NO-Donating Tacrine Hybrid Compounds Improve Scopolamine-Induced Cognition Impairment and Show Less Hepatotoxicity

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Abstract: A series of tacrine−NO donor hybrid compounds are synthesized and evaluated for cholinesterase inhibitory activity, cognition improving activity, and hepatotoxicity. The pharmacological results indicate that hybrid compounds **1**, **2**, and **3a** potently inhibit cholinesterase in vitro and significantly improve the scopolamine-induced cognition impairment, whereas an analogue (**3h**) of **2** without the NO donor moiety does not. Compared to tacrine, **1** and **2** show much less hepatotoxicity. Molecular modeling studies suggest that **2** may interact with the catalytic and the peripheral anionic site of acetylcholinesterase.

Alzheimer's disease (AD[a]) is one of the most common age-related diseases in the world. Though much research effort has been made, it is still incurable. Because of the complex pathophysiology of the disease, developing novel agents with multiple pharmacological effects has become a promising strategy in today's search for new treatment of AD.[1,2] Previously, we have reported several NO donor−tacrine hybrid compounds that simultaneously possess potent cholinesterase (ChE) inhibitory activity, moderate vessel relaxant activity, and hepatoprotective effects and that may be considered as novel anti-AD drug candidates.[3] Among the previously reported hybrid compounds, the amide-linked nitrate−tacrine hybrid **1** (Figure 1) and the amine-linked nitrate−tacrine hybrid **2** (Figure 1) showed the best activities in the in vitro evaluations.[3] Therefore, to investigate the influence of the alkylenediamine side chain of the molecules on their activity, we have designed and synthesized another eight nitrate−tacrine hybrid compounds **3a**−**h** with shorter and longer diamine side chains. All the new target compounds were evaluated for the ChE inhibitory activity in vitro. Furthermore, **1**, **2**, and **3a** were tested in vivo for their ability to improve scopolamine-induced cognition impairment and hepatotoxicity. In addition, with the help of a molecular operating environment (MOE) software, the interaction of **2** with the active sites of acetylcholinesterase (AChE) was simulated and analyzed.

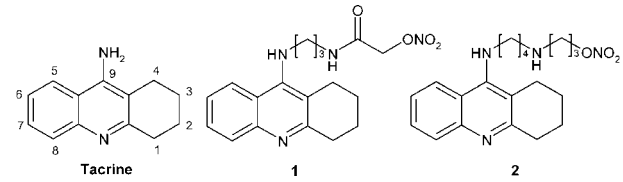
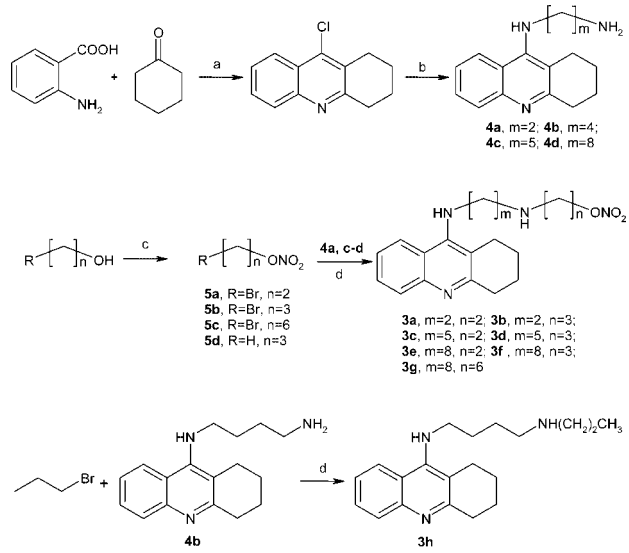


Figure 1. Structures of tacrine, **1**, and **2**.

Scheme 1. General Method for the Synthesis of **3a**−**h**[a]



[a] Reagents and conditions: (a) $POCl_3$, reflux, 3 h; (b) pentanol, $NH_2(CH_2)_mNH_2$, reflux, 18 h; (c) fuming HNO_3, CH_2Cl_2, −5 °C; (d) K_2CO_3, KI, CH_2Cl_2, 24 h, room temp.

