

Letters

Synthesis and Biological Evaluation of NO-Donor-Tacrine Hybrids as Hepatoprotective Anti-Alzheimer Drug Candidates

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Abstract: In search of safer anti-Alzheimer drugs, 14 NO-donor-tacrine hybrids (**1–14**) were synthesized and evaluated for their ability to inhibit cholinesterases and for vasorelaxation effects. Compounds **1–13** showed good cholinesterases inhibitory activities in vitro, while **14**, particularly, was highly selective, preferring butyrylcholinesterase rather than acetylcholinesterase. Four selected compounds (**1**, **9**, **11**, and **14**) moderately relaxed the porcine pulmonary arteries in organ bath. In the hepatotoxicity study, significant hepatotoxicity was caused by tacrine but not by **9**.

Alzheimer's disease (AD^a) is recognized as one of the most severe conditions affecting the aged and is life-threatening for this group of people. The disease is characterized by neuronal loss, synaptic damage, and vascular plaques. Many pathologic factors, such as the deficiency of synaptic acetylcholine (ACh) and other related neurotransmitters, the formation of neuritic and vascular plaque, poor blood supply in the brain, and the inflammation of neurons, are known to cause this disease.¹ Over time, the knowledge about pathogenesis and the development of the neurodegeneration associated with AD has been organized into two main theories, namely, the cholinergic and the amyloid hypotheses, which focus on the loss of cholinergic neurotransmission and the emergence of the toxic amyloid plaque, respectively.² To date, most of the therapeutic strategies for patients with AD are based on these two theories. Tacrine, a reversible acetylcholinesterase inhibitor (AChEI), is one of the

major drugs approved for the clinical treatment of AD.^{3,4} The rationale for its use was related to the elevation of ACh levels that can compensate for the cholinergic deficiency associated with the brain lesions in patients with AD. Nevertheless, the deficiency of tacrine in clinic has been related mainly to elevated liver transaminase levels and dose-related, low-selective peripheral cholinergic effects. And it can halt the progression of AD for only about a year.^{5,6} Thus, the search for tacrine analogues or related new candidates is still of interest to medicinal chemists involved in AD research.

Developing novel agents with multiple effects is one of common strategies in today's search for new treatments of AD.^{7,8} Many such compounds have been reported, such as homodimeric tacrine congeners,⁹ lipoic acid-tacrine hybrids,¹⁰ tacrine-dihydropyridine hybrids,¹¹ galantamine-nitrate derivatives,¹² and so on. The activity of many of these compounds has been improved by the introduction of the second pharmacophores. Here we have designed and synthesized a series of tacrine hybrids with NO-donating nitrate- and diazeniumdiolate (NONOate) moieties connected via an alkylenediamine-type spacer. NO-releasing derivatives have currently come into focus in the treatment of cancer, inflammation, and vascular diseases. Some of them have performed fairly well because NO is a key signaling molecule involved in the regulation of many physiological processes.¹³ NO is also reported to play an important role in the nervous system and some NO donors or NO mimetic molecules have had a positive effect on the treatment of AD.¹⁴ Though the mechanism is not yet clear, two effects of NO might be very important: it increases blood supply and it relieves inflammatory reactions in the brain, both of which are therapeutically beneficial for patients with AD. In the design of the target molecules, an alkylenediamine side chain is introduced at the 9-position of the tacrine-like heterocycle. The side chain is considered to be not only as a spacer between tacrine and the NO donor, but also beneficial for inhibiting the periphery anion site (PAS) of AChE,¹⁰ which is thought to relate to the neurotoxic cascade of AD through AChE-induced amyloid- β aggregation.¹⁵ In addition, a protonatable nitrogen containing side chain of the tacrine-like heterocycle has been suggested to significantly improve the inhibitory potency of cholinesterase (ChE) because of the interaction with the midgorge recognition site.¹⁶ Therefore, the rationale of the target compounds is a synergic action of the NO donor, the tacrine-like heterocycle and the alkylenediamine spacer might yield potent but less toxic drug candidates for the better and safer treatment of AD.

