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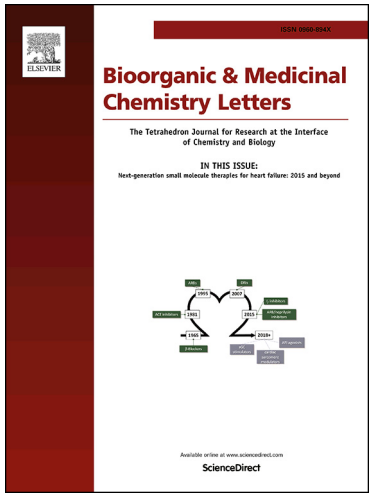

Dual functional cholinesterase and PDE4D inhibitors for the treatment of Alzheimer's disease: design, synthesis and evaluation of tacrine-pyrazolo[3,4-b]pyridine hybrids

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Dual functional cholinesterase and PDE4D inhibitors for the treatment of Alzheimer's disease: design, synthesis and evaluation of tacrine-pyrazolo[3,4-b]pyridine hybrids

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ABSTRACT

A series of tacrine-pyrazolo[3,4-b]pyridine hybrids were synthesised and evaluated as dual cholinesterase (ChE) and phosphodiesterase 4D (PDE4D) inhibitors for the treatment of Alzheimer's disease (AD). Compound **10j**, which is tacrine linked with pyrazolo[3,4-b]pyridine moiety by a six-carbon spacer, was the most potent acetylcholinesterase (AChE) with IC_{50} value of 0.125μM. Moreover, compound **10j** provided a desired balance of AChE and butylcholinesterase (BuChE) and PDE4D inhibition activities, with IC_{50} value of 0.449 and 0.271 μM, respectively. The above results indicated that this hybrid was a promising dual functional agent for the treatment of AD.

Alzheimer's disease (AD) is a neurodegenerative illness characterized by a progressive decline in cognitive function, memory loss, extensive neuronal loss, decrease in cholinergic transmission, and the presence of senile plaques. [1-2] It is the most common single cause of dementia and is also became the world-wide health problem with implications for an increasing number of people and countries. [3] However, up to now, there is no effective treatment is available.[4] The decrease of cholinergic transmission, caused by the loss of basal forebrain cholinergic cells, was supposed to play an important role in the cognitive impairment associated with AD. Studies had dedicated that increase the levels in Ach by inhibiting acetylcholinesterase could effectively improve the cognitive function and memory ability.[3, 5-9] Based on the cholinergic hypothesis, Cholinesterase (ChE) became one of major targets in the current therapy of AD[7] and most of the clinically approved drugs for treatment of AD are acetylcholinesterase inhibitors (AChEIs), such as tacrine, rivastigmine, galantamine and Donepezil (Figure 1).[10-11]

Phosphodiesterase 4 (PDE4) is the primary cAMP-specific hydrolase and is represented by four genes (PDE4A, B, C and D). [12] PDE4D, which is relatively high expression in the frontal cortex[13], has been demonstrated to be the main subtype involved in the process of memory consolidation and LTP.[14] Rolipram (Fig. 1), a typical PDE4D inhibitor, has beneficial effects on hippocampal dependent memory tasks, but PDE4D-deficient mice did not show altered memory with the administration of rolipram, which further demonstrated the crucial role of PDE4D in memory consolidation. [15] Recently, several strategies were proposed to enhance cerebral cyclic adenosine monophosphate (cAMP) for the treatment of neurological disorders by PDEs inhibition.[16-19] The newly developed PDE4D inhibitor GEBR-7b enhanced spatial and object memory performance in mice and rats (Fig. 1).[20-21] In addition, MK-0952 (Fig. 1), another PDE4 inhibitor that enhanced cognition in preclinical studies, prompted clinical trials in patients with mild-to-moderate AD from phase II clinical studies.[22]

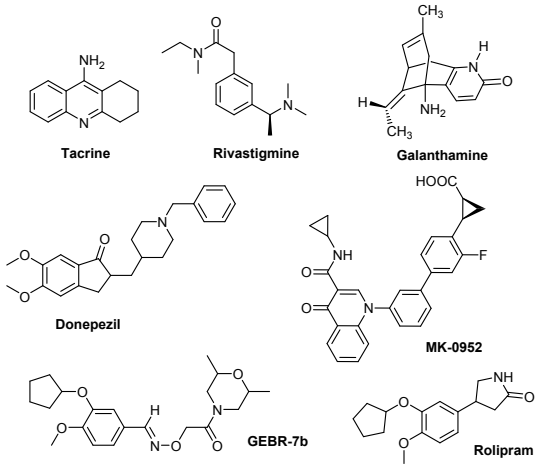


Fig. 1 Structure of tacrine, rivastigmine, galantamine, donepezil, GEBR-7b, MK-0952 and rolipram.