Similar to a previously described protocol,[3] 9-chloro-1,2,3,4-tetrahydroacridine was reacted with different alkylenediamines to produce the intermediate 9-aminoalkylamino-1,2,3,4-tetrahydroacridines **4a**−**d**. The NO donor moiety was prepared by treating bromoalkanols with HNO_3 under ice cooling. The nitrate intermediates **5a**−**d** were reacted with amines **4a**,**c**,**d** in CH_2Cl_2 in the presence of KI and K_2CO_3 to give the target compounds **3a**−**g**. Compound **4b** directly reacted with 1-bromopropane to yield **3h** (Scheme 1).

All the target compounds were screened on their ChE inhibitory activity in vitro using Ellman's assay.[4] The results (Table 1) showed that all the compounds generally retain the ChE inhibitory effect of the parent compound tacrine. Compared to tacrine (IC_{50} = 45.1 nM), the AChE inhibitory activities of **2** (IC_{50} = 5.6 nM), **3a** (IC_{50} = 9.1 nM), and **3e** (IC_{50} = 7.7 nM) are 5- to 6-fold improved while the butyrylcholinesterase (BuChE) inhibitory activities of the target compounds, with the IC_{50} varying from 7.2 to 18.1 nM, remaining at a comparable level. BuChE inhibition has recently been regarded therapeutically beneficial for the treatment of AD, because BuChE, contrary to AChE, increases in the course of the disease and may compensate AChE.[5] The target compounds potently inhibit both of the enzymes more or less nonselectively, which could be favorable since decreasing AChE and increasing BuChE have met approximately at the same level when AD symptoms arise and therapy sets in.[6] Furthermore, no pronounced differences were observed among the ChE inhibitory activities of the target

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[a] Abbreviations: AD, Alzheimer's disease; ChE, cholinesterase; MOE, molecular operating environment; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; CAS, the catalytic active site; PAS, periphery anion site; ASAT, aspartate aminotransferase; HE, hematoxylin and eosin.

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Table 1. Inhibition of AChE and BuChE (IC_{50} Values) and Selectivity Expressed as the Ratio of the Resulting IC_{50} Values

compd	$IC_{50} \pm SEM^a$ (nM) AChE[b]	BuChE[b]	selectivity ratio[c]
tacrine	45.1 ± 6.9	5.1 ± 1.0	8.8
1	28.2 ± 4.0	13.5 ± 3.1	2.1
2	5.6 ± 0.7	9.9 ± 1.1	0.6
3a	9.1 ± 3.8	7.3 ± 4.7	1.4
3b	13.0 ± 3.7	18.1 ± 9.4	0.7
3c	21.6 ± 7.4	13.3 ± 3.1	1.6
3d	15.4 ± 6.4	14.3 ± 3.2	1.1
3e	7.7 ± 0.9	17.0 ± 7.7	0.4
3f	17.1 ± 2.4	14.8 ± 1.9	1.2
3g	19.6 ± 0.7	12.9 ± 0.7	1.5
3h	23.4 ± 5.0	7.2 ± 1.1	3.2

[a] Data are the mean values of at least three determinations. [b] AChE from electric eel and BuChE from equine serum were used. [c] Selectivity ratio = (IC_{50} of AChE)/(IC_{50} of BuChE).

compounds, indicating the length of the side chain does not significantly influence the activity within the range of 4−14 atoms.

