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In vitro antiamyloidogenic properties of 1,4-naphthoquinones

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ABSTRACT

The aim of this study is to find out whether several 1,4-naphthoquinones (1,4-NQ) can interact with the amyloidogenic pathway of the amyloid precursor protein processing, particularly targeting at β-secretase (BACE), as well as at β-amyloid peptide (Aβ) aggregation and disaggregating preformed Aβ fibrils. Compounds bearing hydroxyl groups at the quinoid (**2**) or benzenoid rings (**5**, **6**) as well as some 2- and 3-aryl derivatives (**11**–**15**) showed BACE inhibitory activity, without effect on amyloid aggregation or disaggregation. The halogenated compounds **8** and **10** were selective for the inhibition of amyloid aggregation. On the other hand, 1,4-naphthoquinone (**1**), 6-hydroxy-1,4-naphthoquinone (**4**) and 2-(3,4-dichlorophenyl)-1,4-naphthoquinone (**26**) did not show any BACE inhibitory activity but were active on amyloid aggregation and disaggregation preformed Aβ fibrils. Juglone (5-hydroxy-1,4-naphthoquinone (**3**), and 3-(*p*-hydroxyphenyl)-5-methoxy-1,4-napththoquinone (**19**) were active on all the three targets. Therefore, we suggest that 1,4-NQ derivatives, specially **3** and **19**, should be explored as possible drug candidates or lead compounds for the development of drugs to prevent amyloid aggregation and neurotoxicity in Alzheimer's disease.

1. Introduction

1,4-Naphthoquinones (1,4-NQ) are widely spread in nature and their interest lies on their broad-range biological action though mainly owing to naphthoquinones being redox active organic cofactors and important species in biological systems [1]. Quinones account for one of the largest families of antitumor agents [2]. The basic knowledge on quinone studies has been used to design new anticancer drugs, improving selectivity and providing a more rational therapeutic application of them [3]. The 1,4-NQ scaffold has been identified as a new class of Hsp90 inhibitors [4] which could be useful for the treatment of cancer [5] and numerous neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease, in which protein aggregation is a common etiology [6,7]. Different derivatives of 2-hydroxy-1,4-naphthoquinone ring have shown to activate the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) [8], strategy which could be promising in the treatment of several neurological pathologies [9]. Exposure of neuroblastoma cells and primary cortical neurons to 2-methyl-5-hydroxy-1,4-naphthoquinone (**6**) has shown to provide protection against subsequent oxidative and metabolic insults based upon its ability to activate adaptive cellular stress response pathways [10]. Some naphthoquinone derivatives from the root of *Lithospermum erythrorhizon* [11] have been reported to provide neuroprotection by reducing the release of various proinflammatory molecules from activated microglia. The 2,3,6-trimethyl-1,4-naphthoquinone, a MAO inhibitor which is present in the tobacco plant and smoke, has been identified as neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) C57BL/6 mouse model of neurodegeneration [12]. MK-4, a naphthoquinone with a side chain of four isoprenoid units, which is the predominant form of vitamin K in the brain [13], has shown to prevent oxidative cell death induced by reduced glutathione depletion in primary oligodendrocyte precursors and in primary cortical neurons [14].

β-Amyloid peptide (Aβ) is the major constituent of senile plaques found in the brain of patients with AD, and the aggregation and deposition of the Aβ in neural tissue give rise to the neuronal atrophy that is characteristic of the disease [15]. Decreasing Aβ production or aggregation are two therapeutic approaches for AD. The first one is based primarily on the inhibition of two proteolytic enzymes, β- and γ-secretases, that participate in the processing of the amyloid precursor protein (APP) [16]. The second one lies on inhibiting Aβ aggregation and destabilizing preformed Aβ fibrils [17].

Rifampicin and its derivatives, which possess a naphthohydroquinone or naphthoquinone structure, have demonstrated to

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inhibit Aβ (1–40) aggregation and neurotoxicity in the PC12 cells. Similarly, it has been shown that tetracyclines and doxycycline not only inhibited the Aβ (1–42) fibril formation but also disassembled the preformed fibrils *in vitro* [18,19].

A wide array of anti-amyloid and neuroprotective therapeutic approaches are under investigation on the basis of the hypothesis that Aβ peptide plays a pivotal role in disease onset and progression. Development of β-secretase (BACE) inhibitors and targeting the fibrillary aggregates of Aβ peptide are strategies which are being explored and could promise for acute improvement in AD [20]. Therefore, interventions with agents that reduce amyloid production or limit aggregation, or increase removal might block the cascade of events comprising AD pathogenesis. Based on some reported neuroprotective effects of naphthoquinones, it was of our interest to find out whether several 1,4-NQ can interact with the amyloidogenic pathway of the APP processing, particularly targeting at BACE which plays a crucial role in the rate-limiting step of the amyloid cascade, as well as at Aβ aggregation and destabilizing preformed Aβ fibrils.

