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Synthesis, biological evaluation and molecular modelling of diversely functionalized heterocyclic derivatives as inhibitors of acetylcholinesterase/butyrylcholinesterase and modulators of Ca^{2+} channels and nicotinic receptors

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Abstract—The synthesis and the biological activity of compounds **5**–**40** as inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), as well as modulators of voltage-dependent Ca^{2+} channels and nicotinic receptors, are described. These molecules are tacrine analogues, which have been prepared from polyfunctionalized 6-amino-5-cyano-4*H*-pyrans, 6-amino-5-cyano-pyridines and 5-amino-2-aryl-3-cyano-1,3-oxazoles via Friedländer reaction with selected cycloalkanones. These compounds are moderate acetylcholinesterase and butyrylcholinesterase inhibitors, the BuChE/AChE selectivity of the most active molecules ranges from 10.0 (compound **29**) to 76.9 (compound **16**). Interestingly, the 'oxazolo-tacrine' derivatives are devoid of any activity. All compounds showed an important inhibitory effect on the nicotinic acetylcholine receptor. Most of them also blocked L-type Ca^{2+} channels, and three of them, **64**, **19** and **67**, the non-L type of Ca^{2+} channels. Molecular modelling studies suggest that these compounds might bind at the peripheral binding site of AChE, which opens the possibility to design inhibitors able to bind at both, the catalytic and peripheral binding sites of the enzyme.
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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative, irreversible disorder that is clinically characterized by a progressive loss of several cognitive abilities. It is the most common type of dementia in western societies, which has been causing profound economic and social impact as the ageing population increases.[1] Although many factors have been implicated in AD, its etiology and pathogenesis remain unclear. The 'cholinergic hypothesis'[2] represents one of the most useful approaches involved in the design of new agents for the treatment of AD. This strategy is based on the development of drugs with an acetylcholinesterase (AChE) inhibition profile in order to rectify the deficit of cerebral acetylcholine (ACh).[3] AChE inhibitors such as galanthamine,[4] donepezil,[5] rivastigmine[6] and huperzine[7] increase the brain ACh levels by preventing the degradation of the released neurotransmitter, thereby

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enhancing neurotransmission at cholinergic synapses.[2] Tacrine (THA, Fig. 1) was the first drug approved in USA for the treatment of AD. However, severe adverse effects such as hepatotoxicity represent a drawback. Despite these limitations, THA appears as an important reference and a great synthetic and pharmacological research effort was carried on in order to design safer and more potent tacrine analogues.[8–11]

There is also emerging evidence showing that an altered calcium homeostasis can play a pivotal role in the pathogenesis of AD.[12,13] On the other hand, ligands of nicotinic receptors may be strongly associated to neuroprotectant effects.[14] Thus, we have designed new THA analogues as AChE/BuChE inhibitors and modulators of voltage-dependent Ca^{2+} channels and ligands of nicotinic receptors.

In this context, in the last years we have reported the synthesis and the AChE/BuChE inhibitory activity of a series of THA analogues where the benzene ring has been substituted by a 4*H*-pyran or a pyridine moiety, and the cyclohexyl ring has been contracted or enlarged to a cyclopentane or a cycloheptane, respectively. As a result, we have prepared heterocycles such as cyclopenta[*b*]pyrano[3,2-*e*]pyridine (**A**, $n = 0$), 4*H*-pyrano-[2,3-*b*]quinoline (**A**, $n = 1$), cyclohepta[*b*]-pyrano[3,2-*e*]pyridine (**A**, $n = 2$),[15a,b,c] 6*H*-cyclopenta[*b*][1,8]naphthyridine (**B**, $n = 0$), benzo[*b*][1,8]naphthyridine (**B**, $n = 1$), 6*H*-cyclohepta[*b*][1,8]naphthyridine (**B**, $n = 2$),[15b] furo (and thieno)[2,3-*b*]quinoline (**C**, $n = 1$) and cyclohepta[*e*]furo (and thieno) [2,3-*b*]pyridine (**C**, $n = 2$),[15c,d] (Chart 1). From this work we concluded that all the compounds were moderate inhibitors of AChE/BuChE, with variable selectivity for these enzymes. The best values were observed for compounds **1** [AChE (IC_{50}) (M) $8.68 \pm 0.42 \times 10^{-7}$],[15a] **2** [AChE ($IC_{50}$) (M) $8.22 \pm 1.57 \times 10^{-7}$],[15a] **3** [AChE ($IC_{50}$) (M) $3.77 \pm 0.18 \times 10^{-7}$; BuChE ($IC_{50}$) (M) $> 10^{-4}$][15d] and **4** [AChE (IC_{50}) (M) $3.18 \pm 0.50 \times 10^{-7}$; BuChE ($IC_{50}$) (M) $7.72 \pm 0.50 \times 10^{-7}$][15d] (Chart 1). Curiously, the thieno derivatives, with a similar structure to compounds **3** and **4**, were devoid of any inhibitory activity.[15d]

In relation to these endeavours, Barreiro et al. have recently reported interesting results on pyrazolo [4,3-*d*]pyridine and pyrazolo[3,4-*b*][1,8]naphthyridine analogues.[16]

We have also found that compounds of type **B** (Chart 1), in addition to its ability of inhibiting AChE, behave as modulators or blockers of neuronal nicotinic ACh receptors (nAChRs) as well as neuronal voltage-dependent Ca^{2+} channels.[15c]

Prompted by these results and pursuing our project in this area, we describe here the synthesis and the AChE/BuChE inhibitory activities, as well as the modulation of voltage-dependent Ca^{2+} channels and/or nAChR of compounds **5**–**40** (Chart 2). Finally, molecular modelling calculations are carried out in order to understand the structural basis for the biological activity of compounds **1** and **2** (Chart 1), which are representative of the **A** and **B** classes, trying to obtain data for future endeavours and new rationale synthetic approaches.

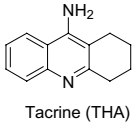


Figure 1.

2. Results and discussion

2.1. Chemistry

2.1.1. Pyrano[3,2-*e*]pyridines, pyrano[2,3-*b*]quinolines and [1,8]naphthyridines. The synthesis of these THA analogues has been achieved by Friedländer reaction[17] of the 6-amino-5-cyano-4*H*-pyran-3-carboxylic acid alkyl esters[18] and 6-amino-5-cyano-pyridine-3-carboxylic acid

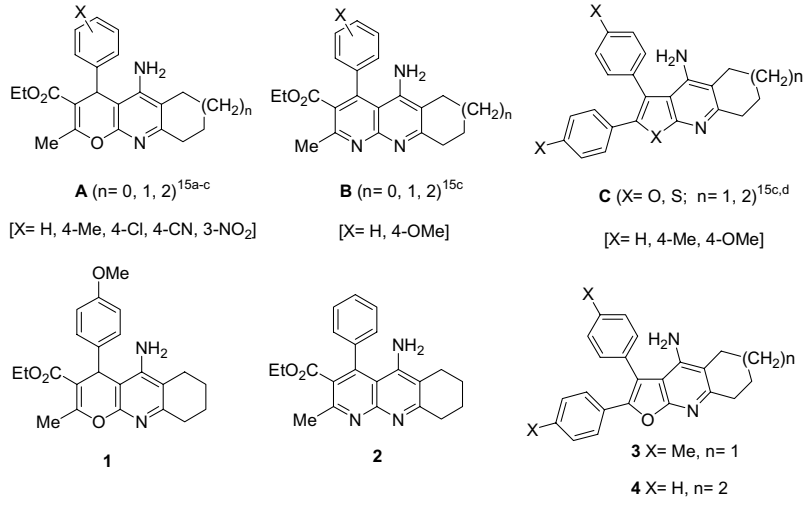
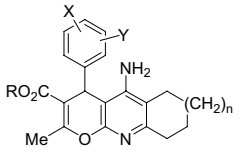
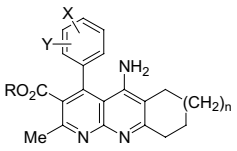


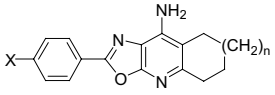
Chart 1.



5 X= 3-OMe, Y= H, R= Et, n= 0
6 X= 3-OMe, Y= H, R= Et, n= 1
7 X= 3-OMe, Y= H, R= Et, n= 2
8 X= 2-OMe, Y= H, R= Et, n= 0
9 X= 2-OMe, Y= H, R= Et, n= 1
10 X= 2-OMe, Y= H, R= Et, n= 2
11 X, Y= 3,4-di-OMe, R= Et, n= 0
12 X, Y= 3,4-di-OMe, R= Et, n= 1
13 X, Y= 3,4-di-OMe, R= Et, n= 2
14 X, Y= H, R= *i*-Pr, n= 1



15 X= H, Y= 3-OMe, R= Et, n= 1
16 X, Y= 3,4-di-OMe, R= Et, n= 1
17 X= H, Y= 4-F, R= Et, n= 1
18 X= H, Y= 4-Cl, R= Et, n= 1
19 X= H, Y= 3-NO_2, R= Et, n= 1
20 X= H, Y= 4-Me, R= Et, n= 2
21 X= H, Y= 3-OMe, R= Et, n= 2
22 X= H, Y= 2-OMe, R= Et, n= 2
23 X, Y= 3,4-di-OMe, R= Et, n= 2
24 X= H, Y= 4-F, R= Et, n= 2
25 X= H, Y= 4-Cl, R= Et, n= 2
26 X= H, Y= 3-NO_2, R= Et, n= 2
27 X, Y= H, R= Et, n= 3
28 X= 4-OMe, Y= H, R= Et, n= 3
29 X, Y= H, R= *i*-Pr, n= 1



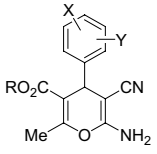
30 X= Cl, n= 0
31 X= OMe, n= 0
32 X= Me, n= 0
33 X= Cl, n= 1
34 X= NO_2, n= 1
35 X= OMe, n= 1
36 X= Me, n= 1
37 X= Cl, n= 2
38 X= NO_2, n= 2
39 X= OMe, n= 2
40 X= Me, n= 2

Chart 2.

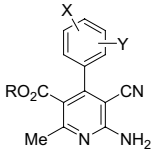
alkyl esters[19] with selected cycloalkanones such as cyclopentanone, cyclohexanone, cycloheptanone and cyclooctanone.

For the synthesis of the pyrano[3,2-*e*]pyridines and pyrano[2,3-*b*]quinolines we required the 6-amino-5-cyano-4*H*-pyrans **41**–**44** (Chart 3). Compounds **41**, **42**, **44** are known and have been prepared as previously reported.[18] 6-Amino-5-cyano-4-(3,4-dimethoxyphenyl)-4*H*-pyran-3-carboxylic acid ethyl ester **43** (Chart 3) is a new compound that we describe here for the first time. It was prepared using the standard protocol, by Michael addition of ethyl acetoacetate to 3,4-dimethoxybenzylidenemalononitrile[20] followed by enol-promoted 5-*exo*-*dig* cyclization into one of the nitrile groups, in 57% yield (Scheme 1).

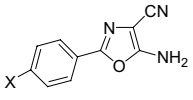
Under standard conditions, using dry 1,2-dichloroethane as solvent and aluminium trichloride (see Experimental part), 6-amino-5-cyano-4*H*-pyran-3-carboxylic acid ethyl or *i*-propyl esters (**41**–**44**) afforded the expected THA analogues (**5**–**14**) employing cyclopentanone, cylohexanone or cycloheptanone as the ketone partner in a typical Friedländer reaction (Scheme 2). Compounds **5**–**10** and **14** are known products, prepared in our laboratory some years ago, and have been



41 X= H, Y= 3-OMe, R= Et
42 X= H, Y= 2-OMe, R= Et
43 X, Y= 3,4-di-OMe, R= Et
44 X, Y= H, R= *i*-Pr
55 X= 4-F, Y= H, R= Et
56 X= 4-Cl, Y= H, R= Et
57 X= 3-NO_2, Y= H, R= Et
58 X= 4-Me, Y= H, R= Et

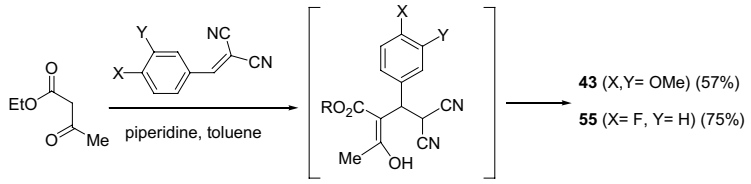


45 X= H, Y= 3-OMe, R= Et
46 X, Y= 3,4-di-OMe, R= Et
47 X= H, Y= 4-F, R= Et
48 X= H, Y= 4-Cl, R= Et
49 X= H, Y= 3-NO_2, R= Et
50 X, Y= H, R= *i*-Pr
51 X= H, Y= 4-Me, R= Et
52 X= H, Y= 2-OMe, R= Et
53 X, Y= H, R= Et
54 X= H, Y= 4-OMe, R= Et

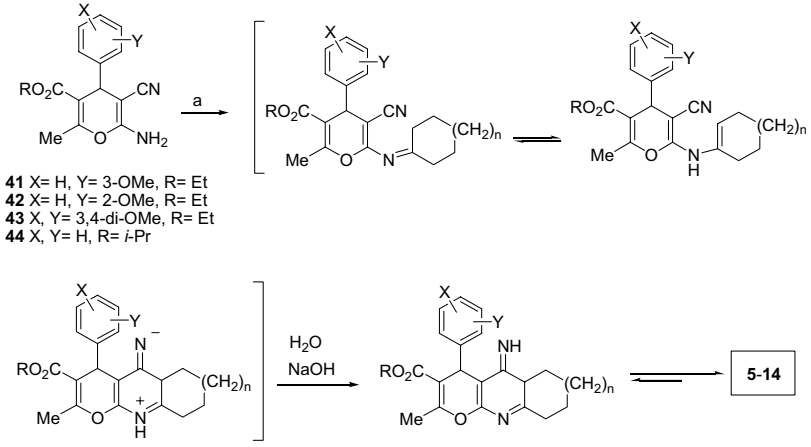


59 X= Cl
60 X= NO_2
61 X= OMe
62 X= Me

Chart 3.



Scheme 1. Synthesis of 4*H*-pyrans **43** and **55**.



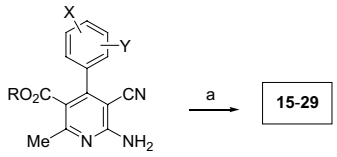
Scheme 2. Synthesis of tacrine-analogues **5**–**14** by Friedländer reaction. Reagents and conditions: (a) cyclopentanone ($n = 0$), cyclohexanone ($n = 1$), cycloheptanone ($n = 2$), $(CH_2)_2Cl_2$, $AlCl_3$, reflux.

obtained as previously mentioned.[15c] Precursor **43** afforded the new THA analogues **11**–**13** in mild conditions and good chemical yield (Scheme 2).

