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Design and synthesis of tacrine–ferulic acid hybrids as multi-potent anti-Alzheimer drug candidates

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Abstract—Five tacrine–ferulic acid hybrids (**6a**–**e**) were designed and synthesized as multi-potent anti-Alzheimer drug candidates. All target compounds have better acetylcholinesterase inhibitory activity and comparable butyrylcholinesterase inhibitory activity in relation to tacrine. Interestingly, **6d** showed a reversible and non-competitive inhibitory action for acetylcholinesterase indicating interaction with the peripheral anionic site, whereas a reversible but competitive inhibitory action for butyrylcholinesterase. The antioxidant study revealed that four target compounds have, compared to Trolox, high ability to absorb reactive oxygen species.
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A case report of what was later named 'Alzheimer's disease' (AD) was first reported by Alois Alzheimer in 1907 at a neurology conference, where he described a 51-year-old woman with rapid and devastating memory degeneration. Although the disease was once considered rare, it is now established as the leading cause of dementia and despite major research efforts not curable. It has been estimated that the incidence of AD varies from 12% to 19% for women and 6% to 10% for men over the age of 65 years[1] and rises exponentially with age.[2] Five pharmaceutical drugs representing cholinesterase inhibitors (ChEIs), namely galantamine, rivastigmine, donepenzil, tacrine (Fig. 1) and an NMDA receptor antagonist (memantine) are applied clinically. However, they can only offer little more than short-term palliative effects and ChEIs suffer from pronounced peripheral side effects.

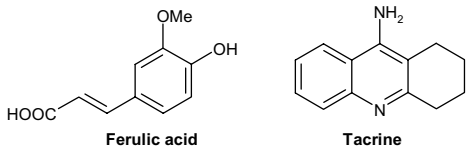

Figure 1. Structures of ferulic acid and tacrine.

The difficulty for developing satisfactory therapy of AD lies in the complex pathophysiology of the disease, which involves numerous pathways. These include deficiency in cholinergic neurotransmission, defective beta-amyloid protein metabolism, abnormalities of glutamatergic, adrenergic, serotonergic and dopaminergic neurotransmission, and the involvement of inflammatory, oxidative and hormonal pathways.[3] Due to the multi-pathogenesis of AD, one of the current strategies to develop novel anti-Alzheimer agents focuses on compounds with multiple potencies.[4] Several, quite diverse attempts have been made and some of them have shown good performance.[5] We here report a series of tacrine–ferulic acid hybrids (**6a**–**e**) as multi-potent anti-Alzheimer drug candidates. The target molecules consist of three parts: the tacrine-like heterocycle, the ferulic acid (Fig. 1) moiety and an alkylenediamine side chain to link the former two parts.

Keywords: Tacrine; Ferulic acid; Cholinesterase inhibitor; Antioxidant; Anti-Alzheimer drug; Hybrid molecule.
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Tacrine was the first approved ChEI by the FDA for the treatment of AD. Its rationale mainly aims to stabilize acetylcholine levels in the synaptic cleft by the inhibition of the main degrading enzyme of the neurotransmitter to maintain neurotransmission. However, because it only targets at the cholinergic dysfunction, without any effect on the other pathologic factors, it can retard the progression of AD for only about a year.[6]

