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Synthesis and Acetylcholinesterase/Butyrylcholinesterase Inhibition Activity of New Tacrine-like Analogues

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Abstract—The synthesis and preliminary results for acetylcholinesterase and butyrylcholinesterase inhibition activity of a series of pyrano[2,3-*b*]quinolines (**2**, **3**) and benzonaphthyridines (**5**, **6**) derivatives are described. These molecules are tacrine-like analogues which have been prepared from readily available polyfunctionalized ethyl [6-amino-5-cyano-4*H*-pyrans and 6-amino-5-cyano-pyridines]-3-carboxylates via Friedländer condensation with selected ketones. These compounds showed moderate acetylcholinesterase inhibition activity, the more potent (**2e**, **5b**) being 6 times less active than tacrine. The butyrylcholinesterase activity of some of these molecules is also discussed.

Introduction

The only therapeutic strategy thas has been shown consistently useful in treating Alzheimer's disease (AD) patients is acetylcholinesterase inhibitors (AChEI). This therapeutic approach is a rational strategy which follows the so-called 'cholinergic hypothesis'.[1] AChEI increase the brain acetylcholine levels by decreasing the metabolic rate of released neurotransmitter, thereby enhancing neurotransmission at cholinergic synapses.[1a] In the last decades, tacrine (**1**) (Chart 1) has been one of the most used and known acetylcholinesterase inhibitors for AD therapy, but not without important side effects. This has prompted a great synthetic and pharmacological effort in order to design more potent and less aggressive tacrine analogues.[1b]

In this context, in a current project under progress in our laboratory, we have been very recently involved in the synthesis and biological evaluation of new acetylcholinesterase inhibitors related to tacrine (**1**); as a result, the preparation of differently substituted 4*H*-pyrano[2,3-*b*]quinoline-3-carboxylic acid derivatives (**A**) has been described (Chart 1).[2]

The central point in this approach was the Friedländer-type[3] reaction of 4*H*-pyran-3-carboxylic acid derivatives (**B**) with the selected ketones, under Lewis-acid catalysis (Scheme 1). Proceeding in this way, compounds **2a–f** and **3** have been obtained in mild reactions conditions and convenient chemical yields.

Continuing with our work in this area, we have now addressed our attention to the analogous cycloannulated [1,8]naphthyridine ring system (**C**) (Chart 1) in order to extend the Friedländer reaction to the corresponding densely functionalized 6-amino-5-cyano-pyridines of type (**D**)[4] (Scheme 2). This would allow us to have a new set of molecules for testing the acetylcholinesterase inhibition activity and to compare their pharmacology with compounds of type **A** (Chart 1) for structure–activity relationship purposes. As in our previous approach,[2] our selection was based on the "conjunctive pharmacomodulation"[5] of tacrine and the naphthyridine ring system. The naphthyridine nucleus has been largely known, synthesized and incorporated into biological active molecules,[6] and appeared to us as an excellent candidate for the 'substitution' of the aromatic A/B rings in tacrine (**1**) (Chart 1).

A search for tacrine-like molecules having the naphthyridine nucleus showed scarce examples, the products

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4a–d (Chart 2), for instance, in a study for the high-affinity choline uptake in rat brain.[7a]

In this paper, we report the synthesis of new tacrine-like analogues of type **C** (**5a–c**: X = H; $n = 0$, 1, 2; **6a–c**: X = *p*-OCH_3; $n = 0$, 1, 2) (Scheme 2), and preliminary pharmacological acetylcholinesterase and butyryl-cholinesterase activity for compounds **2a–f**,[2] **3**,[2] (Scheme 1), **5** and **6**.

Results and Discussion

Chemistry

The synthesis of the target molecules **5** and **6** was achieved starting from pyridines **7** and **8**, respectively, under standard Friedländer reaction conditions,[2] with cyclopentanone, cyclohexanone or cycloheptanone ($n = 0$, 1 and 2, respectively) as the cycloalkanone partner (see Experimental).[8] These pyridines have been described