2. Materials and methods

2.1. Reagents

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and human recombinant β-secretase (BACE) were obtained from Sigma–Aldrich Chemical Company (St. Louis, USA). Fluorogenic BACE (H-RE(EDANS) EVNLDAEFK(DABCYL)R-OH) substrate was obtained from Calbiochem (La Jolla, CA). Aβ (25–35) peptide was purchased from GenScript (New Jersey, USA), 200-mesh copper grids coated with Formvar/carbon film (TAAB Essentials for Microscopy, UK).

2.2. Compound synthesis

1,4-NQ **4** [21], **9** [22], **10** [23] and **26** [24] were synthesized following literature procedures. 1,4-NQ **11**, **12** and **27**, **28** were synthesized from 1,4-naphthoquinone (**1**), 1,4-NQ **13**–**17**, **20** and **21** from 5-hydroxy-1,4-naphthoquinone (juglone, **3**) by direct arylation with boronic acids under dicationic Pd(II)-catalysis as recently described [25]. 1,4-NQ **20** and **21** were synthesized, respectively, by methylation or acetylation of **3** [25]. The rest of 1,4-NQ were commercially available. All 1,4-NQ were dissolved in DMSO and stored frozen at −20 °C.

2.3. Determination of cell viability

The mitochondrial-dependent reduction of MTT to formazan was used to exclude a cytotoxic effect of the 1,4-NQ in SH-SY5Y neuroblastoma cells. Cells were plated in 96-well polystyrene plates with 40,000 cells per well and incubated at 37 °C for 24 h to allow cells to attach. The plates were then incubated with the different 1,4-NQ in a concentration range of 1–10 μM for an additional 24 h. Cell viability was determined by addition of 20 μl of 2 mg/ml MTT to each well. After incubation for 1 h at 37 °C, the medium was removed and 100 μl of DMSO was added to dissolve the formazan crystals. The absorbance was read at 550 nm using a Digiscan Microplate Reader (Assys Hitech, Kornenburg, Austria). The optical density of the formazan formed in the control cells (untreated) was taken as 100% viability.

2.4. β-Secretase (BACE) enzyme activity assay

The assay is based on the secretase-dependent cleavage of a secretase-specific peptide conjugated to the fluorescent reporter molecules and DABCYL, which results in the release of a fluorescent signal. The level of secretase enzymatic activity is proportional to the fluorimetric reaction. BACE assay was carried out at 37 °C using 0.24 U of human recombinant BACE enzyme and 10 μM substrate in 20 mM sodium acetate buffer (pH 4.5) in a final volume of 100 μl. Fluorescence was measured using a FLx800 microplate fluorescence reader (Bio-tek Instruments, Inc.). Wavelengths of excitation and emission were 360 and 528 nm, respectively. The enzyme activity assay was performed in the absence (control reaction), and in the presence of the 1,4-NQ. Before the addition of the substrate the human recombinant BACE enzyme and 1,4-NQ were preincubated at 37 °C for 1 h. The inhibition ratio of BACE activity was calculated from the percentage of control after 1 h of incubation once the substrate was added. Data were expressed as the 50% inhibitory concentration (IC_{50}).

2.5. Preparation of A (25–35) peptide

Aβ (25–35) peptide was dissolved at 1 mM in PBS. For aggregation inhibition experiments, 10 μM Aβ (25–35) peptide was mixed with the 1,4-NQ and incubated at 37 °C for 4 days. For disaggregating experiments, 10 μM Aβ (25–35) peptide was incubated at 37 °C for 4 days to generate fibrils. Preformed fibrils were mixed with the 1,4-NQ for 4 additional days at 37 °C. The degree of Aβ aggregation was determined using both Thioflavin-T (Thio-T) fluorescence and Electron microscopy (EM) analyses. The degree of Aβ disaggregation was determined using Thio-T fluorescence analysis.

2.6. Time-resolved fluorescence spectroscopy

Aliquots from each incubated sample were treated with Thio-T (final concentration 25 μM). Each test sample was shaken for 10 s prior to each measurement. Fluorescence intensity decay was measured by using an apparatus FluoroMax-4® based on the time-correlated single-photon counting method, including a diode laser (Horiba–Jobin–Yvon). The fluorescence spectroscopy technique used in this work measures the time dependence of the emission intensity. Lifetimes represent the average times during which the fluorophore (Thio-T) remains in the first excited electronic state prior to its return to the ground state. An examination of the lifetimes will permit identification of 1,4-NQ as inhibitors of amyloid aggregation and/or disaggregates of amyloid once the fibrils are formed. Samples were excited at 463 nm and the fluorescence intensity decay was registered at 482 nm. The pulses of laser excitation on the sample result in a histogram in the multichannel analyzer, which represents the number of counts in each channel, which in turn corresponds to the sample fluorescence decay profile. The fluorescence was measured until a predetermined signal count of 10,000 was reached. We conducted a control experiment to rule out the possibility that the low values in the Thio-T fluorescence assay might result from a quenching effect by the inhibitor. No significant quenching effect was observed (data not shown). Data were expressed as IC_{50}.

