[*b*]quinolin-9-one[1] (**7ny**) (200 mg, 0.64 mmol), **7py** (175 mg, 87% yield) was obtained. It was transformed into the corresponding hydrochloride as described for **7ay·HCl**, which was crystallized from ethyl acetate/methanol 1/1 (10 mL) to give, after drying under standard conditions, **7py·HCl·2H$_2$O** (130 mg, 53% overall yield) as a yellow solid, mp 259–261 °C (dec). IR (KBr) ν 3700–2000 [max at 3374, 3225 (N–H and O–H st)], 1637, 1584 (Ar–C–C and Ar–C–N st) cm^{-1}. Found: C, 64.57; H, 6.37; N, 6.96; Cl, 9.02; calcd for $C_{21}H_{20}N_2O{\cdot}HCl{\cdot}2H_2O$: C, 64.86; H, 6.48; N, 7.20; Cl, 9.12.

Biochemical studies

AChE inhibitory activity was measured according to the method of Ellman.[40] Briefly, the hydrolysis of acetylcholine by AChE from bovine erythrocytes was evaluated. The activity was measured in phosphate-buffered solution (3 mL, 0.1 M, pH 8.0) at 25 °C with a solution of 5,5′-dithiobisnitrobenzoic acid (DTNB, 100 μL, 0.01 M), enzyme (50 μL, 0.5 units/mL) and inhibitor (30 μL). Acetylthiocholine (SIGMA) was employed as a substrate at a concentration of 8 mM. Assays were performed with at least 10 concentrations of inhibitor and IC_{50} (μM drug concentration that inhibited 50% AChE activity) was calculated.

Neuromuscular studies

Right and left phrenic nerve-hemidiaphragm removed from male Sprague–Dawley rats (250–300 g) were used. Details of the experimental procedures have been previously described.[45] Briefly, rats were lightly anaesthetized with ether and decapitated. After quick dissection, each phrenic-hemidiaphragm preparation was suspended in organ baths of 75 mL volume with Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1. The preparation was bubbled with 5% CO_2 in oxygen and the temperature was maintained at 25 ± 1 °C. Drug effects of AChE compounds on neuromuscular junction were assessed as the ability of reversing the partial blockade induced by *d*-tubocurarine in indirectly elicited twitch responses. The twitches were obtained by stimulating the phrenic nerve with square pulses of 0.5 ms duration at 0.2 Hz and a supramaximal voltage. Neuromuscular blockade was obtained with the addition of *d*-tubocurarine (1–1.5 μM). Study drugs were added when a reduction of twitch response to 70–80% control values was obtained. The effect of each drug was evaluated during 15 min of exposure. To avoid the possible carry-over effects, only one concentration of inhibitor was tested on each preparation. Several drug concentrations were used for each AChE inhibitor. To evaluate the reversal effect of each drug, the antagonism index (AI or % of antagonism[46] was determined for each concentration and the AI_{50} (μM drug concentration that inhibits a 50% *d*-tubocurarine blockade) was calculated. All results were expressed as mean ± standard error of the mean (SEM).

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