The synthesis of amine- and amide-linked nitrate- and NONOate-tacrine hybrids is outlined in Schemes 1 and 2. Because an alkylenediamine side chain was introduced to the hybrids, the synthesis did not utilize tacrine itself as a starting material, but 9-chloro-1,2,3,4-tetrahydroacridine¹⁷ was reacted with alkylenediamines to produce the important intermediates 9-aminoalkylamino-1,2,3,4-tetrahydroacridines **15a,b**. To synthesize the amine-linked tacrine hybrids **5–10**, the bromoalkanol were nitrated by 100% HNO₃ and, subsequently, the bromoalkylnitrates were condensed with **15** to yield the target compounds. Intermediates **15a,b** were acylated by the corresponding bromoacyl chloride to give the halogenated intermediates **16a–d** and then conveniently converted to the amide-linked

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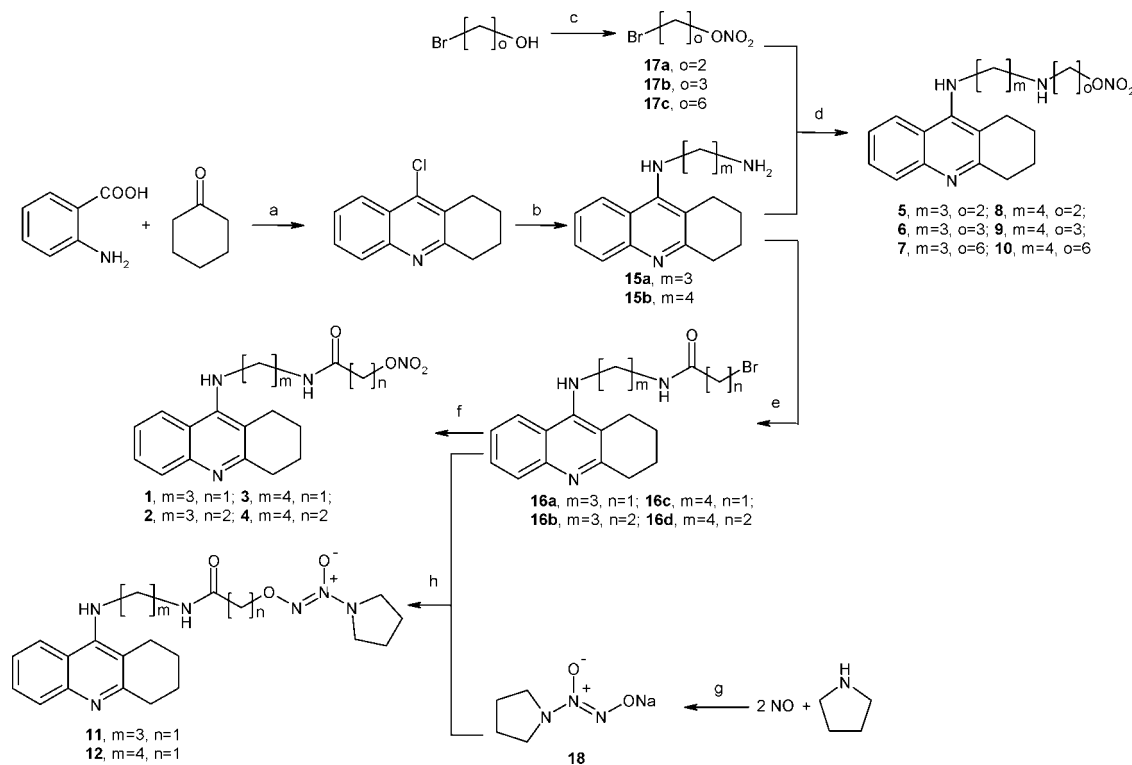
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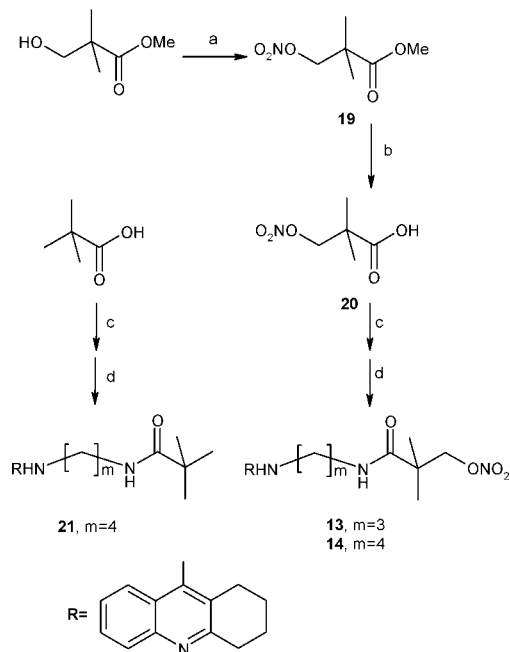
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^a Abbreviations: AD, Alzheimer's disease; ACh, acetylcholine; AChEI, acetylcholinesterase inhibitor; PAS, peripheral anionic site; ChE, cholinesterase; HMPA, hexamethylphosphotriamide; CDI, *N,N'*-carbonyldiimidazole; BuChE, butyrylcholinesterase; ISDN, isosorbide dinitrate; ASAT, aspartate aminotransferase; LDH, lactate dehydrogenase.