2.7. Electron microscopy (EM) analyses

A 3 μl aliquot of each reaction mixture was spotted to 200-mesh copper grids coated with Formvar/carbon film and incubated for 30 s. Afterwards, the peptide was stained with 2% uranyl acetate in water. This solution was wicked off and then the grid was air-dried. Samples were examined using a JEOL JEM-4000 EX transmission electron microscopy.

2.8. Data statistical analyses

All experiments were done at least in three independent experiments and all assays were done in triplicates. Data were analyzed

Table 1
β-Secretase (BACE) inhibitory activity of 1,4-naphthoquinones in a cell-free assay system.

Compound	IC_{50} (μM)
1,4-Naphthoquinone (**1**)	–
2-Hydroxy-1,4-naphthoquinone (**2**)	5.96
5-Hydroxy-1,4-naphthoquinone (**3**)	6.51
6-Hydroxy-1,4-naphthoquinone (**4**)	–
5,8-Dihydroxy-1,4-naphthoquinone (**5**)	9.08
2-Methyl-5-hydroxy-1,4-naphthoquinone (**6**)	12.92
2-Methyl-1,4-naphthoquinone (**7**)	–
2-Bromo-1,4-naphthoquinone (**8**)	–
2-Bromo-5-hydroxy-1,4-naphthoquinone (**9**)	7.86
2-Bromo-8-hydroxy-1,4-naphthoquinone (**10**)	–
2-Phenyl-1,4-naphthoquinone (**11**)	9.33
2-(*p*-Hydroxyphenyl)-1,4-naphthoquinone (**12**)	8.22
2-Phenyl-5-hydroxy-1,4-naphthoquinone (**13**)	12.56
3-Phenyl-5-hydroxy-1,4-naphthoquinone (**14**)	8.98
5-Hydroxy-2-(*p*-hydroxyphenyl)-1,4-naphthoquinone (**15**)	16.09
5-Hydroxy-2-(*p*-methoxyphenyl)-1,4-naphthoquinone (**16**)	–
5-Hydroxy-3-(*p*-methoxyphenyl)-1,4-naphthoquinone (**17**)	–
5-Methoxy-2-(*p*-methoxyphenyl)-1,4-naphthoquinone (**18**)	11.21
3-(*p*-Hydroxyphenyl)-5-methoxy-1,4-naphthoquinone (**19**)	10.31
5-Methoxy-1,4-naphthoquinone (**20**)	–
5-Acetoxy-1,4-naphthoquinone (**21**)	–
2,3-Dibromo-1,4-naphthoquinone (**22**)	–
2,3-Dichloro-1,4-naphthoquinone (**23**)	–
5-Nitro-1,4-naphthoquinone (**24**)	–
2-(*p*-Bromophenyl)-1,4-naphthoquinone (**25**)	–
2-(3,4-Dichlorophenyl)-1,4-naphthoquinone (**26**)	–

using one-way analysis of variance (ANOVA) followed by a Student test. The median inhibition concentration (IC_{50}) was determined using SPSS 11.0.1 statistical software (SPSS Inc., Chicago, USA).

3. Results

3.1. Cell viability

The treatment of neuroblastoma SH-SY5Y cells with the different 1,4-NQ derivatives for 24 h did not show any significant toxicity between 1 and 10 μM in comparison with untreated cells.

3.2. BACE inhibitory activity

A series of 1,4-NQ were tested for their inhibition of BACE activity in a cell-free assay system (Table 1). Our results revealed that the 1,4-NQ **2**, **3**, **5**, **9**, **11**, **12** and **14** had BACE inhibitory activity with IC_{50} values lower than 10 μM.

3.3. Amyloid aggregation process

The effect of 1,4-NQ on β-amyloid aggregation was observed by Thio-T-induced fluorescence assay and EM analysis. Fluorescence decay analysis of the Thio-T in complexes with fibrillar amyloid revealed that the fluorescence lifetime values were significantly higher in comparison with those from amyloid plus **4**, **8** and **24** (Table 2). Fig. 2 shows an analysis of time-resolved fluorescence spectroscopy of **4** which has the lowest IC_{50} in inhibition of amyloid aggregation.

In order to detect the morphologies of Aβ (25–35) aggregates, we used EM to probe the aggregation process in the presence or absence of the most active 1,4-NQ: **4**, **8** and **24**. When Aβ (25–35) was incubated alone for 4 days, there were robust fibrils observed (Fig. 1A). But when Aβ (25–35) was co-incubated with **4** (5 μM) (Fig. 1B), **8** (5 μM) (Fig. 1C) or **24** (1 μM) (Fig. 1D), the number of fibrils decreased.

To evaluate whether or not preformed fibrils were disaggregated, 1,4-NQ were incubated with Aβ preformed fibrils. Compounds **19**, **24** and **4** significantly decreased the fluorescence lifetime values in comparison with those from amyloid alone (Table 2).

4. Discussion

We observed that whereas 1,4-naphthoquinone (**1**) did not show any inhibitory effect, the presence of several substituents allowed for a significant increase of BACE inhibition. Whereas substitution at C-2 by methyl (**7**) was ineffective, the presence of a phenyl ring at C-2 (**11**) increased the inhibition of BACE. The inhibition was further increased by the presence of a *p*-OH substituent at the phenyl ring (**12**). However, other substitution patterns of the phenyl ring such as *p*-bromo (**25**) or 3,4-dichloro (**26**) gave rise to a

Table 2
Effect of 1,4-naphthoquinones on Aβ aggregation by Thio-T-induced fluorescence assay.

