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Synthesis and evaluation of tacrine–E2020 hybrids as acetylcholinesterase inhibitors for the treatment of Alzheimer's disease

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Abstract—Tacrine–E2020 hybrids and some related compounds were prepared and their bioactivities on the Alzheimer's disease were assayed. The optimum hybrid inhibitor **3** is 37-fold more potent and 31-fold more selective than tacrine in vitro.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia among the elderly, is a progressive, degenerative disorder of the brain with a loss of memory, and cognition.[1] The social and economic consequences of AD are alarming due to the notable increase in life expectancy. Tacrine (**1**) is a reversible inhibitor of acetylcholinesterase (AChE) that was launched in 1993 as the first drug for the treatment of AD.[2] The evaluation of the clinical effects of tacrine has shown efficacy in delaying the deterioration of the symptoms of AD, while confirming the adverse events consisting mainly in the elevated liver transaminase levels.[3] The study of tacrine analogs is still of interest to medicinal chemists involved in AD research. In fact, the possibility to design potent and selective bis-tetrahydroaminoacridine[4] and tacrine–huperzine A Hybrids[5] was demonstrated. The other modification of tacrine has been reported.[6–12] E2020 (**2**) is a member of a large family of *N*-benzylpiperidine based AChE inhibitors, which were developed, synthesized, and evaluated by the Eisai Company in Japan, and has been approved for use in the US for the treatment of AD.[13] In this communication we report a new class of tacrine–E2020 hybrids and other related compounds, the optimum inhibitor **3** is much more potent and selective than tacrine (**1**) (Fig. 1).

2. Chemistry

The synthesis of the compound **3** was accomplished as shown in Scheme 1. **11** can be prepared from commercially available 1-benzyl-4-piperidone **8** and malononitrile in 66.4% over three steps, the synthetic method has not been reported before, it can be synthesized on large scale. Reduction of **11** with $LiAlH_4$ provided amine **12** (83%). Intermediate **12** was then converted to the corresponding **13** (72.3%) with the active *p*-nitro-phenyl ester in THF at room temperature, deprotection of **13** with CF_3COOH in CH_2Cl_2 to give **14** in 76.4% yield. 9-Chloro-1,2,3,4-tetrahydroacridine **15** can be prepared from anthranilic acid and cyclohexanone in 89%,[4b] treatment of **15** with **14** in refluxing 1-pentanol provided the hybrid **3** in 81.1%, then it was treated with fumaric acid–EtOH, and the resulting solid were crystallized from EtOH–Et_2O to give the fumaric salt of **3** (70.6%).[14]

The synthesis of the compound **4** was accomplished as shown in Scheme 2. Ethyl piperidine-4-carboxylate **16** reacted with benzoyl chloride and hydrolyzed in 10% NaOH to give **17** (84.6%), Esterification of **17** with 4-nitrophenol in DCC and aminated to provide the amide **18** (71.5%). Reduction of **18** with $LiAlH_4$ provided

