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Discovery of alkenylboronic acids as neuroprotective agents affecting multiple biological targets involved in Alzheimer's disease

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ABSTRACT

Alkenylboronic acids have shown important biological activities that contribute to neuroprotection. We have determined their influence on the β-amyloid (βA) aggregation process, β-secretase and acethylcholinesterase activities on cell-free systems, on the redox and lipid peroxidation status, and on the vulnerability to apoptotic death in an APPswe neuroblastoma cell line, before and after hydrogen peroxide treatment. We have discovered that 2-arylvinylboronic acids and some of their esters possess a set of properties which makes them highly useful as neuroprotective agents affecting multiple biological targets involved in AD. These properties are not paralleled by the related 2-arylboronic acids.

Nowadays, there is a growing interest in the design and synthesis of novel boron-containing compounds, since they are appropriate species for applications in medicinal chemistry. Boronic acids have attracted considerable interest as versatile intermediate compounds in organic synthesis.[1] Due to their stability towards air and moisture, they can be handled with no special care and can be well purified. Moreover, the electrophilic character of the boronic acids allows for interactions with nucleophiles, like the serine or threonine residues of proteases active sites, while possessing good stability under in vivo conditions.[2] For example, *N*-acetyl-D-Phe-Pro1amido-4-guanidino-butyl-1-boronic acid can interact with thrombin, a multifunctional serine protease of the hemostasis system, and consequently inhibit the endothelial secretion of the amyloid precursor protein (APP),[3] whose processing generates the β-amyloid peptide (βA) that forms the core of vascular and cerebral plaques in Alzheimer's disease (AD). In addition, it has been reported that the peptidic boronic acid *t*-butoxycarbonyl-D-Val-Leu-L-boroArg inhibits prolyl oligopeptidases,[4] a class of cytosolic serine proteases which are widely distributed in the brain. The high activity of these proteases is associated with an increased breakdown of neuropeptides, which results in a decline of cognitive functions and accelerates neurodegeneration.[5] Also, several boronic acids derived from thiazolidinediones inhibit the activity of autotaxin,[6] an enzyme with a threonine residue in the active site that is strongly expressed in the brain of AD patients, suggesting their potential usefulness as a treatment or prevention of AD.[7] It has been shown that bortezomib, a boronate peptide used in multiple myeloma therapy, decreases the production of amyloid in patients with primary systemic amyloidosis, thus alleviating organ injury from extracellular deposition of abnormal protein fibrils.[8,9]

It should be stressed that most of the biological studies with boronic acids have been carried out in cancer cells, and few studies have been reported in non-tumoral cells. Though, it is intriguing that certain tumor cells, like thyroid cancer cells, undergo elevation of the amount of glutathione and of the level of mRNA for glutamate cysteine ligase, as a mechanism of resistance against bortezomib-toxicity.[10] Therefore it could be hypothesized that this ability could likely occur in non-tumoral cells under treatment with boronic acids.

On the assumption that the reported pharmacological activities of certain boronic acids might contribute to cell neuroprotection, in the present report we have explored the structure–activity relationship exerted by several boronic acid derivatives on several biological targets involved in neuroprotection. Firstly, we have determined their influence on the βA aggregation process and on the activities of β-secretase (BACE) and acethylcholinesterase (AChE), and secondly the redox and lipid peroxidation status, and the vulnerability to apoptotic neuronal death in a Swedish mutation stably overexpressed SH-SY5Y cell line which resembles the in vivo βA-induced neurotoxicity (APPswe) human cell model of AD.

Since AD is a complex neurodegenerative disorder resulting from multiple molecular abnormalities, strategies to develop new drugs that simultaneously affect multiple biological targets is

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highly important.[11] Much effort is currently being devoted towards the finding of new multitarget drug ligands.[12]

From a structural standpoint, we have focused our attention on vinylboronic acids, a subclass of boronic acids that have been scarcely considered in previous biological studies. The boronic acid derivatives upon study are shown in Figure 1.[13] 2-Phenylvinylboronic acid (**1a**) was chosen as starting point for the SAR studies. Compound **4a** (phenylboronic acid) has been deprived of the C=C bond between the aromatic cycle and the $B(OH)_2$ functional group of **1a**. Compound **2** has the same structure as **1a** but with a saturated cycle (cyclohexyl) instead of an aromatic cycle (phenyl). Compound **3** represents an open-chain analogue (*n*-pentyl) of **2**. Compounds **1b**–**1e** differ from **1a** in the electronic character (electron-withdrawing or electron-donating) of the aromatic moiety. Similarly, **4b** is an electron-withdrawing variant of **4a**. Finally, we have considered variations in the boronic acid moiety of the arylvinylboronic skeleton: the pinacol esters **5a**–**5d**, and the methyliminodiacetic acid (MIDA)-ester **6**.

The disruption of aggregation and/or the destruction of the preformed βA-fibrils by small compounds which can interfere with different aspects of βA assembly, the inhibition of BACE activity and the inhibition of AChE activity, can be cited among the most promising strategies for AD treatment.[14] An efficient inhibitor of βA aggregation should physically block the growth of the fibrils or stabilize the prefibrillar nucleus conformation, as well as interfere with the cytotoxicity of the assembled peptides.

With respect to βA aggregation and disaggregation of preformed βA fibrils, only the arylvinylboronic acids **1a** and **1b** showed activity. Compound **1b** performed particularly well, with IC_{50} values of 3.92 and 7.19 M for inhibition of aggregation and disaggregation, respectively; whereas compound **1a** exhibited IC_{50} values of 15.90 and 26.83 M. These results showed that **1a** and **1b** inhibited the in vitro βA fibrillogenesis. Whether these compounds inhibit βA aggregation by binding hydrophobic β-sheet channels and/or disturbing βA hydrogen bond formation would need further studies.