Compound	Inhibition of Aβ fibrils, IC_{50} (μM)	Disaggregation of Aβ fibrils, IC_{50} (μM)
1,4-Naphthoquinone (**1**)	12.43	11.32
2-Hydroxy-1,4-naphthoquinone (**2**)	–	–
5-Hydroxy-1,4-naphthoquinone (**3**)	11.10	15.49
6-Hydroxy-1,4-naphthoquinone (**4**)	2.75	6.7
5,8-Dihydroxy-1,4-naphthoquinone (**5**)	–	–
2-Methyl-5-hydroxy-1,4-naphthoquinone (**6**)	–	–
2-Methyl-1,4-naphthoquinone (**7**)	–	–
2-Bromo-1,4-naphthoquinone (**8**)	6.44	–
2-Bromo-5-hydroxy-1,4-naphthoquinone (**9**)	17.25	–
2-Bromo-8-hydroxy-1,4-naphthoquinone (**10**)	18.65	–
2-Phenyl-1,4-naphthoquinone (**11**)	–	–
2-(*p*-Hydroxyphenyl)-1,4-naphthoquinone (**12**)	–	–
2-Phenyl-5-hydroxy-1,4-naphthoquinone (**13**)	–	–
3-Phenyl-5-hydroxy-1,4-naphthoquinone (**14**)	–	–
5-Hydroxy-2-(*p*-hydroxyphenyl)-1,4-naphthoquinone (**15**)	–	–
5-Hydroxy-2-(*p*-methoxyphenyl)-1,4-naphthoquinone (**16**)	–	–
5-Hydroxy-3-(*p*-methoxyphenyl)-1,4-naphthoquinone (**17**)	–	–
5-Methoxy-2-(*p*-methoxyphenyl)-1,4-naphthoquinone (**18**)	22.55	–
3-(*p*-Hydroxyphenyl)-5-methoxy-1,4-naphthoquinone (**19**)	16.59	5.05
5-Methoxy-1,4-naphthoquinone (**20**)	–	–
5-Acetoxy-1,4-naphthoquinone (**21**)	–	–
2,3-Dibromo-1,4-naphthoquinone (**22**)	–	–
2,3-Dichloro-1,4-naphthoquinone (**23**)	–	–
5-Nitro-1,4-naphthoquinone (**24**)	4.36	6.28
2-(*p*-Bromophenyl)-1,4-naphthoquinone (**25**)	–	–
2-(3,4-Dichlorophenyl)-1,4-naphthoquinone (**26**)	–	–

