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Bivalent β-Carbolines as Potential Multitarget Anti-Alzheimer Agents

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Alzheimer's disease (AD) is a prevalent neurodegenerative disorder with multifactorial causes that requires multitargeted treatment. Inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) improve cholinergic signaling in the central nervous system and thus AChE inhibitors are well established in the therapy of AD to improve memory disturbances and other cognitive symptoms. On the other hand, AD patients benefit from reduction of pathologic glutamate-induced, Ca^{2+}-mediated excitotoxicity by the *N*-methyl-D-aspartate receptor (NR) antagonist memantine. New drugs that simultaneously affect both cholinergic transmission and glutamate-induced excitotoxicity may further improve AD treatment. While connecting β-carboline units by alkylene spacers in two different series of compounds and subsequent evaluation of their AChE/BChE-inhibitory potential, we found that several of these bivalent β-carbolines were potent NR blockers. The most promising compound was a N^9-homobivalent β-carboline with a nonylene spacer, which displayed IC_{50} values of 0.5 nM for AChE, 5.7 nM for BChE, and 1.4 μM for NR, respectively.

Introduction

Alzheimer's disease (AD[a]) is a chronic and progressive neurodegenerative disorder that affects cognition, behavior, and function and is the most common manifestation of dementia. The World Health Organization estimates that about 18 million people worldwide presently suffer from AD, and this number may approach 34 million by the year 2025. Thus, serious attention has to be paid to the development of effective medications that can delay disease progression and the hospitalization of affected patients. Medicinal AD treatment is currently based on three different types of drugs: extracts from the leaves of *Ginkgo biloba*, AChE inhibitors, and the NMDA receptor antagonist memantine.

β-Carbolines (pyrido[3,4-*b*]indoles) were first discovered in plants and are referred to as harman alkaloids because they were first characterized in *Peganum harmala*.[1] In the human body, they may be formed from the biogenic amines tryptamine and serotonin by condensation with aldehydes or α-keto acids.[2] Several studies have shown that β-carbolines are effective inhibitors of both AChE and BChE.[3−5] AChE inhibitors are proposed as a first line therapy of moderate AD and may symptomatically enhance the cognitive state of patients to some degree, albeit for a limited period of time.[6−8]

Recently (*S*)-glutamate-mediated neurotransmission has come into focus in the search for new antidementia drugs. In the brain, (*S*)-glutamate mediates normal physiological excitatory synaptic transmission, which is important for plastic synaptic changes including long-term potentiation, memory, and learning.[9] Glutamate-activated ionotropic receptors can be distinguished by their selective agonists: NMDA, α-amino-3-hydroxy-5-methyl-isoxazol-4-yl-propionic acid (AMPA), and kainate.[10] Among these, NMDA receptors (NRs) have the highest permeability to calcium. Therefore, pathophysiological conditions causing high extracellular concentrations of glutamate favor excessive calcium influx into neuronal cells through activated NRs, which promotes the production of free radicals and activation of proteolytic processes that contribute to neuronal cell damage. This is called glutamate-induced excitotoxicity, and it is thought to be a major contribution to neurodegenerative disorders like AD,[11] cerebral ischemia and stroke,[12] epilepsy,[13] Huntington disease,[14] amyotrophic lateral sclerosis,[15] Morbus Parkinson,[16] and AIDS-associated dementia.[17]

Functional NRs form heterotetramer complexes assembled from three different classes of subunits, named NR1, NR2, and NR3 (reviewed in ref 18). The human genome encodes one gene for NR1, which occurs in eight splice variants (NR1-1a to NR1-1h) and binds the NR coagonist glycine. There are four NR2 proteins (NR2A-D) that contain the binding site of the (*S*)-glutamate agonist. Functional NRs are composed of multiple NR1 subunits in combination with at least one type of NR2 subunit. Both (*S*)-glutamate and glycine are required for activation of these receptor complexes. NR3A or NR3B subunits can assemble with NR1/NR2 to depress NR responses, but NR3 can also assemble with NR1 to form functional glycine receptors. The cell type-specific subunit composition of NRs determines their pharmacological properties in different areas of the brain.[18]

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[a]Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; BChE, butyrylcholinesterase; NMDA, *N*-methyl-D-aspartate; NR, NMDA receptor; LDH, lactate dehydrogenase.

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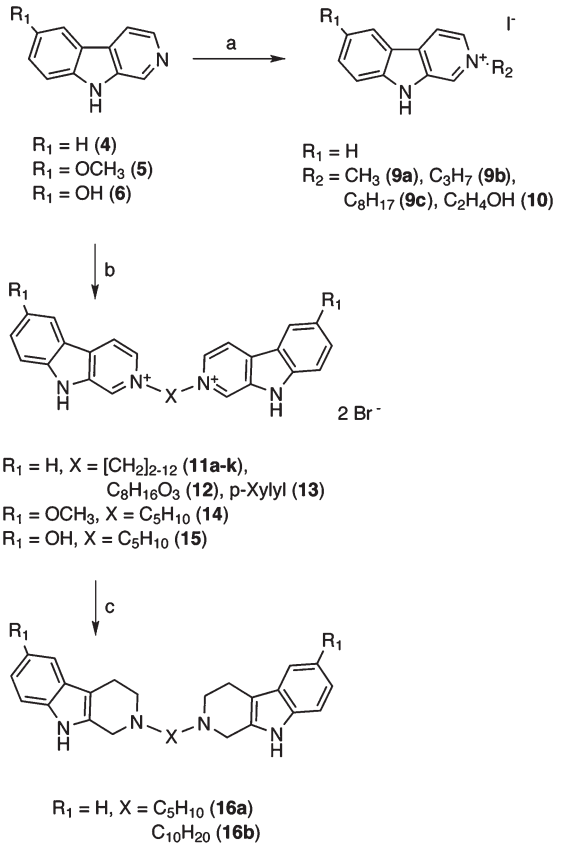
Scheme 1. Synthesis of the N^2-Substituted Monovalent and N^2-Bivalent β-Carbolines (**9**−**16**)[a]



