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Novel conjugates of 4-amino-2,3-polymethylenequinolines and vanillin as potential multitarget agents for AD treatment

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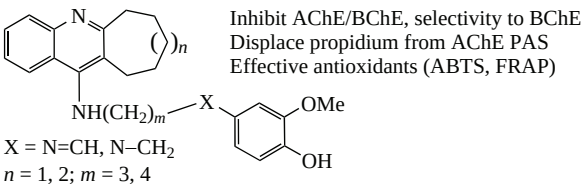
A series of novel conjugates of 4-amino-2,3-polymethylenequinolines and phenolic antioxidant vanillin was synthesized by the condensation of aminoquinolines with vanillin followed by reduction of imines with sodium borohydride. The conjugates effectively inhibit AChE and BChE with preferable BChE inhibition and displace propidium from the PAS AChE. Compounds with aminoalkyl spacer have preferable esterase profile being more potent cholinesterases inhibitors with lower anti-CES activity and are the most potent antioxidants in ABTS and FRAP tests.



Keywords: 4-amino-2,3-polymethylenequinolines, quinolines, vanillin, acetylcholinesterase, butyrylcholinesterase, antioxidants, propidium displacement, ABTS, FRAP, Alzheimer's disease.

The article is dedicated to the memory of academician Nikolay Serafimovich Zefirov (1935–2017) – the outstanding Russian organic and medicinal chemist, the founder of the first course of medicinal chemistry discipline in Russian universities and the Director of the Institute of Physiologically Active Compounds, Russian Academy of Sciences.

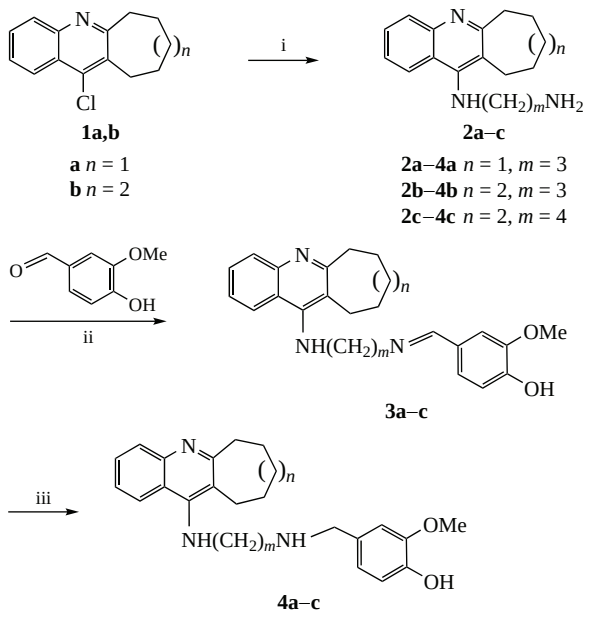
Alzheimer's disease (AD), a progressive and irreversible neurodegenerative disorder,[1] is a complex, multifactorial disease and is characterized by impaired functioning of neurotransmitter systems, deposition of aberrant proteins (beta-amyloid and tau protein), development of oxidative stress, mitochondrial dysfunction, loss of synapses and death of nerve cells, especially cholinergic neurons.[2,3] Currently, cholinergic drugs are the main pharmacotherapeutic group used for the symptomatic treatment of AD.[4] Acetylcholinesterase (AChE) inhibitors increase the activation of cholinergic neurons and stimulate cognitive functions. Inhibition of butyrylcholinesterase (BChE) also contributes to the improvement of cognitive functions. BChE inhibition is particularly effective with the progression of the disease, when the activity of AChE decreases, while the activity of BChE gradually increases.[5,6] It is believed that compounds inhibiting both cholinesterases increase the efficiency of treatment.[7]

Creating multifunctional drugs that have a synergistic effect on a number of biological targets responsible for the pathogenesis of the disease is a promising approach.[8–12] One of the directions is based on the development of hybrid conjugates containing two different pharmacophores connected by a spacer. In the related cases, a well-known anticholinesterase drug molecule is often used as one pharmacophore.[13–17] As for the second pharmacophore, such fragments are introduced that impart neuroprotective and disease-modifying properties to the conjugates, in particular, antiaggregatory and/or antioxidant.[14,15,18,19]