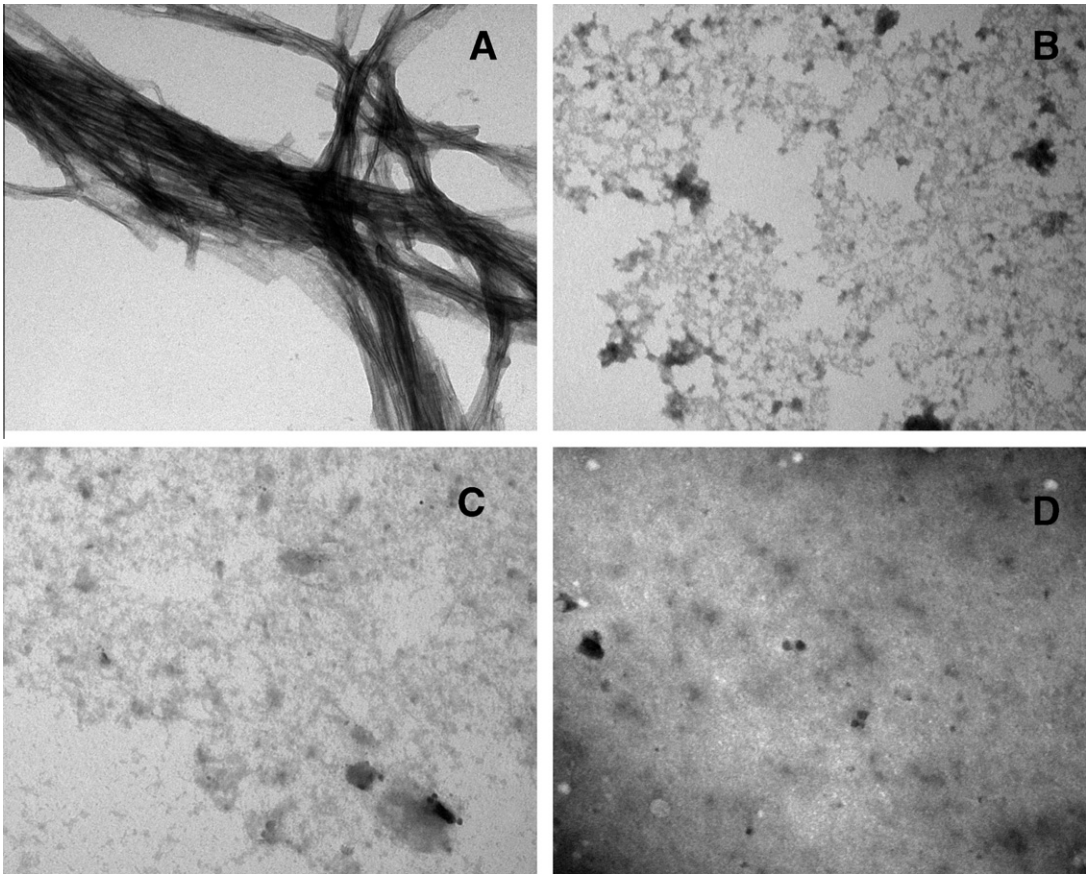

Fig. 1. A representative electron micrograph showing the formation of Aβ fibrils after 4-day incubation as described in material and methods (A). A representative electron micrograph showing the aggregation process in the presence of the most active 1,4-naphthoquinones: **4** (B) **8** (C) and **24** (D). Scale bar, 200 nm.

loss of the activity. Substitution at C-2 by bromo (**8**) did not promote any BACE inhibition, as well as simultaneous substitution by halogen at C-2 and C-3 (**22** and **24**). Substitution of the parent 1,4-naphthoquinone ring by hydroxyl groups rendered interesting compounds. Thus, substitution at C-2 by OH (**2**) afforded a notably active compound. 5-Hydroxy-1,4-napthtoquinone (**3**) was also found to produce BACE inhibition. The simultaneous presence of an extra hydroxyl group at C-8 (**5**) gave rise to a slight loss of activity. Location of the OH group at C-6 (**4**) did not show any inhibition.

Modification of the 5-hydroxy-1,4-naphthoquinone scaffold by the inclusion of substituents at C-2 was then tested. The presence of Br at C-2 (**9**) gave rise to a slight decrease of the inhibition when

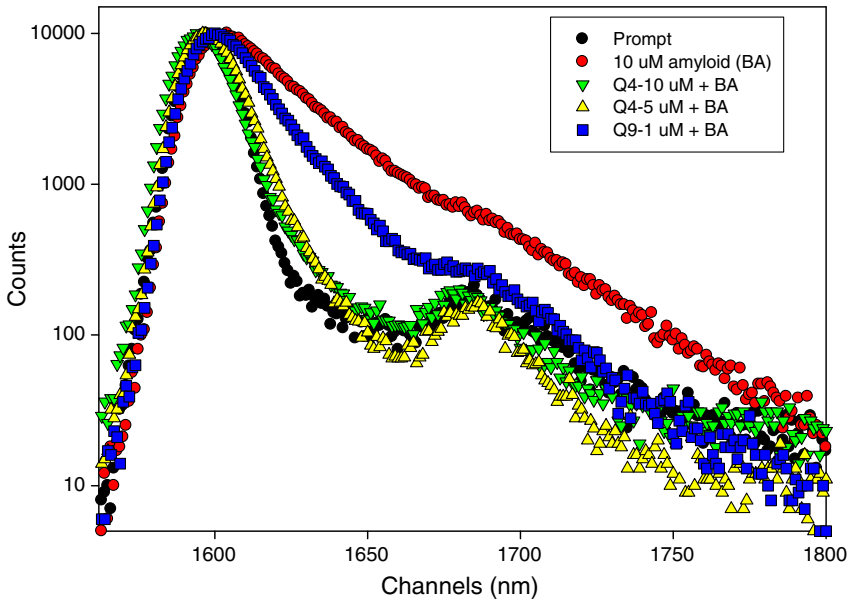

Fig. 2. Inhibition of Aβ aggregation by 6-hydroxy-1,4-naphthoquinone (**4**).

compared with the parent compound (**3**). On the other hand, the presence of a methyl group gave rise to a stronger decrease of the activity (**6**). This was also the case when substituting C-2 with a phenyl group (**13**). Modification of the phenyl ring by a *p*-OH group (**15**) further decreased the inhibition of BACE, and the presence of a *p*-methoxy group (**16**) gave a completely inactive compound.

On the other hand, substitution with a phenyl group at C-3 (**14**) gave a more active compound than substitution with phenyl at C-2 (**13**). Again, modification of the phenyl ring by a *p*-methoxy group (**17**) led to a loss of the activity. No activity was found either for bromo substitution at C-3 (**10**).

The effect of the protection of the OH group of 5-hydroxy-1,4-naphthoquinone was tested. Both 5-methoxy-1,4-naphthoquinone (**20**) and 5-acetoxy-1,4-naphthoquinone (**21**) showed no BACE inhibition at all. This was also the case when introducing aryl substituents either at C-2 or C-3 of the 5-methoxy-1,4-naphthoquinone scaffold (**18** and **19**). Replacement of the OH group at C-5 by a nitro group (**24**) afforded an inactive compound.