R_1 = H (**4**)
R_1 = OCH_3 (**5**)
R_1 = OH (**6**)

R_1 = H
R_2 = CH_3 (**9a**), C_3H_7 (**9b**), C_8H_{17} (**9c**), C_2H_4OH (**10**)

R_1 = H, X = $[CH_2]_{2\text{-}12}$ (**11a-k**), $C_8H_{16}O_3$ (**12**), p-Xylyl (**13**)
R_1 = OCH_3, X = C_5H_{10} (**14**)
R_1 = OH, X = C_5H_{10} (**15**)

R_1 = H, X = C_5H_{10} (**16a**)
$C_{10}H_{20}$ (**16b**)

[a] The monovalent β-carbolines not listed in this scheme have been described elsewhere: 6-methoxy-1,2,3,4-tetrahydro-β-carboline (**2**),[5] 3,4-dihydro-6-methoxy-β-carboline (**3**),[20] 6-methoxy-β-carboline (**5**),[5] 6-hydroxy-β-carboline (**6**),[5] 6-methoxy-1-methyl-β-carboline (**7**),[5] and 6-bromo-β-carboline (**8**).[21] Compounds 1,2,3,4-tetrahydro-β-carboline (**1**) and β-carboline (**4**) were prepared like the 6-methoxy- and 6-hydroxy derivatives.[5] (a) Alkyl iodide, acetone, room temperature; (b) α,ω-dibromoalkane, DMF, reflux; (c) $NaBH_4$, methanol.

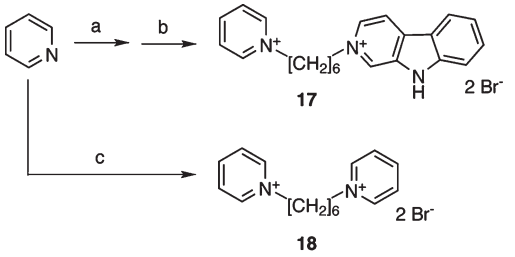
Memantine is the first approved drug of a new class of AD-modifying therapeutics that target the NMDA receptor, and it is recommended for patients with moderate to severe AD.[17,19] Memantine seems to possess neuroprotective activity by inhibiting glutamate-induced excitotoxicity.[17,19]

The overall unsatisfactory efficacy of the medicinal treatment of AD clearly demands a search for new dementia-modifying drugs. Several previous studies have found that monovalent β-carbolines are potent AChE/BChE inhibitors.[3−5] While investigating the AChE/BChE inhibitory potential of bivalent β-carboline compounds, we found that several of these drugs were also potent NMDA receptor blockers. Thus, bivalent β-carboline compounds may provide a novel multitarget approach for the treatment of AD.

Chemistry

In addition to available monovalent β-carbolines, two different series of bivalent β-carbolines were synthesized: one series with a 2−12 carbon spacer between the two pyridine nitrogens (N^2-series) and the other connected via the indole nitrogens (N^9-series). Some monovalent β-carboline derivatives (**1**−**8**) have been previously described.[5,20,21] Further N^2-substituted carbolinium salts (**9a**−**c**, **10**) have been prepared by adding the appropriate alkyl iodide to β-carboline in acetone. To prepare bivalent derivatives **11**−**15** of the N^2-series, we reacted the appropriate β-carbolines **4**−**6** with α,ω-dibromoalkanes in DMF as described for bis-pyridinium salts by Musilek et al.[22] The bivalent derivatives **11d** and **11i** were converted to their partially reduced analogues **16a** and **16b** by using $NaBH_4$ in methanol (Scheme 1).

Scheme 2. Synthesis of N^2-Hetero/Homobivalent Compounds (**17**,**18**)[a]



17

18

[a] (a) 1,6-Dibromohexane, MeCN, room temperature; (b) β-carboline **4**, MeCN; (c) 1,6-dibromohexane, DMF, reflux.

Additionally, we prepared the bivalent bis-pyridinium derivative **18** (Scheme 2) and the β-carboline/pyridine hetero-bivalent compound **17**, the latter by a two-step procedure: ω-bromohexylpyridinium bromide was prepared from pyridine and excessive dibromohexane and then reacted with β-carboline **4** to give **17** (Scheme 2).

The N^9-substituted monovalent β-carbolines **19a**−**b** were prepared by alkylation of the deprotonated β-carboline **4** and were further derivatized with methyl iodide to compounds **20a** and **20b** (Scheme 3). As described for bivalent carbazoles,[23] the synthesis of the symmetrical N^9-bivalent compounds **21a**−**c** was achieved by reaction of an α,ω-dibromoalkane with deprotonated β-carboline **4** in DMSO. **21a**−**c** were converted to quaternary salts **22a**−**c** with methyl iodide in acetone. $NaBH_4$-reduction of **22b** gave partially reduced β-carboline derivative **23** (Scheme 3).