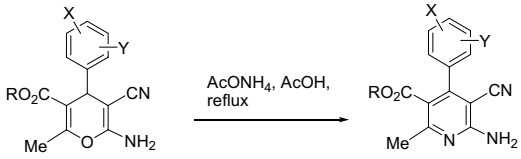
The THA analogues exhibiting the cycloannulated[*b*][1,8]naphthyridine ring system (**15**–**29**) (Chart 2) have also been synthesized from the corresponding 6-amino-5-cyanopyridines (**45**–**54**)[19] (Chart 3) using the Friedländer reaction (Scheme 3).[17]

Very unusually, the Friedländer reaction with cyclopentanone did not afford the expected 6*H*-cyclopenta[*b*][1,8]naphthyridines. In general, this reaction with cyclopentanone has always led to low yields of the envisaged adducts. The reasons for this disappointing result are not clear for us. Conversely, the benzo[*b*][1,8]naphthyridines (**15**–**19**, **29**) (Chart 2) have been prepared successfully from the corresponding aminopyridines **45**–**50** (Chart 3) in the Friedländer reaction (Scheme 3).[17]

Substituted 2-amino-pyridines **45**–**47**, **49** and **50** (Chart 3) are reported here for the first time. They were prepared from the corresponding 4*H*-pyran-3-carboxylic acid ethyl esters **41**, **43**, **55**, **57** and **44** (Chart 3), respectively, by reaction with ammonium acetate in glacial acetic acid (Scheme 4).[19] Moreover, 4*H*-pyran **55** (Chart 3) was previously described in a non-easily available literature report.[21] We have synthesized it in 75% yield, starting from known 4-fluorobenzylidene-



Scheme 3. Synthesis of tacrine-analogues **15**–**29** by Friedländer reaction. Reagents and conditions: (a) cyclohexanone ($n = 1$) or cycloheptanone ($n = 2$) or cyclooctanone ($n = 3$), $(CH_2)_2Cl_2$, $AlCl_3$, reflux.



41 X= H, Y= 3-OMe, R= Et
43 X, Y= 3,4-di-OMe, R= Et
55 X= 4-F, R= Et
57 X= 3-NO_2, R= Et
44 X, Y= H, R= *i*-Pr
58 X= H, Y= 4-Me, R= Et
42 X= H, Y= 2-OMe, R= Et

45 X= H, Y= 3-OMe, R= Et (41%)
46 X, Y= 3,4-di-OMe, R= Et (52%)
47 X= H, Y= 4-F, R= Et (41%)
49 X= H, Y= 3-NO_2, R= Et (53%)
50 X, Y= H, R= *i*-Pr (40%)
51 X= H, Y= 4-Me, R= Et (44%)
52 X= H, Y= 2-OMe, R= Et (31%)

Scheme 4. Synthesis of pyridines **45**–**47**, **49** and **50**–**52**.

malononitrile[22] after condensation with ethyl acetoacetate, using piperidine as catalyst[18] (Scheme 1) (see Experimental part).

6*H*-Cyclohepta[*b*][1,8]naphthyridines (**20**–**26**) (Chart 2) were synthesized from the related aminopyridines **51**, **45**, **52**, **46**–**49** (Chart 3) via Friedländer reaction,[17] using cycloheptanone (Scheme 3). The 4-methyl and 2-methoxy substituted aminopyridines **51** and **52** (Chart 3) are new compounds that were prepared as usual from the known pyrans **58**[18] and **42**,[18] respectively (Scheme 4).

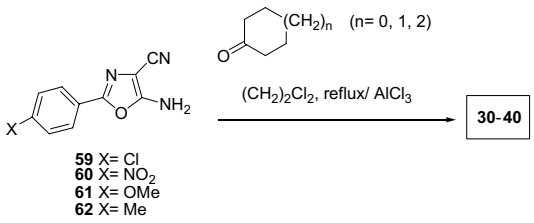
Finally, the 6*H*-cycloocta[*b*][1,8]naphthyridines **27** and **28** (Chart 2) were prepared in a similar process by the condensation of aminopyridines **53**[19] and **54**[19] (Chart 3) with cyclooctanone (Scheme 3).

All the new compounds showed analytical and spectroscopic data in good agreement with these structures (see Experimental part) and with the data we formerly observed and reported in similar compounds.[15]

2.1.2. Cyclopenta[*b*]oxazolo[4,5-*e*]pyridines, oxazolo[5,4-*b*]quinolines and cyclohepta[*b*]oxazolo[4,5-*e*]pyridines. The relevant AChE/BuChE inhibitory activities previously attained with the furo-THA derivatives (compounds of type **C**, Chart 1)[15d] urged us to undertake the synthesis and biological evaluation of new, related derivatives. Therefore, we have designed the oxazolo-THA analogues **30**–**40** (Chart 2) by removing one of the aromatic rings of the furo derivatives **C**[15c] (Chart 1) and incorporating a nitrogen atom instead in the heterocycle.

The synthesis of these THA analogues was also accomplished by Friedländer reaction[17] of the related 2-aryl substituted 5-amino-4-cyano-1,3-oxazoles.[23] These compounds are readily prepared from commercially available aminopropanedinitrile *p*-toluenesulfonate and acid chlorides, as reported by Freeman and Kim.[24] Following this protocol, and for the preliminary tests, we have prepared the known 1,3-oxazoles **59**–**61**[24] and compound **62**, which had not been reported in the literature yet (Chart 3).

The Friedländer reaction of these precursors with cyclopentanone, cyclohexanone and cycloheptanone proceeded as expected to afford compounds **30**–**32**, **33**–**36** and **37**–**40** (Chart 2), respectively, from low to moderate chemical yield (see Experimental part) (Scheme 5). All the new compounds showed excellent analytical and spectroscopic data in good agreement with these structures.



Scheme 5. Synthesis of tacrine-analogues **30**–**40** by Friedländer reaction.

2.2. Biological activity

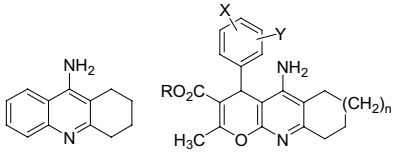
2.2.1. Pyrano[3,2-*e*]pyridines, pyrano[2,3-*b*]quinolines and [1,8]naphthyridines

2.2.1.1. Evaluation of AChE and BuChE. The AChE inhibitory activities in the pyrano[3,2-*e*]pyridines and pyrano[2,3-*b*]quinolines **5**–**14** (see Table 1) were evaluated according to the standard methodology[25,26] (see Experimental part).

For comparative purposes we have also included the IC_{50} (M) for AChE of the formerly evaluated THA analogue **1** (Chart 1).[15] Comparing with THA (IC_{50} $1.8 \pm 0.2 \times 10^{-7}$), the new synthesized compounds manifest lower activity. The most active compound is still **1** (IC_{50} $8.7 \pm 0.42 \times 10^{-7}$)[15b] (around 4–5-fold less active). For the same substituents (3-, 2-methoxy, 3,4-dimethoxy) in the aromatic ring, the cyclohexane-fused compounds ($n = 1$) showed the best activity (**6**, **9**, **12**) and the cyclopentane ($n = 0$) compounds manifested the worst. Considering the same ring size, the 3-methoxy derivatives presented the lowest activities (**5**, **6**, **7**). Hence, we conclude that the 4-methoxy group leads to more active analogues than the 2-, 3-methoxy groups, and the 3,4-dimethoxy substituents do not impart better selectivity. Analysis of compound **14**, with a similar carboxylic acid ester,[15b] reveals that simple conversion of the ethyl into the *i*-propyl group in the carboxylic acid moiety introduced a strong negative effect for the inhibitory activity (from $1.6 \pm 0.1 \times 10^{-6}$ to $>3.0 \times 10^{-5}$).

The AChE inhibitory activity calculated for the [1,8]naphthyridine series of derivatives is presented in

Table 1. AChE IC_{50} (M) data for the inhibitory activity of compounds **5**–**14**



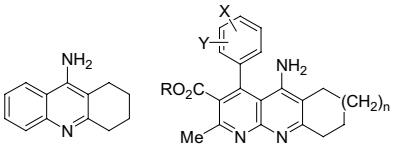
	n	X, Y	R	IC_{50} (M)
Tacrine	—	—	—	$1.8 \pm 0.2 \times 10^{-7}$
1	1	H, 4-OMe	Et	$8.7 \pm 0.4 \times 10^{-7}$
5	0	H, 3-OMe	Et	$>10^{-4}$
8	0	H, 2-OMe	Et	$2.4 \pm 0.6 \times 10^{-5}$
11	0	3,4-Di-OMe	Et	$2.7 \pm 0.1 \times 10^{-5}$
6	1	H, 3-OMe	Et	$>3.0 \times 10^{-5}$
9	1	H, 2-OMe	Et	$7.7 \pm 0.8 \times 10^{-6}$
12	1	3,4-Di-OMe	Et	$4.6 \pm 0.9 \times 10^{-6}$
7	2	H, 3-OMe	Et	$1.3 \pm 0.2 \times 10^{-5}$
10	2	H, 2-OMe	Et	$2.1 \pm 0.3 \times 10^{-5}$
13	2	3,4-Di-OMe	Et	$3.3 \pm 0.4 \times 10^{-5}$
14	1	H, H	*i*-Pr	$>3.0 \times 10^{-5}$

Table 2. For comparative purposes we have also included the IC_{50} (M) for AChE of compound **2** (Chart 1).[15b]

Among these THA analogues, compound **17**, with a 4-fluor substituent in the aromatic ring, is found to be the most active. Actually, it is even more active than the other pyrano[3,2-*e*]pyridines, and pyrano[2,3-*b*]quinolines (Table 1). It is noteworthy that the benzo[*b*][1,8]naphthyridines ($n = 1$) are the most active in this series, regardless of the substituent in the aromatic ring, if we compare with the other inhibitors for $n = 0$, 2 or 3. Very interestingly, as in the pyran-like THA substrates, the isopropyl ester **29** is still less active than the similar ethyl ester THA derivative.[15b]

The most potent compound of each group was assayed to determine its activity on BuChE. Data are given in Table 3. All the compounds inhibited AChE more strongly than BuChE. Overall, the best BuChE/AChE selectivity was found in compound **16** (76.9). These data are very interesting since tacrine's adverse effects are ascribed to its ability to inhibit BuChE, and suggest future structural modifications for activity improvement.

Table 2. AChE IC_{50} (M) data for the inhibitory activity of compounds **15–29**



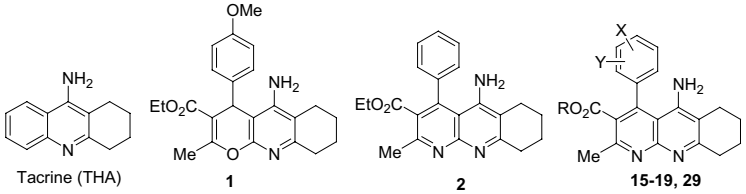
	n	X, Y	R	IC_{50} (M)
Tacrine		—	—	$1.8 \pm 0.2 \times 10^{-7}$
2	1	H, H	Et	$8.2 \pm 1.6 \times 10^{-7}$
15	1	H, 3-OMe	Et	$1.2 \pm 0.3 \times 10^{-6}$
16	1	3,4-Di-OMe	Et	$1.3 \pm 0.3 \times 10^{-6}$
17	1	H, 4-F	Et	$7.1 \pm 1.7 \times 10^{-7}$
18	1	H, 4-Cl	Et	$1.3 \pm 0.2 \times 10^{-6}$
19	1	H, 3-NO_2	Et	$9.3 \pm 1.9 \times 10^{-7}$
20	2	H, 4-Me	Et	$1.3 \pm 0.1 \times 10^{-5}$
21	2	H, 3-OMe	Et	$>3.0 \times 10^{-5}$
22	2	H, 2-OMe	Et	$2.2 \pm 0.5 \times 10^{-5}$
23	2	3,4-Di-OMe	Et	$6.4 \pm 0.7 \times 10^{-6}$
24	2	H, 4-F	Et	$5.4 \pm 1.5 \times 10^{-6}$
25	2	H, 4-Cl	Et	$1.9 \pm 0.3 \times 10^{-5}$
26	2	H, 3-NO_2	Et	$1.4 \pm 0.3 \times 10^{-5}$
27	3	H, H	Et	$1.7 \pm 0.4 \times 10^{-5}$
28	3	H, 4-OMe	Et	$1.7 \pm 0.6 \times 10^{-5}$
29	1	H, H	*i*-Pr	$1.2 \pm 0.2 \times 10^{-6}$

2.2.1.2. $^{45}Ca^{2+}$ uptake into bovine chromaffin cells. Three different types of experiments were performed in order to find out whether the new compounds, at the concentration of 3 μM, had an action on: (i) basal Ca^{2+} uptake, (ii) Ca^{2+} taken up by cells under conditions of depolarization with high K^+ (70 mM with equimolar reduction of Na^+); and (iii) Ca^{2+} uptake by cells stimulated by the nicotinic agonist dimethylphenylpiperazinium (DMPP). The results obtained for some of the compounds with AChE/BuChE inhibitory data reported here, were published before.[15c]

In basal conditions, resting cells took up 781 ± 78 cpm of $^{45}Ca^{2+}$ when incubated in the presence of 1 mM $^{40}Ca^{2+}$ for 5 min (data from five different batches of cells). After 5 min incubation, compounds **24** and **25** affected basal Ca^{2+} uptake (Chart 2) around 20% increase; compounds **20** and **21**increased Ca^{2+} uptake by 37% and 45%, respectively. Other compounds such as **66** (Chart 4),[15b] **18** and **19** (Chart 2) inhibited Ca^{2+} uptake around 20% (Table 4).

Looking for a possible modulatory effect on voltage-dependent Ca^{2+} channels (VDCCs), we analyzed the action of the compounds on $^{45}Ca^{2+}$ entry into K^+-depolarized chromaffin cells. High K^+ stimulation for 5 s induced $^{45}Ca^{2+}$ uptake (in the presence of 1 mM

Table 3. IC_{50} for BuChE inhibition for some selected compounds and the ratio BuChE/AChE



	X, Y	R	AChE (M)	BuChE (M)	BuChE/AChE
Tacrine	—	—	$1.8 \pm 0.2 \times 10^{-7}$	$3.6 \pm 0.4 \times 10^{-8}$	0.2
1			$8.7 \pm 0.4 \times 10^{-7}$	$6.2 \pm 0.6 \times 10^{-6}$	7.1
2			$8.2 \pm 1.6 \times 10^{-7}$	$5.0 \pm 0.6 \times 10^{-6}$	6.1
15	H, 3-OMe	Et	$1.2 \pm 0.3 \times 10^{-6}$	$3.1 \pm 0.2 \times 10^{-5}$	25.4
16	3,4-Di-OMe	Et	$1.3 \pm 0.3 \times 10^{-6}$	$>10^{-4}$	76.9
17	H, 4-F	Et	$7.1 \pm 1.7 \times 10^{-7}$	$1.2 \pm 0.4 \times 10^{-5}$	16.9
18	H, 4-Cl	Et	$1.3 \pm 0.2 \times 10^{-6}$	$5.2 \pm 0.9 \times 10^{-5}$	40.0
19	H, 3-NO_2	Et	$9.3 \pm 1.9 \times 10^{-7}$	$2.1 \pm 0.3 \times 10^{-5}$	22.6
29	H, H	*i*-Pr	$1.2 \pm 0.2 \times 10^{-6}$	$1.2 \pm 0.2 \times 10^{-5}$	10.0

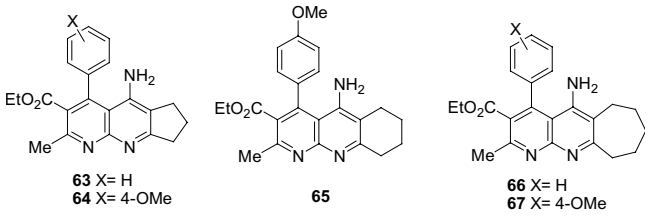
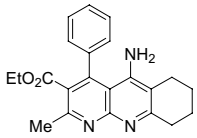


Chart 4.