BACE inhibitors are under intensive study and a lot of peptidomimetic inhibitors have been reported and developed [26,27]. Considering the development of therapeutics for AD, chemical compounds must cross the blood–brain barrier (BBB) and the plasma membrane. The peptidomimetic inhibitors seem to have difficultly crossing the BBB. On the other hand, quinones in general are very small compounds which are relatively advantageous for crossing the BBB. Indeed, recent studies revealed that quinones like plumbagin [10], thymoquinone [28] and coenzyme Q10 [29] are able to traverse the BBB *in vivo*.

It could be speculated that those active 1,4-NQ, by inhibiting the BACE activity, could make predominant the metabolism of APP towards the α-secretase pathway, thus increasing the release of αAPPs [30]. In this regard, the α-secretase activity increases with enhanced membrane fluidity [31] and processing of APP by β-secretase might be explained by alterations in cell membrane fluidity [32]. The activity of BACE is sensitive to oxidative stress [33]. There is convincing evidence that oxidative stress regulates the BACE activity, resulting in Aβ accumulation [34]. Therefore, BACE inhibition by 1,4-NQ could contribute to an improvement of the cell membrane fluidity.

Some of the 1,4-NQ which had shown BACE inhibition were also found to inhibit Aβ aggregation and/or to disaggregate Aβ fibrils (Tables 1 and 2). Among the C-2 substituted 1,4-NQ, the 2-bromoderivative (**8**) inhibited Aβ aggregation but had no effect on disaggregation. However, 2-methyl-1,4-naphthoquinone (**7**), the 2-aryl derivatives (**11**, **12**, **25**, **26**) as well as the dihalogenated derivatives (**22** and **23**) were not active either on aggregation or disaggregation. On the other hand, 1,4-naphthoquinone (**1**), which had not shown any significant BACE inhibition, was found to inhibit Aβ aggregation and also to disaggregate Aβ fibrils.

Among the hydroxylated 1,4-NQ derivatives, 2-hydroxy-1,4-naphthoquinone (**2**) was inactive upon both Aβ aggregation or disaggregation. However, 5-hydroxy-1,4-naphthoquinone (**3**) was slightly active on both targets, and its 2-bromoderivative (**9**) inhibited Aβ aggregation but had no effect on disaggregation. The presence of an extra hydroxyl group (**5**) rendered an inactive compound on both targets. On the other hand, 2-bromo-8-hydroxy-1,4-naphthoquinone (**10**) and 6-hydroxy-1,4-naphthoquinone (**4**), which had not shown any BACE inhibition, were found to inhibit Aβ aggregation, and in addition the latter (**4**) was also found to disaggregate Aβ fibrils.

The 5-methoxyderivatives (**18** and **19**), which had not shown BACE inhibition, were active only against Aβ disaggregation, and in a similar fashion, 5-nitro-1,4-naphthoquinone (**24**) was active both on aggregation and disaggregation of Aβ fibrils.

In the choice of candidate compounds as inhibitors of Aβ aggregation process we follow these criteria: (1) the compounds should be sufficiently hydrophobic to interfere with key hydrophobic interactions in Aβ fibrils; (2) they should still be sufficiently water-soluble to make their utilization straightforward in *in vitro* assays. (3) Low molecular weight and (4) lack of neurotoxicity at the concentrations required for effective inhibition Aβ aggregation. Therefore, the most interesting compounds would be the 1,4-NQ derivatives with IC_{50} values lower than 10 μM.

5. Conclusion

We have found several 1,4-NQ with activities on BACE inhibition, inhibition of Aβ aggregation and disaggregation of Aβ fibrils. 1,4-NQ **2**, **5**, **6** and **11**–**15** were selective for BACE inhibition, with no effect on Aβ aggregation or disaggregation. 1,4-NQ **8** and **10** were selective for the inhibition of Aβ aggregation. 1,4-NQ **1**, **4** and **24** did not show any BACE inhibition but were active on Aβ aggregation and disaggregation. 1,4-NQ **3** and **19** were active on all the three targets. Although these compounds are toxic at high doses and some of them have been evaluated for their ability to prevent the cell growth or kill cancer cells [35], investigations of the possibility that subtoxic doses of them might activate adaptive stress response pathways in neurons that may protect neurons against more severe stress are currently performed and could be promising for finding neuroprotective compounds [10,36]. 1,4-NQ which inhibit BACE activity and/or inhibit Aβ aggregation and/or disaggregate Aβ fibrils could be promising strategies for the prevention and treatment of AD.

In conclusion, we propose that 1,4-NQ derivatives, specially **3** and **19**, should be explored as possible drug candidates or lead compounds for the development of drugs to prevent Aβ aggregation and neurotoxicity in AD.