Scheme 1. General Method for the Synthesis of 1–12^a

^a Reagents and conditions: (a) POCl₃, reflux, 3 h; (b) H₂N(CH₂)_mNH₂, pentanol, reflux, 18 h; (c) 100% HNO₃, CH₂Cl₂, -5 °C; (d) K₂CO₃, CH₂Cl₂, 24 h, rt; (e) Br(CH₂)_mCOCl, CH₂Cl₂, -5 °C, 0.5 h; (f) CH₃CN, AgNO₃, reflux, 8 h; (g) CH₃OH, Na; (h) K₂CO₃, HMPA, 72 h, rt.

Scheme 2. General Method for the Synthesis of 13 and 14^a

^a Reagents and conditions: (a) 100% HNO₃, CH₂Cl₂, 1.5 h, rt; (b) NaOH, ethanol, 3 h, rt; (c) CDI, CH₂Cl₂, 1.5 h, rt; (d) 15, 3 h, rt.

nitrate 1–4 by treatment with AgNO₃ in dry CH₃CN. For the NONOate derivatives 11 and 12, compound 18 was first prepared according to a previously reported protocol¹⁸ and then reacted with 16 in HMPA in the presence of K₂CO₃ to offer the target compounds. Pivalic acid was also used as a further spacer, resulting in 13, 14, and analogue 21. Compound 20¹⁹ or pivalic acid was activated by CDI and then reacted with 15 to give the corresponding product.

Table 1. Inhibition of AChE and BuChE (IC₅₀ Values) and Selectivity Expressed as the Ratio of the Resulting IC₅₀ Values

cmpd	IC ₅₀ (nM) ± SEM ^a		selectivity ratio ^b
	AChE	BuChE	
1	28.2 ± 4.0	13.5 ± 3.1	2.1
2	93.0 ± 37	28.0 ± 14	3.3
3	22.6 ± 7.4	12.1 ± 3.1	1.9
4	45.7 ± 14	15.1 ± 3.2	3.0
5	10.0 ± 2.3	5.2 ± 1.4	1.9
6	6.4 ± 0.5	5.5 ± 1.8	1.2
7	6.3 ± 0.6	21.7 ± 9.7	0.3
8	11.1 ± 2.4	13.8 ± 0.9	0.8
9	5.6 ± 0.7	9.9 ± 1.1	0.6
10	18.1 ± 3.6	10.8 ± 1.5	1.7
11	35.5 ± 3.8	15.3 ± 4.7	2.3
12	50.7 ± 7.5	41.0 ± 5.6	1.2
13	69.5 ± 9.2	5.5 ± 1.3	12.6
14	226.0 ± 91	7.3 ± 2.0	31.0
21	21.6 ± 2.7	36.5 ± 3.7	0.6
tacrine	45.1 ± 6.9	5.1 ± 1.0	8.8

^a Data is the mean of at least three determinations. ^b Selectivity ratio = (IC₅₀ of AChE)/(IC₅₀ of BuChE).