Table 4. Effect of the different compounds on $^{45}Ca^{2+}$ uptake into bovine chromaffin cells (% inhibition concerning a control without any drug); all the compounds were tested at the concentration of 3 μM[a]



2

Compound	Basal	70 mM K^+	100 μM DMPP
63	−0.8 ± 5.7[b]	24.3 ± 6.4	51.6 ± 4.8
64	−8.1 ± 5	14.9 ± 2.4	71.1 ± 3.8
2	4.3 ± 4.3	19.7 ± 4.6	52.2 ± 2.2
65	10.6 ± 6	10.3 ± 5.6	76.0 ± 4.1
66	22.2 ± 4.1	11.7 ± 7.2	69.7 ± 3.2
67	7.1 ± 4.5	39.2 ± 6	82.3 ± 2.9
15	5.8 ± 2.1	15.5 ± 3	49.7 ± 9.1
16	9.1 ± 9.3	26 ± 0.8	99.6 ± 0.2
17	14.2 ± 7.6	38.5 ± 7.9	63.0 ± 7.5
18	20.6 ± 4.2	34.4 ± 3.3	83.4 ± 4.6
19	25.1 ± 8.9	18.4 ± 6.1	61.5 ± 8.2
20	−36.6 ± 12	23.8 ± 2.8	68.1 ± 4
21	−45.3 ± 11.3	8.9 ± 3.6	61.7 ± 7.5
22	4.5 ± 6.9	16.9 ± 2.7	89.5 ± 3.9
23	4.0 ± 6.3	9.2 ± 4.8	96.8 ± 3.6
24	−16.9 ± 4	17.2 ± 4.4	70.6 ± 3.2
25	−19.0 ± 7.6	26.9 ± 2.7	62.9 ± 3.7
26	6.1 ± 5.4	12.7 ± 9.4	74.4 ± 5.5
27	9.0 ± 6.2	10.3 ± 1.8	81.9 ± 4.8
28	7.5 ± 5.4	19.6 ± 3.8	73.3 ± 4.3
29	13.8 ± 9.6	9.7 ± 10.9	81.8 ± 12.1

[a] Data are expressed as the means+SEM of at least three different cultures in quadruplicate.

[b] Negative values mean an increase in $^{45}Ca^{2+}$ uptake.

$^{40}Ca^{2+}$) of 401 ± 14 cpm per 2×10^5 cells (16 individual wells data from three different batches of cells). In basal conditions for this time of incubation (5 s in Krebs-HEPES solution containing 5 μCi mL^{-1} $^{45}Ca^{2+}$ plus 1 mM $^{40}Ca^{2+}$), cells retained 85 ± 1 cpm (16 individual wells data from different batches of cells). Thus, the mean ratio evoked/basal Ca^{2+} uptake was about 5-fold for K^+-depolarization. Most compounds inhibited Ca^{2+} entry around 20%, which could indicate a blockade of the L-type Ca^{2+} channels, since the proportion of this subtype of channels is about 20% of the total in bovine chromaffin cells. When the experiments were repeated in the presence of 3 μM nifedipine, a specific blocker of L-type Ca^{2+} channels, most compounds did not exert an additional blockade. Only **64** (Chart 4),[15b] **19** (Chart 2) and **67** (Chart 4)[15b] presented an additional inhibition of 25%, 19% and 39%, respectively, which could indicate an effect on the other subtypes of Ca^{2+} channels (N, P/Q) (Table 4).

Stimulation with the nicotinic receptor agonist DMPP (100 μM during 5 s) induced $^{45}Ca^{2+}$ uptake (in the presence of 1 mM $^{40}Ca^{2+}$) of 455 ± 48 cpm per 2×10^5 cells (12 individual wells data from three different batches of cells). After 15 min incubation all the compounds blocked Ca^{2+} uptake induced by nicotinic stimulation with DMPP, with inhibition values ranging from 50% to almost 100% (compound **16**) (Table 4).

2.2.2. Cyclopenta[*b*]oxazolo[4,5-*e*]pyridines, oxazolo[5,4-*b*]quinolines and cyclohepta[*b*]oxazolo[4,5-*e*]pyridines

2.2.2.1. Evaluation of AChE. We have noted that these substrates were particularly insoluble in most of the usual solvents, which was a real problem in order to carry out biological evaluation (see below). With the purpose to solve this, we have also prepared the hydrochlorides of compounds **33**–**35** and **37**–**39**, but their conversion into salts did not improve the solubility either.

Due to these solubility difficulties, we only obtained the following data (% inhibition) for the AChE inhibition at 10 μM concentration for a limited number of substrates [(**39**): 14.1 ± 0.2%; (**32**) 27.5 ± 2.9%; (**30**): 39.9 ± 4.4%; (**37**): 45.6 ± 5.8%]. Then, we tested the 1,3-oxazole precursors and observed the best value (63.2%) for compound **60** (Chart 3) at 10 μM concentration. These low results prevented us to investigate at any other different concentrations.

2.3. Molecular modelling

2.3.1. Pyrano[3,2-*e*]pyridines, pyrano[2,3-*b*]quinolines and [1,8]naphthyridines. Despite the variety of chemical modifications introduced into the basic core of the AChE inhibitors examined here and their high structural similarity with THA, the most potent compounds are ~4-fold less active than THA. Moreover, it is also intriguing that the mechanism of AChE inhibition strikingly varies from competitive to noncompetitive type due to apparently minor structural modifications,[15b] thus suggesting that these compounds cannot be easily accommodated in the AChE active site. Therefore, the putative binding mode of compounds **1** and **2**, which are representative members of the **A**- and **B**-like ligands (see Chart 1) and are amongst the most potent AChE inhibitors (see above) was investigated by means of molecular dynamics simulations. To this end, compounds **1** and **2** were initially positioned into the binding site by superposing the N and NH_2 groups of the central ring to those of THA in the X-ray crystallographic structure of the *Torpedo californica* AChE–THA complex (PDB entry 1ACJ).[27] The aromatic and alicyclic rings were then placed over those of THA (*direct* binding mode; **d**) or by rotating the molecule 180°

through the N···NH_2 axis (*inverse* binding mode; **i**). Therefore, four (**1***R*-**d**, **1***R*-**i**, **1***S*-**d** and **1***S*-**i**) and two (**2-d** and **2-i**) starting binding modes were examined for compounds **1** and **2**, respectively. For each binding mode, a molecular dynamics (MD) simulation was performed to explore the structural and energetic properties of the drug–enzyme complex using the simulation protocol adopted in our previous studies[11,28] (see Experimental part).

The root-mean square deviation (rmsd) profiles determined for the subset of residues that form the wall of the active site was in all cases larger than 2.4 Å, thus indicating the occurrence of relevant local distortions in the protein as a consequence of the binding of these molecules. As a reference, the corresponding rmsd determined for the binding of THA and huprine Y, a mixed tacrine–huperzine A derivative, was only around 0.6 Å.[28] For three complexes of compound **1** (**1***R*-**d**, **1***R*-**i** and **1***S*-**d**) and one complex of compound **2** (**2-d**) the rmsd increased steadily along the trajectory, it being larger than 3 Å at the end of the simulation. For the two remaining binding modes (**1***S*-**i** and **2-i**) the rmsd remained stable at ~2.5 Å along the simulation. The energetic analysis also pointed out that these latter binding modes were also those leading to the most stable complexes. The greater stability of the binding modes **1***S*-**i** and **2-i** is mostly due to the internal energy of the enzyme (by more than 20 kcal/mol with respect to the other binding modes). For compound **1** the interaction energy between inhibitor and enzyme also favours the binding mode **1***S*-**i**. For compound **2**, the enzyme–drug interaction term slightly favoured the binding mode **2-d**, but this was counterbalanced by both the solvation free energy and the destabilization in the internal energy of the enzyme, which favour binding mode **2-i**.

The preceding results suggest that the binding site of AChE is not flexible enough as to easily accommodate compounds **1** and **2**. For compound **1**, the methoxybenzene unit interferes with the stacking interaction between the aminoacridine unit of THA and the rings of Trp84 and Phe330, thus forcing the drug to reorient inside the binding pocket (see Fig. 2; top). Such a disrupting effect is less marked in compound **2**, since the stacking interaction is roughly preserved along the simulation (see Fig. 2; bottom). Nevertheless, unfavourable steric contacts due to the ester side chain forces the displacement of the inhibitor to the position occupied by THA, thus leading to the loss of specific interactions such as the hydrogen bonding between the NH group of THA and the C═O group of His440. Overall, it seems that the chemical groups present in **1** and **2** constrain these compounds to occupy a large fraction of (or even to exceed) the allowable molecular size of the binding pocket. Thus, whereas the cavity at the catalytic binding site is estimated to be ~340 $Å^3$, the van der Waals volumes of compounds **1** and **2** are ~330 and ~300 $Å^3$, which are noticeably larger than the van der Waals volume of tacrine (~170 $Å^3$). Finally, since attachment of those bulky substituents might difficult the pass through the gorge to reach the catalytic binding site, we suggest that these compounds, which act

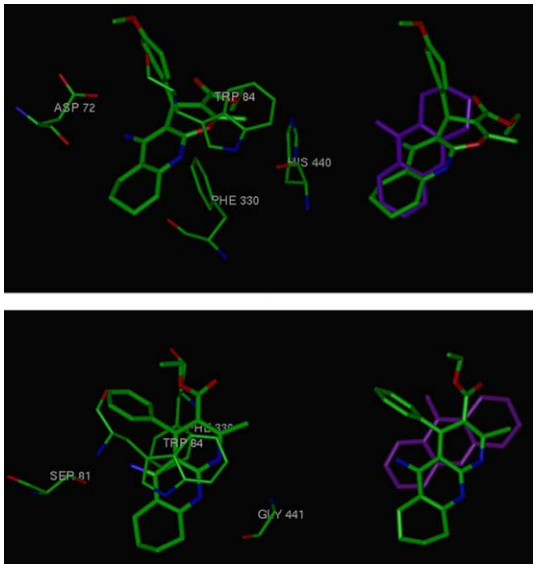
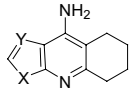


Figure 2. Representation of the binding mode of compounds **1** (**1***S*-**i**; top) and **2** (**2-i**; bottom) found from molecular dynamics simulations. Superposition of the inhibitor with THA (cyano) obtained by fitting the enzyme's backbone atoms for the AChE-inhibitor complex and the X-ray crystallographic structure of the AChE–THA complex (1ACJ) is also displayed. Hydrogens are not shown for the sake of clarity.

through a noncompetitive mechanism,[15b] might exert their inhibitory activity by interacting at the peripheral binding site of AChE, which is postulated to be Trp279, as it has already been noted for propidium,[29] which is a selective ligand for the peripheral site. This finding might be of particular relevance in view of recent studies pointing out that inhibitors binding to the peripheral site might play a role in controlling the formation of amyloid fibrils.[30]

2.3.2. Cyclopenta[*b*]oxazolo[4,5-*e*]pyridines, oxazolo[5,4-*b*]quinolines and cyclohepta[*b*]oxazolo[4,5-*e*]pyridines. The preceding results show that the different oxazolo-THA compounds **30**–**40** are devoid of any AChE inhibitory activity, a result that is in contrast with the inhibitory activity exhibited by the furo derivatives **3** and **4** (see Introduction). Such a drastic reduction in the inhibitory potency must be related to the replacement of the aromatic ring in the related furo derivatives (**C**, Chart 1) by a nitrogen atom in compounds **30**–**40**. Based on the molecular modelling results presented above for the 4*H*-pyrano[2,3-*b*]quinoline and benzo[*b*][1,8]naphthyridine derivatives, as well as on structure–activity studies of 11*H*-indeno[1,2-*b*]quinolin-10-ylamine analogues of THA,[31] we suggest that compounds **3** and **4** might also bind to the AChE peripheral site. Therefore, it is reasonable to expect that their binding should be mediated by a cation-π interaction between the protonated ligand and the ring of Trp279. Thus, the reduced activity of **30**–**40** might be due to the increased acidity of these compounds due to the electron-withdrawing nature of the oxazole ring.



68 X= NH, Y= CH
69 X= O, Y= CH
70 X= O, Y= N

Chart 5.

To explore this possibility, we determined the well depth of the electrostatic potential[32] generated in the vicinity of the N atom in the central ring for THA and for compounds **68**–**70** (Chart 5). The energy of the minimum in THA (−76.8 kcal/mol) is similar to that found for compounds **68** (−75.0 kcal/mol) and **69** (−75.4 kcal/mol). However, a lower (in absolute term) value of −68.7 kcal/mol is found for the oxazolo compound **70**. Therefore, the reduced activity of compounds **30**–**40** might be related to their increased acidity, which hinders the formation of the cation-π interaction with Trp279.

3. Conclusions

In this work we have reported the synthesis, the AChE and BuChE inhibitory activities, as well as the modulatory effect on nAChRs and VDCCs of a series of tacrine analogues **5**–**40**. 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. Overall, the substitution of a benzene ring in tacrine by pyran or pyridine rings generates analogues with strong capacity to inhibit AChE, with lower values, but very close to those shown by tacrine (**1**). With respect to modulation of VDCC and nAChR in the bovine chromaffin cell, [1,8]naphthyridines seem to have an important inhibitory effect on nAChR. Furthermore, most of the members of this family could be blocking L-type Ca^{2+} channels, and three of them, **64**,[15b] **67**[15b] and **19**, non-L type Ca^{2+} channels. Molecular modelling results suggest that the binding of these compounds to the AChE catalytic site might be hampered by unfavourable steric interactions. As a result, there might be a displacement of the drug to the peripheral binding site, leading then to a noncompetitive inhibitory mechanism. This finding could be of interest to explore new inhibitors able to bind at both catalytic and peripheral binding sites of AChE.

4. Experimental part

4.1. General methods

Reactions were monitored by TLC, using precoated silica gel aluminium plates containing a fluorescent indicator (Merck, 5539). Detection was accomplished 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_2SO4 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). ^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. Known 4*H*-pyrans (**41**, **42**, **44**, **56**–**58**),[18] pyridines (**45**–**47**, **49**, **50**)[19] and oxazoles (**59**–**61**)[24] have been synthesized as described.

4.2. General method for the synthesis of 6-amino-4-aryl-5-cyano-4*H*-pyran-3-carboxylic acid ethyl esters

To a solution of the corresponding aldehyde (1 equiv) in dry toluene (1 mL/mmol), under argon, malononitrile (1 equiv) and a catalytic amount of piperidine were added. The mixture was stirred at rt for 3–7 h. The solvent was evaporated and the crude was purified by column cromathography eluting with mixtures of hexane/ethyl acetate. Next, to a solution of the arylidenemalononitrile in dry toluene, ethyl acetoacetate (1 equiv) and a catalytic amount of piperidine were added. The mixture was stirred at rt for 2 h, and the precipitated solid was isolated by filtration, washed with cold toluene, dried and recrystallized.

4.3. General method for the synthesis of 6-amino-4-aryl-5-cyano-pyridine 3-carboxylic acid ethyl esters

To a solution of ammonium acetate (10 equiv) in acetic acid (1.25 mL/mmol) the appropriate ethyl 6-amino-5-cyano-4*H*-pyran-3-carboxylate (1 equiv) was added. The mixture was refluxed (116 °C) for 3–7 h. Then, the reaction was cooled and the solvent was evaporated. The crude was treated with an aqueous saturated solution of sodium bicarbonate until pH 7 and extracted with ethyl acetate. The organic phase was dried with sodium sulfate, filtered and the solvent was removed. The crude was purified by column chromatography eluting with mixtures of dichloromethane/ethyl acetate. The isolated product was recrystallized from mixtures of hexane/dichloromethane.