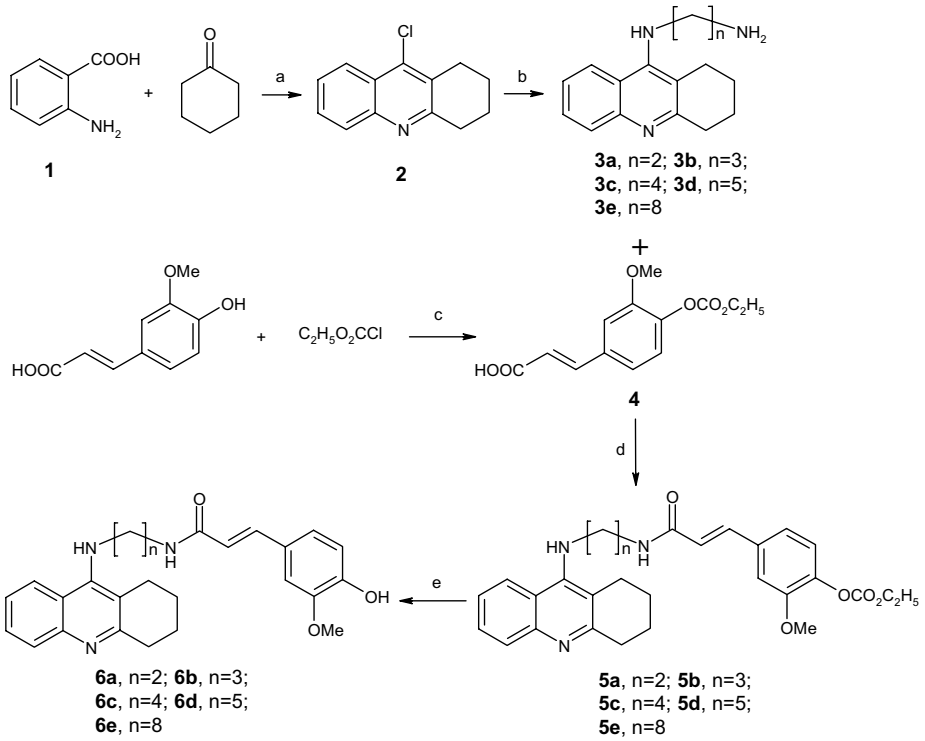
More and more attention is paid to oxidative stress for its role in the progression of AD.[7] A recent report suggested that oxidative stress is involved in the early stage of the pathologic cascade and represents a key factor to initiate the aggregation of β-amyloid and τ-protein hyperphosphorylation.[8] Accordingly, many antioxidants were observed to be able to attenuate the syndrome of AD and prevent the progression of the disease.[9]

Ferulic acid (Fig. 1) is one of the dominating natural phenolic acids and occurs, often together with caffeic acid, in the secondary metabolite spectrum of important economic and medicinal plants such as wheat (*Triticum aestivum*) or eucalyptus (*Eucalyptus globulus*). Furthermore, many secondary metabolites contain ferulic acid substructures or ferulic acid esters and showed very potent anti-oxidative activity depending on the molecular structure of this part.[10] It was shown in an in vivo study in mice that long-term administration of ferulic acid induced resistance to $A_{\beta 1-42}$ toxicity in the brain, this chemopreventive effect could also be proved in an behavioural assay.[11] Therefore, connecting ferulic acid to a tacrine template via an alkylenediamine side chain may yield a novel class of target molecules, which might exert a synergic action of the cholinesterase (ChE) inhibitory activity originating from the tacrine template and the antioxidant activity originating from the ferulic acid moiety. Besides, according to our previous investigation,[12] an introduction of an alkylenediamine side chain at 9-position of the tacrine-like heterocycle may also contribute to a reduction of the hepatotoxicity of tacrine and enhance the ChE inhibitory activity. In the context of tacrine's hepatotoxicity,[13] the formation of a hybrid with ferulic acid seems to be of special interest, since also hepatoprotective effect has been described for ferulic acid.[14]

Because the target molecules consist of three parts, the synthetic strategy is firstly to prepare the heterocycle, then to introduce the side chain, and finally to connect it to the ferulic acid moiety. According to a previously reported protocol,[15] 9-chloro-1,2,3,4-tetrahydroacridine (**2**) was first prepared by the cyclization of anthranilic acid with cyclohexanone. In order to introduce the side chain, different alkylenediamines reacted with 9-chloro-1,2,3,4-tetrahydroacridine to generate the 9-aminoalkylamino-1,2,3,4-tetrahydroacridines **3a**–**e**. We had tried direct coupling of **3a**–**e** to ferulic acid in the presence of DCC/DMAP or treating CDI-activated ferulic acid with **3a**–**e**. However, many by-products were formed during the reaction because of the possibility of inter-molecular side reactions (such as the reaction of the phenolic OH-group of ferulic acid with another ferulic acid molecule), thus the target compounds could hardly be isolated. Therefore, the phenol hydroxyl functional group of ferulic acid was at first protected by reacting with ethyl chloridocarbonate to give the ester intermediate **4**. Compound **4** was then coupled to **3a**–**e** in the presence of DCC/DMAP. Finally, the protection group was removed by the treatment with 95% ethanol/aminoethanol to yield the target compounds (Scheme 1).

