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Design, synthesis and in vitro evaluation of benzothiazole-based ureas as potential ABAD/17β-HSD10 modulators for Alzheimer's disease treatment

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ABSTRACT

Amyloid-beta peptide (Aβ) has been recognized to interact with numerous proteins, which may lead to pathological changes in cell metabolism of Alzheimer's disease (AD) patients. One such known metabolic enzyme is mitochondrial amyloid-binding alcohol dehydrogenase (ABAD), also known as 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10). Altered enzyme function caused by the Aβ-ABAD interaction, was previously shown to cause mitochondrial distress and a consequent cytotoxic effect, therefore providing a feasible target in AD drug development. Based on previous frentizole derivatives studies, we report two novel series of benzothiazolyl ureas along with novel insights into the structure and activity relationships for inhibition of ABAD. Two compounds (**37**, **39**) were identified as potent ABAD inhibitors, where compound **39** exhibited comparable cytotoxicity with the frentizole standard; however, one-fold higher cytotoxicity than the parent riluzole standard. The calculated and experimental physical chemical properties of the most potent compounds showed promising features for blood–brain barrier penetration.

Alzheimer's disease (AD) is the most common form of dementia in the elderly, characterized by the slow deterioration of cognitive functions.[1] The accumulation of amyloid-beta peptide (Aβ) and its formation into plaques, along with the formation of neurofibrillary tangles, highlights the pathological changes in affected brain regions with progressed AD. These hallmarks have become commonly monitored markers, however the initiating events still remain unclear.[2] Even though a precise mechanism of Aβ-induced toxicity has not been fully understood, several studies have reported synaptic and mitochondrial Aβ accumulation and dysfunction in early stages of AD development.[2–4] Furthermore, it has been described that Aβ interacts with various mitochondrial proteins, resulting in enhanced oxidative stress, energy misbalance and overall cell toxicity.[2,5–8]

Amyloid-binding alcohol dehydrogenase (ABAD), also known as 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10), is one of the proteins which was identified to interact directly with Aβ at nanomolar concentrations.[9,10] Moreover, it has been reported that the interaction between Aβ and ABAD promotes oxidative stress and mitochondrial dysfunction, consequently resulting in cell death.[5] Cell based assays and transgenic mice experiments demonstrated that the overexpression of both Aβ and ABAD show enhanced cell cytotoxicity, reduced levels of ATP and COX activity along with impaired energy metabolism in mice. Conversely, the overexpression of Aβ with inactive ABAD displayed less cytotoxicity and transgenic (ABAD) mice when compared with non-transgenic

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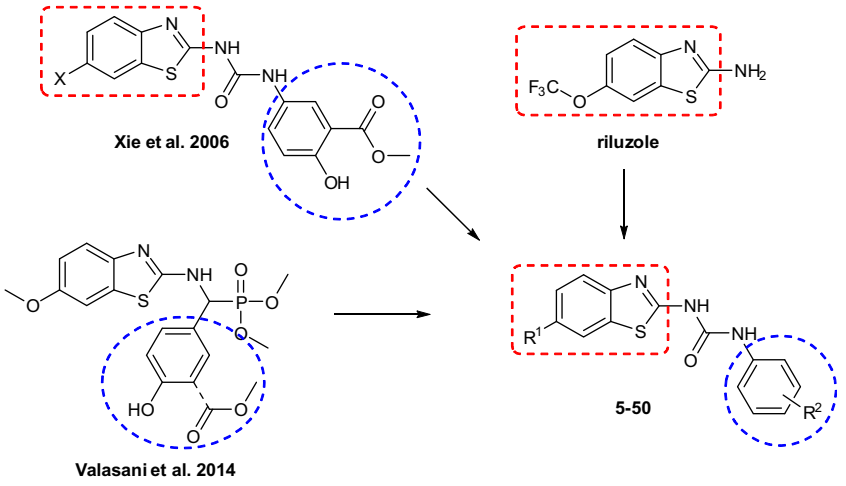
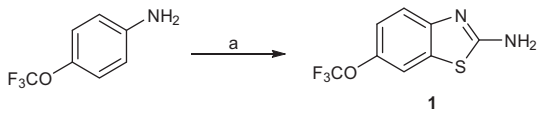


Scheme 1. Design of benzothiazole-based urea ABAD inhibitors.