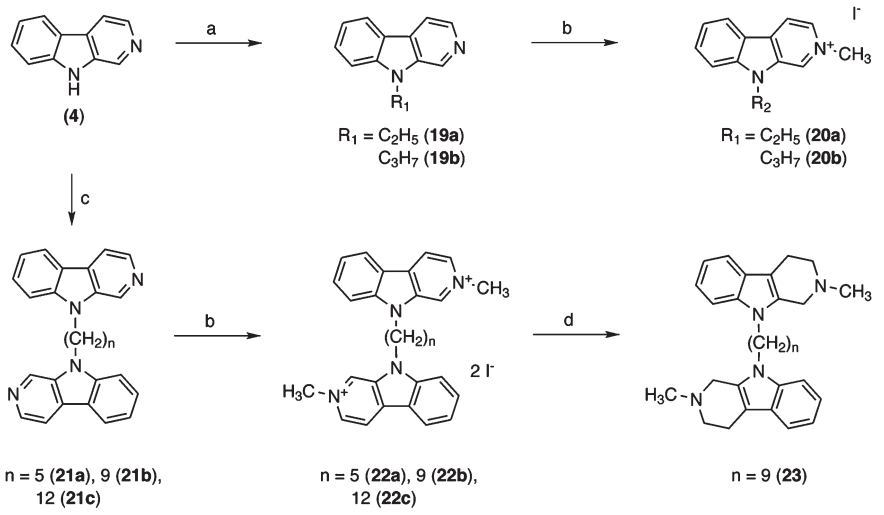
Results and Discussion

As discussed below, some monovalent (**1**−**8**) and the mono- and bivalent β-carbolines listed in Table 1 were screened for their inhibitory activities on AChE, BChE, and NMDA receptors. Known inhibitors of either the AChE/BChE enzymes or the NMDA receptor were used as references in both assays. The most active AChE/BChE and NR inhibitors were also tested for their general cytotoxicity. These results are summarized in Table S1 of the Supporting Information.

Bivalent β-Carbolines as AChE/BChE Inhibitors. Depending on the spacer length, the N^2-bivalent β-carbolines showed moderate to strong inhibitory properties for both AChE and BChE. Compounds **11a**−**11e**, **14**, and **15** with a spacer of less than six atoms showed moderate activities, whereas compounds **11f**−**11k** with a spacer of more than six atoms displayed stronger activities in the nanomolar range for both cholinesterases. Substituents at the aromatic residue (**14**, **15**) and replacement of the tricyclic aromatic residues with pyridinium (**17**, **18**), partial reduction (**16a**−**b**), and variation of the spacer (**12**, **13**) reduced the activities of the compounds.

The N^9-bivalent β-carbolines without a permanent positive charge (**21a**−**c**) generally showed very low AChE/BChE-inhibitory activities. By contrast, methylation at position 2, introducing a permanent positive charge in the structure,

Scheme 3. Synthesis of the N^9-Monovalent/-Bivalent β-Carbolines (**19**−**23**)[a]



[a] (a) NaH, alkyl iodide, DMF; (b) methyl iodide, acetone, room temperature; (c) NaOH, 1,6-dibromohexane, DMSO; (d) $NaBH_4$, methanol.

Table 1. Cholinesterase and NMDA Receptor Inhibitory Activities of Compounds Tested in This Study[a]