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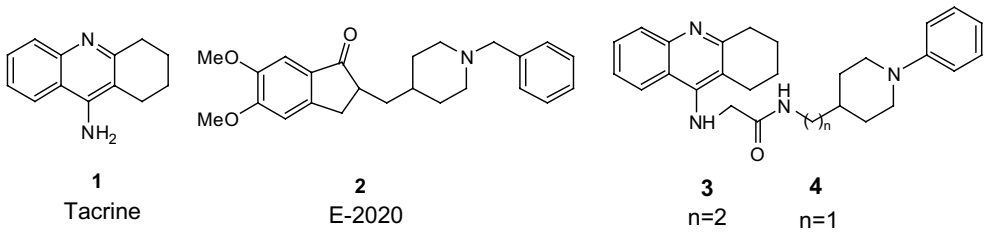
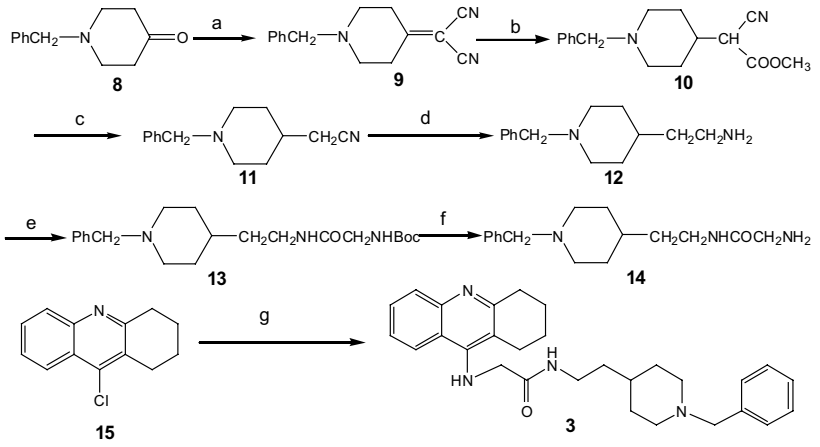
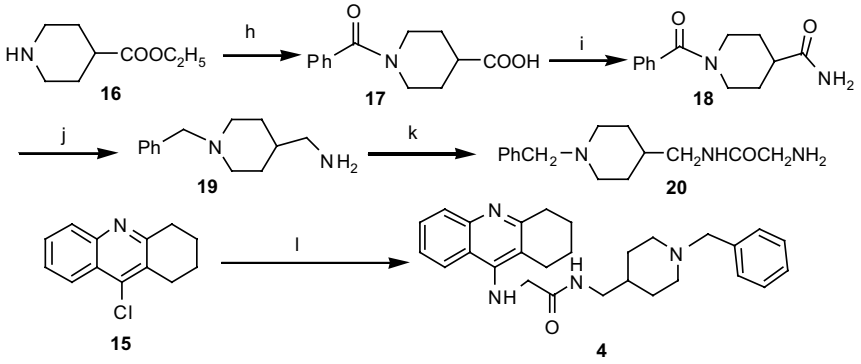


Figure 1. Structure of tacrine, E-2020, and their hybrids.



Scheme 1. Synthesis of the hybrid **3**. Reagents and conditions: (a) $CH_2(CN)_2$, CH_2Cl_2, rt, 90.3%; (b) $NaBH_4$, HCl/CH_3OH, 85.5%; (c) 10% NaOH/HCl, DMF, reflux, 86%; (d) $LiAlH_4$, rt, 83%; (e) $BocNHCH_2$ COO–Ph–NO_2-*p*, THF, 72.3%; (f) CF_3COOH/CH_2Cl_2, rt, 76.4%; (g) **14**, 1-pentanol, reflux, 81.1%.



Scheme 2. Synthesis of the hybrid **4**. Reagents and conditions: (h) C_6H_5COCl/Et_3N, 10% NaOH, 84.6%; (i) *p*-NO_2–Ph–OH/DCC, $NH_3{\cdot}H_2O$, 71.5%; (j) $LiAlH_4$/THF, reflux, 73.6%; (k) $BocNHCH_2COO$–Ph–NO_2-*p*/THF, CF_3COOH/CH_2Cl_2, 52.4%; (l) **20**, 1-pentanol, reflux, 76.9%.

amine **19** (73.6%). Intermediate **19** reacted with the active *p*-nitro-phenyl ester in THF, then deprotection of Boc group with CF_3COOH in CH_2Cl_2 to give **20** (52.4%). Treatment of **15** with **20** in refluxing 1-pentanol provided the hybrid **4** in 76.9%, which was treated with fumaric acid–EtOH to give the fumaric salt of **4** (73.2%).

3. Biological results and discussion

With completion of the synthesis, in vitro AChE and BuChE inhibitory activities of the hybrids and some related compounds as compared with tacrine (**1**) were measured according to the method of Ellman et al.[15] using rat cortex homogenate (AChE) and rat serum (BuChE).