To determine the therapeutic potential of **6a**–**e** for the treatment of AD, the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities, respectively, were measured for the target compounds as well as for the free amine intermediates **3a**–**e** using the Ellman's assay.[16] The results are given in Table 1. All the target compounds showed better AChE inhibitory activity than tacrine (IC_{50} = 45 nM) with the IC_{50} values varying from 4.4 to 38.6 nM. Particularly, the potency of **6c** and **6d** (IC_{50} = 7.6 nM and 4.4 nM) is 6- and 10-fold improved, respectively. As for BChE, all the tested compounds also showed comparable activity to tacrine (IC_{50} = 5.1 nM) with the IC_{50} values varying from 5.9 to 34.1 nM. The results of the amine intermediates showed a trend consistent with the target compounds, indicating that the ferulic acid moiety might not obviously affect the extent of ChE inhibitory activity. Amongst all the target compounds, **6c** and **6d** showed high activity toward both AChE and BChE, suggesting the optimal distance between the tacrine-like heterocycle and the ferulic acid moiety is 6–7 atoms long. This result seems consistent with the previous reports of lipoic acid/tacrine,[17] huperzine A/tacrine[18] and lipoic acid/quinazolinimines hybrids, the latter ones representing a novel class of BChE-selective compounds.[19]

Because **6d** showed the highest activities towards both AChE and BChE, it was selected for kinetic measurements, in order to gain information about the mode of inhibition and binding of the novel inhibitors. The mechanism of inhibition was analyzed by recording substrate–velocity curves in the absence and presence of different concentrations of **6d** using 25, 50, 90, 150, 226 and 450 μM of substrate for both BChE and AChE curves. The concentrations of **6d** were varied between 1 and 10 nM comprising 1, 2, 4, 8 and 10 nM, respectively. Figures 2 and 3 show the Lineweaver–Burk plots, which are reciprocal rates versus reciprocal substrate concentrations for the different inhibitor concentrations resulting from the substrate–velocity curves for AChE and BChE. For AChE, with increasing inhibitor concentrations, the v_{max} value (i.e., the reciprocal of the Y intercept) is obviously decreased. In contrast the K_m values (i.e., the negative reciprocal of the X intercept) are not changed with different inhibitor concentrations. For BChE, a different Lineweaver–Burk plot from AChE is observed. In this Lineweaver–Burk plot the K_m values differ with the different inhibitor concentrations, while the v_{max} value remains unchanged. The Lineweaver–Burk plot for AChE clearly shows reversible and non-competitive inhibition by tacrine–ferulic acid hybrids, meaning that these compounds do not compete for the same active site as the substrate acetylcholine. Based



Scheme 1. General method for the synthesis of **6a**–**e**. Reagents and conditions: (a) $POCl_3$, reflux, 3 h; (b) 3 equiv $H_2N(CH_2)_nNH_2$, pentanol, reflux, 18 h; (c) 1 equiv NaOH, water, 0 °C; (d) 1.2 equiv DCC, equimolar **3a**–**e**, cat. DMAP, CH_2Cl_2, rt; (e) 95% ethanol, 1 equiv $H_2NCH_2CH_2OH$, 3 h, rt.

