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A Small Molecule Targeting the Multifactorial Nature of Alzheimer's Disease**

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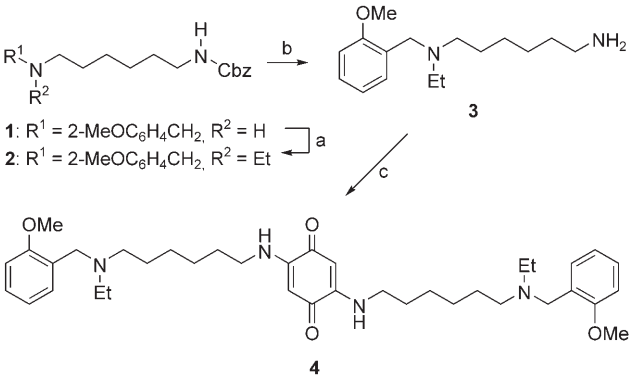
Alzheimer's disease (AD) is a complex multifactorial syndrome unlikely to arise from a single causal factor, but due to a number of different but related biological alterations that contribute to its pathogenesis.[1] None of the presently marketed drugs, although valuable in improving cognitive, behavioral, and functional impairments, alter AD progression,[2] and the goal of an effective disease-modifying treatment is still unmet. Nowadays, mechanism-based drug-design approaches are mainly directed toward two proteins, amyloid β (Aβ) and tau. Aβ is the core constituent of senile plaques, one of the key pathological characteristics of AD, whereas phosphorylated tau is the main component of neurofibrillary tangles, the other hallmark lesion of AD.[3] Despite enormous research effort, no new molecules that interfere with the biochemical pathways of either Aβ or tau have been approved so far for the treatment of AD. This failure is probably due to the weakness of the classic drug-discovery approach based on the "one molecule, one target" paradigm, which might prove inadequate when the molecular complexity of the disease is addressed. In fact, the multifactorial nature of AD and the current lack of an accepted unitary theory to account for AD neurodegeneration have formed the basis of a growing consensus that single compounds are required that are able to interact with several molecular targets in the neurotoxic cascade (the innovative "one molecule, multiple targets" paradigm).[4,5]

Herein, we report on a new drug candidate (memoquin (**4**), patent pending) derived from a program[5–9] aimed at creating multifunctional molecules to interfere with different key target points of AD neurodegeneration. Compound **4** was designed rationally by incorporating a 1,4-benzoquinone functionality as a radical scavenger into the polyamine skeleton of a previously reported series of cholinergic derivatives.[7] A straightforward synthetic strategy was developed (Scheme 1), and the biological profile of **4** was then explored in detail by means of both in vitro and in vivo assays to assess its therapeutic potential for combating AD.



Scheme 1. Synthesis of **4**: a) $(EtO)_2SO_2$, toluene, reflux, 76%; b) HBr/CH_3COOH, room temperature, 2 h, 98%; c) 1,4-benzoquinone, EtOH, room temperature, 6 h, 17%. Cbz = $C_6H_5CH_2OCO$.

The antioxidant properties of **4** were verified initially by testing in vitro its ability to neutralize free radicals.[10] Compound **4** was able to decrease the formation of free radicals with an inhibition percentage of 44.1 ± 3.7, which is slightly lower than that shown by trolox ($(57.6 \pm 0.9)\%$). Moreover, **4** was a good substrate for the enzyme NAD(P)H:-quinone oxidoreductase 1 (NQO1), with apparent V_{max} and K_M values quite similar to those of menadione for reduction by the enzyme (Table 1). These results show that **4** is itself a fairly good antioxidant, and, more interestingly, that **4** might be transformed by NQO1 from the quinone into the hydroquinone. Therefore, we suggest that the oxidized and reduced forms of **4** can coexist in vivo. The reduced form was demonstrated to be the actual antioxidant species in the case of other 1,4-benzoquinone derivatives, such as coenzyme Q and idebenone.[11] To further validate this molecular mechanism, **4** was also tested against the formation of reactive oxygen species (ROS) in SH-SY5Y neuroblastoma cells.[12] At a concentration of 3 μM, **4** did not cause any marked difference in ROS formation from that in untreated cells

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Table 1: Activities of **4** and reference compounds toward molecular targets investigated.[a]