	cholinesterase inhibition			NR inhibition	
compd	AChE IC_{50} [nM] ($pIC_{50} \pm$ SEM)	BChE IC_{50} [nM] ($pIC_{50} \pm$ SEM)	selectivity (IC_{50}(BChE)/ IC_{50}(AChE)	L12-G10 IC_{50} [μM] ± SD (excitotoxicity [%] at 25 μM)	L13-E6 IC_{50} [μM] ± SD (excitotoxicity [%] at 25 μM)
9a	> 10000	540 (6.267 ± 0.068)	< 0.1	nd (96.4 ± 2.2)	nd (94.4 ± 1.5)
9b	845 (6.073 ± 0.030)	2305 (5.637 ± 0.099)	2.7	nd (139.0 ± 7.0)	nd (121.0 ± 3.9)
11a	430 (6.367 ± 0.077)	319 (6.496 ± 0.063)	0.7	nd (83.6 ± 10.2)	nd (85.2 ± 1.9)
11b	7661 (5.116 ± 0.139)	652 (6.186 ± 0.029)	0.1	24.8 ± 7.4 (43.8 ± 9.5)	17.3 ± 4.9 (40.5 ± 14.5)
11c	278 (6.556 ± 0.158)	78 (7.109 ± 0.199)	0.3	nd (70.1 ± 2.5)	nd (88.5 ± 0.3)
11d	595 (6.225 ± 0.063)	48 (7.311 ± 0.088)	0.1	20.5 ± 6.1 (49.7 ± 1.5)	16.3 ± 1.5 (45.8 ± 7.9)
11e	248 (6.605 ± 0.070)	186 (6.730 ± 0.081)	0.8	2.2 ± 0.6 (2.1 ± 1.7)	6.5 ± 0.5 (15.0 ± 3.9)
11f	81 (7.091 ± 0.068)	65 (7.188 ± 0.026)	10.8	6.1 ± 1.5 (11.5 ± 2.5)	17.8 ± 5.2 (31.0 ± 5.5)
11g	113 (6.947 ± 0.076)	75 (7.120 ± 0.170)	0.7	3.5 ± 2.4 (3.6 ± 2.8)	6.0 ± 0.6 (7.5 ± 2.0)
11h	125 (6.903 ± 0.074)	40 (7.392 ± 0.052)	0.3	13.5 ± 5.6 (28.2 ± 12.5)	12.3 ± 3.2 (30.4 ± 0.9)
11i	63 (7.201 ± 0.040)	49 (7.309 ± 0.055)	0.8	9.1 ± 1.4 (10.4 ± 2.1)	5.9 ± 2.7 (8.6 ± 4.0)
11j	92 (7.035 ± 0.053)	18 (7.741 ± 0.041)	0.3	5.1 ± 0.8 (15.5 ± 2.7)	10.7 ± 0.7 (36.0 ± 12.1)
11k	86 (7.064 ± 0.024)	22 (7.665 ± 0.042)	0.3	2.6 ± 1.1 (2.6 ± 0.6)	1.8 ± 0.7 (6.3 ± 1.4)
12	3141 (5.503 ± 0.042)	195 (6.709 ± 0.023)	0.1	60.9 ± 6.9 (80.1 ± 8.8)	nd (89.3 ± 3.5)
13	147 (6.834 ± 0.110)	473 (6.358 ± 0.223)	3.2	nd (58.3 ± 6.4)	nd (76.3 ± 10.5)
14	> 10000	319 (6.496 ± 0.063)	< 0.03	29.4 ± 2.5 (61.2 ± 4.0)	nd (65.3 ± 4.4)
15	4261 (5.371 ± 0.371)	1629 (5.788 ± 0.131)	0.4	22.7 ± 6.3 (63.2 ± 9.6)	nd (75.8 ± 2.1)
16a	> 1000	> 1000		nd (104.2 ± 7.7)	nd (124.2 ± 0.8)
16b	> 1000	2895 (5.538 ± 0.073)	< 3	nd (103.6 ± 6.5)	nd (101.4 ± 11.6)
17	564 (6.249 ± 0.034)	6331 (5.199 ± 0.040)	11.2	nd (87.0 ± 4.2)	nd (92.3 ± 6.7)
18	> 10000	> 10000		nd (103.0 ± 7.7)	nd (92.0 ± 3.4)
19a	> 1000	> 10000		nd (95.8 ± 8.6)	nd (89.7 ± 3.1)
20a	2567 (5.591 ± 0.103)	2019 (5.695 ± 0.029)	0.8	nd (97.3 ± 1.8)	nd (93.6 ± 1.8)
21a	> 10000	> 1000		nd (74.7 ± 5.4)	nd (90.5 ± 1.4)
21b	> 10000	> 1000		nd (92.6 ± 4.3)	nd (111.3 ± 4.0)
21c	> 10000	> 10000		nd (91.8 ± 9.1)	nd (97.6 ± 1.7)
22a	280 (6.553 ± 0.055)	19 (7.728 ± 0.043)	0.1	nd (69.7 ± 7.5)	nd (100.3 ± 2.3
22b	0.5 (9.308 ± 0.082)	5.7 (9.295 ± 0.074)	11.4	1.4 ± 0.2 (−1.5 ± 1.8)	2.9 ± 1.1 (4.4 ± 2.4)
22c	1.2 (8.910 ± 0.047)	4.0 (8.402 ± 0.045)	3.3	4.4 ± 0.4 (12.1 ± 3.8)	5.6 ± 0.1 (24.8 ± 7.1)
23	27 (7.569 ± 0.029)	38 (7.424 ± 0.029)	1.4	nd (91.0 ± 11.0)	nd (89.3 ± 10.2)
tacrine	45 (7.346 ± 0.034)	5 (8.304 ± 0.043)	0.1	4.9 ± 1.3 (25.4 ± 2.4)	44.0 ± 2.1 (86.6 ± 4.9)
galantamine	636 (6.197 ± 0.052)	8404 (5.076 ± 0.034)	13.2	nd (96.8 ± 7.5)	nd (93.7 ± 8.4)
memantine	> 10000	> 10000		5.6 ± 1.3 (11.4 ± 0.3)	5.5 ± 1.1 (11.3 ± 2.1)
ketamine	> 10000	> 10000		6.1 ± 1.2	3.2 ± 0.4

[a] Cholinesterase assays: IC_{50} > 1000 nM, at 10 μM 50-60% enzyme inhibition; IC_{50} > 10000 nM, at 10 μM < 50% enzyme inhibition. Excitotoxicity assays: nd, not determined.

strongly increased the activities of the resulting compounds with spacers longer than 5 atoms (**22a**−**c**) to IC_{50} values in the lower nanomolar range (e.g., **22b** IC_{50} on AChE 0.5 nM). Interestingly, partial reduction of compound **22b** to **23** resulted in a moderate loss of AChE/BChE-inhibitory activity, but in a total loss of NR-inhibitory activity (discussed below).