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References

[1] D. Tauraite, V. Razumas, E. Butkus, Lipophilic 1,4-naphthoquinone derivatives: synthesis and redox properties in solution and entrapped in the aqueous cubic liquid-crystalline phase of monoolein, Chem. Phys. Lipids 159 (2009) 45–50.
[2] J.A. Valderrama, P. Colonelli, D. Vasquez, M.F. Gonzalez, J.A. Rodriguez, C. Theoduloz, Studies on quinones. Part 44: novel angucyclinone *N*-heterocyclic analogues endowed with antitumoral activity, Bioorg. Med. Chem. 16 (2008) 10172–10181.
[3] R.C. Montenegro, A.J. Araujo, M.T. Molina, J.D. Marinho Filho, D.D. Rocha, E. Lopez-Montero, M.O. Goulart, E.S. Bento, A.P. Alves, C. Pessoa, M.O. de Moraes, L.V. Costa-Lotufo, Cytotoxic activity of naphthoquinones with special emphasis on juglone and its 5-*O*-methyl derivative, Chem. Biol. Interact. 184 (2010) 439–448.
[4] M.K. Hadden, S.A. Hill, J. Davenport, R.L. Matts, B.S. Blagg, Synthesis and evaluation of Hsp90 inhibitors that contain the 1,4-naphthoquinone scaffold, Bioorg. Med. Chem. 17 (2009) 634–640.
[5] T.J. Turbyville, E.M. Wijeratne, M.X. Liu, A.M. Burns, C.J. Seliga, L.A. Luevano, C.L. David, S.H. Faeth, L. Whitesell, A.A. Gunatilaka, Search for Hsp90 inhibitors with potential anticancer activity: isolation and SAR studies of radicicol and monocillin I from two plant-associated fungi of the Sonoran desert, J. Nat. Prod. 69 (2006) 178–184.
[6] F. Dou, W.J. Netzer, K. Tanemura, F. Li, F.U. Hartl, A. Takashima, G.K. Gouras, P. Greengard, H. Xu, Chaperones increase association of tau protein with microtubules, Proc. Natl. Acad. Sci. USA 100 (2003) 721–726.
[7] H.Y. Shen, J.C. He, Y. Wang, Q.Y. Huang, J.F. Chen, Geldanamycin induces heat shock protein 70 and protects against MPTP-induced dopaminergic neurotoxicity in mice, J. Biol. Chem. 280 (2005) 39962–39969.
[8] S. Sundriyal, B. Viswanad, P. Ramarao, A.K. Chakraborti, P.V. Bharatam, New PPARgamma ligands based on barbituric acid: virtual screening, synthesis and receptor binding studies, Bioorg. Med. Chem. Lett. 18 (2008) 4959–4962.