Tacrine, the first inhibitor of both AChE and butylcholinesterase (BuChE) approved by the FDA for the treatment of AD, was withdrawn from the market due to its side effects such as hepatotoxicity. [23] Nevertheless, medicinal

chemists remain interested in researching tacrine analogues or related new candidates as the excellent inhibition activity. [24-27] Pyrazolo[3,4-b]pyridine, a novel scaffold reported as potent and selective PDE4 inhibitors, may could be bind to peripheral anionic binding site (PAS) of AChE.[28] Taking into account the importance of PDE4 and AChE inhibition in AD therapeutics, we designed, synthesized and evaluated a series of new tacrine-pyrazolo[3,4-b]pyridine hybrids hybrids as dual functional ligands for the treatment of Alzheimer's disease (Fig. 2). In this paper, We variated the length of the linker chain between the tacrine and pyrazolo[3,4-b]pyridine moiety to obtain a hybrid with the desired balance of AChE and BuChE and PDE4D inhibition activities.

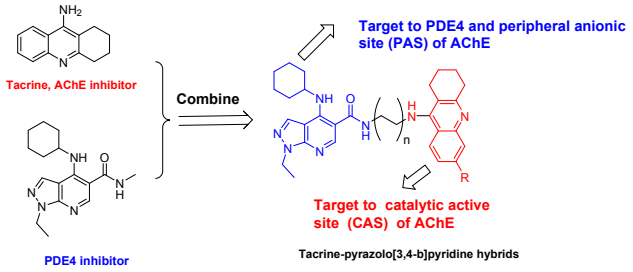
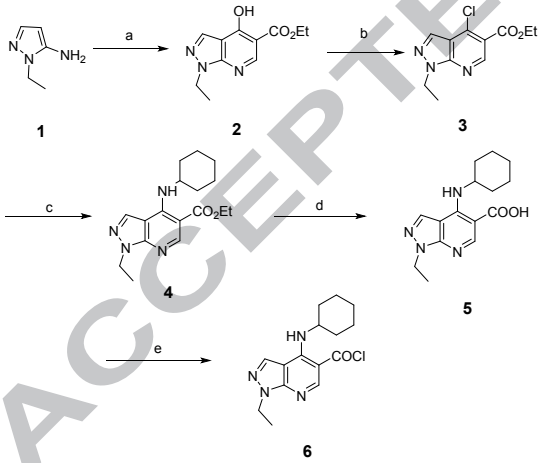


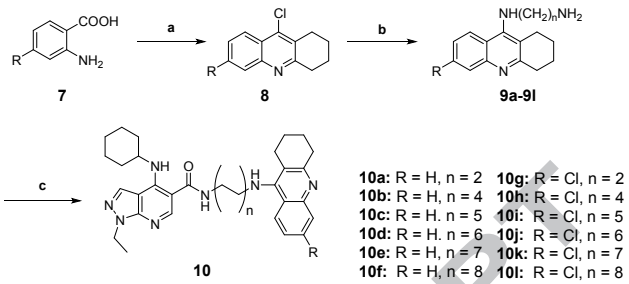
Fig. 2. Design strategy of tacrine-pyrazolo[3,4-b]pyridine hybrids

Routes for the synthesis of the key intermediate **6**, target compounds **10a-l** are summarized in Scheme 1 and Scheme 2. Commercially available compound **1** was refluxed with diethyl ethoxymethylenemalonate (EMME) at high temperature for 12 hours to get compound **2**, and it was refluxed with phosphorus oxychloride, now intermediate **3** has been obtained. Triethylamine was added to a mixed solution of intermediate **3** and cyclohexylamine, and stirred at room temperature for 10 hours to obtain **4**. After hydrolysis, it was reacted with phosphorus oxychloride to obtain an acid chloride intermediate **6**.



Scheme 1. Synthesis of intermediate **6**. Reagents and conditions: (a) EMME, reflux 12h; (b) $POCl_3$, 120 °C, reflux 12h; (c) cyclohexylamine, TEA, rt, 10h; (d) NaOH, CH_3CH_2OH, rt, 10h; (e) $POCl_3$, reflux 2h.