Table 1. IC_{50} values of **3a**–**e** and **6a**–**e** against AChE and BChE

Compound	IC_{50} (nM) ± SEM[a]	
	AChE[b]	BChE[c]
3a	54.8 ± 10.4	12.3 ± 4.1
3b	69.5 ± 14.7	5.5 ± 1.4
3c	23.6 ± 5.0	7.1 ± 1.1
3d	3.2 ± 0.9	5.0 ± 0.7
3e	3.5 ± 1.4	1.4 ± 0.4
6a	38.6 ± 9.7	34.1 ± 8.1
6b	33.0 ± 8.3	27.0 ± 9.9
6c	7.6 ± 1.9	5.9 ± 0.7
6d	4.4 ± 1.7	6.7 ± 1.6
6e	9.6 ± 2.1	12.7 ± 2.6
Tacrine	45.1 ± 6.9	5.1 ± 1.0

[a] Data is the mean of at least three determinations.
[b] E.C. 3.1.1.7, type VI-S, from Electric Eel.
[c] E.C. 3.1.1.8, from equine serum.

on the analysis of the chemical structure of the target compounds as well as the model of the enzyme, we postulate that, rather than the catalytic binding site, the binding site of the test compound might be the peripheral anionic site (PAS), which is reportedly a second binding site of AChE, at the lip of the gorge and around 18 Å away from the active site.[20] Molecular modelling studies are necessary to validate these assumptions though. Given the relatively narrow gap of the active site and the considerable steric demand of the target compounds, the hybrids may be inclined to bind at the PAS rather than the active site. This would be a highly desirable therapeutic effect, since the neurotoxic cascade is mediated through AChE-induced Aβ aggregation,[21,22] the PAS of AChE being the structural motif responsible for Aβ-peptide fibril formation catalyzed by AChE.[23]

In contrast, the Lineweaver–Burk plot for BChE shows reversible and competitive inhibition by tacrine–ferulic acid hybrids, revealing that these compounds compete for the same active site as the substrate acetylcholine. The different mechanism of the inhibitory action may be due to the difference of the structures of the two cholinesterases. Because AChE presents several aromatic residues that are replaced by smaller aliphatic ones in BChE, BChE has a larger electrostatic gradient than AChE,[24] which makes it possible to allow a large molecule to approach the active site at the bottom of the gorge.

In order to prove the antioxidant capacities of the hybrid molecules in comparison to tacrine, the ability of compounds **6a**–**6e** to reduce the amount of peroxyl radicals was determined by the ORAC (oxygen radical absorbance capacity) assay.[25] Ferulic acid itself was selected as positive control, whereas tacrine was also tested to serve as a negative standard (Table 2). The compounds' ability to scavenge radicals is expressed as Trolox equivalent, that is, their relative ability (at concentrations between 0.5 and 10 μM) compared to the highly potent compound Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid). Ferulic acid itself is in exact accordance with literature data, a potent radical scavenger of peroxyl radicals.[26] After connecting the ferulic acid to the tacrine moiety the antioxidant activity decreased, but nevertheless all hybrids tested showed moderate to good antioxidant activity. Interestingly,

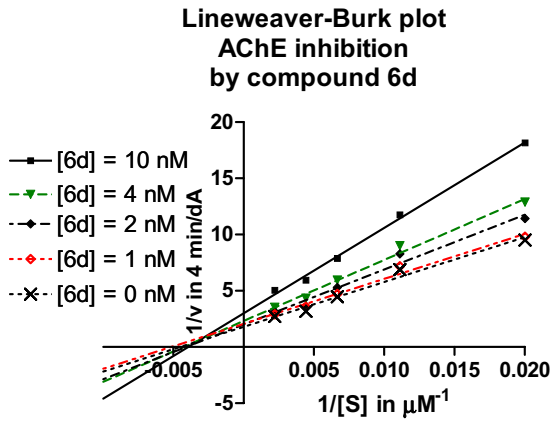


Figure 2. Lineweaver–Burk plots resulting from substrate–velocity curves of AChE activity with different substrate concentrations (50–450 μM) in the absence and presence of 1, 2, 4 and 10 nM **6d**.