4.4. General method for the Friedländer reaction

Aluminium chloride (1.2–1.7 equiv) was suspended in dry 1,2-dichloroethane (10 mL/mmol) at rt under argon. The corresponding ethyl 6-amino-4-aryl-5-cyano-4*H*-pyran-3-carboxylic acid derivatives (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). After the reaction was complete (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 mixtures as eluent to provide pure compounds.

4.5. 6-Amino-5-cyano-4-(3,4-dimethoxyphenyl)-2-methyl-4*H*-pyran-3-carboxylic acid ethyl ester (43)

This compound was prepared following the general method for the synthesis of 4*H*-pyrans. To a solution of *m*,*p*-dimethoxybenzylidenemalononitrile[20] (1.00 g, 4.67 mmol) in dry toluene (23 mL) at rt under argon, ethyl acetoacetate (592 μL, 4.67 mmol) and piperidine (six drops) were added. After the reaction was complete and following the work-up, purification of the resultant crude afforded compound **43** (909 mg, 57%): mp 134–136 °C; IR (KBr) *v* 3397, 3326, 3223, 2934, 2193, 1693, 1675, 1649, 1606, 1512, 1463, 1448, 1418, 1369, 1334, 1267, 1063, 1022 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.82–6.70 (m, 3H, C_6H_3), 4.44 (s, 2 H, NH_2), 4.41 (s, 1H, H4), 4.05 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.87, 3.85 (2s, 6H, $2\times OCH_3$), 2.36 [s, 3H, $CH_3C(2)$], 1.12 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 165.9 (C=O), 157.3 (C6), 156.3 (C2), 148.8 (C3′), 148.1 (C4′), 136.5 (C1′), 119.6 (C6′), 118.9 (C≡N), 111.2, 110.9 (C5′, C2′), 108.1 (C3), 62.4 (C5), 60.6 (OCH_2CH_3), 55.8 ($2\times OCH_3$), 38.3 (C4), 18.3 [$CH_3C(2)$], 13.9 (CH_3CH_2O); MS (APIES+) m/z 367 [$(M+Na)^+$, 100], 345 [$(M+1)^+$, 20]. Anal. Calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13. Found: C, 63.04; H, 6.12; N, 8.40.

4.6. 6-Amino-5-cyano-4-(4-fluorophenyl)-2-methyl-4*H*-pyran-3-carboxylic acid ethyl ester (55)

This compound was prepared following the general method for the synthesis of 4*H*-pyrans. To a stirred solution of *p*-fluorobenzylydenemalonitrile[22] (1.00 g, 5.81 mmol) in dry toluene (25 mL) at rt under argon, ethyl acetoacetate (737 μL, 5.81 mmol) and piperidine (seven drops) were added. After the reaction was complete and following the work-up, purification of the resultant crude rendered compound **55**[21] (1.31 g, 75%): mp 154–157 °C; IR (KBr) *v* 3408, 3341, 2194, 1695, 1679, 1649, 1610, 1508, 1368, 1337, 1267, 1059 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.28–7.15 (m, 2H, H2′, H6′), 7.05–6.95 (m, 2H, H3′, H5′), 4.49 (s, 2H, NH_2), 4.46 (s, 1 H, H4), 4.06 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 2.39 [s, 3H, $CH_3C(2)$], 1.12 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 165.8 (C=O), 164.5, 159.6 ($J_{C4'-F} = 246$ Hz, C4′), 157.4 (C6), 156.9 (C2), 139.7, 139.6 ($J_{C1'-F} = 3$ Hz, C1′), 129.3, 129.1 ($J_{C2'-F} = 8$ Hz, $2\times$C2′), 118.8 (C≡N), 115.7, 115.3 ($J_{C3'-F} = 22$ Hz, $2\times$C3′), 108.0 (C3), 62.5 (C5), 60.8 (OCH_2CH_3), 38.2 (C4), 18.5 [$CH_3C(2)$], 14.0 (CH_3CH_2O); MS (APCI+) m/z 303 [$(M+1)^+$, 100], 287 (5), 257 (6), 229 (30), 207 (14). Anal. Calcd for $C_{16}H_{15}FN_2O_3$: C, 63.55; H, 5.00; N, 9.27. Found: C, 63.80; H, 5.30; N, 9.51.

4.7. 6-Amino-5-cyano-4-(3-methoxyphenyl)-2-methyl-3-pyridinecarboxylic acid ethyl ester (45)

This compound was obtained following the general method for the synthesis of 6-amino-5-cyano-pyridines. To a stirred solution of ammonium acetate (1.53 g, 19.60 mmol) in acetic acid (25 mL), pyran **41** (625 mg, 1.99 mmol) was added. After the reaction was complete and following the work-up, the crude was purified to produce compound **45** (255 mg, 41%): mp 165–167 °C; IR (KBr) *v* 3404, 3315, 3170, 2979, 2218, 1705, 1650, 1602, 1562, 1493, 1469, 1459, 1376, 1286, 1252, 1178, 1159, 1094, 1079, 1069 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.38 (dd, $J = 7.8$, 7.9 Hz, 1H, H5′), 7.02–6.86 (m, 3H, H2′, H3′, H6′), 5.42 (s, 2H, NH_2), 3.98 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.82 (s, 3H, OCH_3), 2.51 [s, 3H, $CH_3C(2)$], 0.90 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 167.3 (C=O), 160.6 (C6), 159.5 (C3′), 159.0 (C2), 153.7 (C4), 136.7 (C1′), 129.7 (C5′), 120.2 (C6′), 120.0 (C3), 115.9 (C=N), 115.4 (C4′), 113.2 (C2′), 88.9 (C5), 61.3 (OCH_2CH_3), 55.3 (OCH_3), 23.5 [$CH_3C(2)$], 13.5 (CH_3CH_2O); MS (APCI+) m/z 312 [$(M+1)^+$, 100], 284 (25), 266 (14). Anal. Calcd for $C_{17}H_{17}N_3O_3$: C, 65.8S; H, 5.80; N, 13.50. Found: C, 65.37; H, 5.82; N, 13.78.

4.8. 6-Amino-5-cyano-4-(3,4-dimethoxyphenyl)-2-methyl-3-pyridinecarboxylic acid ethyl ester (46)

This compound was prepared following the general method for the synthesis of 6-amino-5-cyano-pyridines. To a stirred solution of ammonium acetate (980 mg, 12.72 mmol) in acetic acid (16 mL), pyran **43** (438 mg, 1.27 mmol) was added. After the reaction was complete and following the work-up, the crude was purified to afford compound **46** (226 g, 52%): mp 171–174 °C; IR (KBr) *v* 3417, 3329, 3183, 2978, 2215, 1726, 1657, 1604, 1556, 1516, 1463, 1365, 1325, 1276, 1256, 1238, 1175, 1143 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.96–6.87 (m, 3H, C_6H_3), 5.43 (s, 2H, NH_2), 4.01 (q, $J = 7,1$ Hz, 2H, $CO_2CH_2CH_3$), 3.92, 3.88 (2s, 6H, $2\times OCH_3$), 2.49 [s, 3H, $CH_3C(2)$], 0.96 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.2 (C=O), 161.5 (C6), 160.7 (C2), 154.2 (C4), 151.3 (C4′), 149.9 (C3′), 138.7 (C1′), 121.0 (C6′), 120.8 (C3), 117.5 (C≡N), 111.3, 111.2 (C2′, C5′), 89.8 (C5), 61.1 (OCH_2CH_3), 57.9 ($2\times OCH_3$), 23.5 [$CH_3C(2)$], 18.2 (CH_3CH_2O); MS (APCI+) m/z 342 (M^+, 100), 314 (71), 296 (12), 280 (6), 254 (6). Anal. Calcd for $C_{18}H_{19}N_3O_4$: C, 63.33; H, 5.61; N, 12.31. Found: C, 63.12, H, 5.68, N, 12.33.

4.9. 6-Amino-5-cyano-4-(4-fluorophenyl)-2-methyl-3-pyridinecarboxylic acid ethyl ester (47)

This compound was obtained following the general method for the synthesis of 6-amino-5-cyano-pyridines. To a stirred solution of ammonium acetate (2.55 g, 33.08 mmol) in acetic acid (30 mL), pyran **55** (1.00 g, 3.31 mmol) was added. After the reaction was complete and subsequently to the work-up, the crude was purified to give compound **47** (404 mg, 41%): mp 239–241 °C; IR

(KBr) ν 3384, 3317, 3182, 2222, 1714, 1654, 1606, 1567, 1512, 1283, 1184, 1162 cm^{-1}; ^1H [$(CD_3)_2CO$, 200 MHz] δ 7.61–7.38 (2m, 4H, C_6H_4), 6.77 (s, 2H, NH_2), 4.11 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 2.58 [s, 3H, $CH_3C(2)$], 1.05 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 167.7 (C=O), 163.2, 159.9 ($J_{C4'-F} = 249$ Hz, C4′), 161.3 (C6), 159.4 (C2), 153.3 (C4), 132.5 (C1′), 130.4, 130.3 ($J_{C2'-F} = 8$ Hz, 2×C2′), 117.2 (C3), 116.4, 116.1 ($J_{C3'-F} = 23$ Hz, 2×C3′), 116.3 (C≡N), 89.8 (C5), 61.9 (OCH_2CH_3), 24.0 [$CH_3C(2)$], 14.0 (CH_3CH_2O); MS (APIES+) m/z 300 [$(M+1)^+$, 50], 322[$(M+Na)^+$, 100]. Anal. Calcd for $C_{16}H_{15}FN_2O_3$: C, 64.21; H, 4.71; N, 14.04. Found: C, 64.02, H, 4.55, N, 13.93.

4.10. 6-Amino-5-cyano-2-methyl-4-(3-nitrophenyl)-3-pyridinecarboxylic acid ethyl ester (49)

This compound was prepared following the general method for the synthesis of 6-amino-5-cyano-pyridines. To a stirred solution of ammonium acetate (1.08 g, 14.12 mmol) in glacial acetic acid (18 mL), pyran **57** (465 mg, 1.41 mmol) was added. After the reaction was complete and following the work-up, the resultant crude was purified to afford compound **49** (245 mg, 53%): mp 152–155 °C; IR (KBr) ν 3394, 3327, 3172, 2222, 1716, 1658, 1561, 1532, 1353, 1278, 1178, 1074 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.36 (m, 1H, H4′), 8.22 (m, 1H, H2′), 7.71–7.66 (m, 2H, H5′, H6′), 5.47 (s, 2H, NH_2), 4.01 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 2.55 [s, 3H, $CH_3C(2)$], 0.95 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 166.8 (C=O), 161.8 (C6), 158.9 (C2), 151.3 (C4), 148.1 (C3′), 137.4 (C1′), 134.0 (C6′), 129.8 (C5′), 124.2, 123.1 (C2′, C4′), 119.5 (C3), 115.3 (C≡N), 88.7 (C5), 61.5 (OCH_2CH_3), 23.9 [$CH_3C(2)$], 13.6 (CH_3CH_2O); MS (APIES−) m/z 325 [$(M-1)^-$, 100], 253 (12), 237 (52), 205 (23). Anal. Calcd for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17. Found: C, 59.01, H, 4.60, N, 17.32.

4.11. 6-Amino-5-cyano-2-methyl-4-phenyl-3-pyridinecarboxylic acid *i*-propyl ester (50)

This compound was prepared following the general method for the synthesis of 6-amino-5-cyano-pyridines. To a stirred solution of ammonium acetate (2.58 g, 33.52 mmol) in acetic acid (40 mL), pyran **44** (1.00 g, 3.35 mmol) was added. After the reaction was complete and following the work-up, the resultant crude was purified to provide compound **50** (395 mg, 40%): mp 222–224 °C; IR (KBr) ν 3393, 3328, 3204, 2978, 2927, 2217, 1713, 1663, 1632, 1561, 1282, 1184, 1110 cm^{-1}; ^1H ($CDCl_3$, 200 MHz) δ 7.55–7.42 (m, 3H, 2×H2′, H4′), 7.37–7.27 (m, 2H, 2×H3′), 5.39 (s, 2H, NH_2), 4.85 [h, $J = 6.2$ Hz, 1H, $CO_2CH(CH_3)_2$], 2.50 [s, 3H, $CH_3C(2)$], 0.91 [d, 6H, $CO_2CH(CH_3)_2$]; ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 166.8 (C=O), 160.4 (C6), 158.9 (C2), 153.6 (C4), 135.7 (C1′), 129.4 (C4′), 128.6, 128.0 (2×C2′, 2×C3′), 120.6 (C3), 115.9 (C≡N), 89.0 (C5), 69.1 [$OCH(CH_3)_2$], 23.4 [$CH_3C(2)$], 21.1 [$(CH_3)_2CHO$]; MS (APIES+) m/z 296 [$(M+1)^+$, 100], 318 [$(M+Na)^+$, 15], 254 (6). Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 68.97; H, 6.09; N, 14.17.

4.12. 6-Amino-5-cyano-2-methyl-4-(4-methylphenyl)-3-pyridinecarboxylic acid ethyl ester (51)

This compound was prepared following the general method for the synthesis of 6-amino-5-cyano-pyridines. To a stirred solution of ammonium acetate (2.22 g, 28.86 mmol) in acetic acid (30 mL), pyran **58** (861 mg, 2.89 mmol) was added. After the reaction was complete and following the work-up, the resultant crude was purified to afford compound **51** (372 mg, 44%): mp 253–256 °C; IR (KBr) ν 3382, 3347, 3185, 2980, 2222, 1713, 1653, 1561, 1514, 1461, 1370, 1280, 1254, 1184, 1073 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.24 (s, 4H, C_6H_4), 5.35 (s, 2H, NH_2), 3.98 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 2.50 [s, 3H, $CH_3C(2)$], 2.40 [s, 3H, $CH_3C(4')$], 0.90 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (DMSO-d_6, 50.2 MHz) δ 166.8 (C=O), 159.7/159.5 (2C, C6, C2), 153.5 (C4), 138.5 (C4′), 133.1 (C1′), 128.8 (2×C3′), 127.6 (2×C2′), 117.8 (C3), 115.3 (C≡N), 79.0 (C5), 60.5 (OCH_2CH_3), 23.0 [$CH_3C(2)$], 20.7 [$CH_3C(4')$], 13.2 (CH_3CH_2O); MS (APCI+) m/z 296 [$(M+1)^+$, 100], 268 (50), 250 (32), 224 (5). Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 68.95, H, 6.00, N, 14.30.

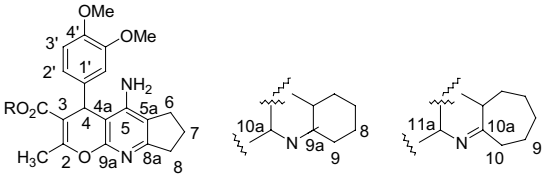
4.13. 6-Amino-5-cyano-4-(2-methoxyphenyl)-2-methyl-3-pyridinecarboxylic acid ethyl ester (52)

This compound was prepared following the general method for the synthesis of 6-amino-5-cyano-pyridines. To a stirred solution of ammonium acetate (12.33 g, 0.16 mol) in acetic acid (90 mL), pyran **42** (3.45 g, 10.98 mmol) was added. After the reaction was complete and following the work-up, the crude was purified to give compound **52** (1.05 g, 31%): mp 200–203 °C; IR (KBr) ν 3388, 3321, 3181, 2222, 1711, 1653, 1560, 1496, 1461, 1285, 1268, 1248, 1180 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.40 (ddd, $J_{3'-4'} = 8.3$ Hz, $J_{4'-5'} = 7.8$ Hz, $J_{4'-6'} = 1.9$ Hz, 1H, H4′), 7.12 (dd, $J_{5'-6'} = 7.5$ Hz, $J_{4'-6'} = 1.9$ Hz, 1H, H6′), 7.00 (m, 2H, H3′, H5′), 5.36 (s, 2H, NH_2), 3.94 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.82 (s, 3H, OCH_3), 2.54 [s, 3H, $CH_3C(2)$], 0.85 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 166.1 (C=O), 161.2 (C6), 158.7 (C2), 156.0 (C2′), 151.8 (C4), 130.9, 129.3 (C4′, C6′), 125.1 (C1′), 120.6 (C5′), 120.1 (C3), 116.0 (C≡N), 111.0 (C3′), 90.4 (C5), 61.0 (OCH_2CH_3), 55.6 (OCH_3), 23.9 [$CH_3C(2)$], 13.4 (CH_3CH_2O); MS (APCI+) m/z 312 [$(M+1)^+$, 100], 240 (8), 645 [$(2\times M+Na)^+$, 15]. Anal. Calcd for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.29, H, 5.72, N, 13.43.