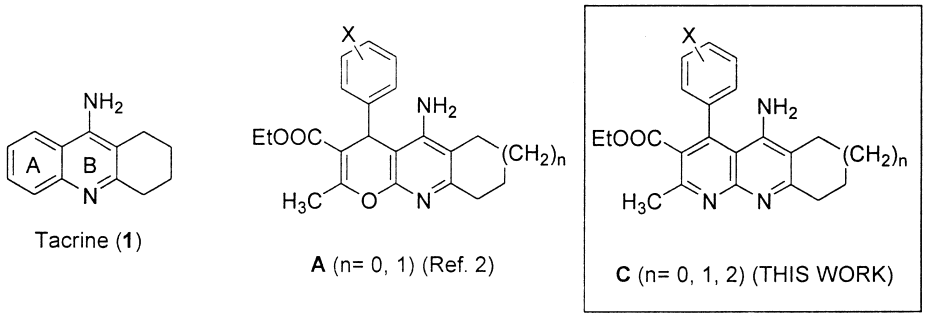
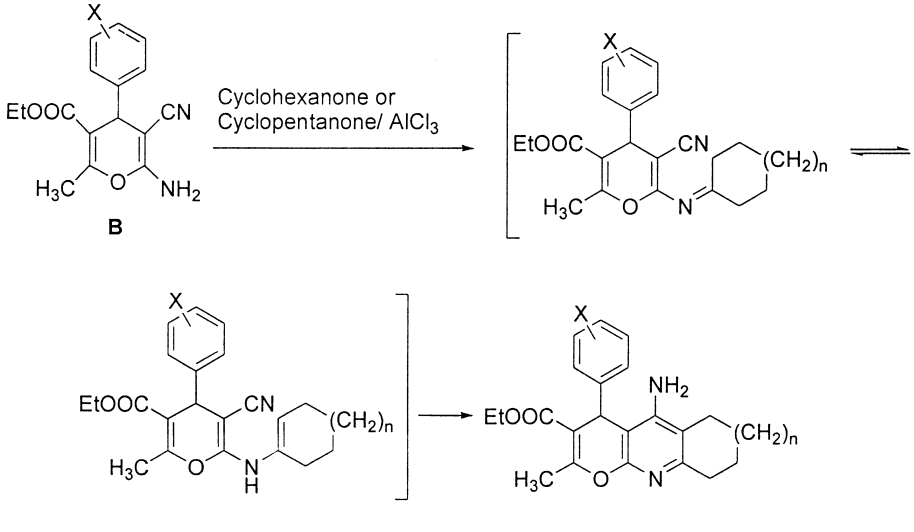
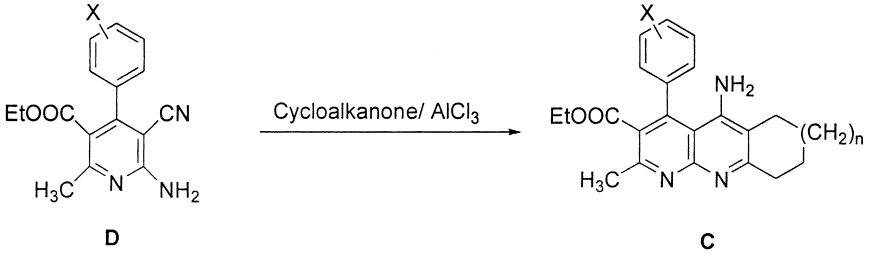


Chart 1.



Scheme 1. Mechanism for the Friedländer reaction, and synthesis of the tacrine-like analogues **2** and **3**.



Scheme 2. Strategy for the synthesis of new tacrine-like analogues of type **C**.

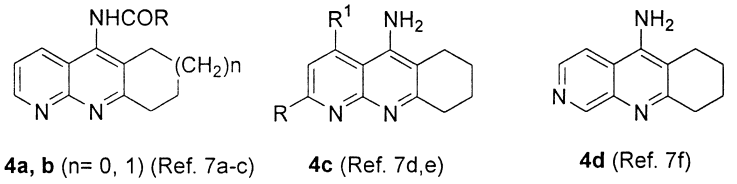
before and are readily prepared from the corresponding 4*H*-pyran-3-carboxylic acid derivatives (**9**, **10**)[9] by reaction with ammonium acetate in glacial acetic acid; these compounds (**7**, **8**) showed spectroscopic data in good agreement with the reported ones.[4] For the initial experiments, and as in compounds **2** and **3**, the selection of the substituents at the aromatic ring has been prompted by the easy availablility of pyrans of type (**B**) (Scheme 1) and by the AChE activity observed for compound **2e** (Scheme 1) (see below); in addition, the size of the cycloannulated ring was also a major concern. In overall, this has moved us to select and prepare compounds **5a–c** (X = H, $n = 0$, 1, 2; lead product) and **6a–c** (X = *p*-OCH_3, $n = 0$, 1, 2) (Scheme 3).