Using scopolamine and an eight-arm radial maze is an accepted standard protocol in behavioral pharmacology for the evaluation of ChE inhibitors as anti-AD drug candidates.[7,8] To determine the in vivo activity of the target compounds, we used cognition impaired adult rats (after scopolamine administration) as animal model to measure the cognition improving effects of **1**, **2**, and **3a**. Measuring was performed in an eight-arm radial maze. After the rats were adapted to the maze and trained to be able to quickly find the bait at the end of each arm, they were treated with scopolamine (0.05 mg/100 g body weight, ip). Scopolamine distinctly blocks muscarinic cholinergic receptors and thus impaires the animals' cognition significantly. Then tacrine (1.978 μmol/100 g body weight) and **1**, **2**, and **3a** (equimolar dose), which are supposed to compensate for the cholinergic dysfunction in the central nervous system by inhibiting ChEs, were administrated to the animals. To determine whether the nitrate group of the target molecule has an influence on the in vivo activity, 1-propylnitrate (**5d**) and **3h**, which is an analogue of **2** without the nitrate group, were also selected for in vivo testing in the assay applied alone and in combination, respectively. Two parameters, the number of errors made during the exploration and the total exploration time, were recorded before, 20, 60, and 180 min after scopolamine administration to determine the cognition improving activity of the selected compounds.

The results show that, compared to the group treated with scopolamine (scop) alone, the performance of groups **1**, **2**, and **3a** was significantly improved, as indicated by decreased numbers of errors and shorter exploration time (Figure 2). Compared to tacrine (tac), **1** and **2** showed an approximately equal activity. However, tacrine given without additional scopolamine caused a significant slow-down of a rat's performance in the maze, whereas **1**, **2**, and **3a** given alone had no influence on a rat's behavior at all. All these findings indicate advantages of the NO-donating hybrid compounds in comparison to tacrine.

Interestingly, the nonhybrid **3h**, though it showed comparable ChE inhibitory activity to **2** in vitro, did not show any improving effects in the in vivo assay in the dose range applied. This suggests that the nitrate group of **2**, which was reported to possess vessel relaxant activity,[3] may also contribute to the overall procognitive effect of the hybrids, though the mechanism is not clear yet. Administrating **3h** with the nitrate **5d** or administrating **5d** alone did not show significant activity in vivo,

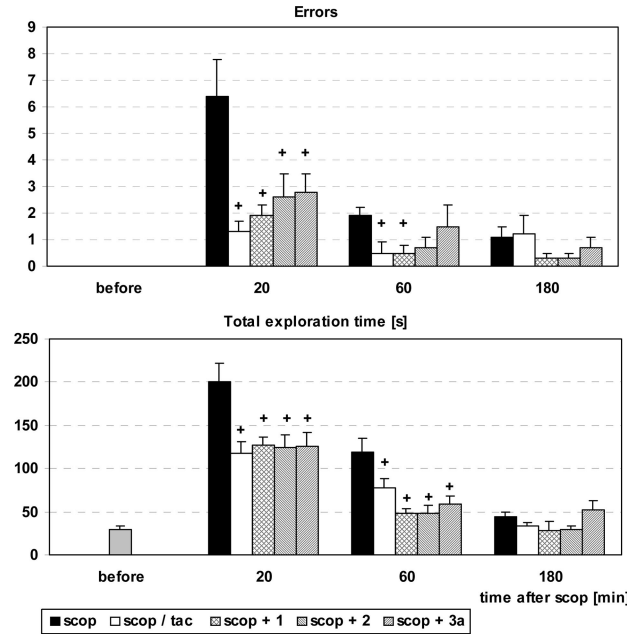

Figure 2. Number of errors rats made during the search for baits and exploration time needed to find all food baits before, 20, 60, and 180 min after the administration of scopolamine, **1**, **2**, and **3a**: (+) significant difference to scopolamine (scop) (*t* test, $p \leq 0.05$).