The ChE inhibitory activity of 1–14 was measured in vitro using Ellman's assay^{20,21} (Table 1). With the exception of 14 (226.0 nM), all target compounds showed good inhibitory activity against AChE with IC₅₀ values varying from 5.2 to 93 nM. Compared to tacrine (45 nM), the potency of 6, 7, and 9 (6.4, 6.3, and 5.6 nM) is 7 ~ 8-fold improved. The effect levels of all compounds against butyrylcholinesterase (BuChE) were also similar to tacrine (5.1 nM) with IC₅₀ values varying from 5.2 to 41.0 nM. These results seem consistent with the previous reports of lipoic acid-tacrine hybrids¹⁰ and huperzine A-tacrine hybrids.¹⁶ Among all of the compounds, compound 6 and 9 showed potent inhibitory effects on both AChE and BuChE, while 14 seems special, showing a significant selectivity toward BuChE with a 31-fold selective ratio. BuChE inhibition has

recently been regarded as therapeutically beneficial for AD, because BuChE seems able to act as an alternative to compensate AChE for processing of AD.²² The selectivity of **14** is likely due to the large steric hindrance of the spacer. At the midgorge level AChE and BuChE differ in their amino acid composition, because AChE presents several aromatic residues that are replaced by smaller aliphatic ones in BuChE. These structural differences are supposed to be responsible for the larger electrostatic gradient and for the narrower void along the AChE gorge with respect to BuChE.²³ Given the considerable steric demand of **14**, it seems understandable that **14** binds to BuChE much more easily than to AChE. To determine the influence of the nitrate group on the selectivity of BuChE, compound **21** was also synthesized and screened for its ability to inhibit ChEs. The results showed the ability of **21** to inhibit AChE (21.6 nM) is significantly improved compared to **14** (226.0 nM), while the ability to inhibit BuChE is reduced. Thus, the selectivity toward BuChE disappears, which indicates the nitrate group of **14** may be necessary for developing such selectivity, though whether the influence of the nitrate group originates from the electrostatic effect or the steric hindrance remains unclear.

The poor blood supply to the brain has recently been considered an important pathologic factor initiating the progression of AD. NO donors can significantly increase blood supply by relaxing vessels.²⁴ To determine their vasorelaxation effects, compounds **1**, **9**, **11**, and **14**, which effectively inhibited ChEs in vitro and stand for the amide-linked nitrate derivatives, amine-linked nitrate derivatives, NONOate derivatives, and pivalic acid-linked derivatives, respectively, were selected for use in an in vitro vascular relaxation assay (organ bath) using PGF_{2α}-precontracted porcine pulmonary arteries. Isosorbide dinitrate (ISDN) and tacrine were chosen as positive and negative controls, respectively. The results are shown in Figure 1. Expectedly, ISDN showed the strongest activity with an EC₅₀ value of 0.42 μM. Compared to tacrine, compounds **1**, **9**, and **14** showed moderate vasorelaxation effects with EC₅₀ values varying from 3.68 to 10.47 μM, while the activity of **11** is relatively low, with an EC₅₀ value of 22.54 μM. In this tacrine-NONOate hybrid, the NONOate moiety is protected prodrug-like and, thus, can not spontaneously release NO under the in vitro conditions of the organ bath.

The hepatotoxicity is a main limitation for the clinical use of tacrine. It is reported to elevate levels of liver transaminase and decrease albumin concentration.²⁵ NO production in the liver is usually increased in response to acute insult with hepatotoxins and may become a mediator of the tissue damage.²⁶ Some NO donors, like V-PYRRO/NO²⁷ and nitrate,²⁸ are reportedly able to prevent the liver from injury. To determine whether the introduction of a NO donor could improve the safety