4.14. 5-Amino-4-cyano-2-(4-methylphenyl)-1,3-oxazole (62)

To a solution of aminopropanedinitrile *p*-toluenesulfonate (AMNT) (4.68 g, 18.5 mmol) in dry 1-methyl-2-pyrrolidinone (40 mL) at rt *p*-toluyl chloride (3.74 mL, 28 mmol) was added. The mixture was stirred for 74 h,

the solvent evaporated and the crude was dissolved in ethyl acetate, washed with an 10% aqueous solution of $NaHCO_3$, dried with anhydrous Na_2SO_4 and concentrated in vacuum. The resultant crude was submitted to chromatography with dichloromethane/ethyl acetate (8: 2) to give compound **62** (2.332 g, 67%): mp 255–260 °C; IR (KBr) ν 3300, 3140, 2200, 1640, 1595, 1485, 1420 cm^{-1}; ^1H NMR (DMSO-d_6 300 MHz, 75 °C) δ 7.8 (s, 2H, NH_2), 7.6 (d, $J = 8.1$ Hz, 2H), 7.3 (d, $J = 8.1$ Hz, 2H), 2.3 (s, 3H, CH_3); MS (70 eV) m/z (70 eV) 199 $[M]^+$ (100), 171 $[M-CO]^+$ (39), 156 (58), 155 $[M-(NH_2CO)]^+$ (7), 119 (71), 118 (79), 117 [p-$CH_3PhCN]^+$ (9), 103 (17), 91 [p-$CH_3Ph]^+$ (27), 77 (17), 65 (17), 54 [(M−CO)-p-$CH_3PhCN]^+$ (1), 44 $[NH_2CO]^+$ (10). Anal. Calcd for $C_{11}H_9N_3O$: C, 66.32; H, 4.55; N, 21.09. Found: C, 66.60; H, 4.82; N, 21.37.



4.15. 5-Amino-4,6,7,8-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methyl-cyclopenta[*b*]pyrano[3,2-*e*]pyridine-3-carboxylic acid ethyl ester (11)

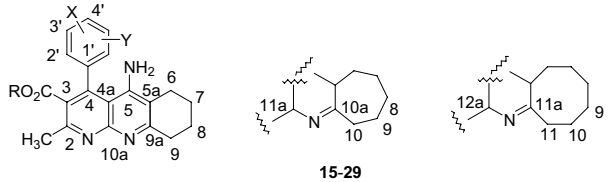
This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (263 mg, 1.97 mmol) in dry 1,2-dichloroethane (12 mL) at rt under argon, pyran **43** (400 mg, 1.16 mmol) and cyclopentanone (175 μL, 1,97 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to produce compound **11** (285 mg, 64%): mp 72–73 °C; IR (BrK) ν 3467, 1685, 1644, 1513, 1282, 1274, 1212, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.88–6.71 (m, 3H, C_6H_3), 4.83 (s, 1H, H4), 4.14 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.01 (s, 2H, NH_2), 3.81, 3.78 (2s, 6H, 2×OCH_3), 2.88 (m, 2H, H8), 2.59 (m, 2H, H6), 2.43 [s, 3H, $CH_3C(2)$], 2.11 (m, 2H, H7), 1.27 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 167.0 (C=O), 162.2 (C8a), 159.2 (C9a), 156.6 (C2), 149.4 (C5), 148.6, 148.3 (C3′, C4′), 136.7 (C1′), 120.7 (C6′), 118.2 (C5a), 111.9 (C5′), 111.2 (C2′), 106.9 (C3), 100.0 (C4a), 60.2 (OCH_2CH_3), 56.0, 55.9 (2×OCH_3), 38.2 (C4), 34.2 (C8), 27.0 (C6), 22.3 (C7), 19.6 [$CH_3C(2)$], 14.3 (CH_3CH_2O); MS (APIES+) m/z 411 $[(M+1)^+$, 100], 433 $[(M+Na)^+$, 10], 843 $[(2\times M+Na)^+$, 20]. Anal. Calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.38; N, 6.82. Found: C, 67.04; H, 6.60; N, 6.75.

4.16. 5-Amino-6,7,8,9-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methyl-4*H*-pyrano[2,3-*b*]quinoline-3-carboxylic acid ethyl ester (12)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (198 mg, 1.48 mmol) in dry 1,2-dichloroethane (9 mL) at rt under argon, pyran **43** (300 mg, 0.87 mmol) and cyclohexanone (154 μL, 1.48 mmol) were added. After the reaction was complete and following the work-up, the crude was purified to afford product **12** (319 mg, 86%): mp 77–79 °C; IR (BrK) ν 3420, 3393, 2933, 1706, 1642, 1573, 1513, 1449, 1377, 1266, 1224, 1141, 1095, 1060, 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.86–6.71 (m, 3H, C_6H_3), 4.80 (s, 1H, H4), 4.17 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.07 (s, 2H, NH_2), 3.80, 3.78 (2s, 6H, 2×OCH_3), 2.73 (m, 2H, H9), 2.43 [s, 3H, $CH_3C(2)$], 2.26 (m, 2H, H6), 1.79 (m, 4H, H7, H8), 1.26 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 167.1 (C=O), 159.3 (C9a), 154.4 (C2), 154.0 (C10a), 150.6 (C5), 149.3, 148.2 (C3′, C4′), 136.6 (C1′), 120.8 (C6′), 113.7 (C5a), 111.7 (C5′), 111.1 (C2′), 106.9 (C3), 99.8 (C4a), 60.4 (OCH_2CH_3), 56.0, 55.9 (2×OCH_3), 38.3 (C4), 32.6 (C9), 23.0, 22.7, 22.5 (C6, C7, C8), 19.8 [$CH_3C(2)$], 14.4 (CH_3CH_2O); MS (APIES+) m/z 425 $[(M+1)^+$, 100], 871 $[(2\times M+Na)^+$, 30]. Anal. Calcd for $C_{24}H_{28}N_2O_5$: C, 67.91; H, 6.65; N, 6.60. Found: C, 67.75; H, 6.94; N, 6.90.

4.17. 5-Amino-4,6,7,8,9,10-hexahydro-4-(3,4-dimethoxyphenyl)-2-methyl-cyclohepta[*b*]pyrano[3,2-*e*]pyridine-3-carboxylic acid ethyl ester (13)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (139 mg, 1.05 mmol) in dry 1,2-dichloroethane (9 mL) at rt under argon, pyran **43** (300 mg, 0.87 mmol) and cycloheptanone (123 μL, 1.05 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to give compound **13** (205 mg, 54%): mp 180–183 °C; IR (BrK) ν 3440, 3401, 2924, 1710, 1643, 1568, 1513, 1455, 1263, 1229, 1213, 1076 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.89–6.72 (m, 3H, C_6H_3), 4.81 (s, 1H, H4), 4.14 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.09 (s, 2H, NH_2), 3.82, 3.78 (2s, 6H, 2×OCH_3), 2.89 (m, 2H, H10), 2.46 [s, 3H, $CH_3C(2)$], 2.44 (m, 2H, H6), 1.80 (m, 2H, H9), 1.67 (m, 4H, H7, H8), 1.28 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 167.1 (C=O), 160.6 (C10a), 159.2 (C2), 153.9 (C11a), 149.7 (C5), 149.3, 148.2 (C3′, C4′), 136.5 (C1′), 120.8 (C6′), 119.1 (C5a), 111.6 (C5′), 111.0 (C2′), 106.9 (C3), 100.5 (C4a), 60.4 (OCH_2CH_3), 56.0, 55.9 (2×OCH_3), 38.7, 38.4 (C10, C4), 32.2 (C9), 26.9 (C7), 26.2 (C8), 25.8 (C6), 19.7 [$CH_3C(2)$], 14.4 (CH_3CH_2O); MS (APIES+) m/z 439 $[(M+1)^+$, 100], 301 (5). Anal. Calcd for $C_{25}H_{30}N_2O_5 \cdot H_2O$: C, 65,77; H, 7,06; N, 6.14. Found: C, 66.01; H, 7,04; N, 6.47.



4.18. 5-Amino-6,7,8,9-tetrahydro-4-(3-methoxyphenyl)-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (15)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (340 mg, 2.55 mmol) in dry 1,2-dichloroethane (9 mL) at rt under argon, pyridine **45** (265 mg, 0.85 mmol) and cyclohexanone (221 μL, 2.13 mmol) were added. After the reaction was complete and following the work-up, the crude was purified to give compound **15** (241 mg, 73%): mp 152–154 °C; IR (KBr) ν 3446, 2934, 1725, 1637, 1570, 1546, 1432, 1288, 1248 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.41 (dd, $J = 7.6$ Hz, 7.9 Hz, 1H, H5′), 7.04–6.90 (m, 3H, H2′, H3′, H6′), 4.61 (s, 2H, NH_2), 4.02 (q, $J = 7.5$ Hz, 2H, $CO_2CH_2CH_3$), 3.82 (s, 3H, OCH_3), 3.10 (m, 2H, H9), 2.69 [s, 3H, $CH_3C(2)$], 2.38 (m, 2H, H6), 1.89 (m, 4H, H7, H8), 0.99 (s, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.0 (C=O), 161.6 (C9a), 159.5 (C3′), 156.4 (C2), 154.0 (C10a), 149.5 (C5), 144.4 (C4), 138.2 (C1′), 129.9 (C5′), 126.8 (C3), 121.0 (C6′), 115.0 (C4′), 114.1 (C2′), 111.5 (C5a), 106.1 (C4a), 61.2 (OCH_2CH_3), 55.3 (OCH_3), 33.7 (C9), 23.5, 23.4 [C6, $CH_3C(2)$], 22.5, 22.3 (C7, C8), 13.7 (CH_3CH_2O); MS (APIES+) m/z 392 [$(M+1)^+$, 100], 805 [$(2\times M+Na)^+$, 30]. Anal. Calcd for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73. Found: C, 70.82; H, 6.64; N, 10.72.

4.19. 5-Amino-6,7,8,9-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (16)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (113 mg, 0.85 mmol) in dry 1,2-dichloroethane (4 mL) at rt under argon, pyridine **46** (116 mg, 0.34 mmol) and cyclohexanone (93 μL, 1.00 mmol) were added. After the reaction was complete and following the work-up, the crude was purified to give product **16** (39 mg, 33%): mp 107–110 °C; IR (KBr) ν 3460, 3413, 2933, 1726, 1617, 1569, 1544, 1514, 1462, 1313, 1252 cm^{-1}; ^1H ($CDCl_3$, 200 MHz) δ 6.95–6.87 (m, 3H, C_6H_3), 4.61 (s, 2H, NH_2), 4.04 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.95, 3.87 (2s, 6H, $2\times OCH_3$), 3.10 (m, 2H, H9), 2.69 [s, 3H, $CH_3C(2)$], 2.39 (m, 2H, H6), 1.90 (m, 4H, H7, H8), 1.03 (s, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) 168.4 (C=O), 161.9 (C9a), 156.3 (C2), 154.4 (C10a), 149.6, 149.3, 149.0 (C5, C4′, C3′), 144.3 (C4), 129.2 (C1′), 127.4 (C3), 121.3 (C6′), 112.1 (C5′), 111.4 (C5a), 111.0 (C2′), 106.6 (C4a), 61.3 (OCH_2CH_3), 56.1, 56.0 ($2\times OCH_3$), 34.0 (C9), 23.6 (C6), 23.5 [$CH_3C(2)$], 22.6, 22.5 (C8, C7), 13.9 (CH_3CH_2O); MS (APIES+) m/z 422 [$(M+1)^+$, 100], 865 [$(2\times M+Na)^+$, 20]. Anal. Calcd for $C_{24}H_{27}N_3O_4\cdot H_2O$: C, 65.59; H, 6.65; N, 9.56. Found: C, 65.54; H, 6.79; N, 9.49.

4.20. 5-Amino-4-(4-fluorophenyl)-6,7,8,9-tetrahydro-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (17)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (167 mg, 1.25 mmol) in dry 1,2-dichloroethane (5 mL) at rt under argon, pyridine **47** (150 mg, 0.50 mmol) and cyclohexanone (130 μL, 1.25 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to afford compound **17** (52 mg, 28%): mp 110–112 °C; IR (KBr) ν 3414, 2927, 1725, 1626, 1570, 1544, 1509, 1223 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.42–7.13 (2m, 4H, C_6H_4), 4.21 (s, 2H, NH_2), 4.01 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.04 (m, 2H, H9), 2.68 [s, 3H, $CH_3C(2)$], 2.35 (m, 2H, H6), 1.89 (m, 4H, H7, H8), 1.01 (s, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.1 (C=O), 165.6, 160.6 ($J_{C4'-F} = 251$ Hz, C4′), 162.4 (C9a), 156.3 (C2), 154.8 (C10a), 148.9 (C5), 143.4 (C4), 133.3 (C1′), 131.1, 130.9 ($J_{C2'-F} = 8$ Hz, $2\times C2'$), 127.4 (C3), 116.1, 115.6 ($J_{C3'-F} = 22$ Hz, $2\times C3'$), 111.8 (C5a), 106.5 (C4a), 61.3 (OCH_2CH_3), 34.2 (C9), 23.7 (C6), 23.5 [$CH_3C(2)$], 22.7, 22.5 (C7, C8), 13.8 (CH_3CH_2O); MS (APIES+) m/z 380 [$(M+1)^+$, 100], 781 [$(2\times M+Na)^+$, 30]. Anal. Calcd for $C_{18}H_{20}N_2O_5\cdot 2H_2O$: C, 63.60; H, 6.31; N, 10.12. Found: C, 63.34; H, 6.66; N, 10.51.

4.21. 5-Amino-4-(4-chlorophenyl)-6,7,8,9-tetrahydro-2-methyl-benzo[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (18)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (116 mg, 0.87 mmol) in dry 1,2-dichloroethane (4 mL) at rt under argon, pyridine **48** (110 mg, 0.35 mmol) and cyclohexanone (90 μL, 0.87 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to produce compound **18** (122 mg, 88%): mp 119–122 °C; IR (KBr) ν 3482, 3413, 2937, 2862, 1727, 1632, 1571, 1540, 1493, 1314, 1269, 1219 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.47 (d, $J_{2'-3'} = 8.6$ Hz, 2H, $2\times H2'$), 7.33 (d, $J_{2'-3'} = 8.6$ Hz, 2H, $2\times H3'$), 4.22 (s, 2H, NH_2), 4.01 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.03 (m, 2H, H9), 2.68 [s, 3H, $CH_3C(2)$], 2.35 (m, 2H, H6), 1.88 (m, 4H, H7, H8), 1.00 (s, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.0 (C=O), 162.5 (C9a), 156.3 (C2), 154.6 (C10a), 148.8 (C5), 143.2 (C4), 135.8, 135.5 (C1′, C4′), 130.4 ($2\times C2'$), 128.9 ($2\times C3'$), 127.1 (C3), 111.8 (C5a), 106.3 (C4a), 61.4 (OCH_2CH_3), 34.2 (C9), 23.7 (C6), 23.5 [$CH_3C(2)$], 22.7, 22.5 (C7, C8), 13.8 (CH_3CH_2O); MS (APIES+) m/z 396 [$(M+1)^+$, 100], 813 [$(2\times M+Na)^+$, 15]. Anal. Calcd for $C_{22}H_{22}ClN_3O_2$: C, 66.81; H, 5.61; N, 10.63. Found: C, 66.58; H, 5.90; N, 10.35.