[9] X. Zhao, Z. Ou, J.C. Grotta, N. Waxham, J. Aronowski, Peroxisome-proliferator-activated receptor-gamma (PPARgamma) activation protects neurons from NMDA excitotoxicity, Brain Res. 1073–1074 (2006) 460–469.
[10] T.G. Son, S. Camandola, T.V. Arumugam, R.G. Cutler, R.S. Telljohann, M.R. Mughal, T.A. Moore, W. Luo, Q.S. Yu, D.A. Johnson, J.A. Johnson, N.H. Greig, M.P. Mattson, Plumbagin, a novel Nrf2/ARE activator, protects against cerebral ischemia, J. Neurochem. 112 (2010) 1316–1326.
[11] K.N. Nam, M.S. Son, J.H. Park, E.H. Lee, Shikonins attenuate microglial inflammatory responses by inhibition of ERK, Akt, and NF-kappaB: neuroprotective implications, Neuropharmacology 55 (2008) 819–825.
[12] K.P. Castagnoli, S.J. Steyn, J.P. Petzer, C.J. Van der Schyf, N. Castagnoli Jr., Neuroprotection in the MPTP Parkinsonian C57BL/6 mouse model by a compound isolated from tobacco, Chem. Res. Toxicol. 14 (2001) 523–527.
[13] H.H. Thijssen, M.J. Drittij-Reijnders, Vitamin K status in human tissues: tissue-specific accumulation of phylloquinone and menaquinone-4, Br. J. Nutr. 75 (1996) 121–127.
[14] J. Li, H. Wang, P.A. Rosenberg, Vitamin K prevents oxidative cell death by inhibiting activation of 12-lipoxygenase in developing oligodendrocytes, J. Neurosci. Res. 87 (2009) 1997–2005.
[15] K. Blennow, M.J. de Leon, H. Zetterberg, Alzheimer's disease, Lancet 368 (2006) 387–403.
[16] B. De Strooper, L. Umans, F. Van Leuven, H. Van Den Berghe, Study of the synthesis and secretion of normal and artificial mutants of murine amyloid precursor protein (APP): cleavage of APP occurs in a late compartment of the default secretion pathway, J. Cell Biol. 121 (1993) 295–304.
[17] F. Yang, G.P. Lim, A.N. Begum, O.J. Ubeda, M.R. Simmons, S.S. Ambegaokar, P.P. Chen, R. Kayed, C.G. Glabe, S.A. Frautschy, G.M. Cole, Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo, J. Biol. Chem. 280 (2005) 5892–5901.
[18] G. Forloni, L. Colombo, L. Girola, F. Tagliavini, M. Salmona, Anti-amyloidogenic activity of tetracyclines: studies in vitro, FEBS Lett. 487 (2001) 404–407.
[19] H. Kim, B.S. Park, K.G. Lee, C.Y. Choi, S.S. Jang, Y.H. Kim, S.E. Lee, Effects of naturally occurring compounds on fibril formation and oxidative stress of beta-amyloid, J. Agric. Food Chem. 53 (2005) 8537–8541.
[20] S. Salloway, J. Mintzer, M.F. Weiner, J.L. Cummings, Disease-modifying therapies in Alzheimer's disease, Alzheimers Demen. 4 (2008) 65–79.
[21] T.R. Kelly, N.D. Parekh, E.N. Trachtenberg, Regiochemical control in the Diels-Alder reaction of substituted naphthoquinones. The directing effects of C-6 oxygen substitutents, J. Organic Chem. 47 (1982) 5009–5013.
[22] T.N. Van, N. De Kimpe, Synthesis of 6H-naphtho[2,3-c]chromene-7,12-diones via palladium-catalyzed intramolecular cyclization of 2-bromo-3-aryloxymethyl-1,4-naphthoquinones, Tetrahedron 59 (2003) 5941–5946.
[23] L.F. Tietze, R.R. Singidi, K.M. Gericke, Total synthesis of the proposed structure of the anthrapyran metabolite delta-indomycinone, Chem.-Eur. J. 13 (2007) 9939–9947.
[24] N. Maugel, B.B. Snider, Efficient synthesis of the tetracyclic aminoquinone moiety of marmycin A, Org. Lett. 11 (2009) 4926–4929.
[25] M.T. Molina, C. Navarro, A. Moreno, A.G. Csaky, Arylation of benzo-fused 1,4-quinones by the addition of boronic acids under dicationic Pd(II)-catalysis, Org. Lett. 11 (2009) 4938–4941.
[26] M.C. Maillard, R.K. Hom, T.E. Benson, J.B. Moon, S. Mamo, M. Bienkowski, A.G. Tomasselli, D.D. Woods, D.B. Prince, D.J. Paddock, T.L. Emmons, J.A. Tucker, M.S. Dappen, L. Brogley, E.D. Thorsett, N. Jewett, S. Sinha, V. John, Design, synthesis, and crystal structure of hydroxyethyl secondary amine-based peptidomimetic inhibitors of human beta-secretase, J. Med. Chem. 50 (2007) 776–781.
[27] R.K. Hom, A.F. Gailunas, S. Mamo, L.Y. Fang, J.S. Tung, D.E. Walker, D. Davis, E.D. Thorsett, N.E. Jewett, J.B. Moon, V. John, Design and synthesis of hydroxyethylene-based peptidomimetic inhibitors of human beta-secretase, J. Med. Chem. 47 (2004) 158–164.
[28] A.A. Al-Majed, F.A. Al-Omar, M.N. Nagi, Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus, Eur. J. Pharmacol. 543 (2006) 40–47.
[29] X. Yang, G. Dai, G. Li, E.S. Yang, Coenzyme Q10 reduces beta-amyloid plaque in an APP/PS1 transgenic mouse model of Alzheimer's disease, J. Mol. Neurosci. 41 (2010) 110–113.
[30] Z. Mei, B. Situ, X. Tan, S. Zheng, F. Zhang, P. Yan, and P. Liu, Cryptotanshinone upregulates alpha-secretase by activation PI3K pathway in cortical neurons. Brain Res. June 2 (2010) [Epub ahead of print].
[31] E. Kojro, G. Gimpl, S. Lammich, W. Marz, F. Fahrenholz, Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha-secretase ADAM 10, Proc. Natl. Acad. Sci. USA 98 (2001) 5815–5820.
[32] C.A. von Arnim, B. von Einem, P. Weber, M. Wagner, D. Schwanzar, R. Spoelgen, W.L. Strauss, H. Schneckenburger, Impact of cholesterol level upon APP and BACE proximity and APP cleavage, Biochem. Biophys. Res. Commun. 370 (2008) 207–212.
[33] E. Tamagno, P. Bardini, A. Obbili, A. Vitali, R. Borghi, D. Zaccheo, M.A. Pronzato, O. Danni, M.A. Smith, G. Perry, M. Tabaton, Oxidative stress increases expression and activity of BACE in NT2 neurons, Neurobiol. Dis. 10 (2002) 279–288.
[34] J. Apelt, M. Bigl, P. Wunderlich, R. Schliebs, Aging-related increase in oxidative stress correlates with developmental pattern of beta-secretase activity and beta-amyloid plaque formation in transgenic Tg2576 mice with Alzheimer-like pathology, Int. J. Dev. Neurosci. 22 (2004) 475–484.
[35] S. Sugie, K. Okamoto, K.M. Rahman, T. Tanaka, K. Kawai, J. Yamahara, H. Mori, Inhibitory effects of plumbagin and juglone on azoxymethane-induced intestinal carcinogenesis in rats, Cancer Lett. 127 (1998) 177–183.
[36] V. Calabrese, C. Cornelius, C. Mancuso, G. Pennisi, S. Calafato, F. Bellia, T.E. Bates, A.M. Giuffrida Stella, T. Schapira, A.T. Dinkova Kostova, E. Rizzarelli, Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity, Neurochem. Res. 33 (2008) 2444–2471.