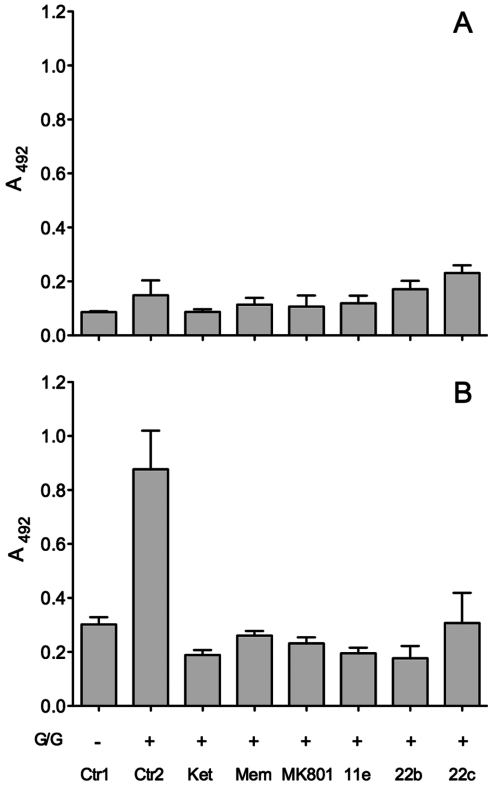


Figure 1. Assay of NR-mediated cytotoxicity. (A) L12-G10 cells were cultured under conditions of low expression of NR (i.e., no dexamethasone added to the medium). Cells were treated with the following compounds: Ket, ketamine (100 μM); Mem, memantine (20 μM); MK801 (50 nM); **11e** (20 μM); **22b** (20 μM); **22c** (20 μM). G/G: activation of NR with (*S*)-glutamate/glycine (10 μM each). (B) L12-G10 cells were cultured in the presence of 4 μM dexamethasone to induce NR expression. Cells were treated with compounds as in (A).

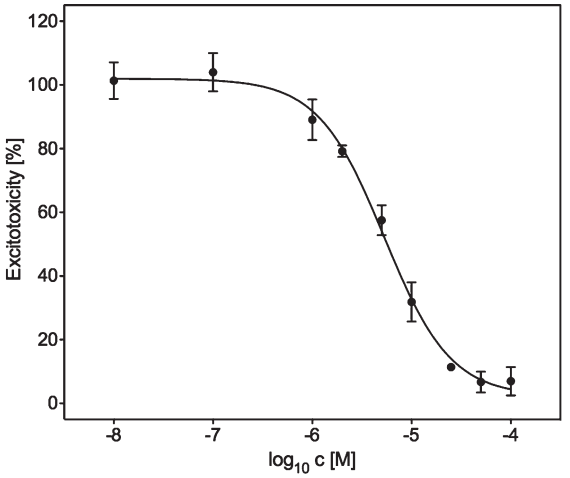


Figure 2. Inhibition of NMDA receptor-mediated cell toxicity by memantine. Different concentrations of compound were applied to L12-G10 cells, and NMDA receptors were activated by the addition of (*S*)-glutamate and glycine. Memantine had an IC_{50} value of 5.6 μM in this experiment.

There is a debate whether a cholinesterase inhibitor for the treatment of AD should be selective for AChE or BChE or instead nonselective. AChE activity decreases progressively in certain brain regions from mild to severe stages of AD to reach 10−15% of normal values, while BChE activity remains unchanged.[24] Accordingly, strong AChE selectivity may be disadvantageous for the treatment of AD and lead to lower efficacy of AD treatment.[25] The new homobivalent, quaternary β-carbolines reported in this study (e.g., **22a**−**c**) are generally nonselective for either ChE. On the other hand, **22b** showed IC_{50} values against AChE and BChE of 0.5 nM and 5.7 nM, respectively, which is a 100- to 1000-fold higher AChE/BChE-inhibitory activity compared to the corresponding monomeric, quaternary β-carbolines (for example, compare **9a** and **22b** in Table 1; see also ref 5). Thus, bivalent AChE/BChE inhibitors may be considered superior to monovalent compounds as candidates in anti-AD drug research.

Bivalent β-Carbolines as NMDA Receptor Blockers. L12-G10 and L13-E6 cells, which express the human NR1-1a/NR2A and NR1-1a/NR2B subunits, respectively, were used to establish an assay of NR-mediated, glutamate-induced excitotoxicity. The assay relies on the quantification of lactate dehydrogenase (LDH) activity released from damaged cells. In transfected L(tk−) cells, we observed an increase of cell damage in response to dexamethasone induction of NR1-1a/NR2A and NR1-1a/NR2B expression, respectively, in the presence of NR agonists (*S*)-glutamate and glycine (compare parts A and B of Figure 1). This glutamate-induced excitotoxicity was effectively blocked by known NR-specific antagonists such as ketamine, memantine, or MK-801 (Figure 1B). This indicated that the assay was capable of identifying new NR-selective compounds as potential novel inhibitors of glutamate-induced excitotoxicity.

As mentioned before, glutamate-induced excitotoxicity was determined by the quantification of LDH activity that accumulated in the medium supernatant of cells treated with NR agonists. Thus, if cells were incubated with potential NR inhibitors, direct inhibitory effects of the test compounds on LDH may have mimicked excitotoxicity caused via NMDA receptors. To exclude such false results, we tested all relevant compounds for direct effects on LDH. Therefore we performed the excitotoxicity assay in the presence of 20 μM of test compounds but in the absence of NR agonists and then lysed all cells with triton X-100 and determined LDH activity in the extracts. Under these conditions, none of the compounds described in this study showed significant direct inhibition of LDH (see Table S2 of the Supporting Information for examples).