Target	Memoquin (**4**)	Reference compound
NQO1	$V_{max} = 3.48$ mM min^{-1} mg^{-1}; $K_M = 12.7$ μM	$V_{max} = 7.29$ mM min^{-1} mg^{-1}; $K_M = 1.20$ μM[b]
hAChE	$IC_{50} = (1.55 \pm 0.11)$ nM; $K_i = (2.60 \pm 0.48)$ nM	$IC_{50} = (23.1 \pm 4.8)$ nM; $K_i = (20.5 \pm 3.3)$ nM[c]
Aβ(1–40) aggregation (hAChE-induced)	$IC_{50} = (28.3 \pm 0.30)$ μM	$IC_{50} \gg 100$ μM[c]
Aβ(1–42) self-aggregation	$IC_{50} = (5.93 \pm 0.33)$ μM	$IC_{50} = (60.3 \pm 11.2)$ μM[d]
BACE-1	$IC_{50} = (108 \pm 23)$ nM	$IC_{50} = (18 \pm 2)$ nM[e]

[a] Reference compounds (see the Supporting Information for chemical structures): [b] Menadione. [c] Donepezil.[8] [d] Tetracycline. [e] Statine derivative.

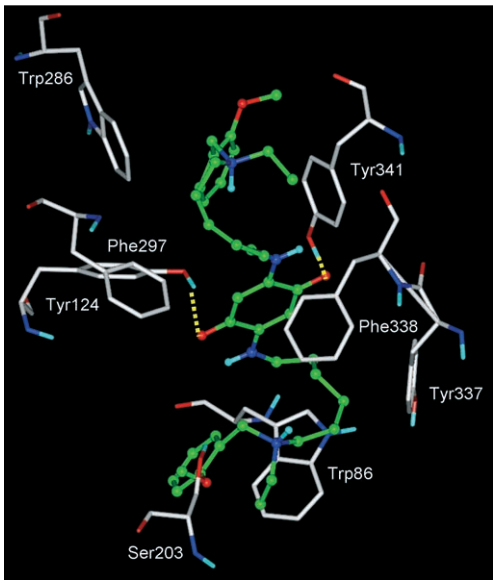

Figure 1. A docking model of **4** to human AChE: The contacts of the benzylammonium ends of **4** with residues Trp86 and Trp286 at the catalytic and peripheral sites of the enzyme, respectively, are shown. Possible favorable contacts of the quinone moiety with the tyrosine residues Tyr124 and Tyr341 of the gorge are also shown (H bonds, yellow dashed lines).

((75.5±8.0)% for cells treated with **4**, (100 ± 6)% for untreated cells). Conversely, when the same experiment was repeated after pretreating the SH-SY5Y cells with sulforaphane, a potent inducer of NQO1,[13] **4** produced a remarkable inhibitory effect on ROS formation relative to that in the untreated cells ((56.6±4.0)% and (104 ± 11)%, respectively). NQO1 activity is increased in the hippocampal pyramidal neurons of AD patients and colocalizes closely with AD pathology, which supports the hypothesis of its role as an antioxidant system that is upregulated in response to the oxidative stress of the AD process.[14,15]

Compound **4** is a potent inhibitor of the activity of human acetylcholinesterase (AChE), with IC_{50} and K_i values 15 and 8 times lower, respectively, than those obtained for donepezil (Table 1), the most potent marketed AChE inhibitor (AChEI).[16] The Lineweaver–Burk plot (see the Supporting Information) revealed that the inhibition was of a mixed type, and we interpreted this result as evidence of the interaction of **4** with both the catalytic and the accessory peripheral anionic site (PAS) of the enzyme. Docking simulations (Figure 1) confirmed that **4** is able to bind simultaneously both the catalytic site and the PAS of AChE.

AChE can induce Aβ aggregation, and AChEIs that bind to the PAS can block this proaggregating action.[16,17] Compound **4** showed a dose-dependent inhibitory effect on AChE-induced Aβ(1–40) aggregation (Table 1; Figure 2a). Remarkably, none of the marketed AChEIs tested under the same conditions showed any antiaggregating activity.[16,18]

Besides the ability to inhibit AChE-induced Aβ(1–40) aggregation, which is likely to be relevant in the brain of AD patients,[19] **4** also inhibited in vitro the self-assembly of Aβ(1–42), which is the most amyloidogenic Aβ fragment found in the AD plaques, in a dose-dependent manner (Table 1; Figure 2b). When tested at an equimolar concentration (50 μM) with Aβ(1–42), **4** was able to inhibit fibril formation by (95.5 ± 0.4)%, whereas the marketed AD drug galantamine did not show any significant inhibitory activity under the same experimental conditions. For comparison, the inhibitory activity of tetracycline[20] under the same experimental conditions (50 μM, Aβ/tetracycline 1:1) was (44.9 ± 12.1)%.