The heteroannulation reaction proceeded smoothly to provide the desired compounds **5** (**5a**: 15% ($n = 0$); **5b**: 60% ($n = 1$); **5c**: 57% ($n = 2$)) and **6** (**6a**: 15% ($n = 0$); **6b**: 74% ($n = 1$); **6c**: 80% ($n = 2$)) (Scheme 2) from moderate to good yields, the cyclopentanone giving the worst of the results (15%); in spite of several trials changing the reaction time or the ratio of reagents, we were unable to improve these yields. All new compounds showed excellent analytical and spectroscopic data in good accordance the proposed structure.[10] In the IR spectra bands for N–H (NH_2) (3440 cm^{-1}), conjugated C=O, C–O (ArCOOEt) (1700, 1300–1200 cm^{-1}) and aromatic C=C (1600–1500 cm^{-1}) vibrations were observed; very significantly, the sharp and intense CN conjugate band at 2000 cm^{-1} was absent. In the ^1H NMR spectra, in addition to the aromatic, methyl at C2 and the ethoxycarbonyl protons, or the methoxy singlet for compounds of type **6**, we analyzed singlets at approximately 4.20 ppm for two protons which were assigned to NH_2; depending of the case ($n = 0$, 1 and 2), for products **5** or **6**, a set of high field protons (6, 8 or 10), between 3.00 and 2.00 ppm, were observed. In agreement with these data, in the ^{13}C NMR spectra, with the help of the DEPT, and ^1H–^{13}C HMQC experiments, we could analyze the carbons for the cycloalkane annulated moiety (in the range of 35–20 ppm), the $COOCH_2CH_3$ signals at 144, 61 and 13 ppm, respectively, and the $C(2)H_3$ at 23 ppm; the analysis of the aromatic carbons was performed by these techniques and by comparison with the reported data for [1,8]naphthyridines and the known shifts effects of substituents.[11] The routine mass spectra of these new molecules showed in all the cases the molecular peak with intense values, and very typical fragmentation peaks at $(M^+ + 1) - 28$, corresponding to the loss of HCN.[11]

Biological evaluation

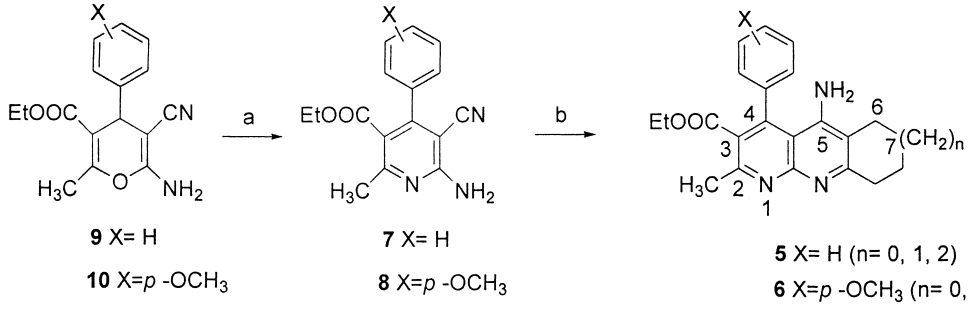
According to the standard methodology (see Experimental) we obtained the following data for the acetylcholinesterase inhibition activity (see Table 1). Comparing with tacrine (**1**) (IC_{50} $1.3 + 0.12 \times 10^{-7}$ M), we can see that all the compounds examined were of lower activity, the more active compounds being **5b** and **2e** (around 6–7 times less active); these compounds have cyclohexane rings onto the aromatic nucleus, with X = H or *p*-OCH_3, respectively. Between the pyrano-like tacrine analogues (compounds **2** and **3**), the cyclopenta-annulated analogue **3** was the less active. In the cyclohexa-annulated analogues, the more active corresponded to product **2e** with the strongest electron donor power; however, the substituents electronic abilities do not seem to play a determinant effect, as compound **2b** (X = *p*-CH_3) or **2f** (X = *m*-NO_2) have a similar IC_{50} value.