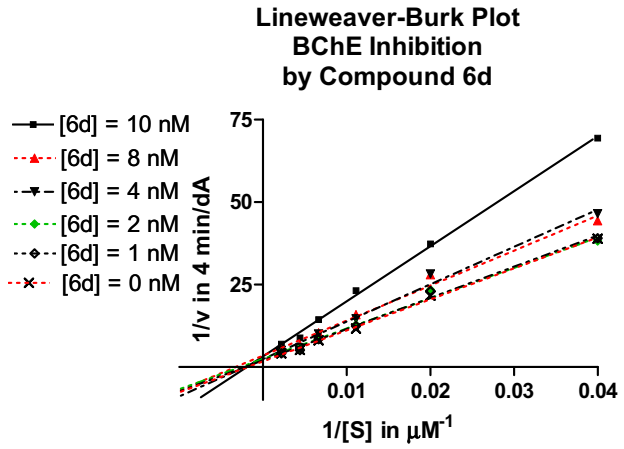


Figure 3. Lineweaver–Burk plots resulting from substrate–velocity curves of BChE activity with different substrate concentrations (25–450 μM) in the absence and presence of 1, 2, 4 and 10 nM **6d**.

Table 2. Oxygen radical absorbance capacity (ORAC, Trolox equiv, 0.5–10 μM) by tacrine and its hybrids with ferulic acid

Compound	Trolox equivalents	SD
6a (hybrid, $n = 2$)	1.3	±0.04
6b (hybrid, $n = 3$)	2.0	±0.1
6c (hybrid, $n = 4$)	0.4	±0.03
6d (hybrid, $n = 5$)	1.5	±0.01
6e (hybrid, $n = 8$)	1.4	±0.04
Tacrine	0.2	±0.04
Ferulic acid	3.4	±0.1

the activity of the hybrids not only depends on the ferulic acid substructure, but also on the chain length of the connecting linker.

In summary, we have designed and synthesized five novel hybrid compounds (**6a**–**e**) with an antioxidant ferulic acid moiety connected to the tacrine template via an alkylenediamine-type spacer. All compounds effectively inhibited ChEs in vitro. Two target compounds (**6c** and **6d**), in particular, showed 6- and 10- fold higher AChE inhibitory activity compared to tacrine. The inhibitory mechanism of **6d** was analyzed by determining the Lineweaver–Burk plots. Interestingly, the results indicated that the hybrid exerts a reversible and non-competitive inhibitory action for AChE, whereas a reversible but competitive inhibitory action for BChE. The previous result strongly suggests the inhibition of the PAS of AChE and therefore the possibility to inhibit Aβ-peptide fibril formation also, but this has to be unambiguously proven in additional investigations. All the target compounds were screened for their antioxidant activity using the ORAC-Fluorescein assay. Compared to Trolox, most of the target compounds, except **6c**, showed a high ability to absorb reactive oxygen species. The results suggest that these tacrine–ferulic acid hybrids may be considered to be a kind of novel anti-Alzheimer's drug candidates, which are multi-target-directed ligands with not only ChE inhibitory (both acting at the active centre and the PAS of AChE) yet also antioxidant activity.

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Supplementary data

Supplementary data is available online. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.073.

References and notes

1. Seshadri, S.; Wolf, P. A.; Beiser, A.; Au, R.; McNulty, K.; White, R.; D'Agostino, R. B. *Neurology* **1997**, *49*, 1498.
2. Evans, D. A.; Funkenstein, H. H.; Albert, M. S.; Scherr, P. A.; Cook, N. R.; Chown, M. J.; Hebert, L. E.; Hennekens, C. H.; Taylor, J. O. *J. Am. Med. Assoc.* **1989**, *262*, 2551.
3. Doraiswamy, P. M. *CNS Drugs* **2002**, *12*, 811.
4. Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 347.
5. Decker, M. *Mini-Rev. Med. Chem.* **2007**, *7*, 221.
6. Watkins, P. B.; Zimmerman, H. J.; Knapp, M. J.; Gracon, S. I.; Lewis, K. W. *J. Am. Med. Assoc.* **1994**, *271*, 992.
7. Smith, M. A.; Rottkamp, C. A.; Nunomura, A.; Raina, A. K.; Perry, G. *Biochim. Biophys. Acta* **2000**, *1502*, 139.
8. Nunomura, A.; Castellani, R. J.; Zhu, X.; Moreira, P. I.; Perry, G.; Smith, M. A. *J. Neuropathol. Exp. Neurol.* **2006**, *65*, 631.
9. Liu, Q.; Xie, F.; Rolston, R.; Moreira, P. I.; Nunomura, A.; Zhu, X.; Smith, M. A.; Perry, G. *Mini-Rev. Med. Chem.* **2007**, *7*, 171.
10. Heilmann, J.; Çalis, I.; Kirmizbekmez, H.; Schühly, W.; Harput, S.; Sticher, O. *Planta Med.* **2000**, *66*, 746.
11. Yan, J.; Cho, J.; Kim, H.; Kim, K.; Jung, J.; Huh, S.; Suh, H.; Kim, Y.; Song, D. *Br. J. Pharmacol.* **2001**, *133*, 89.
12. Fang, L.; Appenroth, D.; Decker, M.; Kiehntopf, M.; Roegler, C.; Deufel, T.; Fleck, C.; Peng, S.; Zhang, Y.; Lehmann, J. *J. Med. Chem.* **2008**, *51*, 717.