To further validate the excitotoxicity assay, we recorded dose−response curves for ketamine, memantine, and MK-801. A representative result is shown in Figure 2 for memantine. The IC_{50} values determined in this assay were 6.1 ± 1.2 μM, 5.6 ± 1.3 μM, and 6.4 ± 1.8 nM for ketamine, memantine, and MK-801, respectively.

We tested the compounds listed in Table 1 for their ability to block glutamate-induced excitotoxicity. The monovalent β-carbolines were generally inactive in this assay, where inactivity was defined as less than 15% inhibition of glutamate-induced excitotoxicity at a 50 μM final concentration of compound. Remarkable NR-inhibitory activity was observed for N^2-bivalent β-carbolines **11a**−**k**, except for compounds **11a** and **11c**, and N^9-bivalent β-carbolines **22b**−**c**. The activities of the compounds seemed to depend on the presence of two quaternary nitrogens (compare compounds **11d** and **16a** or compounds **22b** and **23** for example), the spacer length, and the β-carboline structure (i.e., compare active compound **11e** with β-carboline/pyridine heterobivalent

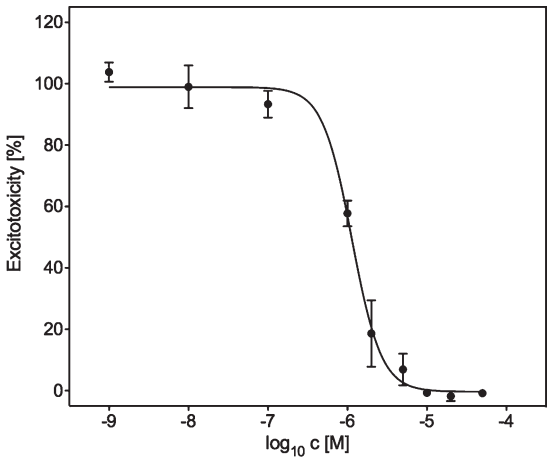


Figure 3. Inhibition of NMDA receptor-mediated cell toxicity by compound **22b**. Different concentrations of compound were applied to L12-G10 cells, and NMDA receptors were activated by the addition of (*S*)-glutamate and glycine. Compound **22b** had an IC_{50} value of 1.4 μM in this experiment.

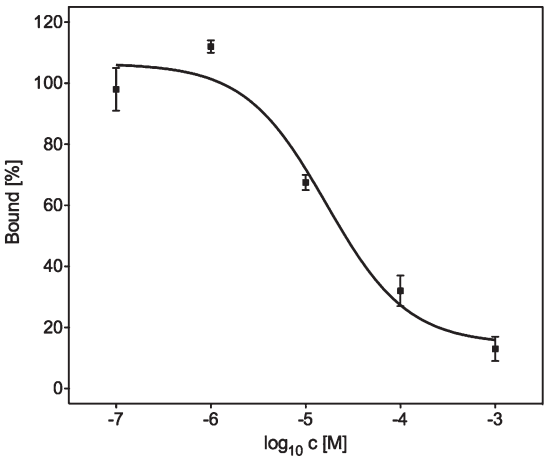


Figure 4. Competitive receptor binding assay. NR2B-specific binding of [^{3}H]ifenprodil to isolated membranes prepared from L13-E6 cells[28] was determined in the presence of increasing concentrations of compound **11e**.

compound **17** and homobivalent pyridine compound **18**, which were inactive). The NR-inhibitory activities of both the N^2-bivalent β-carbolines (**11a**−**k**) and N^9-bivalent β-carbolines (**22a**−**c**) were most significant for compounds bearing a spacer length longer than five carbons, although compounds with spacer lengths longer than nine atoms could not be reproducibly tested due to insufficient solubility of the compounds in the cell culture medium. A representative dose−activity relationship is shown in Figure 3 for the N^9-bivalent β-carboline compound **22b** (IC_{50} = 1.4 μM).

Because the NR-inhibitory activity of the bivalent β-carbolines strictly depended on the permanent positive charges, one could speculate that the compounds interact with the polyamine binding site on either NR1 or NR2 subunits. It is worth noting that under our assay conditions (10 μM (*S*)-glutamate/glycine in a cell culture medium at pH 8.1), spermine at 10 μM or 100 μM final concentration had no effect on glutamate-induced excitotoxicity on L12-G10 cells (excitotoxicity at 100 μM spermine 108.5 ± 4.8%, n = 3). The effects of spermine on NMDA receptors is complex and includes glycine-dependent and glycine-independent stimulation, as well as voltage-dependent inhibition and modulation of agonist (glutamate) binding (reviewed in ref 26). It has been suggested that the voltage-dependence of the spermine block may be due to both a direct open-channel block and a charge-screening effect, meaning that the positive charges of spermine interact with negative charges near the channel mouth, thereby interfering with ion flow.[26] We have tested whether the NR-inhibitory activity of compound **22b** is suppressed by preincubation of L12-G10 cells with spermine. This was obviously not the case (Figure S1, Supporting Information), suggesting that our bivalent β-carbolines may act by a mechanism different from the spermine inhibition of NR-mediated excitotoxicity.