In the pyridine-like tacrine analogues of type **5** (X = H) the most active corresponded to the cyclohexaannulated derivative **5b**, while in compounds **6** (X = *p*-OCH_3), the



4a, b (n= 0, 1) (Ref. 7a-c) **4c** (Ref. 7d,e) **4d** (Ref. 7f)

Chart 2.



9 X= H
10 X=*p* -OCH_3
7 X= H
8 X=*p* -OCH_3
5 X= H (n= 0, 1, 2)
6 X=*p* -OCH_3 (n= 0,

Scheme 3. Synthesis of tacrine-like compounds **5** and **6**. Reagents: (a) for X = H (ref 4); for X = *p*-OCH_3 (ref 4); (b) cycloalkanone (cyclopentanone ($n = 0$), cyclohexanone ($n = 1$), cycloheptanone ($n = 1$)), $(CH_2)_2Cl_2$, reflux/$AlCl_3$.

most active is the cyclohepta-annulated analogue. In any case, in compounds **5** or **6**, the most active corresponded to the aryl, unsubstituted, cyclohexa-annulated derivative **5b**.

The most potent compound of each group was assayed to determine their activity on butyrylcholinesterase. Data are also given in Table 1. All the compounds tested inhibit more strongly AchE than BuChE, the most active derivative being compound **5b**, which was also the most active as AChE inhibitor. Note that compound **2a** showed a strong selectivity for AChE. For the other 'pyranoquinolines', compounds **2e** and **3** are 10-fold and 100-fold more selective, respectively. For the 'benzonaphtyridines', compounds **5b** and **6c** showed selectivities in the range of 10-fold. These data are very interesting as it is well known that the tacrine's adverse side effects are due to its ability to inhibit BuChE, and suggest future structural modifications for activity improvement.

Finally, a series of experiments were carried out in order to determine the type of antagonism in compounds **2a**, **2e**, **3**, **5b** and **6c**. The data are shown in Table 2. It is important to note that the most active AChE and BuChE inhibitors (**2e** and **5b**) are non-competitive inhibitors.

Table 1. IC_{50} (M) values for activities on acetylcholinesterase and butyrylcholinesterase of compounds **2**, **3**, **5** and **6**. Results are expressed in (x±SEM)

Compound	AChE inhibition (IC_{50}) (M)	BuChE inhibtion (IC_{50}) (M)
Tacrine	$1.3\pm0.12\times10^{-7}$	$4.39\pm0.17\times10^{-8}$
Pyranoquinolines		
Cyclohexa- ($n=1$)		
2a (X = H)	$1.56\pm0.05\times10^{-6}$	$>3\times10^{-3}$
2b (X = *p*-CH_3)	$1.82\pm0.11\times10^{-6}$	
2c (X = *p*-Cl)	$1.93\pm0.16\times10^{-6}$	
2d (X = *p*-CN)	$3.47\pm0.79\times10^{-6}$	
2e (X = *p*-OCH_3)	$8.68\pm0.42\times10^{-7}$	$6.18\pm0.60\times10^{-6}$
2f (X = *m*-NO_2)	$1.89\pm0.76\times10^{-6}$	
Cyclopenta ($n=0$)		
3 (X = H)	$3.90\pm0.68\times10^{-6}$	$3.35\pm2.09\times10^{-4}$
Benzonaphthyridines		
5a (X = H) Cyclopenta ($n=0$)	$1.19\pm0.07\times10^{-5}$	
5b (X = H) Cyclohexa ($n=1$)	$8.22\pm1.57\times10^{-7}$	$5.03\pm0.57\times10^{-6}$
5c (X = H) Cyclohepta ($n=2$)	$2.10\pm0.11\times10^{-5}$	
6a (X = *p*-OCH_3) Cyclopenta (n = 0)	$1.37\pm0.28\times10^{-5}$	
6b (X = *p*-OCH_3) Cyclohexa ($n=1$)	$8.97\pm1.55\times10^{-6}$	
6c (X = *p*-OCH_3) Cyclohepta ($n=2$)	$1.35\pm0.71\times10^{-6}$	$8.06\pm2.60\times10^{-5}$

Table 2. Effects on the drugs on the V_{max} and K_m values of enzyme compared to control values in the absence of inhibitors