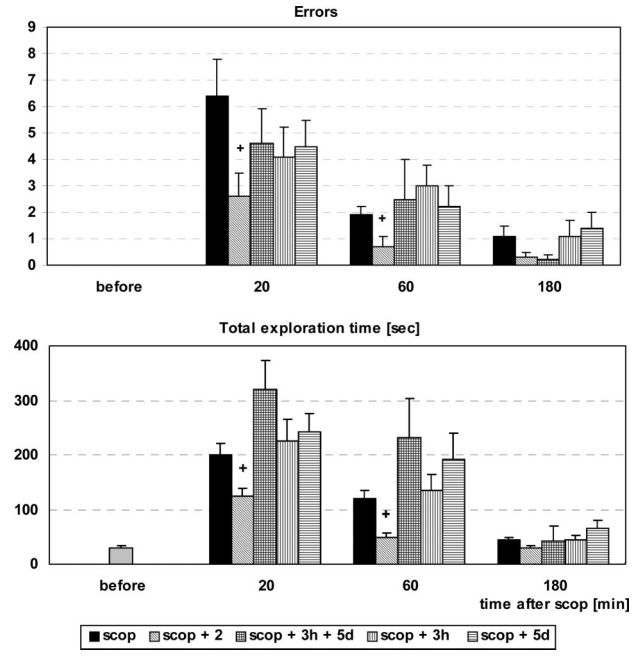

Figure 3. Number of errors rats made during the search for baits and exploration time needed to find all food baits before, 20, 60, and 180 min after the administration of scopolamine, **2**, **3h**, and **5d**: (+) significant difference to scopolamine (scop) (*t* test, $p \leq 0.05$).

indicating that the administration of the combination of equimolar parts of NO donor and tacrine in one hybrid molecule is clearly superior to a simple mixture of both parts (Figure 3).

The serious hepatotoxicity of tacrine is the main limitation for its clinical use.[9] To determine the hepatotoxicity, rats were treated with the highest tolerated dose of tacrine (5.93 μmol/100 g body weight) or equimolar doses of **1** and **2**. The determination of ASAT and albumin concentration in serum samples was performed before (intraindividual control), 24, and 36 h after administration using an Abbot Architect ci 16200 analyzer according to the instructions of the manufacturer. The

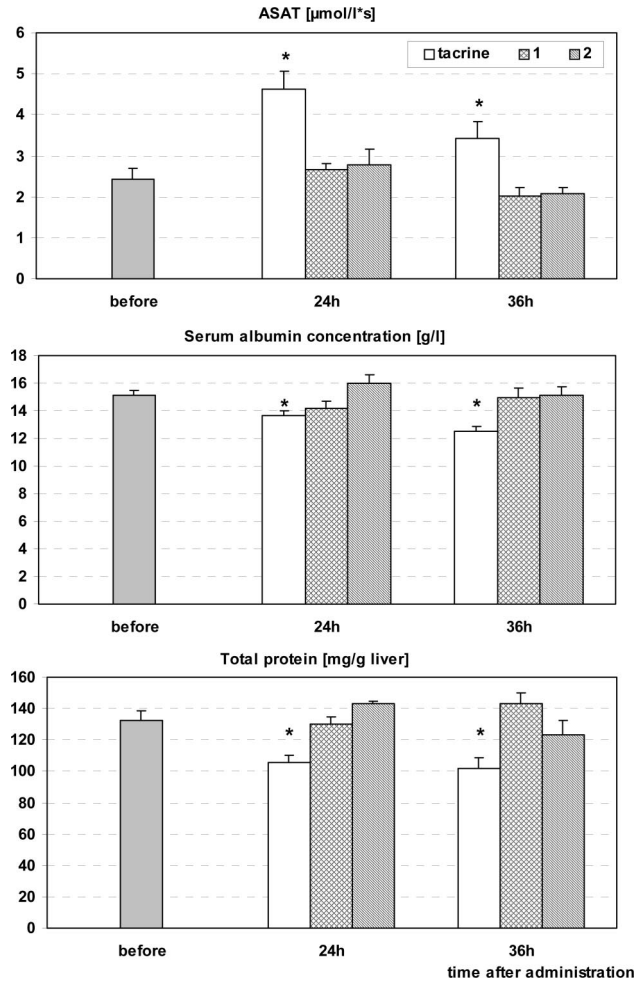

Figure 4. ASAT activity, serum albumin concentration, and total protein in the liver before and after the administration of tacrine, **1**, or **2**: (∗) significant difference to values before administration (*t* test, $p \leq 0.05$).

results (Figure 4) demonstrate significant hepatotoxic effects caused by tacrine concerning ASAT activity and albumin concentration in serum and protein content in liver tissue. In contrast, none of these parameters are affected significantly by the hybrid compounds **1** and **2**.