4.22. 5-Amino-6,7,8,9-tetrahydro-2-methyl-4-(3-nitrophenyl)-benzo[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (19)

This compound was obtained following the general method for the Friedländer reaction. To a stirred solution of $AlCl_3$ (116 mg, 0.87 mmol) in dry 1,2-dichloroethane (3 mL) at rt under argon, pyridine **49** (81 mg, 0.25 mmol) and cyclohexanone (90 μL, 0.87 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to afford

compound **19** (55 mg, 54%): mp 214–217 °C; IR (KBr) ν 3460, 3420, 2924, 2852, 1724, 1630, 1570, 1531, 1462, 1351, 1315, 1269, 1222 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ ($CDCl_3$, 300 MHz) δ 8.40–8.30 (m, 2H, H2′, H4′), 7.80–7.68 (m, 2H, H5′, H6′), 4.06 (s, 2H, NH_2), 4.02 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.08 (m, 2H, H9), 2.72 [s, 3H, $CH_3C(2)$], 2.38 (m, 2H, H6), 1.91 (m, 4H, H7, H8), 1.11 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 169.0 (C=O), 162.8 (C9a), 156.5 (C2), 154.8 (C10a), 150.2 (C5), 147.8 (C3′), 142.7 (C4), 139.0 (C1′), 134.5 (C6′), 129.3 (C5′), 123.6 (C2′, C4′), 127.1 (C3), 111.8 (C5a), 106.3 (C4a), 61.2 (OCH_2CH_3), 33.7 (C9), 23.3, 23.2 [$CH_3C(2)$, C6)], 22.1 (C7, C8), 13.4 (CH_3CH_2O); MS (APIES+) m/z 407 [$(M+1)^+$, 100]. Anal. Calcd for $C_{22}H_{22}N_4O_4$: C, 65.01;H, 5.46; N, 13.78. Found: C, 64.73; H, 5.62; N, 13.58.

4.23. 5-Amino-7,8,9,10-tetrahydro-2-methyl-4-(4-methylphenyl)-6*H*-cyclohepta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (20)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (91 mg, 0.68 mmol) in dry 1,2-dichloroethane (4 mL) at rt under argon, pyridine **51** (100 mg, 0.34 mmol) and cycloheptanone (80 μL, 0.68 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to produce compound **20** (102 mg, 77%): mp 158–161 °C; IR (KBr) ν 3469, 3420, 2924, 1725, 1631, 1570, 1543, 1226, 1092 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.28 (s, 4H, C_6H_4), 4.40 (s, 2H, NH_2), 3.99 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.18 (m, 2H, H10), 2.69 [s, 3H, $CH_3C(2)$], 2.58 (m, 2H, H6), 2.43 [s, 3H, $CH_3C(4')$], 1.81 (m, 4H, H7, H9), 1.60 (m, 2H, H8), 0.98 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.3 (C=O), 168.0 (C10a), 156.0 (C2), 154.5 (C11a), 148.0 (C5), 144.8 (C4), 139.1 (C4′), 134.2 (C1′), 129.3 (2×C3′), 128.8 (2×C2′), 127.7 (C3), 116.9 (C5a), 107.4 (C4a), 61.2 (OCH_2CH_3), 39.7 (C10), 31.8 (C9), 27.0, 26.4, 26.0 (C7, C8, C9), 23.4 [$CH_3C(2)$], 21.3 [$CH_3C(4')$], 13.7 (CH_3CH_2O); MS (APCI+) m/z 390 [$(M+1)^+$, 100], 362 (2). Anal. Calcd for $C_{24}H_{27}N_3O_2{\cdot}3H_2O$: C, 64.99; H, 7.50; N, 9.47. Found: C, 65.16; H, 7.76; N, 9.56.

4.24. 5-Amino-7,8,9,10-tetrahydro-4-(3-methoxyphenyl)-2-methyl-6*H*-cyclohepta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (21)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (160 mg, 1.20 mmol) in dry 1,2-dichloroethane (5 mL) at rt under argon, pyridine **45** (150 mg, 0.48 mmol) and cycloheptanone (142 μL, 1.20 mmol) were added. After the reaction was complete and following the work-up, the crude was purified to afford compound **21** (103 mg, 53%): mp 125–128 °C; IR (KBr) ν 3436, 2924, 1724, 1631, 1568, 1543, 1286, 1224, 1093 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.43–7.38 (dd, $J = 7.8$ Hz, 7.9 Hz, 1H, H5′), 7.02–6.86 (m, 3H, H2′, H3′, H6′, H2′), 7.05–6.90 (m, 3H, C_6H_3), 4.53 (s, 2H, NH_2), 4.01 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.82 (s, 3H, OCH_3), 3.18 (m, 2H, H10), 2.69 [s, 3H, $CH_3C(2)$], 2.60 (m, 2H, H6), 1.81 (m, 4H, H7, H9), 1.60 (m, 2H, H8), 0.98 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.2 (C=O), 167.9 (C10a), 159.6 (C3′), 156.4 (C2), 154.3 (C11a), 148.4 (C5), 144.6 (C4), 138.4 (C1′), 130.0 (C5′), 127.6 (C3), 121.3 (C6′), 117.1 (C5a), 115.2 (C4′), 114.4 (C2′), 107.1 (C4a), 61.4 (OCH_2CH_3), 55.5 (OCH_3) 39.5 (C10), 32.0 (C9), 27.1 (C7), 26.5, 26.1 (C6, C8), 23.6 [$CH_3C(2)$], 13.9 (CH_3CH_2O); MS (APCI+) m/z 406 [$(M+1)^+$, 100], 390 (6), 378 (85), 362 (25). Anal. Calcd for $C_{24}H_{27}N_3O_3{\cdot}H_2O$: C, 68.07; H, 6.90; N, 9.92. Found: C, 68.27; H, 6.60; N, 9.68.

4.25. 5-Amino-7,8,9,10-tetrahydro-4-(2-methoxyphenyl)-2-methyl-6*H*-cyclohepta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (22)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (320 mg, 2.40 mmol) in dry 1,2-dichloroethane (10 mL) at rt under argon, pyridine **52** (300 mg, 0.96 mmol) and cycloheptanone (283 μL, 2.40 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to give compound **22** (248 mg, 64%): mp 87–90 °C; IR (KBr) ν 3478, 3400, 2923, 1724, 1630, 1567, 1543, 1495, 1224, 1086 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.51 (ddd, $J_{4'-6'} = 1.8$ Hz, 1H, H4′), 7.23 (dd, $J_{5'-6'} = 7.5$ Hz, $J_{4'-6'} = 1.8$ Hz, 1H, H6′), 7.03 (m, 2H, H3′, H5′), 4.53 (s, 2H, NH_2), 3.96 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.73 (s, 3H, OCH_3), 3.20 (m, 2H, H10), 2.70 [(t, $J = 7.2$ Hz, 3H, $CH_3C(2)$], 2.63 (m, 2H, H6), 1.82 (m, 4H, H7, H9), 1.60 (m, 2H, H8), 1.00 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.3 (C=O), 167.8 (C10a), 157.1 (C2′), 156.5 (C2), 154.5 (C11a), 148.5 (C5), 142.2 (C4), 131.1, 130.2 (C4′, C6′), 127.6 (C3), 126.4 (C1′), 120.8 (C5′), 116.8 (C5a), 111.5 (C3′), 108.2 (C4a), 61.2 (OCH_2CH_3), 56.0 (OCH_3) 39.7 (C10), 32.0 (C9), 27.1 (C7), 26.5 (C8), 26.1 (C6), 23.7 [$CH_3C(2)$], 13.9 (CH_3CH_2O); MS (APCI+) m/z 406 [$(M+1)^+$, 100], 334 (2). Anal. Calcd for $C_{24}H_{27}N_3O_2{\cdot}2H_2O$: C, 68.07; H, 6.90; N, 9.92. Found: C, 68.28; H, 6.75; N, 9.83.

4.26. 5-Amino-7,8,9,10-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methyl-6*H*-cyclohepta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (23)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (66 mg, 0.49 mmol) in dry 1,2-dichloroethane (3 mL) at rt under argon, pyridine **46** (100 mg, 0.29 mmol) and cycloheptanone (59 μL, 0.50 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to produce compound **23** (96 mg, 76%): mp 105–108 °C; IR (KBr) ν 3476, 3375, 2923, 1724, 1628, 1567, 1513, 1253, 1234,

1140 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.95–6.89 (m, 3H, C_6H_3), 4.58 (s, 2H, NH_2), 4,03 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.95/3.82 (2s, 6H, 2×OCH_3), 3.20 (m, 2H, H10), 2.68 [s, 3H, $CH_3C(2)$], 2.61 (m, 2H, H6), 1.82 (m, 4H, H7, H9), 1.61 (m, 2H, H8), 1.03 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 168.2 (C=O), 167.8 (C10a) 155.8 (C2), 154.3 (C11a), 149.4, 148.7, 147.9 (C4′, C3′, C5), 144.3 (C4), 129.0 (C1′), 128.8 (C3), 121.2 (C6′), 116.7 (C5a), 112.0 (C5′), 110.7 (C2′), 107.2 (C4a), 61.1 (OCH_2CH_3), 55.9, 55.8 (2×OCH_3), 39.5 (C10), 31.7 (C9), 26.8 (C7), 26.2 (C8), 25.8 (C6), 23.2 [$CH_3C(2)$], 13.7 (CH_3CH_2O); MS (APIES+) m/z 436 [$(M+1)^+$, 100]. Anal. Calcd for $C_{25}H_{29}N_3O_4 \cdot 2H_2O$: C, 63.68; H, 7.05; N, 8.91. Found: C, 63.90; H, 6.96; N, 8.71.

4.27. 5-Amino-4-(4-fluorophenyl)-7,8,9,10-tetrahydro-2-methyl-6*H*-cyclohepta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (24)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (133 mg, 1.00 mmol) in dry 1,2-dichloroethane (5 mL) at rt under argon, pyridine **47** (150 mg, 0.50 mmol) and cycloheptanone (118 μL, 1.00 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to afford compound **24** (138 mg, 70%): mp 165–168 °C; IR (KBr) ν 3487, 3417, 2924, 1725, 1629, 1567, 1509, 1224 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.41–7.14 (m, 4H, C_6H_4), 4.32 (s, 2H, NH_2), 4.02 (q, $J = 7.0$ Hz, 2H, $CO_2CH_2CH_3$), 3.17 (m, 2H, H10), 2.69 [s, 3H, $CH_3C(2)$], 2.60 (m, 2H, H6), 1.81 (m, 4H, H7, H9), 1.61 (m, 2H, H8), 1.02 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.4 (C=O), 168.2 (C10a), 165.6, 160.6 ($J_{C4'-F} = 251$ Hz, C4′), 156.1 (C2), 154.6 (C11a), 147.8 (C5), 143.6 (C4), 133.3, 133.2 ($J_{C1'-F} = 4$ Hz, C1′), 131.1, 131.0 ($J_{C2'-F} = 8$ Hz, 2×C2′), 128.0 (C3), 117.3 (C5a), 116.1, 115.6 ($J_{C3'-F} = 22$ Hz, 2×C3′), 107.4 (C4a), 61.5 (OCH_2CH_3), 39.8 (C10), 31.9 (C9), 27.1 (C7), 26.5 (C8), 26.1 (C6), 23.5 [$CH_3C(2)$], 13.9 (CH_3CH_2O); MS (APCI+) m/z 394 [$(M+1)^+$, 100], 366 (2). Anal. Calcd for $C_{23}H_{24}FN_3O_2 \cdot H_2O$: C, 67.12; H, 6.37; N, 10.22. Found: C, 66.85; H, 6.21; N, 10.24.

4.28. 5-Amino-4-(4-chlorophenyl)-7,8,9,10-tetrahydro-2-methyl-6*H*-cyclohepta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (25)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (85 mg, 0.64 mmol) in dry 1,2-dichloroethane (3 mL) at rt under argon, pyridine **48** (100 mg, 0.32 mmol) and cycloheptanone (74 μL, 0.64 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to afford compound **25** (97 mg, 75%): mp 96–99 °C; IR (KBr) ν 3496, 3401, 3242, 2890, 1718, 1651, 1573, 1546, 1494, 1281, 1229 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.46 (d, $J_{2'-3'} = 8.6$ Hz, 2H, 2×H2′), 7.34 (d, $J_{2'-3'} = 8.6$ Hz, 2H, 2×H3′), 4.30 (s, 2H, NH_2), 4.01 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.16 (m, 2H, H10), 2.68 [s, 3H, $CH_3C(2)$], 2.58 (m, 2H, H6), 1.80 (m, 4H, H7, H9), 1.59 (m, 2H, H8), 1.01 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.4 (C=O), 168.0 (C10a), 156.1 (C2), 154.4 (C11a), 147.6 (C5), 143.2 (C4), 135.6, 135.4 (C1′, C4′), 130.4 (2×C2′), 128.8 (2×C3′), 127.6 (C3), 117.2 (C5a), 107.1 (C4a), 61.4 (OCH_2CH_3), 39.7 (C10), 31.8 (C9), 27.0 (C7), 26.3 (C8), 26.0 (C6), 23.4 [$CH_3C(2)$], 13.7 (CH_3CH_2O); MS (APIES+) m/z 410 [$(M+1)^+$, 100], 432 [$(M+Na)^+$, 60], 382 (90). Anal. Calcd for $C_{23}H_{24}ClN_3O_2 \cdot 2H_2O$: C, 61.95; H, 6.33; N, 9.42. Found: C, 62.05; H, 6.34; N, 9.18.

4.29. 5-Amino-7,8,9,10-tetrahydro-2-methyl-4-(3-nitrophenyl)-6*H*-cyclohepta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (26)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (57 mg, 0.43 mmol) in dry 1,2-dichloroethane (3 mL) at rt under argon, pyridine **49** (70 mg, 0.21 mmol) and cycloheptanone (51 μL, 0.43 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to afford compound **26** (78 mg, 86%): mp 182–185 °C; IR (KBr) ν 3519, 3420, 2924, 2853, 1724, 1623, 1566, 1531, 1349, 1269 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.38–8.28 (m, 2H, H2′, H4′), 7.80–7.64 (m, 2H, H5′, H6′), 4.07 (s, 2H, NH_2), 4.00 (q, $J = 7.1$ Hz, 2H, $CO_2CH_2CH_3$), 3.18 (m, 2H, H10), 2.70 [s, 3H, $CH_3C(2)$], 2.57 (m, 2H, H6), 1.81 (m, 4H, H7, H9), 1.60 (m, 2H, H8), 0.99 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 169.0 (C=O), 167.7 (C10a), 156.2 (C2), 154.5 (C11a), 147.9 (C3′), 146.9 (C5), 141.7 (C4), 139.2 (C1′), 134.9 (C6′), 129.6 (C5′), 127.5 (C3), 124.0, 123.9 (C2′, C4′), 117.8 (C5a), 106.9 (C4a), 61.6 (OCH_2CH_3), 39.9 (C10), 31.8 (C9), 27.0 (C7), 26.3 (C8), 26.0 (C6), 23.6 [$CH_3C(2)$], 13.8 (CH_3CH_2O); MS (APCI+) m/z 421 [$(M+1)^+$, 100]. Anal. Calcd for $C_{23}H_{24}N_4O_4$: C, 65.70; H, 5.75; N, 13.32. Found: C, 65.45; H, 6.02; N, 13.34.