To further explore the antiamyloidogenic profile of **4**, we also investigated its ability to act as an inhibitor of BACE-1, an enzyme involved in Aβ production from the amyloid precursor protein.[21,22] Compound **4** was found to inhibit BACE-1 activity in a concentration-dependent manner with an IC_{50} value of (108 ± 23) nM, whereas a previously reported statine-based derivative had an IC_{50} value of (18 ± 2) nM[23] (Table 1).

To test the action of **4** on AD neurodegeneration in vivo, we used the anti-NGF transgenic mouse (AD11 mouse), which has been shown to be a comprehensive animal model for AD.[24,25] The ability of **4** to prevent AD-like neurodegeneration was tested at three stages: at 2 months of age, which corresponds to a very early stage of neurodegeneration; at 6 months of age, when the neurodegeneration is not established completely; and at 15 months of age, when the neurodegeneration is full-blown.[26] At all ages, **4** rescued the cholinergic deficit in the basal forebrain, as quantified by stereological counts of the number of cholineacetyltransferase (ChAT)-positive neurons (Figure 3a).

The initial appearance of Aβ in dystrophic neurites of the hippocampus of AD11 mice at 6 months of age[26] evolves, by 15 months of age, into a frank accumulation of extracellular Aβ deposits.[26,27] The administration of **4** caused a complete reduction in Aβ expression in 6-month-old AD11 mice and a marked decrease in Aβ deposits in 15-month-old AD11 mice (Figure 3b).

To determine whether **4** was also able to prevent or rescue tau hyperphosphorylation in the cortex, neurostereological counts were performed to determine the number of neurons labeled abnormally by the monoclonal antibody against

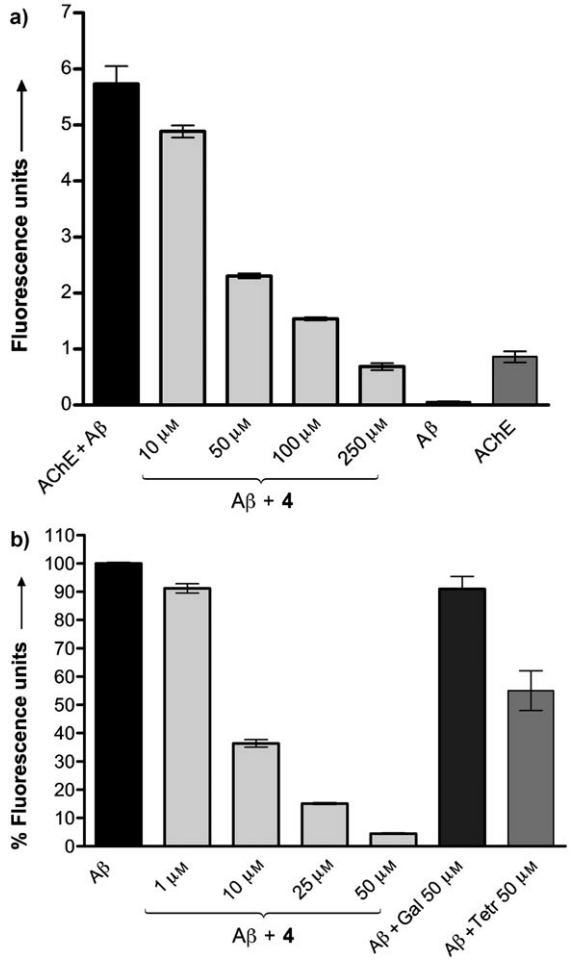

Figure 2. a) Inhibition of AChE-induced Aβ(1–40) aggregation by the thioflavin T method. Compound **4** was used at different concentrations (10–250 μM) and showed an IC_{50} value of (28.3 ± 0.3) μM. b) Inhibition of Aβ(1–42) self-aggregation by the thioflavin T method. Compound **4** was used at different concentrations (1–50 μM) and showed an IC_{50} value of (5.93 ± 0.33) μM. For comparison, inhibition by galantamine (Gal) and tetracycline (Tetr) is also reported.

phosphotau AT8. Tau hyperphosphorylation was either completely (at 2 and 6 months of age) or partially (at 15 months of age) prevented by the administration of **4** (Figure 3c).