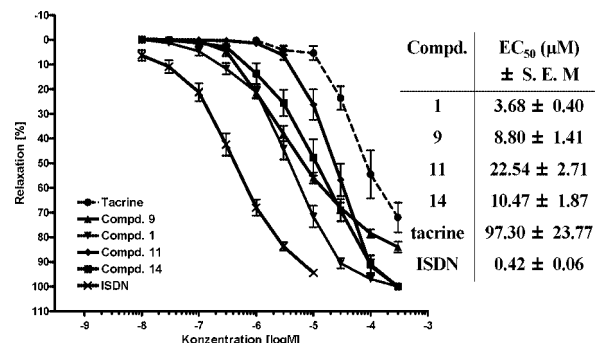


Figure 1. Concentration–response curves for the vasorelaxant effects of tacrine, ISDN, and compounds **1**, **9**, **11**, and **14** on PGF_{2α} (3 mM)-precontracted porcine pulmonary arteries in vitro. Data are means ± SEM from 4 to 6 independent experiments.

of the parent compound, compound **9** was chosen to be tested its hepatotoxicity. After equimolar doses of tacrine and **9** were administered, levels of aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH) and albumin in serum as well as the concentration of protein in liver tissue were investigated (Table 2). Compared to the control, significant hepatotoxicity was caused by tacrine, as indicated by the increased activity of the ASAT and LDH as well as by the reduced concentration of albumin and protein in liver tissue. In contrast, the values in compound **9** group appeared to remain unchanged. Clearly, compound **9** did not show any hepatotoxicity, but tacrine did.

In summary, we have designed and synthesized 14 novel tacrine hybrid compounds with NO-donating nitrate and NONOate moieties connected to the tacrine template via an alkylenediamine-type spacer. All compounds effectively inhibited ChEs in vitro. Three target compounds (**6**, **7**, and **9**), in particular, showed 7 ~ 8-fold higher AChE inhibitory activity compared to tacrine, while compound **14** was found to be selective toward BuChE rather than AChE. Compounds **1**, **9**, **11**, and **14** moderately relaxed the porcine pulmonary arteries in in vitro vasorelaxation experiments (organ bath), aided by the NO donor part of the molecule. In the in vivo hepatotoxicity studies, tacrine, but not compound **9**, was found to cause serious hepatotoxicity. The results suggest that these NO-donor-tacrine hybrids, especially compound **9**, may be considered to be novel more potent and safer anti-Alzheimer's drug candidates.

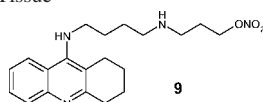
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Supporting Information Available: Experimental procedures for the synthesis of **1–14** and detailed procedures of pharmacologi-

Table 2. Influence of Equimolar Doses (5.93 μmol/100 g b.wt.) of Tacrine and **9** on the Activity of ASAT and LDH, as well as the Albumin Concentration in Serum, and Protein Concentration in Liver Tissue^a

time	serum						liver tissue	
	ASAT [μmol/s]		albumin [g/l]		LDH [μmol/s]		protein [mg/g]	
	tacrine	compd 9	tacrine	compd 9	tacrine	compd 9	tacrine	compd 9
before	2.5 ± 0.3	2.1 ± 0.2	16.3 ± 0.5	16.7 ± 0.5	4.2 ± 0.8	4.6 ± 0.6	138 ± 1	
12 h	4.5 ± 0.8 ^b	2.3 ± 0.2	16.0 ± 0.4	16.6 ± 0.5	5.5 ± 0.9	4.4 ± 0.6	n.d.	n.d.
20 h	3.4 ± 0.3	2.8 ± 0.4	15.0 ± 0.2 ^b	17.1 ± 0.6	10.0 ± 3.2 ^b	5.6 ± 0.3	118 ± 4	143 ± 1
36 h	3.1 ± 0.4	2.1 ± 0.2	13.1 ± 0.3 ^b	15.1 ± 0.7	6.5 ± 1.5	4.1 ± 0.4	92 ± 2 ^b	123 ± 9

^a Results are compared to control values before the administration. ^b Statistical significant difference to control values before administration (Student's *t*-test, *p* ≤ 0.05, *n* = 3–6).