Compound	V_{max}	K_m (μM)	Antagonism
Control ($n=4$)	0.39±0.04	134.24±23.13	
2a (X = H)	0.41±0.03 NS	222.58±13.59 *	Competitive
2e (X = *p*-OCH_3)	0.05±0.01 **	119.19±12.24 NS	Non-competitive
|**3** (X = H)	0.40±0.03 NS	217.28±14.19 *	Competitive
5b (X = H)	0.11±0.01 **	182.95±28.33 NS	Non-competitive
6c (X = *p*-OCH_3)	0.42±0.01 NS	228.49±22.25 *	Competitive

NS = not significant, *$p<0.05$, ** $p<0.01$

Conclusions

In summary, we have reported the synthesis and preliminary results for acetylcholinesterase and butyrylcholinesterase inhibition activity of a series of pyrano-[2,3-*b*]quinolines (**2**, **3**) and benzonaphthyridines (**5**, **6**) derivatives. These molecules have been prepared from readily available polyfunctionalized ethyl [4*H*-pyrans and 6-amino-5-cyanopyridines]-3-carboxylates via Friedländer condensation with selected ketones. The most active compounds in these series correspond to pyrano- or pyridine-like tacrines with saturated cyclohexane rings. The type of the substituent at the aromatic ring in C4 does not seem to have a deep influence on the inhibitory activity. In overall, the substitution of a benzene ring in tacrine (**1**) by pyran or pyridine ring gives analogues with strong acetylcholinesterase inhibition, with lower values, but very close to those shown by tacrine (**1**).

These data give support to our initial hypothesis,[2] and prompt us to continue this project. Work is now in progress to extend this chemistry to other related precursors, test the biological activity and perform molecular modelling in order to have a clearer picture of the (enzyme)receptor–substrate interactions.

Experimental

General methods

Reactions were monitored by TLC using precoated silica gel aluminium plates containing a fluorescent indicator (Merck, 5539). Detection was done by UV (254 nm) followed by charring with sulfuric-acetic acid spray, 1% aqueous potassium permanganate solution or 0.5% phosphomolybdic acid in 95% EtOH. Anhydrous Na_2SO_4 was used to dry organic solutions during work-ups and the removal of solvents was carried out under vacuum with a rotary evaporator. Flash column chromatography was performed using silica gel 60 (230–400 mesh, Merck) and dichloromethane/methanol mixtures as eluent unless otherwise stated. ^1H spectra were recorded with a Varian VXR-200S spectrometer, using tetramethylsilane as internal standard and ^{13}C NMR spectra were recorded with a Bruker WP-200-SY. Compounds **2a**–**f** and **3** have been prepared according to our previously described protocol.[2]

General method for the Friedländer reaction

Aluminium chloride (1.2–1.7 equiv) was suspended in dry 1,2-dichloroethane (10 mL/mmol) at room temperature under argon. The corresponding 6-amino-5-cyanopyridines **7**[4] and **8**[4] (1 equiv) and the ketone (cyclopentanone, cyclohexanone and cycloheptanone; 1.2–1.7 equiv) were added. The reaction mixture was

heated under reflux (10–24 h). When the reaction was over (TLC analysis), a mixture of THF/H_2O (2:1) was added at rt. An aqueous solution of sodium hydroxide (10%) was added dropwise to the mixture until the aqueous solution was basic. After stirring for 30 min, the mixture was extracted three times with dichloromethane. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. The resultant solid was purified by silica gel flash chromatography using methanol/dichloromethane (6/94) as eluent to give pure compounds **5a**, **5b**, **5c**, **6a**, **6b** and **6c**, respectively. These compounds showed good elemental analysis in agreement with structures.