The synthesis route of the target compounds **10a-l** are summarized in Scheme 2. Intermediate **9a-l** was synthesized according to the reported procedure. [29-30] Firstly, using anthranilic acid or 2-amino-4-chlorobenzoic acid as the starting materials, $POCl_3$ was added dropwise to the mixed solution of the starting materials and cyclohexanone at 0°C, then recrystallized to give compound **8**. Dissolving intermediate **8**, KI and diamines of different carbon chains in n-pentanol. They were refluxed at 160°C for 12 hours to give **9a-l**. Finally, in the presence of anhydrous dichloromethane, compound **9a-l** and intermediate **6** were stirred for 12 hours under room temperature to give the target compound **10a-l.**



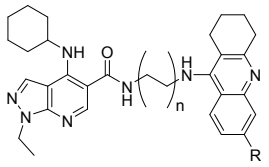
Scheme 2. Synthesis of **10a-l**. Reagents and conditions: (a) cyclohexanone, $POCl_3$, 0 °C-reflux; (b) diamine, KI, 1-pentanol, 160 °C; (c) **6**, CH_2Cl_2, TEA, rt, 12h. 69% for **10a**, 68% for **10b**, 73% for **10c**, 65% for **10d**, 68% for **10e**, 71% for **10f**, 78% for **10g**, 75% for **10h**, 70% for **10i**, 65% for **10j**, 72% for **10k**, 75% for **10l**.

The AChE and BuChE inhibitory effects of the hybrids were determined by the spectroscopic method described by Ellman et al. using tacrine as the standard.[31] AChE (E.C.3.1.1.7) and BuChE (E.C. 3.1.1.8) were obtained from electric eel and equine serum, respectively. The IC_{50} values for AChE and BuChE inhibition are summarized in Table 1. Most of the hybrids demonstrated potent inhibitory activity against AChE and BuChE with IC_{50} values in the micromolar to sub-micromolar range. The results of eeAChE inhibition of compounds showed that the AChE inhibitory potency was closely related to the length of the alkylene chain. It appears that a 6-carbon spacer seems to be the proper length for a linker between the two pharmacophore groups in both series. Compounds **10d** and **10j** gave the most active AChE inhibitory activities with IC_{50} value of 0.149 and 0.125μM, respectively. Increasing or decreasing the length of carbon linker resulted a slightly decreasing the inhibitory activities (**10c**, n = 5, IC_{50} = 0.358 μM ; **10e**, n = 7, IC_{50} = 0.316μM ; **10i**, n = 5, IC_{50} = 0.306μM ; **10k**, n = 5, IC_{50} = 0.0.391μM). However, the same trend was not observed in BuChE inhibition. Almost all the compounds showed potent activity against BuChE. Additionally, some compounds exhibited almost equal inhibitory activity with AChE. Based on these results, these hybrids should be good dual AChE/BChE inhibitors to treat AD.

As PDE4D is one of the key subtypes participating in LTP, the inhibition of PDE4D2 by the tacrine-pyrazolo[3,4-b]pyridine hybrids were determined using a previously described method, with rolipram as the reference compounds. [32] The results in Table 1 indicated that most of the hybrids exhibited more potent or similar inhibitory activities against PDE4D2 compared to rolipram. The results of PDE4D2 inhibition of compounds showed that 6-chlorin substituted group in tacrine was beneficial to the inhibitory activities of PDE4D2, for example, compound **10f** (IC_{50} = 0.025 μM) was more potent than **10a** (IC_{50} = 0.041 μM) by 1.5-fold improvement. Similar to the SAR of AChE inhibitory activities, a structure-activity relationship analysis showed that the PDE4D2 inhibitory potency was closely related to the length of the alkylene chain. 2-carbon spacer was more favorable for the activity than the other positions. For example , hybrids **10a** and **10g** demonstrated the most potent PDE4D2 inhibitory activities with IC_{50} value of 0.041 and 0.0.025μM, respectively, which are about 10-fold more active than rolipram. When the length of the alkyl chain was changed from 2 to 8, the activity against PDE4D2 decreased slightly. The hybrids **10b**, **10c**, **10d, 10e** and **10f**, with four, five, six, seven, and eight carbon spacers between tacrine and tacrine-pyrazolo[3,4-b]pyridine hybrids unit (**10b**, n = 4, IC_{50} = 0.108 μM ; **10c**, n = 6, IC_{50} = 0.477μM ; **10d**, n = 7, IC_{50} = 0.501μM ; **10e**, n = 8, IC_{50}

= 1.071μM) had slightly weak inhibitory activities than 10a . The same tread was also observed in 6- chlorinetacrine-pyrazolo[3,4-b]pyridine series compounds. Considering the results in inhibiting AChE and BuChE, compound **10j** provided a desired balance of AChE and BuChE and PDE4D inhibition activities, with IC_{50} value of 0.125, 0.449 and 0.271 μM, respectively.