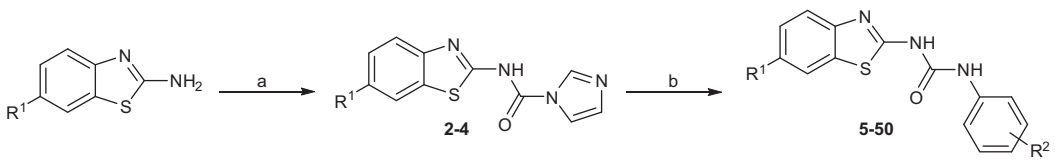


Scheme 2. Synthesis of 4-(trifluoromethoxy)aniline **1**. Reagents and conditions: (a) Br_2, KSCN, CH_3COOH.

mice do not display these changes.[7,10] Lim et al. reported an ABAD inhibition study where the compound (AG18051) appeared to reduce the levels of Aβ-induced oxidative stress and mitochondrial respiration impairment, as well as alleviate the Aβ-induced down-regulation of ABAD activity.[11] Whereas elevated levels of ABAD have been associated with AD pathology.[10,12] Reduced levels of ABAD has been reported in brain of Parkinson's disease patients.[13] These findings suggest that both ABAD-Aβ interaction and ABAD itself may represent a viable objective for deeper understanding of AD pathogenesis in context of Aβ-induced toxicity. Consequently, it may also aid in the development of novel AD therapeutics.

There has been a limited number of reported compounds acting as ABAD or ABAD-Aβ interaction inhibitors.[14,15] In 2006, Xie et al. described the marketed drug frentizole acting as a poor inhibitor of ABAD-Aβ interaction (IC_{50} ~200 μM) along with a novel series of synthesised frentizole analogues displaying a 30-fold increase in improved potency (IC_{50} ~6.5 μM).[16] A more recent study reported two phosphonate analogues of these previously discussed compounds, which also exhibited moderate to weak ABAD inhibition (IC_{50} 53 μM and 342 μM).[17,18] Thus, our aim of the study was focused on generating more potent inhibitors based around the benzothiazole scaffold. To this end, a first series was designed around a 6-halogen-benzothiazole urea scaffold with various phenyl ring substitution patterns including those previously reported (Scheme 1).[16–18] Furthermore, many benzothiazole analogues have been shown to possess various biological activities in the central nervous system.[19] Riluzole, a well-known drug with neuroprotective properties, has a similar benzothiazolyl core, however its neuroprotective mechanism of action is not still completely understood.[20] Riluzole is now an FDA approved drug to treat amyotrophic lateral sclerosis, and several other studies report riluzole exhibiting neuroprotective properties in other neurological disorders (e.g. Parkinson's disease, Huntington's disease or cerebral ischemia).[21–23] Riluzole possesses a wide range of mechanisms of action including anti-glutamate activity, Na^+ and Ca^{2+} channel blockage, GABAergic modulation[20] throughout the modulation of the excitatory cascade.[24] As Aβ is known to disturb cell ionic homeostasis on various levels including calcium balance,[25] it can be hypothesized that a riluzole moiety may partially mitigate such Aβ-induced ionic homeostasis misbalance. Thus, a second series of compounds was designed with a riluzole core moiety and further combined with a urea linker and substituted phenyl moiety to more closely address the effect of phenyl ring substitution variations (Scheme 1). The urea linker was selected based on compounds that were found to be the most potent in perturbing the ABAD-Aβ interaction.[16] Additionally, the phenyl substitutions were based upon the first series of compounds, where oxygen-based substitutions with additional halogen substitutions were selected. The second series includes both overlapping substitutions of the first compound series (to confirm possible lead structures and validate structure-activity relationship). More importantly additional variations of the phenyl ring were made to more exhaustively explore possible phenyl ring variations and structure-activity relationship necessary for ABAD inhibition.

While commercially available 6-flouro and 6-chloro substituted benzo[*d*]thiazole-2-amines were used in the first series of compounds, 6-(trifluoromethoxy)benzo[*d*]thiazol-2-amine (riluzole) was prepared from its corresponding *para*-substituted aniline derivative (Scheme 2). Therefore, 4-(trifluoromethoxy)aniline was treated with potassium thiocyanate and bromine to afford 6-(trifluoromethoxy)benzo[*d*]thiazol-2-amine (**1**) in excellent yield (94%).[26]



Scheme 3. Synthesis of benzothiazolyl ureas **5**–**50**. Reagents and conditions: (a) CDI, DCM, reflux; (b) Ar-NH_2, MeCN, reflux.