It has been reported that the binding site of the subtype NR2B-specific inhibitor ifenprodil is located close to the spermine binding site on the extracellular amino terminal regulatory domain but can be clearly distinguished in binding assays with spermine or ifenprodil ligands.[27] We also tested the activity of bivalent β-carbolines in a recently established assay of [^{3}H]ifenprodil binding to isolated membranes prepared from L13-E6 cells.[28] In this assay, we used the N^2-bivalent, permanently charged β-carboline **11e**, which inhibits glutamate-induced excitotoxicity with an IC_{50} of 6.5 μM. In this receptor binding assay, compound **11e** significantly competed at concentrations above 10 μM (K_i = 20 μM; Figure 4), indicating that at least part of the NR-inhibitory effect may be mediated via the ifenprodil binding site of the receptor.

Conclusions

The N^2-bivalent β-carbolines and N^9-bivalent β-carbolines described in this study were identified as potent inhibitors of both AChE/BChE enzymes and NMDA receptors. It is worth noting that structure−activity profiles were similar in both test systems, i.e., the most potent AChE/BChE inhibitors (**22b**) also proved to be the most active NMDA receptor blockers. The inhibitory activity of quaternary N^9-bivalent β-carbolines on both AChE and BChE was increased by a factor of about 1000 compared to their monovalent analogs. For example, substance **22b** displayed IC_{50} values of 0.5 and 5.7 nM for AChE and BChE, respectively, compared to substance **20a** (IC_{50} values 2657 and 2019 nM). These novel homobivalent compounds reached inhibitory activities on the NMDA receptor similar to the established NR blocker memantine (1.4 μM for **22b** vs 5.6 μM for memantine in our assay), whereas the monovalent compounds did not show any activity. Thus, homobivalent β-carbolines may represent interesting leads in the development of multitarget treatment of Alzheimer's disease.

Experimental Section

General Information. Methods. Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus. ^{1}H and ^{13}C NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 and 63 MHz) and Advance 400 spectrometer (400 and 100 MHz), respectively. Elemental analyses were performed on a Hereaus Vario EL III apparatus (Elementar Analysensysteme GmbH, Germany) at the Institute of Organic Chemistry, University of Jena, Germany, and results were within ±0.4% of the theoretical values. Furthermore, all compounds were checked by TLC and showed single spots. Taken together, both features ensure purities ≥95%. TLC was performed on silica gel F254 plates (Merck). High resolution mass spectrometry

(HRMS) data were determined on a TSQ Quantum AM spectrometer (Therma Electron Corporation). IR data were obtained from a Magna-IR FT-IR spectrometer system 550 by Nicolet (WI).

The preparation of the following derivatives was described earlier: 6-methoxy-1,2,3,4-tetrahydro-β-carboline (**2**),[5] 6-methoxy-β-carboline (**5**),[5] 6-hydroxy-β-carboline (**6**),[5] 6-methoxy-1-methyl-β-carboline (**7**),[5] 6-bromo-β-carboline (**8**),[21] and 3,4-dihydro-6-methoxy-β-carboline (**3**).[20] 1,2,3,4-Tetrahydro-β-carboline (**1**) and β-carboline (**4**) were prepared analogously to the 6-methoxy and 6-hydroxy derivatives.[5] The insertion of substituents at position 9 (**19a**−**b**) was achieved as previously described.[5,29] Protocols for the preparation of the target compounds and intermediates together with their physical and spectral data (NMR, elemental analysis, HRMS) are given in the Supporting Information.

Synthesis of the N^2-Bivalent Compounds (11−**15): General Procedure.** A solution of 8.2 mmol β-carboline (**4**, **5**, **6**) and 3.7 mmol α,ω-dibromoalkane in 10 mL DMF was refluxed for 4−6 h under nitrogen as previously described.[22] After cooling to room temperature, 40 mL of acetone was added and the precipitated solids were isolated by filtration and dried in vacuo.

Synthesis of the Quaternary Salts (9, 10, 20, 22): General Procedure. A 10-fold molar excess of alkyl iodide was added to a solution of the respective β-carboline derivative (**4**, **19**, **21**) in acetone. Under nitrogen, the mixture was stirred at room temperature for 24 h. The precipitated solids were isolated by filtration and dried in vacuo.

For Example: 2-Methyl-9-[9-(2-methyl-β-carboline-9-yl)nonyl]-β-carboline-2-ium Diiodide (22b). From **21b** and methyl iodide; yield 98% slightly yellow crystals; mp 250−251 °C, ^{1}H NMR: 400 MHz (DMSO-d_6) δ = 1.17−1.30 (m, 10H, $NCH_2CH_2(C\underline{H}_2)_5$), 1.78−1.85 (mc, 4H, 2 × $NCH_2C\underline{H}_2$), 4.51 (s, 6H, 2 × $N^+C\underline{H}_3$), 4.55−4.59 (t, J = 7.3, 4H, 2 × $NC\underline{H}_2$), 7.47−7.51 (dt, J = 0.6, 7.8, 2H, 2 × 6), 7.83−7.87 (dt, J = 1.0, 8.2, 2H, 2 × 7), 7.92−7.95 (d, J = 8.5, 2H, 2 × 8), 8.52−8.53 (d, J = 7.9, 2H, 2 × 5), 8.67−8.68 (dd, J = 0.9, 6.5, 2H, 2 × 4), 8.82−8.84 (d, J = 6.4, 2H, 2 × 3), 9.69 (s, 2H, 2 × 1). ^{13}C NMR: 100 MHz (DMSO-d_6) δ = 26.70 ($N(CH_2)_2(\underline{C}H_2)_5$), 29.16 (2 × $NCH_2\underline{C}H_2$), 44.07 (2 × $N^+\underline{C}H_3$), 48.31 (2 × $\underline{N}CH_2$), 111.84 (aromatic), 118.10 (aromatic), 119.45 (aromatic), 122.15 (aromatic), 124.24 (aromatic), 130.01 (aromatic), 131.85 (aromatic), 132.47 (aromatic), 133.95 (aromatic), 135.78 (aromatic), 144.50 (aromatic). Anal. ($C_{33}H_{38}I_2N_4$) C,H,N.