Table 1. In vitro inhibition of AChE, BuChE and PDE4D activities of tacrine-pyrazolo[3,4-b]pyridine hybrids **10a-l**.



10a-l

compounds	n	R	IC_{50}(μM)±SD[a]		SI[e]	IC_{50}(μM)±SD
			AChE[c]	BuChE[d]		PDE4D2[b]
10a	2	H	0.243 ± 0.023	0.891 ± 0.084	3.67	0.041 ± 0.007
10b	4	H	0.333 ± 0.015	0.889 ± 0.010	2.67	0.108 ± 0.003
10c	5	H	0.358 ± 0.018	0.264 ± 0.028	0.73	0.477 ± 0.015
10d	6	H	0.149 ± 0.009	0.332 ± 0.009	2.23	0.501 ± 0.012
10e	7	H	0.316 ± 0.015	0.324 ± 0.009	1.02	1.071 ± 0.081
10f	8	H	0.392 ± 0.020	0.320 ± 0.003	0.82	1.307 ± 0.020
10g	2	Cl	0.412 ± 0.013	0.713 ± 0.020	6.54	0.025 ± 0.001
10h	4	Cl	0.335 ± 0.030	1.283 ± 0.020	3.82	0.087 ± 0.009
10i	5	Cl	0.306 ± 0.023	0.434 ± 0.053	1.42	0.308 ± 0.003
10j	6	Cl	0.125 ± 0.010	0.449 ± 0.062	3.60	0.271 ± 0.011
10k	7	Cl	0.391 ± 0.019	0.245 ± 0.004	0.63	0.934 ± 0.021
10l	8	Cl	0.812 ± 0.003	0.314 ± 0.065	4.30	1.077 ± 0.034
Tacrine	-	-	0.110 ± 0.007	0.216 ± 0.018	1.96	/
Rolipram	/	/	/	/	/	0.671 ± 0.03

[a]Result are the mean of three independent experiments(n=3) ± SD

[b]Result are the mean of three independent experiments(n=3) ± SD

[C]AChE from electriceel was used

[d]BuChE from equine serum was used.

[e]Selectivity ratio: IC_{50} (BuChE)/ IC_{50} (AChE)

In summary, this study involved the design, synthesis and biological evaluation of a novel series of tacrine-pyrazolo[3,4-b]pyridine hybrids as dual cholinesterase (ChE) and phosphodiesterase 4D (PDE4D) inhibitors for the treatment of Alzheimer's disease (AD). Most of the target compounds displayed excellent inhibition of AChE and BuChE and PDE4D activity . The results indicated that both the AChE and PDE4D2 inhibitory potency were closely related to the length of the alkylene chain. Anomy them, compound **10j,** which is tacrine linked with pyrazolo[3,4-b]pyridine moiety by a six-carbon spacer, was the most potent acetylcholinesterase (AChE) with IC_{50} value of 0.125μM. Moreover, compound **10j** provided a desired balance of AChE and butylcholinesterase (BuChE) and PDE4D inhibition activities, with IC_{50} value of 0.449 and 0.271 μM, respectively. The above results indicated that this hybrid was a promising dual functional agent for the treatment of AD.

Acknowledgments

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Graphical Abstract

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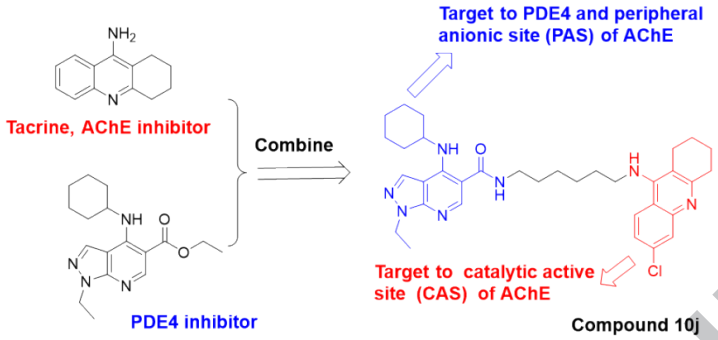

	IC_{50} (μM)
AChE	0.125
BuChE	0.449
PDE4D2	0.271