Compound **4** was tested finally in different animal models to assess its ability to rescue the behavioral deficits linked to attention and memory. The oral and intraperitoneal administration of **4** rescued the behavioral impairment, as assessed by object-recognition tests. Remarkably, besides its effectiveness in vivo, **4** showed other promising properties, such as good oral bioavailability, efficacy in crossing the blood–brain barrier, and a favorable safety profile. In addition, it is tolerated well after prolonged administration. The

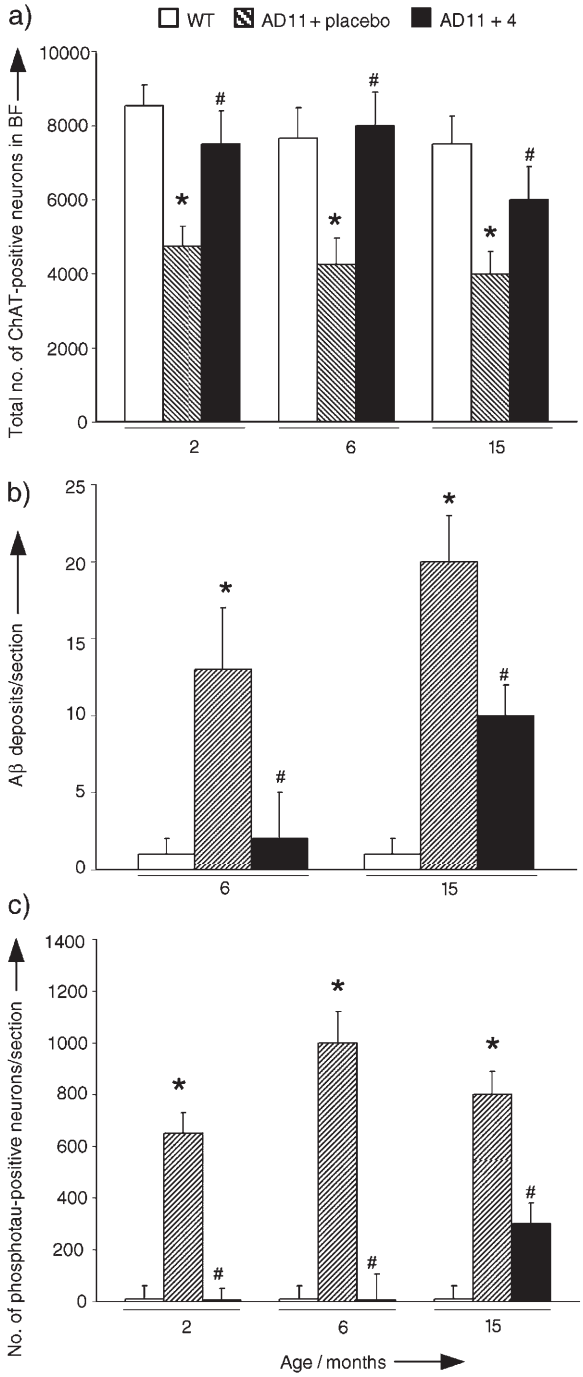

Figure 3. a) Amelioration of the cholinergic deficit in AD11 mice. The administration of **4** restores the number of cholinergic neurons in the basal forebrain (BF) of 2-, 6-, and 15-month-old AD11 mice. b) Effect of **4** on Aβ accumulation in AD11 mice. At 6 months of age, Aβ is localized in deposits which form clusters in the hippocampus of AD11 mice. These deposits are absent in control mice. At this age, treatment with **4** decreases the number of Aβ-positive clusters. In 15-month-old AD11 mice, Aβ is localized in clusters and in extracellular plaques; these clusters and plaques are absent in control mice. At this age, treatment with **4** partially decreases the number of Aβ plaques. The graph shows the number of clusters/plaques per histological section at the two different ages. c) Effect of **4** on the accumulation of hyperphosphorylated tau in AD11 mice. In AD11 mice, hyperphosphorylated tau accumulates in the somatodendritic compartment. No accumulation was seen in age-matched wild-type mice or in animals treated with **4**. The graph shows the neurostereological counts used to quantify the difference in the number of neurons that expressed phosphorylated tau in the three treatment groups. In all experiments, **4** (7 $mg\,kg^{-1}\,day^{-1}$) was administered intraperitoneally for 15 days to AD11 mice of different ages ($n = 4$ per treatment group). $* p < 0.05$ versus wild-type (WT) mice; $\# p < 0.05$ versus AD11 mice treated with a placebo.

detailed pharmacological characterization of **4** will be reported elsewhere.

In conclusion, in a setting in which available drugs against AD appear to be palliative rather than curative, memoquin (**4**) is able to confront AD neurodegeneration on a different level. This compound shows activity against the formation of ROS and the processing and aggregation of Aβ peptides, and inhibits AChE. It can therefore be considered a breakthrough drug candidate to face the biological complexity of AD.

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