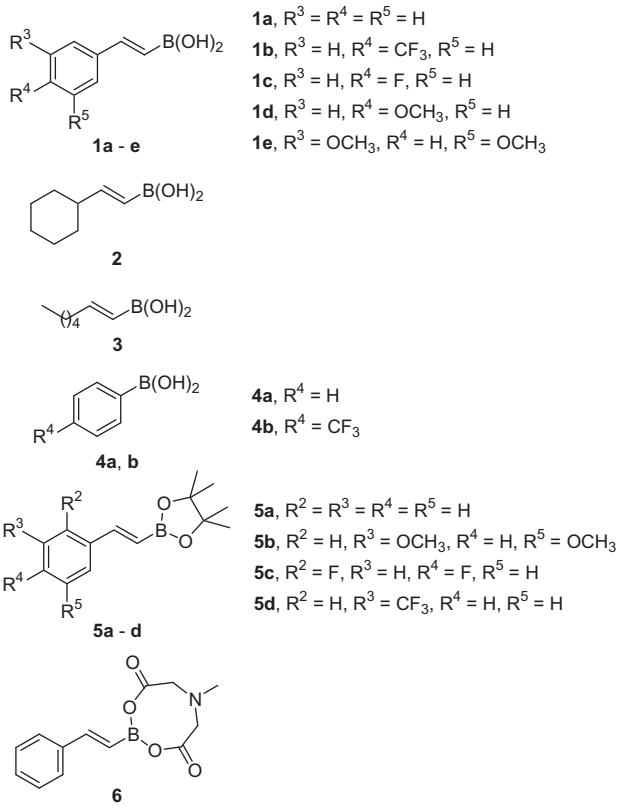

Figure 1. Chemical structures of the boronic acids and esters considered in this study.

Among all compounds tested, only the MIDA-ester **6** exhibited some BACE inhibition (IC_{50} = 136.15 μM), and none of them were active in inhibiting AChE activity.

Numerous studies have documented the roles of lipid peroxidation-derived aldehydes, as malondialdehyde (MDA),[15] in contributing to neuronal dysfunction in neurodegenerative diseases associated with oxidative stress.[16] In addition, in vivo studies have shown that increased lipid peroxidation leads to up-regulation of BACE expression, which may lead to an increased βA1-42 production.[17] These observations strengthen the notion that lipid peroxidation is a potential therapeutic target in the course of early AD.

Glutathione (GSH) depletion is recognized as a significant contributor to the pathogenesis of a variety of neurodegenerative disorders such as AD.[18] The glutathione redox cycle is a major endogenous protective system and an important component of the antioxidant machinery of the nervous system.[19] The synthesis of GSH occurs by two sequential ATP-dependent reactions in the cytoplasm, and is catalyzed by γ-glutamylcysteine synthetase and by GSH synthetase. In neurons, cysteine uptake is an important step for GSH synthesis because neurons cannot import extracellular GSH directly. As a reductant, GSH maintains intracellular sulfhydryl-containing proteins in the reduced and active form, either by the reduction of potentially toxic peroxides, or by the action of thiol-disulfide exchange reactions. The GSH–glutathione disulfide (GSSG) redox pair can readily interact with most of the physiologically relevant redox couples, undergoing reversible oxidation or reduction reactions, thereby maintaining the appropriate redox balance in the cell. Therefore, GSH depletion exacerbates cell damage due to generation of reactive oxygen species (ROS) which are capable of oxidizing key proteins, lipids, and DNA in the cell, eventually triggering cell death. Strategies that increase either GSH content or GSH/GSSG redox state have been shown to act in a neuroprotective manner, suggesting that augmentation of the available GSH pool may be a promising therapeutic target for neurodegeneration.[20]

The APP695-transfected neuroblastoma SH-SY5Y cell line was used to examine the MDA content and the reduced GSH/GSSG ratio before and after exposing cells to hydrogen peroxide (25 μM) insult, and the caspase-3 activity after oxidative stress exposure. First of all, the mitochondrial-dependent reduction of MTT to formazan was used to exclude a cytotoxic effect of the tested compounds in these cells. None of the compounds showed any toxicity at 10 μM after 24 h treatment.

In the absence of hydrogen peroxide insult, all the boronic acids exhibited a good profile in lowering the MDA levels and in enhancing the GSH content (Table 1). A maximum and significant decrease of the MDA levels was shown for compounds **1b**, **1c** and **2**. Arylvinylboronic acid **1b**, substituted in the *p*-position of the aryl ring with a CF_3 group, was highly active both in MDA lowering and in GSH enhancement. Wider variations were observed in the GSH/GSSG ratio along the series.

Whereas the pre-treatment of the cells with **1a** did not show a significant difference with respect to the control, a high increase of the GSH/GSSG ratio was observed in the presence of the strong electron-withdrawing *p*-CF_3 group (**1b**). This effect of the *p*-CF_3 group on the arylvinylboronic skeleton (**1a** vs **1b**) was not paralleled in the arylboronic series (**4a** vs **4b**). In comparison with **1a**, the MIDA-ester **6** showed a significant increase of the GSH/GSSG ratio, and the corresponding pinacol ester **5a** exhibited a very high increase of this value, which was the maximum of all the tested compounds.

The increase in the GSH/GSSG ratio could be derived from reduction of GSSG levels by glutathione reductase or from de novo synthesis of GSH. No marked alteration in GSSG amount was ob-