Ethyl 5-amino-6,7,8-trihydro-2-methyl-4-phenylcyclopenta-[*b*][1,8]naphthyridine-3-carboxylate (5a). Following the general method, compound **7** (150 mg, 0.53 mmol) ($AlCl_3$ (142.2 mg, 1.07 mmol), $Cl_2CH_2CH_2Cl_2$ (6 mL), cyclopentanone (94 μL, 1.07 mmol)) afforded **5a** (28.4 mg, 15%): mp 209–211 °C; IR (KBr) ν 3430, 1710, 1605, 1575, 1550, 1530, 1470, 1420, 1405, 1350, 1250, 1190, 1065, 680 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.49–7.37 (m, 5H, C_6H_5), 4.10 (s, 2H, NH_2), 3.96 (q, 2H, $J=7.2$ Hz, $CO_2CH_2CH_3$), 3.12 (m, 2H, H8), 2.69 (m, 5H, H6, CH_3), 2.16 (m, 2H, H7), 0.93 (t, 3H, $J=7.2$ Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.6 (C8a), 168.1 (C2), 156.9 (C9a), 155.9 (C5), 147.1 (C4), 144.9 (C=O), 137.2 (*C*1′), 129.1 (*C*(4′)H Ar), 128.9 (2×*C*H Ar), 128.5 (2×*C*H Ar), 126.9 (C3), 116.6 (C4a), 106.9 (C5a), 61.2 (OCH_2), 35.2 (C8), 27.5 (C6), 23.4 (CH_3–Ar), 22.0 (C7), 13.6 (CH_3CH_2); MS (APCI+) *m*/*z* 348 ($(M+1)^+$, 82), 334 (4), 320 (100), 276 (8). Anal. calcd for $C_{21}H_{21}N_3O_2$: C, 72.60; H, 6.09; N, 12.10. Found: C, 72.45; H, 6.23; N, 12.09.

Ethyl 5-amino-6,7,8,9-tetrahydro-2-methyl-4-phenylbenzo-[1,8]naphthyridine-3-carboxylate (5b). Following the general method, compound **7** (70 mg, 0.25 mmol) ($AlCl_3$ (39.8 mg, 0.30 mmol), $Cl_2CH_2CH_2Cl_2$ (3 mL), cyclohexanone (31 μL, 0.30 mmol)) afforded **5b** (54 mg, 60%): mp 184–186 °C; IR (KBr) ν 3435, 2880, 1700, 1600, 1595, 1540, 1515, 1470, 1415, 1355, 1345, 1285, 1245, 1195, 1080, 1050, 680 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.50–7.35 (m, 5H, C_6H_5), 4.44 (s, 2H, NH_2), 3.96 (q, 2H, $J=7.2$ Hz, $CO_2CH_2CH_3$), 3.07 (m, 2H, H9), 2.68 (s, 3H, CH_3), 2.36 (m, 2H, H6), 1.88 (m, 4H, H7, H8), 0.94 (t, 3H, $J=7.2$ Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 168.2 (C2), 162.1 (C9a), 156.3 (C10a), 154.6 (C5), 149.1 (C4), 144.5 (C=O), 137.4 (*C*1′), 129.1 (*C*(4′)H Ar), 129.0 (2×*C*H Ar), 128.7 (2×*C*H Ar), 127.0 (C3), 111.6 (C4a), 106.4 (C5a), 61.2 (OCH_2), 34.1 (C9), 23.6 (CH_3–Ar), 23.5 (C6), 22.7, 22.5 (C7, C8), 13.7 (CH_3CH_2); MS (APCI+) *m*/*z* 362 ($(M+1)^+$, 100), 334 (3). Anal. calcd for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63. Found: C, 73.12; H, 7.13; N, 11.21.

Ethyl 5-amino-6,7,8,9,10-pentahydro-2-methyl-4-phenyl-cyclohepta[*b*][1,8]naphthyridine-3-carboxylate (5c). Following the general method, compound **7** (70 mg, 0.25 mmol) ($AlCl_3$ (56.4 mg, 0.42 mmol), $Cl_2CH_2CH_2Cl_2$ (3 mL), cycloheptanone (50 μL, 0.42 mmol)) afforded **5c** (53.6 mg, 57%): mp 96–98 °C; IR (KBr) ν 3435, 2910, 1720, 1625, 1560, 1535, 1310, 1270, 1215, 1080, 1050, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.48–7.36 (m, 5H, C_6H_5), 4.35 (s, 2H, NH_2), 3.95 (q, 2H, $J=7.2$ Hz, $CO_2CH_2CH_3$), 3.14 (m, 2H, H10), 2.68 (s, 3H, CH_3), 2.57 (m, 2H, H9), 1.79 (m, 4H, H7, H8), 1.58 (m, 2H, H6), 0.93 (t, 3H, $J=7.2$ Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 168.2 (C2, C10a), 156.0 (C11a), 154.6 (C5), 147.8 (C4), 144.6 (C=O), 137.4 (*C*1′), 129.0 (C(H)4′) 128.9 (2×*C*H Ar), 128.6 (2×*C*H Ar),127.8 (C3), 117.0 (C4a), 107.2 (C5a), 61.2 (OCH_2), 39.8 (C10), 31.8(C7), 27.0 (C6), 26.4 (C8), 26.0 (C9), 23.4 (CH_3–Ar), 13.7 (CH_3CH_2); MS (APCI+) *m*/*z* 376 ($(M+1)^+$, 100), 348 (3). Anal. calcd for $C_{23}H_{25}N_3O_2$: C, 70.21; H, 6.92; N, 10.68. Found: C, 70.05; H, 7.06; N, 10.77.