In this work we describe the synthesis and evaluation of biological activity of novel conjugates of cyclic homologues of anticholinesterase agent tacrine, which are connected to the phenolic antioxidant molecule of vanillin[20–22] by two types of spacers, namely, imino- and aminoalkyl of various length. This work included studying the esterase profile of the conjugates, *i.e.*, their inhibitory activity against AChE, BChE, and a structurally related enzyme, carboxylesterase (CES),[23] assessment of propidium displacement from the peripheral anionic site (PAS) of AChE from *Electrophorus electricus* (*Ee*AChE) responsible for β-amyloid binding as a measure of their potential ability to block AChE-induced aggregation of β-amyloid, and determination of antioxidant activity in ABTS and FRAP assays.

The synthesis was performed according to Scheme 1. Conjugates **3a**–**c** were prepared by the reaction of aminoquinolines **2a**–**c** with vanillin. The required aminoquinolines, in turn, were obtained by condensation of known[16] chlorinated derivatives **1a**,**b** with diamines according to the reported method.[24] Reduction of imines **3a**–**c** with sodium borohydride in methanol led to conjugates **4a**–**c**, in which pharmacophores are linked by an aminoalkyl spacer (see Scheme 1). The synthesized hybrid compounds based on 4-amino-2,3-polymethylenequinolines **3**,**4** had different size of the aliphatic cycle and two types of spacers (for synthetic details, see Online Supplementary Materials).

Esterase profile studies showed that novel conjugates **3** and **4** effectively inhibit both cholinesterases (Table 1). Like tacrine, the conjugates are more selective to BChE compared with AChE, being in general less active against both cholinesterases. In the test series, an aliphatic ring size and length of the spacer do not



1a,**b** **2a**–**c**

a *n* = 1 **2a**–**4a** *n* = 1, *m* = 3
b *n* = 2 **2b**–**4b** *n* = 2, *m* = 3
2c–**4c** *n* = 2, *m* = 4

3a–**c**

4a–**c**

Scheme 1 *Reagents and conditions*: i, $H_2N(CH_2)_mNH_2$, PhOH, 180 °C; ii, 3-MeO-4-HOC_6H_3CHO, PhMe, reflux; iii, $NaBH_4$, MeOH.

change essentially the inhibitory activity for AChE and BChE. At the same time, there is a tendency to some increase in anti-AChE and especially anti-BChE activity when replacing an iminoalkyl spacer (conjugates **3**) with an aminoalkyl one (**4**), which was previously observed for conjugates of tacrine homologues with BHT.[19] Replacement of iminoalkyl spacer with aminoalkyl one also leads to a decrease in the ability of the conjugates to inhibit CES, which hydrolyzes most of ester-containing medicines,[25] *i.e.*, to improve their esterase profile.

Propidium is a selective ligand for the PAS of AChE responsible for Aβ binding, which showed a significant decrease in AChE-induced Aβ aggregation (82% at 100 μM).[26,27] This observation has served as the basis for the fluorescent evaluation[18] of competitive propidium displacement from the PAS of AChE as a screening method to assess the potential ability of compounds to bind PAS and thus block the pro-aggregation activity of AChE.[28] *Ee*AChE was used owing to its high degree of purification, high activity, lower cost than human AChE, and essential congruence of the two structures.[18]

It can be seen in Table 1 that all compounds at a concentration of 20 μM reduced fluorescence intensity by 14–24% and more effectively displaced propidium from the PAS of AChE compared to the reference compound donepezil. Conjugates **3a**,**4a** with seven-membered aliphatic ring have the higher degree of propidium displacement (23–25%) compared to derivatives with eight-membered ring (14–17%). Thanks to binding to the PAS of AChE, conjugates **3**,**4** can potentially block AChE-induced aggregation of β-amyloid, thus having an additional positive disease modification effect.

Primary antioxidant activity of conjugates **3**,**4** was determined spectrophotometrically with two methods: ABTS radical cation ($ABTS^{\bullet+}$) scavenging assay[29] as described[18] and FRAP assay (ferric reducing antioxidant power).[16,30] Trolox, the soluble vitamin E analogue, was used as a reference antioxidant.