For morphological studies liver tissue was stained with hematoxylin and eosin (HE). Complete pericentral necrosis and distinct fatty degeneration of the hepatocytes of the surrounding intermediate and periportal zones were seen 24 h after administration of tacrine (compare parts A and B of Figure 5). In contrast, after administration of **2** no major histopathological changes were seen (Figure 5C). These findings give further evidence for the absence of hepatotoxic effects of **2**.

It is well-known that AChE has a dumbbell-shape active site gorge composed of two active sites: the catalytic active site (CAS) at the bottom and the peripheral active site (PAS) at the lip.[10] It has been shown that PAS not only contributes to the hydrolysis of ACh but also is responsible for the AChE-induced β-amyloid aggregation.[11] Dual inhibitors toward CAS and PAS are regarded as improved agents for the treatment of AD, expanding the therapeutic spectrum of AChE inhibitors. Some previous studies have revealed that a protonatable nitrogen-containing side chain connected to the tacrine template may be helpful for the parent drug to obtain a PAS inhibitory effect.[12] To determine whether our target compounds can interact with both active sites of AChE, **2** was selected to

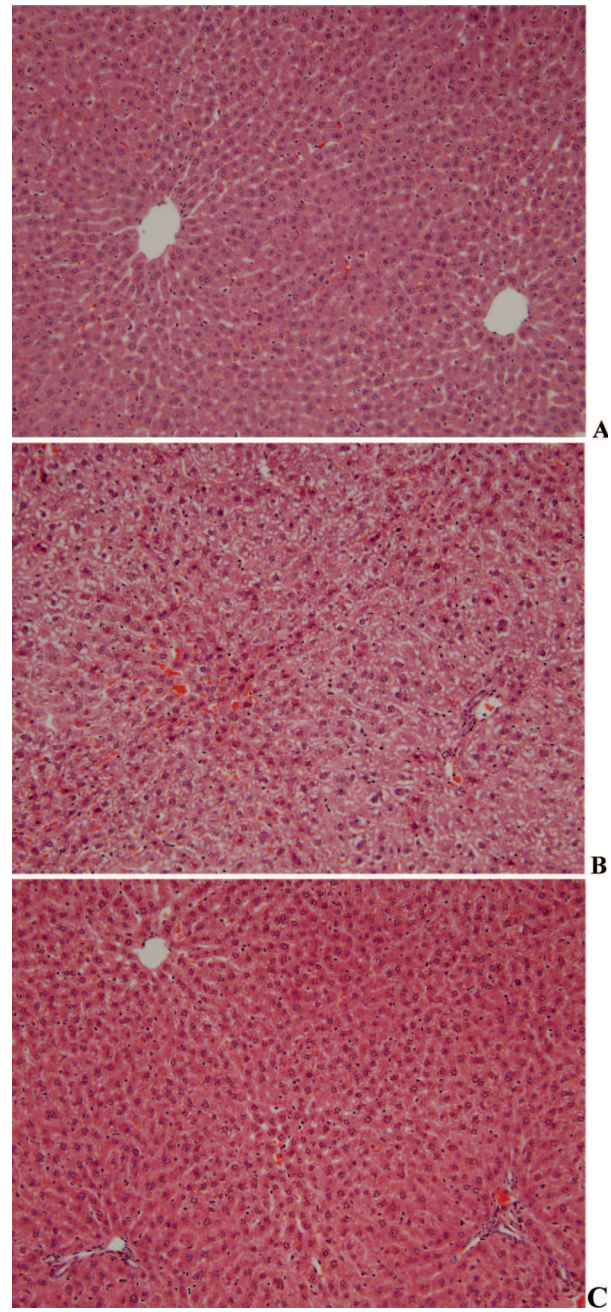

Figure 5. Histomorphological appearance of livers of female rats after treatment with the solvent only (controls) (A) or 24 h after administration of tacrine (B) or **2** (C): HE, original magnification × 200.