cal investigations (ChE inhibition assay, vascular relaxation study, and hepatotoxicity study). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Francotte, P.; Graindorge, E.; Boveriea, S.; de Tullioa, P.; Pirotea, B. New Trends in the Design of Drugs against Alzheimer's Disease. *Curr. Med. Chem.* **2004**, *11*, 1757–1778.
- (2) Scarpini, E.; Scheltens, P.; Feldman, H. Treatment of Alzheimer's Disease: Current Status and New Perspectives. *Neurology* **2003**, *2*, 539–547.
- (3) Knapp, M. J.; Knopman, D. S.; Soloman, P. R.; Pendlebury, W. W.; Davis, C. S.; Gracon, S. I. A 30-week Randomized Controlled Trial of High-Dose Tacrine in Patients with Alzheimer's Disease. *J. Am. Med. Assoc.* **1994**, *271*, 985–991.
- (4) Crismon, M. L. Tacrine: First Drug Approved for Alzheimer's Disease. *Ann. Pharmacother.* **1994**, *28*, 744–751.
- (5) Watkins, P. B.; Zimmerman, H. J.; Knapp, M. J.; Gracon, S. I.; Lewis, K. W. Hepatotoxic Effects of Tacrine Administration In Patients with Alzheimer's Disease. *J. Am. Med. Assoc.* **1994**, *271*, 992–998.
- (6) Ames, D. J.; Bhathal, P. S.; Davies, B. M.; Fraser, J. R. E.; Gibson, P. R.; Roberts, S. Heterogeneity of Adverse Hepatic Reactions to Tetrahydroaminoacridine. *N. Z. J. Med.* **1990**, *20*, 193–195.
- (7) Decker, M. Recent Advances in the Development of Hybrid Molecules/Designed Multiple Compounds with Antiamnesic Properties. *Mini-Rev. Med. Chem.* **2007**, *7*, 221–229.
- (8) Youdim, M. B. H.; Buccafusco, J. J. Multi-Functional Drugs for Various CNS Targets in the Treatment of Neurodegenerative Disorders. *Trends Pharmacol. Sci.* **2005**, *26*, 27–35.
- (9) (a) Hu, M. K.; Wu, L. J.; Hsiao, G.; Yen, M. H. Homodimeric Tacrine Congeners as Acetylcholinesterase Inhibitors. *J. Med. Chem.* **2002**, *45*, 2277–2282. (b) Carlier, P. R.; Chow, E. S.-H.; Han, Y.; Liu, J.; Yazal, J. E.; Pang, Y.-P. Heterodimeric Tacrine-Based Acetylcholinesterase Inhibitors: Investigating Ligand-Peripheral Site Interactions. *J. Med. Chem.* **1999**, *42*, 4225–4231. (c) Carlier, P. R.; Du, D.-M.; Han, Y.-F.; Liu, J.; Pang, Y.-P. Potent Easily Synthesized Huperzine A-Tacrine Hybrid Acetylcholinesterase Inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2335–2338.
- (10) Rosini, M.; Andrisano, V.; Bartolini, M.; Bolognesi, M. L.; Hrelia, P.; Minarini, A.; Tarozzi, A.; Melchiorre, C. Rational Approach To Discover Multipotent Anti-Alzheimer Drugs. *J. Med. Chem.* **2005**, *48*, 360–363.
- (11) Marco-Contelles, J.; León, R.; de los Ríos, C.; Guglietta, A.; Terencio, J.; López, M. G.; García, A. G.; Villarroja, M. Novel Multipotent Tacrine-Dihydropyridine Hybrids with Improved Acetylcholinesterase Inhibitory and Neuroprotective Activities as Potential Drugs for the Treatment of Alzheimer's Disease. *J. Med. Chem.* **2006**, *49*, 7607–7610.
- (12) Perissutti, E.; Fiorino, F.; Severino, B.; Frecentese, F.; Massarelli, P.; Nencini, C.; Santagada, V.; Caliendo, G. Synthesis of 6 β -D-Glucosyl and 6-Nitroxy (–)-Galanthamine Derivatives as Acetylcholinesterase Inhibitors. *Pharmazie* **2007**, *62*, 403–405.
- (13) Megson, I. L.; Webb, D. J. Nitric Oxide Donor Drugs: Current Status and Future Trends. *Expert Opin. Invest. Drugs* **2002**, *11*, 587–601.