Ethyl 5-amino-6,7,8-trihydro-4-(*p*-methoxyphenyl)-2-methylcyclopenta[*b*][1,8] naphthyridine-3-carboxylate (6a). Following the general method, compound **8** (70 mg, 0.22 mmol) ($AlCl_3$ (60 mg, 0.45 mmol), $Cl_2CH_2CH_2Cl_2$ (3 mL), cyclopentanone (40 μL, 0.45 mmol)) afforded **6a** (12.7 mg, 15%): mp 216–219 °C; IR (KBr) ν 3420, 1705, 1610, 1580, 1555, 1530, 1495, 1265, 1225, 1195, 1155, 1065, 680; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.32–6.97 (A_2B_2, 4H, C_6H_4), 4.17 (s, 2H, NH_2), 4.02 (q, 2H, $J=7.2$ Hz, $CO_2CH_2CH_3$), 3.87 (s, 3H, *p*-OCH_3), 3.13 (m, 2H, H8), 2.68 (m, 5H, H6, CH_3), 2.16 (m, 2H, H7), 1.01 (t, 3H, $J=7.2$ Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) 170.5 (C8a), 168.3 (C2), 160.2 (C4′), 156.9 (C9a), 155.9 (C5), 147.3 (C4), 144.8 (C=O), 130.2 (C2′), 129.0 (C1′), 127.5 (C3), 116.5 (C4a), 114.1 (C3′), 107.4 (C5a), 61.2 (OCH_2CH_3), 55.4 (CH_3OAr) 35.3 (C8), 27.5 (C6), 23.5 (CH_3Ar), 22.1 (C7), 13.8 (CH_3CH_2) ; MS (APCI+) *m*/*z* 378 ($(M+1)^+$, 100), 306 (3). Anal. calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 69.22; H, 6.46; N, 11.12.

Ethyl 5-amino-6,7,8,9-tetrahydro-4-(*p*-methoxyphenyl)-2-methylbenzo[1,8]naphthyridine-3-carboxylate (6b). Following the general method, compound **8** (80 mg, 0.26 mmol) ($AlCl_3$ (51.4 mg, 0.39 mmol), $Cl_2CH_2CH_2Cl_2$ (3 mL), cyclohexanone (40 μL, 0.39 mmol)) **6b** (74.9 mg, 74%): mp 187–191 °C; IR (KBr) ν 3485, 2945, 1730, 1630, 1570, 1545, 1515, 1440, 1415, 1385, 1370, 1315, 1290, 1270, 1255, 1220, 1180, 1080 and 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.20–6.86 (A_2B_2, 4H, C_6H_4), 4.45 (s, 2H, NH_2), 3.89 (q, 2H, $J=7.2$ Hz, $CO_2CH_2CH_3$), 3.75 (s, 3H, p-OCH_3), 2.93 (m, 2H, H9), 2.55 (s, 3H, CH_3), 2.26 (m, 2H, H6), 1.76 (m, 4H, H7, H8), 0.89 (t, 3H, $J=7.2$ Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) 168.2 (C2), 161.3 (C9a), 160.2 (C4′), 156.4 (C10a), 154.0 (C5), 149.8 (C4), 144.5 (C=O), 130.1 (2×C2′), 128.6 (C1′), 127.6 (C3), 114.1 (C3′), 111.4 (C4a), 106.6 (C5a), 61.2 (OCH_2), 55.3 (OCH_3), 33.6 (C9), 23.5 (C6), 23.4 (CH_3–Ar), 22.5 and 22.3 (C7 and C8), 13.8 (CH_3CH_2); MS (APCI+) *m*/*z* 392 [$(M+1)^+$, 100], 364 (7). Anal. calcd for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.45; H, 6.70; N, 10.84.