Synthesis of the N^9-Bivalent Compounds (21a−**c): General Procedure.** According to ref 23, 85 mmol of comminuted NaOH and 1.5 mmol α,ω-dibromoalkane were added to a solution of 3.0 mmol β-carboline **4** in 7.5 mL of DMSO. The mixture was stirred at room temperature for 4 h, and 10 mL of water was added and the aqueous layer was extracted with chloroform. The combined organic phases were washed with 10% NaOH solution, dried over $MgSO_4$, and evaporated. The product was purified by column chromatography with chloroform/methanol (12/1) as the mobile phase.

Synthesis of the Partially Reduced Compounds (16a−**b, 23): General Procedure.** According to refs 30,31, a solution of 0.35 mmol of the respective quaternary β-carboline (**11d**,**11i**,**22b**) in 50 mL of methanol was treated with 10 mmol of $NaBH_4$ slowly under nitrogen. The mixture was stirred for 2−4 h at room temperature, the organic phase was evaporated, and 10 mL of water was added. The aqueous phase was then extracted with dichloromethane. The organic phase was dried over $MgSO_4$, evaporated, and dried in vacuo.

Pharmacological Studies. AChE and BChE Assays. Inhibition of AChE and BChE by test compounds was analyzed using the Ellman assay as described previously.[5,32] AChE (EC3.1.1.7, Type V−S, from electric eel) and BChE (EC3.1.1.8, from equine serum) were purchased from Sigma-Aldrich (Steinheim, Germany) with a purity of ≥60% protein by biuret. 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine (ATC), and butyrylthiocholine iodides (BTC) were obtained from Fluka (Buchs, Switzerland). Galantamine hydrobromide, which was used as reference substance for cholinesterase inhibition, was purchased from JANSSEN-CILAG GmbH (Neuss, Germany).

Stock solutions of test compounds were prepared in water or ethanol. At least five different concentrations of the test compound were measured at 25 °C at 412 nm, and each concentration was tested in triplicate. The inhibition curves were obtained by plotting percentage enzyme activity (100% for the reference) versus logarithm of the test compound concentration.

Assay of Glutamate-Induced Excitotoxicity. L12-G10 and L13-E6 cells are derivatives of L(tk−) cells that express cDNAs of the human NR1-1a/NR2A and NR1-1a/NR2B subunits of the NMDA receptor (NR), respectively.[33] Untransfected L(tk−) cells were purchased from ECACC (Salisbury, UK). The cells were cultured in Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum, a mixture of penicillin and streptomycin (100U/100 μg/mL; Biochrom, Germany), and 100 μM ketamine to reduce excitotoxicity generated by background NR expression prior to induction. Ketamine and memantine were provided by Sigma and prepared as 100 mM stock solutions in PBS. Stock solutions of test compounds were prepared at 50 mM in DMSO. Glutamate-induced excitotoxicity was measured as described[33] with modifications. Cells were seeded into 96-well microtiter plates at 1 × 10^4/well. After 24 h of cultivation at 37 °C and 5% CO_2, the expression of NR was induced by the addition of 4 μM dexamethasone. After additional 16 h of incubation, cells were washed three times with MEM containing 1% bovine albumin to remove residual ketamine. The cells were preincubated for 30 min with 200 μL of phenol-red-free MEM containing the test compounds (final DMSO content 0.1%). NR-mediated excitotoxicity was then triggered by adding 10 μM of both (*S*)-glutamate and glycine. The LDH assay was performed 4 h after the addition of glutamate/glycine according to the manual provided with Roche's Cytotoxicity Detection Kit (LDH). All tests were done in triplicate and are expressed as mean values ± SD. Baseline excitotoxicity (0%) was defined as the LDH release after addition of glutamate and glycine but in the presence of 100 μM ketamine. Conversely, 100% excitotoxicity was defined as the LDH release induced by glutamate/glycine in the absence of ketamine. IC_{50} values were calculated using the GraphPad Prism 5.0 software package.

To exclude direct inhibition of LDH by the test compounds, cells were incubated with test compounds as described above but in the absence of NR agonists. Then all cells were lysed by addition of 1% triton X-100 and LDH activity was determined (see Table S2, Supporting Information).

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Supporting Information Available: Synthetic procedures and spectral data for intermediates and target compounds; details of the AChE and BChE assays; specificity controls assay of glutamate-induced excitotoxicity. This material is available free of charge via the Internet at http://pubs.acs.org.

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