According to data in Table 1, all conjugates exhibit high $ABTS^{\bullet+}$ scavenging activity at the level of the standard antioxidant Trolox and the parent antioxidant vanillin, and even exceed them. Changes in the size of the aliphatic ring in the tacrine fragment of the molecule as well spacer length have practically no effect on the radical-scavenging activity of the compounds. Meantime, replacement of the iminoalkyl spacer (conjugates **3**) by the aminoalkyl one (conjugates **4**) enhanced radical-scavenging activity. All conjugates also effectively reduce Fe^{3+}, activity of hybrids **3** is at the level of the parent vanillin pharmacophore, while hybrids **4** with aminoalkyl spacer are about twice more potent.

In summary, the novel conjugates based on tacrine cyclic homologues and vanillin effectively inhibit both cholinesterases

Table 1 Esterase profile of hybrids **3**,**4**, their ability to displace propidium, and antioxidant activities.

Compound	Inhibitory activity against:			Propidium displacement (%)[a]	$ABTS^{\bullet+}$-scavenging activity, TEAC[b] and (IC_{50}, μM)	Ferric reducing antioxidant power, TE[c]
	Human RBC AChE, IC_{50} (μM)	Human serum BChE, IC_{50} (μM)	Porcine liver CES (%)[a]			
3a	4.25 ± 0.07	0.548 ± 0.034	10.8 ± 1.0	24.5 ± 1.9	0.92 ± 0.04 (19.6 ± 1.3)	0.46 ± 0.02
4a	3.01 ± 0.17	0.357 ± 0.021	9.1 ± 0.8	23.1 ± 2.0	1.30 ± 0.03 (15.8 ± 1.2)	0.80 ± 0.04
3b	3.95 ± 0.30	0.772 ± 0.007	22.3 ± 2.2	16.5 ± 1.4	1.1 ± 0.03 (16.3 ± 1.2)	0.47 ± 0.02
4b	3.65 ± 0.10	0.449 ± 0.037	14.1 ± 2.5	14.7 ± 1.2	1.31 ± 0.04 (15.1 ± 1.1)	0.80 ± 0.03
3c	5.91 ± 0.31	0.694 ± 0.060	13.0 ± 1.3	16.7 ± 1.3	0.6 ± 0.03 (29.1 ± 1.3)	0.46 ± 0.04
4c	4.55 ± 0.08	0.609 ± 0.02	10.4 ± 0.7	17.5 ± 1.5	1.33 ± 0.05 (15.1 ± 0.8)	0.81 ± 0.03
Tacrine	0.74 ± 0.05	0.037 ± 0.002	n.a.[d]	4.4 ± 0.6	n.a.	n.a.
Donepezil	0.040 ± 0.004	19.2 ± 3.0	n.a.	10.1 ± 0.6	n.d.[e]	n.d.
Trolox	n.d.	n.d.	n.d.	n.d.	1.0 (20.1 ± 1.2)	1.0
Vanillin	[11.2 ± 1.5%][a]	[10.6 ± 1.0%][a]	n.a.	n.d.	0.45 (44.7 ± 2.4)	0.46 ± 0.02

[a] % inhibition at 20 μM compound concentration. [b] TEAC (Trolox equivalent antioxidant capacity) was determined from the ratio of the slopes of the concentration–response curves test compound / Trolox. [c] TE (Trolox equivalents) – values calculated as the ratio of the concentrations of Trolox and the test compound, resulting to the same effect. [d] n.a. – not active. [e] n.d. – not determined. Data are expressed as mean ±SEM, *n* = 3.

with preferable BChE inhibition, displace propidium from the PAS AChE and are effective antioxidants in ABTS and FRAP tests. The structure of the spacer, namely the presence of an imine or amine fragment in it, largely determines the spectrum of pharmacological activity of the molecules. Compounds with aminoalkyl spacer have preferable esterase profile: they are more potent cholinesterases inhibitors with lower anti-CES activity and are the most potent antioxidants. Overall, the developed conjugates combine in one molecule anticholinesterase, antioxidant and antiaggregant properties. Thus, they have the potential for treating symptoms as well as exerting disease-modifying effects and show promise for further development and optimization as multitarget anti-AD agents.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.09.005.

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