4.30. 5-Amino-6,7,8,9,10,11-hexahydro-2-methyl-4-phenylcycloocta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (27)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (96 mg, 0.72 mmol) in dry 1,2-dichloroethane (4 mL) at rt under argon, pyridine **53** (100 mg, 0.36 mmol) and cyclooctanone (91 mg, 0.72 mmol) were added. After the reaction was complete and following work-up, the resultant crude was purified to give product **27** (111 mg, 79%): mp 174–176 °C; IR (KBr) ν 3485, 3414, 2923, 1724, 1629, 1566, 1542, 1442, 1325, 1274, 1078 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.48–7.44 (m, 3H, H4′, 2×H2′), 7.41–7.33 (m, 2H, 2×H3′), 4.43 (s, 2H, NH_2), 3.95 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.11 (m, 2H, H11), 2.72 (m, 2H, H6), 2.68 [s, 3H, $CH_3C(2)$], 1.89 (m, 2H, H10),

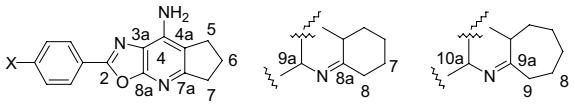
1.58 (m, 2H, H7), 1.47 (m, 2H, H9), 1.33 (m, 2H, H8), 0.92 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.1 (C=O), 166.1 (C11a), 156.3 (C2), 154.7 (C12a), 148.7 (C5), 144.6 (C4), 137.1 (C1′), 129.1 (C4′), 128.9, 128.6 (2×C2′, 2×C3′), 127.4 (C3), 114.3 (C5a), 106.7 (C4a), 61.2 (OCH_2CH_3), 36.8 (C11), 30.9 (C10), 27.2 (C7), 26.5 (C9), 26.2 (C8), 24.6 (C6), 23.5 [$CH_3C(2)$], 13.7 (CH_3CH_2O); MS (APIES+) m/z 390 [$(M+1)^+$, 100]. Anal. Calcd for $C_{24}H_{27}N_3O_2{\cdot}2H_2O$: C, 70.72; H, 7.18; N, 10.32. Found: C, 70.47; H, 7.05; N, 10.48.

4.31. 5-Amino-6,7,8,9,10,11-hexahydro-4-(4-methoxyphenyl)-2-methyl-cycloocta[*b*][1,8]naphthyridine-3-carboxylic acid ethyl ester (28)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (171 mg, 1.28 mmol) in dry 1,2-dichloroethane (7 mL) at rt under argon, pyridine **54** (200 mg, 0.64 mmol) and cyclooctanone (162 mg, 1.28 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to afford product **28** (191 mg, 71%): mp 169–172 °C; IR (KBr) ν 3481, 3412, 2926, 1722, 1620, 1567, 1513, 1251, 1223 cm^{-1}; ^1H NMR($CDCl_3$, 200 MHz) δ 7.32 (d, $J = 8.9$ Hz, 2H, 2×H2′), 6.99 (d, 2H, 2×H3′), 4.54 (s, 2H, NH_2), 4.00 (q, $J = 7.2$ Hz, 2H, $CO_2CH_2CH_3$), 3.85 (s, 3H, OCH_3), 3.12 (m, 2H, H11), 2.73 (m, 2H, H6), 2.67 [s, 3H, $CH_3C(2)$], 1.90 (m, 2H, H10), 1.59 (m, 2H, H7), 1.47 (m, 2H, H9), 1.33 (m, 2H, H8), 1.00 (t, 3H, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 168.4 (C=O), 166.5 (C11a), 160.0 (C4′), 156.0 (C2), 155.1 (C12a), 148.4 (C5), 144.4 (C4), 130.2 (2×C2′), 129.0 (C1′), 127.7 (C3), 114.1 (C5a), 114.0 (2×C3′), 107.2 (C4a), 61.2 (OCH_2CH_3), 55.3 (OCH_3), 37.0 (C11), 30.9 (C10), 27.2 (C7), 26.5 (C9), 26.1 (C8), 24.6 (C6), 23.5 [$CH_3C(2)$], 13.8 (CH_3CH_2O); MS (APIES+) m/z 420 [$(M+1)^+$, 100], 392 (6). Anal. Calcd for $C_{25}H_{29}N_3O_3$: C, 71.58; H, 6.97; N, 10.02. Found: C, 71.31; H, 7.20; N, 10.15.

4.32. 5-Amino-6,7,8,9,10,11-hexahydro-2-methyl-4-phenyl-cycloocta[*b*][1,8]naphthyridine-3-carboxylic acid *i*-propyl ester (29)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (226 mg, 1.69 mmol) in dry 1,2-dichloroethane (7 mL) at rt under argon, pyridine **50** (200 mg, 0.68 mmol) and cyclohexanone (175 μL, 1.69 mmol) were added. After the reaction was complete and following the work-up, the resultant crude was purified to give product **29** (121 mg, 48%): mp 200–201 °C; IR (KBr) ν 3480, 3436, 2978, 2933, 2862, 1720, 1628, 1568, 1543, 1496, 1442, 1372, 1314, 1274, 1225, 1103, 1076 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.51–7.35 (m, 5H, C_6H_5), 4.87 [h, 1H, $J = 6.2$ Hz, $CO_2CH(CH_3)_2$], 4.23 (s, 2H, NH_2), 3.02 (m, 2H, H9), 2.68 [s, 3H, $CH_3C(2)$], 2.33 (m, 2H, H6), 1.87 (m, 4H, H7, H8), 0.96 [d, 6H, $CO_2CH(CH_3)_2$]; ^{13}C NMR ($CDCl_3$, 50.2 MHz) δ 167.7 (C=O), 162.1 (C9a), 156.2 (C2), 154.6 (C10a), 149.1 (C5), 144.2 (C4), 137.4 (C1′), 129.1, 128.7 (2×C2′, 2×C3′, C4′), 127.3 (C3), 111.5 (C5a), 106.5 (C4a), 69.0 [$CO_2CH(CH_3)_2$], 34.2 (C9), 23.6 (C6), 23.4 [$CH_3C(2)$], 22.7, 22.6 (C7, C8), 21.3 [$CO_2CH(CH_3)_2$]; MS (APIES+) m/z 376 [$(M+1)^+$, 100], 773 [$(2\times M+Na)^+$, 35]. Anal. Calcd for $C_{23}H_{25}N_3O_2$: C, 73.58; H, 6.71; N, 11.19. Found: C, 73.71; H, 7.00; N, 11.18.



30-40

4.33. 4-Amino-2-(4-chlorophenyl)-6,7-dihydro-5*H*-cyclopenta[*b*]oxazolo[4,5-*e*]pyridine (30)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.18 g, 1.36 mmol) in dry chlorobenzene (20 mL) at rt under argon, 1,3-oxazole **59** (0.20 g, 0.92 mmol) and cyclopentanone (122 μL, 1.37 mmol) were added. The reaction was complete 9 days later. After the work-up, the resultant crude was purified to produce compound **30** (49.4 mg, 19%): mp 308–315 °C; IR (KBr) ν 3360, 3310, 3200, 2890, 2840, 1630, 1610, 1470, 1330, 1240 cm^{-1}; ^1H NMR (DMSO-d_6 300 MHz) δ 8.07 (d, $J = 8.7$ Hz, 2H, H2′, H6′), 7.64 (d, 2H, H3′, H5′), 6.60 (br s, 2H, NH_2), 2.83 (m, 2H, H7), 2.72 (m, 2H, H5), 2.05 (m, 2H, H6); MS (70 eV) m/z 287 $[M+2]^+$ (40), 285 $[M]^+$ (100), 139 (15), 119 (31), 113 [p-$Cl^{37}Ph]^+$ (5), 111 [p-$Cl^{35}Ph]^+$ (15). Anal. Calcd for $C_{15}H_{12}ClN_3O$: C, 63.05; H, 4.23; N, 14.71. Found: C, 62.95; H, 4.25; N, 14.58.

4.34. 4-Amino-6,7-dihydro-2-(4-methoxyphenyl)-5*H*-cyclopenta[*e*]oxazolo[4,5-*b*]pyridine (31)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.37 g, 2.79 mmol) in dry 1,2-dichloroethane (20 mL) at rt under argon, 1,3-oxazole **61** (0.30 g, 1.39 mmol) and cyclopentanone (247 μL, 2.79 mmol) were added. The reaction was complete 13 days later. After the work-up, the resultant crude was purified to give compound **31** (160 mg, 35%): mp 277–285 °C (dec); IR (KBr) ν 3390, 3340, 3190, 2940, 2860, 1650, 1620, 1500, 1260, 1180 cm^{-1}; ^1H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 8.03 (d, $J = 8.6$ Hz, 2H, H2′, H6′), 7.13 (d, 2H, H3′, H5′), 6.26 (br s, 2H, NH_2), 3.86 (s, 3H, OCH_3), 2.85 (m, 2H, H7), 2.75 (m, 2H, H5), 2.11 (m, 2H, H6); MS (70 eV) m/z 281 $[M]^+$ (100), 280 (45), 266 (27), 238 (7), 140 (6), 119 (11), 92 (8), 77 (4), 65 (6). Anal. Calcd for $C_{16}H_{15}N_3O_2{\cdot}\frac{1}{2}H_2O$: C, 66.19; H, 5.56; N, 14.47. Found: C, 66.38; H, 5.12; N, 14.69.

4.35. 4-Amino-6,7-dihydro-2-(4-methylphenyl)-5*H*-cyclopenta[*e*]oxazolo[5,4-*b*]pyridine (32)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.53 g, 4 mmol) in dry 1,2-dichloroethane (20 mL) at rt under argon, 1,3-oxazole **62** (0.40 g, 2 mmol) and cyclopentanone (355 μL, 4 mmol) were added. The reaction was refluxed during 286 h. After the work-up, the resultant crude was purified to afford compound **32** (46.7 mg, 9%): mp 278–288 °C; IR (KBr) *v* 3410, 3360, 3200, 2950, 2880, 1670, 1635, 1520, 1480, 1355, 1305 cm^{-1}; ^1H NMR (DMSO-d_6 60 °C, 300 MHz) δ 7.98 (d, $J = 8.4$ Hz, 2H, H2′, H6′), 7.38 (d, 2H, H3′, H5′), 6.30 (s, 2H, NH_2), 2.85 (m, 2H, H7), 2.76 (m, 2H, H5), 2.40 (s, 3H, CH_3), 2.08 (m, 2H, H6); MS (70 eV) *m*/*z* 265 $[M]^+$ (100), 147 (4), 132 (8), 119 (28), 91 [*p*-$CH_3Ph]^+$ (15), 77 (6), 65 (18). Anal. Calcd for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.70; H, 6.00; N, 15.61.

4.36. 4-Amino-2-(4-chlorophenyl)-5,6,7,8-tetrahydrooxazolo[5,4-*b*]quinoline (33)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.18 g, 1.38 mmol) in dry chlorobenzene (17 mL) at rt under argon, 1,3-oxazole **59** (0.20 g, 0.91 mmol) and cyclohexanone (142 μL, 1.37 mmol) were added. After 24 h the reaction was complete. Following the work-up, the resultant crude was purified to give compound **33** (111.6 mg, 41%): 305–313 °C; IR (KBr) *v* 3380, 3300, 3200, 2920, 2850, 1630, 1605, 1460, 1430, 1320, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.11 (d, $J = 8.5$ Hz, 2H, H2′, H6′), 7.47 (d, 2H, H3′, H5′), 4.75 (br s, 2H, NH_2), 2.93 (m, 2H, H_8), 2.52 (m, 2H, H_5), 1.90 (m, 4H, H6, H7); MS (70 eV) *m*/*z* 301 [M+2] (36), 299 $[M]^+$ (100), 284 (12), 271 (32), 139 (7), 113 [*p*-$Cl^{37}Ph]^+$ (2), 111 [*p*-$Cl^{35}Ph]^+$ (6), 106 (7), 79 (6). Anal. Calcd for $C_{16}H_{14}ClN_3O$: C, 64.11; H, 4.71; N, 14.02. Found: C, 64.40; H, 4.90; N, 14.31. **33·HCl**: mp 287–288 °C; IR (KBr) *v* 3294, 3131, 2934, 1658, 1628, 1484, 1447, 1427, 1089 cm^{-1}; MS (70 eV) *m*/*z* 301 $[M+2-HCl]^+$ (47), 299 $[M-HCl]^+$ (100), 284 (19), 271 (48), 139 (7), 137 (4), 113 [*p*-$Cl^{37}Ph]^+$ (2), 111 [*p*-$Cl^{35}Ph]^+$ (6), 106 (7), 79 (6), 66 (3). Anal. Calcd for $C_{16}H_{14}ClN_3O.HCl$: C, 57.15; H, 4.49; N, 12.49. Found: C, 56.88; H, 4.58; N, 12.33.

4.37. 4-Amino-5,6,7,8-tetrahydro-2-(4-nitrophenyl)-oxazolo[5,4-*b*]quinoline (34)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.18 g, 1.32 mmol) in dry 1,2-dichloroethane (17 mL) at rt under argon, 1,3-oxazole **60** (0.2 g, 0.88 mmol) and cyclohexanone (136 μL, 1.31 mmol) were added. After 98 h the reaction was complete. Following the work-up, the resultant crude was purified to give **34** (94.7 mg, 35%): mp 319–326 °C; IR (KBr) *v* 3400, 3320, 3205, 2920, 2850, 1650, 1610, 1590, 1515, 1340 cm^{-1}; MS (70 eV) *m*/*z* 310 $[M]^+$ (100), 295 (20), 282 (60), 263 (23), 236 (18), 133 (6) 104 (6). Anal. Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.05. Found: C 62.15; H, 4.45; N, 17.97. **34·HCl**: mp 304–305 °C (MeOH; dec); IR (KBr) *v* 3410, 2944, 1659, 1601, 1520, 1446, 1345 cm^{-1}; MS (70 eV) *m*/*z* 310 $[M^+-HCl]$ (100), 295 (8), 282 (31), 263 (11), 252 (3), 236 (9), 133 (7), 104 (8), 79 (9), 65 (7). Anal. Calcd for $C_{16}H_{14}N_4O_3·HCl·1\frac{1}{2}H_2O$: C, 51.41; H, 4.85; N, 14.98. Found: C, 51.56; H, 4.78; N, 14.71.