perform the docking study. The crystal structure of AChE (Protein Data Bank code 2CKM) was obtained from the Protein Data Bank. The active site was calculated with the "site finding" function of MOE. The docking results showed that **2** can adopt different binding modes within the active gorge of AChE (Figure 6). A potent interaction between the heterocycle moiety and residues Trp84 and Phe330 was observed through a $\pi-\pi$ interaction. Besides, **2** also presented a direct interaction with residue Asp72 through the formation of an H-bond between the secondary amine group at the side chain and the corresponding residue. Interestingly, with regard to the PAS, it was found that the end of the side chain is exactly located at the lip of the gorge, directly toward the PAS. Moderate interaction is therefore gained through a hydrophobic action of the side chain and the residue Trp279, indicating that the target compound might act as a dual inhibitor toward CAS and PAS. The side

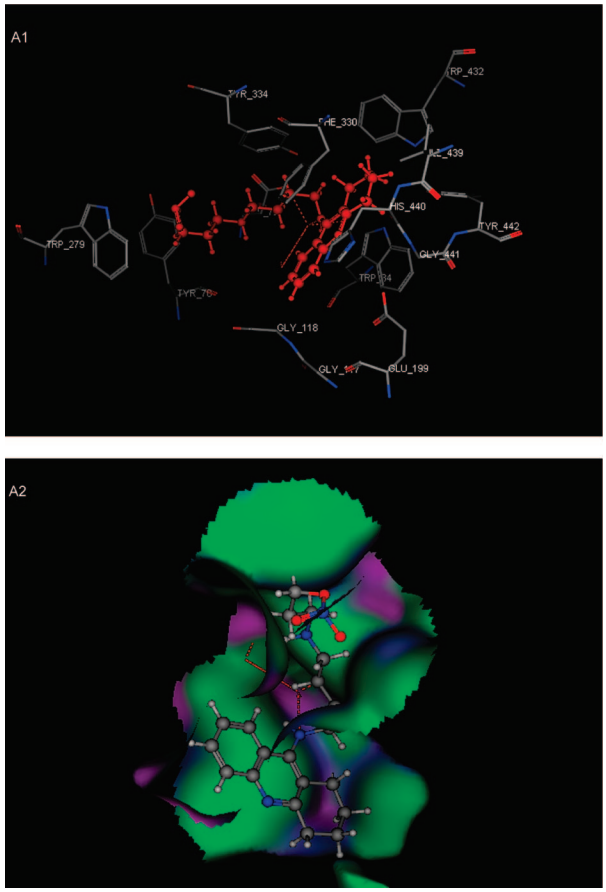

Figure 6. (A1) Docked complex of **2** and AChE (Protein Data Bank code 2CKM). Red is the ligand. (A2) Interaction surface of **2** and AChE (Protein Data Bank code 2CKM). Pictures were generated by MOE.

chain seems to play an important role in this action. The eight-atom long chain exactly covers the distance between the two active sites and possibly allows the compound to block them simultaneously.

In summary, we have designed and synthesized a series of amine-linked tacrine−NO donor hybrid compounds. All of the compounds effectively inhibited ChEs in vitro. Evaluated by the scopolamine-induced cognition impairment animal model, **1**, **2**, and **3a** showed significant cognition improving activity whereas the analogue **3h**, which is without the nitrate group, did not. This result indicates that the nitrate group of **2** not only may contribute to the vessel relaxant activity as previously reported[3] but also is essential for the ChE inhibitory effect. Additional hepatotoxicity studies confirmed the tacrine-induced liver damage whereas the hybrid compounds **1** and **2** did not show obvious signs of hepatotoxicity. Furthermore, the docking study showed that **2** may block the CAS and the PAS, acting as a dual-site inhibitor. Altogether, the distinct ChE inhibitory activity and cognition enhancing effects of the new nitrate−tacrine hybrid substances and the absence of hepatotoxicity qualify them as potential novel anti-AD drug candidates.

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Supporting Information Available: Experimental procedures for the synthesis of **3a**−**h**, detailed procedures of pharmacological investigations, and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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