Ethyl 5-amino-6,7,8,9,10-pentahydro-4-(*p*-methoxyphenyl)-2-methylcyclohepta[*b*][1,8] naphthyridine-3-carboxylate (6c). Following the general method, compound **8** (80 mg, 0.26 mmol) [$AlCl_3$ (51.4 mg, 0.39 mmol), $Cl_2CH_2CH_2Cl_2$ (3 mL), cycloheptanone (46 μL, 0.39 mmol)] afforded **6c**

(83.3 mg, 80%): mp 90–93 °C; IR (KBr) ν 3490, 2930, 1735, 1630, 1570, 1545, 1515, 1445, 1320, 1290, 1255, 1230, 1180, 1090 and 1025 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.34–6.97 (A_2B_2, 4H, C_6H_4), 4.50 (s, 2H, NH_2), 4.02 (q, 2H, $J=7.2$ Hz, $CO_2CH_2CH_3$), 3.87 (s, 3H, *p*-OCH_3), 3.17 (m, 2H, H10), 2.68 (s, 3H, CH_3), 2.60 (m, 2H, H9), 1.81 (m, 4H, H7 and H8), 1.61 (m, 2H, H6), 1.02 (t, 3H, $J=7.2$ Hz, $CO_2CH_2CH_3$), 0.93 (t, 3H, $J=7.2$ Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50 MHz) 168.3 (C10a), 168.0 (C2), 160.2 (C4′), 156.0 (C11a), 154.5 (C5), 148.1 (C4), 144.5 (C=O), 129.0 (C(H)4′), 130.2 (2×C2′), 129.0 (C1′), 128.1 (C3), 116.9 (C4a), 114.0 (2×C3′) 107.6 (C5a), 61.2 (OCH_2), 55.4 (OCH_3), 39.7 (C10), 31.8 (C7), 27.0 (C6), 26.4 (C8), 26.0 (C9), 23.3 (CH_3–Ar), 13.8 (CH_3CH_2); MS (APCI+) *m*/*z* 406 ($(M+1)^+$, 100). Anal. calcd for $C_{24}H_{27}N_3O_3$: C, 65.29; H, 7.08; N, 9.52. Found: C, 65.31; H, 7.34; N, 9.58.

Biological studies

Acetylcholinesterase (AChE) inhibitory activity was evaluated spectrophotometrically at 25 °C by the method of Ellman,[12] using AChE from bovine erythrocytes and acetylthiocholine iodide (0.53 mM) as substrate. The reaction took place in a final volume of 3 mL of 0.1 M phosphate-buffered solution pH 8.0, containing 0.025 unit of AChE and 333 μM 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB) solution used to produce the yellow anion of 5-thio-2-nitrobenzoic acid. Inhibition curves with different derivatives were performed in triplicate by incubating with at least 12 concentrations of inhibitor for 15 min. One triplicate sample without inhibitor was always present to yield the 100% of AChE activity. The reaction was stopped by the addition of 100 μL 1 mM eserine, and the colour production was measured at 412 nm.

Butyrylcholinesterase (BuChE) inhibitory activity determinations were carried out similarly, using 0.035 units of human serum BuChE and 0.56 mM butyrylthiocholine instead of AChE and acetylthiocholine in a final volume of 1 mL.

The drug concentration producing the 50% of AChE or BuChE activity inhibition (IC_{50}) was calculated by non-linear regression. Results are expressed as mean±SEM of at least four experiments.

DTNB, acetylthiocholine and the enzymes were purchased from Sigma, and eserine from Fluka.

To asses if some drugs behaved as competitive or non-competitive inhibitors of the enzyme, kinetic studies were performed in the following manner: Substrate hydrolysis rates were measured at 25 °C during 1–3 min, using six concentrations of substrate ranging from 50 to 400 μM in the presence or absence of a single concentration of inhibitor. Hydrolysis rates were plotted against substrate concentration in the presence of the inhibitory drug. V_{max} and K_M values were derived by fitting data to a rectangular hyperbola by using non-linear least squares regression program. Differences in the V_{max} and K_M values in the presence or absence of inhibitory compounds were assessed using the *t*-test ($p<0.05$ considered significant). The inhibition was considered competitive when the V_{max} was maintained and the K_M value increases. On the contrary, when the K_M was maintained and the V_{max} value decreases, the inhibition was considered non-competitive.

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