13. Gracon, S. I.; Knapp, M. J.; Berghoff, W. G.; Pierce, M.; de Jong, R.; Lobbestael, S. J.; Symons, J.; Dombey, S. L.; Luscombe, F. A.; Kraemer, D. *Alzheimer Dis. Assoc. Disord.* **1998**, *12*, 93.
14. Wang, H.; Peng, R. X. *Acta Pharmacol. Sin.* **1994**, *15*, 81.
15. Carlier, P. R.; Han, Y. F.; Chow, E. S.; Li, C. P.; Wang, H.; Lieu, T. X.; Wong, H. S.; Pang, Y. P. *Bioorg. Med. Chem.* **1999**, *7*, 351.
16. Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
17. Rosini, M.; Andrisano, V.; Bartolini, M.; Bolognesi, M. L.; Hrelia, P.; Minarini, A.; Tarozzi, A.; Melchiorre, C. *J. Med. Chem.* **2005**, *48*, 360.
18. Gemma, S.; Gabellieri, E.; Huleatt, P.; Fattorusso, C.; Borriello, M.; Catalanotti, B.; Butini, S.; De Angelis, M.; Novellino, E.; Nacci, V.; Belinskaya, T.; Saxena, A.; Campiani, G. *J. Med. Chem.* **2006**, *49*, 3421.
19. Decker, M.; Kraus, B.; Heilmann, J. *Bioorg. Med. Chem.* **2008**, *16*, 4252.
20. Pang, Y.-P.; Quiram, P.; Jelacic, T.; Hong, F. *J. Biol. Chem.* **1996**, *271*, 23646.
21. Inestrosa, N. C.; Alvarez, A.; Calderon, F. *Mol. Psychiatry* **1996**, *1*, 359.
22. Piazzi, L.; Rampa, A.; Bisi, A.; Gobbi, S.; Belluti, F.; Cavalli, A.; Bartolli, M.; Andrisano, V.; Valenti, P.; Recanatini, M. *J. Med. Chem.* **2003**, *46*, 2279.
23. De Ferrari, G. V.; Canales, M. A.; Shin, I.; Weiner, L. M.; Silman, I.; Inestrosa, N. C. *Biochemistry* **2001**, *40*, 10447.
24. Campiani, G.; Fattorusso, C.; Butini, S.; Gaeta, A.; Agnusdei, M.; Gemma, S.; Persico, P.; Catalanotti, B.; Savini, L.; Nacci, V.; Novellino, E.; Holloway, W.; Greig, N. H.; Belinskaya, T.; Fedorko, J. M.; Saxena, A. *J. Med. Chem.* **2005**, *48*, 1919.
25. Dávalos, A.; Gómez-Cordovés, C.; Bartolomé, B. *J. Agric. Food Chem.* **2004**, *52*, 48.
26. Trombino, S.; Serini, S.; Di Nicuolo, F.; Celleno, L.; Ando, S.; Picci, N.; Calviello, G.; Palozza, P. *J. Agric. Food Chem.* **2004**, *528*, 2411.