- (14) Thatcher, G. R. J.; Bennett, B. M.; Reynolds, J. N. Nitric Oxide Mimetic Molecules as Therapeutic Agents in Alzheimer's Disease. *Curr. Alzheimer Res.* **2005**, *2*, 171–182.
- (15) Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. β -Amyloid Aggregation Induced by Human Acetylcholinesterase: Inhibition Studies. *Biochem. Pharmacol.* **2003**, *65*, 407–416.
- (16) 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. Discovery of Huperzine A-Tacrine Hybrids as Potent Inhibitors of Human Cholinesterases Targeting Their Midgorg Recognition Sites. *J. Med. Chem.* **2006**, *49*, 3421–3425.
- (17) Carlier, P. R.; Han, Y. F.; Chow, E. S.-H.; Li, C. P.-L.; Wang, H.; Lieu, T. X.; Wong, H. S.; Pang, Y.-P. Evaluation of Short-Tether Bis-THA AChE Inhibitors. A Further Test of the Dual Binding Site Hypothesis. *Bioorg. Med. Chem.* **1999**, *7*, 351–357.
- (18) Velázquez, C.; Rao, P. N. P.; Knaus, E. E. Novel Nonsteroidal Anti-Inflammatory Drugs Possessing a Nitric Oxide Donor Diazen-1-ium-1,2-diolate Moiety: Design, Synthesis, Biological Evaluation, and Nitric Oxide Release Studies. *J. Med. Chem.* **2005**, *48*, 4061–4067.
- (19) Decker, M.; Koenig, A.; Glusa, E.; Lehmann, J. Synthesis and Vasorelaxant Properties of Hybrid Molecules Out of NO-Donors and the Beta-Receptor Blocking Drug Propranolol. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4995–4997.
- (20) Decker, M. Novel Inhibitors of Acetyl- and Butyrylcholinesterase Derived from the Alkaloids Dehydroevodiamine and Rutacarpine. *Eur. J. Med. Chem.* **2005**, *40*, 305–313.
- (21) Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95.
- (22) Giacobini, E. *Cognitive Enhancing Drugs*; Birkhäuser Verlag: Basel, Boston, Berlin, 2004; pp 11–36.
- (23) Saxena, A.; Redman, A. M. G.; Jiang, X.; Lockridge, O.; Doctor, B. P. Differences in Active Site Gorge Dimension of Cholinesterases Revealed by Binding of Inhibitors to Human Butyrylcholinesterase. *Biochemistry* **1997**, *36*, 14642–14651.
- (24) Duncan, A. J.; Heales, S. J. R. Nitric Oxide and Neurological Disorders. *Mol. Aspects Med.* **2005**, *26*, 67–96.
- (25) Gracon, S. I.; Knapp, M. J.; Berghoff, W. G.; Pierce, M.; de Jong, R.; Lobbstaël, S. J.; Symons, J.; Dombey, S. L.; Luscombe, F. A.; Kraemer, D. Safety of Tacrine: Clinical Trials, Treatment IND, and Postmarketing Experience. *Alzheimer Dis. Assoc. Disord.* **1998**, *12*, 93–101.
- (26) Liu, J.; Waalkes, M. P. Nitric Oxide and Chemically Induced Hepatotoxicity: Beneficial Effects of the Liver-Selective Nitric Oxide Donor, V-PYRRO/NO. *Toxicology* **2005**, *208*, 289–297.
- (27) Liu, J.; Qu, W.; Saavedra, J. E.; Waalkes, M. P. The Nitric Oxide Donor, O₂-vinyl 1-(Pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO), Protects against Cadmium-Induced Hepatotoxicity in Mice. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 18–24.
- (28) Futter, L. E.; al-Swayeh, O. A.; Moore, P. K. A Comparison of the Effect of Nitroparacetamol and Paracetamol on Liver Injury. *Br. J. Pharmacol.* **2001**, *132*, 10–12.

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