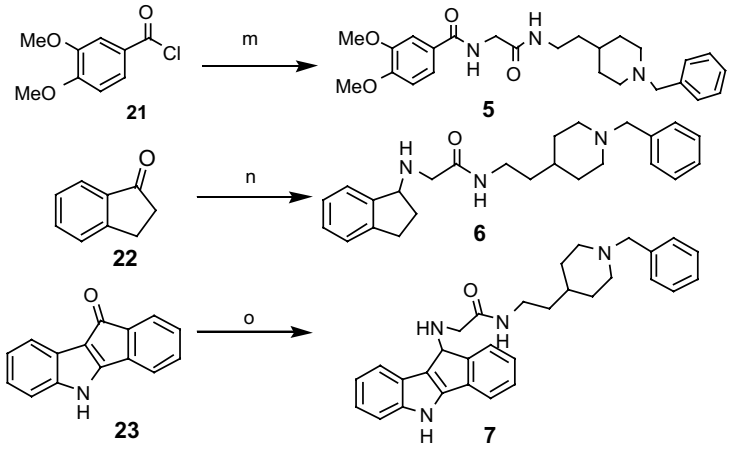
As can be seen (Table 1), tacrine–E2020 hybrids **3** and **4** are more potent for AChE inhibition than tacrine (**1**).[5c] The optimum **3** is easily synthesized, has nanomolar affinity for AChE ($IC_{50} = 6.0$ nM), and is 37-fold more potent and 31-fold more selective than tacrine (**1**). In the related compounds, **6** is similar to tacrine for AChE inhibition, **5** and **7** are weak inhibitors. However, their selectivity is higher than tacrine (Scheme 3).

4. Molecular modeling

To understand the recognition of **3** and to enable rational design of new derivatives, we examined the binding modes of **3** in AChE, the hybrid **3** is a long

Table 1. Cholinesterase inhibition IC_{50} (nM)

Compd	AChE	BuChE	Selectivity for AChE
3	6.0 ± 0.7	76 ± 8.2	12.7
4	10.2 ± 1.1	12.4 ± 1.3	1.2
5	662 ± 38	19,200 ± 134	29.0
6	230 ± 18	6920 ± 57	30.1
7	869 ± 41	17,800 ± 113	20.5
Tacrine (**1**)	223 ± 11	92 ± 2	0.4



Scheme 3. Synthesis of some related compounds **5**–**7**. Reagents and conditions: (m) **14**, Et_3N/CH_2Cl_2, 82.0%; (n) **14**, $Ti(O\text{-}iPr)_4/NaBH_4$, 46.0%; (o) **14**, $Ti(O\text{-}iPr)_4/NaBH_4$, 35.2%.

chain molecule, our hypothesis was to assume that its binding to AChE shares some or all of the features that modulate the binding of E-2020, certainly there maybe the tacrine ring of the hybrid **3** at the catalytic site and the phenyl group at the peripheral site. Binding free energies were calculated through autodock soft. We found the free energy of binding $\Delta G = -15.84$ kcal/mol with the tacrine ring at the peripheral site and $\Delta G = -14.42$ kcal/mol with the tacrine ring at the catalytic site, which exhibited the former state with AChE much more stable than the latter. Accordingly, modeling of the interaction **3** with the enzyme was based on the crystallographic structures of AChE complexes with E-2020.[16] The figure was plotted by insight II soft (left) and ligplot soft (right), respectively.

As seen in Figure 2, the hybrid **3** makes principal interactions along the active-site gorge of the enzyme