4.38. 4-Amino-5,6,7,8-tetrahydro-2-(4-methoxyphenyl)-oxazolo[5,4-*b*]quinoline (35)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.19 g, 1.39 mmol) in dry 1,2-dichloroethane (14 mL) at rt under argon, 1,3-oxazole **61** (0.20 g, 0.93 mmol) and cyclohexanone (145 μL, 1,4 mmol) were added. After 216 h the reaction was complete. Following the work-up, the resultant crude was purified to afford product **35** (316.7 mg, 52%): mp 270–276 °C; IR (KBr) *v* 3370, 3300, 3190, 2900, 2860, 1635, 1610, 1490, 1460, 1430, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.12 (d, $J = 8.9$ Hz, 2H, H2′, H6′), 7.00 (d, 2H, H3′, H5′), 4.72 (br s, 2H, NH_2), 2.92 (m, 2H, H8), 2.53 (m, 2H, H5), 1.91 (m, 4H, H6, H7); MS (70 eV) *m*/*z* 295 $[M]^+$ (100), 280 (17), 267 (26), 147 (7), 134 (13), 106 (9), 77 (9). Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 65.78; H, 6.33; N, 10.93. Found: C, 65.64; H, 6.40; N, 10.83. **35·HCl**: mp 262–263 °C (MeOH); IR (KBr) *v* 3455, 3286, 3193, 2945, 1659, 1623, 1603, 1583, 1555, 1499, 1449, 1424, 1247, 1178 cm^{-1}. Anal. Calcd for $C_{17}H_{17}N_3O_2·HCl·2H_2O$: C, 55.51; H, 6.03; N, 11.42. Found: C, 55.51; H, 6.25; N, 11.34.

4.39. 4-Amino-5,6,7,8-tetrahydro-2-(4-methylphenyl)-oxazolo[5,4-*b*]quinoline (36)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.54 g, 4 mmol) in dry 1,2-dichloroethane (35 mL) at rt under argon, 1,3-oxazole **62** (0.40 g, 2 mmol) and cyclohexanone (416 μL, 4 mmol) were added. After 192 h the reaction was complete. Following the work-up, the resultant crude was purified to give compound **36** (452.6 mg, 81%): 267–273 °C; IR (KBr) *v* 3384, 3333, 3213, 2939, 2854, 1649, 1624, 1499, 1474, 1444, 1429 cm^{-1}; ^1H NMR (DMSO-d_6 300 MHz) δ 7.99 (d, $J = 7.9$ Hz, 2H, H2′, H6′), 7.39 (d, 2H, H3′, H5′), 6.37 (br s, 2H, NH_2), 2.74 (m, 2H, H8), 2.48 (m, 2H, H5), 2.39 (s, 3H, CH_3), 1.77 (m, 4H, H6, H7); MS (70 eV) *m*/*z* 279 $[M]^+$ (100), 264 (19), 251 (44), 133 (6), 118 (7), 106 (6), 91 (8), 79 (6), 65 (5). Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.25; H, 6.40; N, 14.99.

4.40. 4-Amino-2-(4-chlorophenyl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[*e*] oxazolo[4,5-*b*]pyridine (37)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.18 g, 1.38 mmol) in dry chlorobenzene (20 mL) at rt under argon, 1,3-oxazole **59** (0.20 g, 0.92 mmol) and cycloheptanone (161 μL, 1.36 mmol) were added. After 96 h the reaction was complete. Following the work-up, the resultant crude was purified to give compound **37** (111.2 mg, 39%): mp 301–310 °C; IR (KBr) *v* 3360, 3320, 3200, 2900, 2820, 1640, 1610, 1460, 1320, 1080 cm^{-1}; ^1H NMR (DMSO-d_6 300 MHz) δ 8.09 (d, $J = 8.5$ Hz, 2H, H2′, H6′), 7.66 (d, 2H, H3′, H5′), 6.42 (br s, 2H, NH_2), 2.88 (m, 2H, H9), 2.74 (m, 2H, H5), 1.80 (m, 2H, H7), 1.58 (m, 2H, H6), 1.51 (m, 2H, H8); MS (70 eV) *m/z* 315 $[M+2]^+$ (34), 313 $[M]^+$ (100), 298 (26), 284 (48), 272 (9), 259 (11), 139 (17), 113 [*p*-$Cl^{37}Ph]^+$ (5), 111 [*p*-$Cl^{35}Ph]^+$ (15). Anal. Calcd for $C_{17}H_{16}ClN_3O$: C, 65.07; H, 5.14; N, 13.39. Found: C, 64.85; H, 5.12; N, 13.10. **37·HCl**: mp 301–302 °C (MeOH); IR (KBr) *v* 3435, 2924, 2852, 1661, 1623, 1484 cm^{-1}; MS (70 eV) *m/z* 315 $[(M+2)-HCl^+]$ (34), 313 $[M-HCl]^+$ (100), 298 (24), 284 (52), 272 (4), 259 (11), 119 (14), 113 [*p*-$Cl^{37}Ph]^+$ (6), 111 [*p*-$Cl^{35}Ph]^+$ (16), 102 (10), 92 (11), 75 (11), 65 (17). Anal. Calcd for $C_{17}H_{16}ClN_3O{\cdot}HCl{\cdot}H_2O$: C, 55.45; H, 5.20; N, 11.41. Found: C, 55.78; H, 5.51; N, 11.34.

4.41. 4-Amino-6,7,8,9-tetrahydro-2-(4-nitrophenyl)-5*H*-cyclohepta[*e*]oxazolo[4,5-*b*]pyridine (38)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.46 g, 3.5 mmol) in dry 1,2-dichloroethane (30 mL) at rt under argon, 1,3-oxazole **60** (0.40 g, 1.74 mmol) and cycloheptanone (410 μL, 3.5 mmol) were added. The reaction was complete 14 days later. After the work-up, the resultant crude was purified to give compound **38** (327 mg, 58%): mp 310–319 °C; IR (KBr) *v* 3460, 3400, 3280, 2960, 2890, 1665, 1635, 1600, 1350 cm^{-1}; MS (70 eV) *m/z* 324 $[M]^+$ (100), 309 (27), 295 (46), 277 (16), 249 (20), 237 (7), 119 (8), 104 (8), 92 (7), 76 (9), 65 (7). Anal. Calcd for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4,97; N, 17.27. Found: C, 63.10; H, 5.12; N, 16.99. **38·HCl**: mp 289–290 °C (MeOH; dec); IR (KBr) *v* 3395, 3337, 3219, 2924, 2851, 1653, 1623, 1590, 1520, 1477, 1342 cm^{-1}; MS (70 eV) *m/z* 324 $[M^+-HCl]$ (100), 309 (17), 296 (29), 295 (38), 277 (10), 249 (16), 237 (6), 119 (11), 104 (10), 91 (9), 80 (6), 76 (12), 65 (11). Anal. Calcd for $C_{17}H_{16}N_4O_3{\cdot}HCl$: C, 56.59; H, 4.74; N, 15.58. Found: C, 56.84; H, 4.98; N, 15.78.

4.42. 4-Amino-6,7,8,9-tetrahydro-2-(4-methoxyphenyl)-5*H*-cyclohepta[*e*]oxazolo[4,5-*b*]pyridin (39)

This compound was prepared following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.19 g, 1.39 mmol) in dry 1,2-dichloroethane (20 mL) at rt under argon, 1,3-oxazole **61** (0.20 g, 0.93 mmol) and cycloheptanone (149 μL, 1.39 mmol) were added. After 189 h the reaction was complete. Following the work-up, the resultant crude was purified to give compound **39** (144.9 mg, 50%): mp 257–262 °C; IR (KBr) *v* 3420, 3350, 3220, 3030, 2940, 2870, 1650, 1630, 1505, 1470 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.10 (d, $J = 9$ Hz, 2H, H2′, H6′), 7.00 (d, 2H, H3′, H5′), 4.74 (br s, 2H, NH_2), 3.86 (s, 3H, OCH_3), 3.01 (m, 2H, H9), 2.66 (m, 2H, H5), 1.87 (m, 2H, H7), 1.70 (m, 2H, H8), 1.63 (m, 2H, H6); MS (70 eV) *m/z* 309 $[M]^+$ (100), 294 (20), 280 (36), 268 (7), 255 (5), 135 (7), 119 (6), 92 (5), 77 (5), 65 (3). Anal. Calcd for $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58. Found: C, 69.71; H, 5.98; N, 13.57. **39·HCl**: mp 248–249 °C; MS (70 eV) *m/z* 309 $[M^+-HCl]^+$ (100), 294 (32), 280 (50), 268 (11), 255 (9), 135 (8), 119 (7), 92 (5), 77 (5), 65 (4).

4.43. 4-Amino-6,7,8,9-tetrahydro-2-(4-methylphenyl)-5*H*-cyclohepta[*e*]oxazolo[4,5-*b*]pyridine (40)

This compound was obtained following the general method for the Friedländer reaction. To a stirred suspension of $AlCl_3$ (0.54 g, 4 mmol) in dry 1,2-dichloroethane (20 mL) at rt under argon, 1,3-oxazole **62** (0.40 g, 2 mmol) and cycloheptanone (574 μL, 4 mmol) were added. After 190 h the reaction was complete. Following the work-up, the resultant crude was purified to give product **40** (418.9 mg, 71%): mp 284–290 °C; IR (KBr) *v* 3378, 3340, 3212, 2915, 2848, 1654, 1622, 1499, 1481, 1331 cm^{-1}; ^1H NMR (DMSO-d_6 70 °C, 300 MHz) δ 7.99 (d, $J = 8.0$ Hz, 2H, H2′, H6′), 7.39 (d, 2H, H3′, H5′), 6.17 (br s, 2H, NH_2), 2.91 (m, 2H, H9), 2.74 (m, 2H, H5), 1.80 (m, 2H, H7), 1.57 (m, 4H, H6, H8); MS (70 eV) *m/z* 293 $[M]^+$ (100), 278 (28), 264 (46), 252 (9), 239 (9), 119 (11), 91 (9), 65 (6). Anal. Calcd for $C_{18}H_{19}N_3O$: C, 73.70; H, 6.53; N, 14.32. Found: C, 73.48; H, 6.86; N, 14.33.

4.44. Pharmacological studies

4.44.1. Evaluation of AChE activity. The assessment of the compounds AChE inhibitory activity was performed following the spectrometric method of Rappaport et al.[25] using AChE from electric eel (*Torpedo californica*) and acetylcholine chloride (29.5 mM) as a substrate. The reaction took place in a final volume of 2.5 mL of an aqueous solution containing 0.78 U of AChE and 1.9 mM *m*-nitrophenol to produce a yellow colour, which fades out as a function of the enzymatic activity. Inhibition curves were plotted from the incubating data with the different compounds for 30 min; a sample without any compound was always present to determine the 100% of enzymatic activity. After 30 min incubation, the disappearance of yellow colour by *m*-nitrophenol was evaluated by measuring the absorbance at 405 nm in a spectrophometric plate reader (iEMS Reader MF, Labsystems). The concentration of com-

pound able to produce 50% inhibition of the AChE activity (IC_{50}) was calculated by transforming the values of absorbance to Rappaport enzymatic activity units extrapolating from a calibration curve previously obtained. Data are means ± SE of three different experiments in triplicate at least.

4.44.2. Evaluation of BuChE activity. The assessment of the compounds BuChE inhibitory activity was determined following the method of Ellman et al.[26] using BuChE from human serum and butyryl thiocholine chloride (5 mM) as a substrate. The reaction took place in a final volume of 1 mL of a phosphate buffer solution at pH 7.2 containing 0.035 U of BuChE and 0.25 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), which produces the yellow anion 5-thio-2-nitrobenzoic acid. Inhibition curves were plotted from the incubating data with the different compounds for 15 min; a sample without any compound was always present to determine the 100% of enzymatic activity. After 15 min incubation, the production of colour as an indication of enzymatic activity was evaluated by measuring the absorbance at 412 nm in a spectrophometric plate reader (iEMS Reader MF, Labsystems).

4.44.3. Cell isolation and culture of bovine chromaffin cells. Bovine adrenomedullary chromaffin cells were isolated following standard methods[33] after accomplishing some modifications.[34] Cells were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum, 10 μM cytosine arabinoside, 10 μM fluorodeoxyuridine, 50 IU mL^{-1} penicillin and 50 μg mL^{-1} streptomycin. Cells were plated at a density of 2×10^5 cells/well in 96-multiwell Costar plates and were used 1–5 days after plating. Medium was replaced after 24 h and then after 2–3 days.

4.44.4. Evaluation of $^{45}Ca^{2+}$ uptake. $^{45}Ca^{2+}$ uptake studies were carried out in cells after 2–3 days in culture following standard methods.[35] Before the experiment, cells were washed twice with 0.1 mL Krebs-HEPES solution with the following composition (in mM): NaCl 140, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 1, glucose 11, HEPES 10, at pH 7.2 and 37 °C. $^{45}Ca^{2+}$ uptake in chromaffin cells was studied by incubating the cells at 37 °C with $^{45}CaCl_2$ at a final concentration of 5 μCi mL^{-1} in Krebs-HEPES (basal uptake), high K^+ concentration solution (Krebs-HEPES containing 70 mM KCl with isosmotic reduction of NaCl) or 100 μM dimethylphenylpiperazinium (DMPP) in Krebs-HEPES. This incubation was carried out during 5 min for basal uptake or 5 s for high K^+ or DMPP, in the absence (control) or in the presence of the drugs. At the end of the incubation period the test medium was rapidly aspirated and the uptake reaction was ended by adding 0.1 mL of a cold Ca^{2+}-free Krebs-HEPES containing 10 mM $LaCl_3$. Finally, cells were washed again five times with 0.1 mL of Ca^{2+}-free Krebs-HEPES containing 10 mM $LaCl_3$ and 2 mM EGTA, at 15 s intervals. To measure radioactivity retained by chromaffin cells, the cells were scraped with a plastic pipette tip while adding 0.1 mL of 10% trichloroacetic acid. Scintillation fluid (3.5 mL; Optiphase Hisafe II, EGG Instruments) was added and the samples counted in a Packard beta counter. Results are expressed as % of Ca^{2+} taken up by control cells.

4.45. Molecular modelling

The simulation system used in MD simulations consisted of the enzyme, the drug and a cap (with a radius of 25 Å) of TIP3P[36] water molecules centred on the ligand. The enzyme was modelled in its physiologically active form with neutral His440 and deprotonated Glu327, which form together with Ser200 the catalytic triad. The standard ionization state at neutral pH was considered for the ionizable residues, but for Asp392 and Glu443, which were neutral, and His471, which was protonated, according to previous numerical titration studies.[37] The AMBER (amber99 parm file) all-atom force field[38] was used for the enzyme. For the drug, HF/6-31G(d) RESP charges were used, and the van der Waals parameters were taken from those defined for related atoms in the force field.[39] After minimization, the whole system was partitioned into a mobile and a rigid region. The former consisted of the ligand, all the residues having at least an atom at a distance less than 14 Å from the ligand and all the water molecules. After equilibration (0.3 ns), a 2 ns MD simulation (at 298 K) was carried out. A cutoff of 11 Å for nonbonded interactions, together with SHAKE and an integration time step of 2 fs, were used.

The structural stability of the complexes was examined from the rmsd plots determined for the subset of residues that form the walls of the binding pocket with regard to the crystallographic structure. To examine the energetic stability of the complexes, a MM/PB-SA analysis was performed by using 100 snapshots collected regularly along the last 500 ps of the MD simulation. Accordingly, the stability of each complex was estimated from the addition of the internal energy (including the contributions due to both inhibitor and enzyme and their interaction energy) and the solvation free energy. The former term was determined by averaging the corresponding energy values as determined with the AMBER force field. The second was estimated by using a Poisson–Boltzmann calculation for the electrostatic component and a surface-area dependent term for the nonelectrostatic contribution. The solute was assigned a dielectric of 1, the solvent dielectric was set to 78.4, and the dielectric boundary was defined using a 1.4 probe sphere and van der Waals radii taken from the AMBER force field. A focusing approach (final grid spacing of 0.5 point/Å) was employed. The nonelectrostatic term was determined using a surface tension for all the atoms equal to 5.4 cal $Å^{-2}$.[40] Solvation calculations were performed with the CMIP program.[41]

The molecular electrostatic potentials of compounds **63**–**65** were determined at the HF/6-31G(d) level of theory by using the MOPETE program.[42]

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