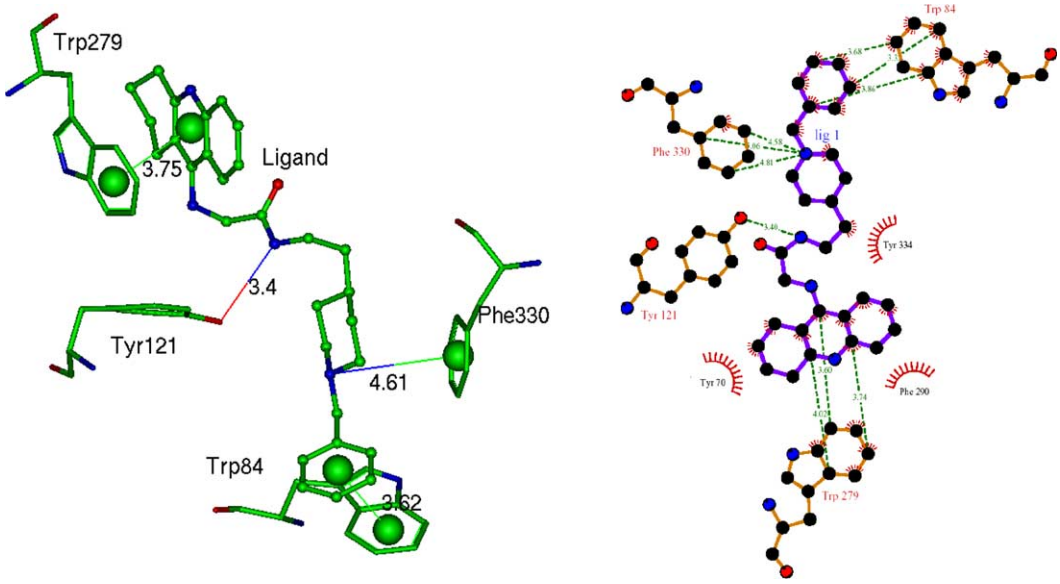


Figure 2. Plot of the main interactions between AChE and ligand **3**.

through its four major functional groups: the benzyl moiety, the piperidine nitrogen, the nitrogen–hydrogen of the amide, and the acridine moiety. Near the bottom of the gorge, one face of the benzyl ring displays classic parallel π–π stacking with the six-membered ring of the Trp84 indole, the ring-to-ring distance is 3.62 Å between Trp84 and the benzyl ring. In the constricted region, halfway up the gorge, the charged nitrogen of the piperidine ring makes a cation–π interaction with the phenyl ring of Phe330, with the distances of 4.61 Å between the nitrogen and the aromatic ring. The nitrogen–hydrogen of the amide makes an hydrogen bond with the oxygen of Tyr121, the distance is 3.4 Å. At the top of the gorge the acridine ring stacks against the indole of Trp279, in the peripheral binding site, by a classical π–π interaction with the distances of 3.75 Å.

In the homolog the molecular chain length of **3** is 16.8 Å, it approaches in the distance of the two tacrine-binding sites,[4a] which is 16 Å, **3** derives its potency and selectivity from simultaneous binding to the peripheral and catalytic sites of AChE.

5. Conclusion

In summary, the hybrids of tacrine–E2020 and some related compounds were synthesized and their bioactivities on AD were assayed. The optimum inhibitor **3** is much more potent and selective than tacrine. The modeling studies clearly indicate that ligand **3** is nicely accommodated by AChE, engaging in appropriate hydrogen bond interactions. It is useful for the modification of the hybrids and design of new AChE inhibitors. Further studies on hybrids are in progress and will be reported in due course.

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14. **3** (fumaric salt) mp 186–188 °C (d) ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 8.24 (t, 1H, J = 5.6 Hz), 8.18 (d, 1H, J = 8.4 Hz), 7.88 (d, 1H, J = 8.5 Hz), 7.73 (t, 1H, J = 7.2 Hz), 7.45 (t, 1H, J = 7.2 Hz), 7.37–7.26 (m, 5H), 7.14 (br s, 1H), 6.58 (s, 2H), 4.35 (s, 2H), 3.68 (s, H), 3.14 (q, 2H, J = 6.5 Hz), 2.97 (br s, 2H), 2.90 (br d, 2H, J = 12.1 Hz), 2.69 (br s, 2H), 2.15 (t, 2H, J = 10.0 Hz), 1.83–1.81 (m, 4H), 1.64 (d, 2H, J = 11.7 Hz), 1.35–1.21 (m, 5H) ^{13}C NMR (100 MHz DMSO-d_6, ppm): 168.3, 166.8, 154, 152.8, 140.4, 134.8, 134.5, 131.3, 129.9, 128.3, 127.9, 124.5, 124.2, 121.8, 116.7, 112.3, 60.7, 52.1, 49.4, 36.2, 35.3, 31.6, 30.1, 29.4, 23.6, 21.7, 20.9; IR (KBr): 3291, 2942, 1714, 1677, 1276, 1188, 983, 630 cm^{-1}: EI-MS MS (*m*/*z*): 456 (M^+). Anal. Calcd for $C_{29}H_{36}N_4O{\cdot}C_4H_4O_4$: C 69.21, H 7.04, N 9.78. Found: C 68.92, H 7.01, N 9.74.
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