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# Synthesis and structure-activity relationship study of multi-target triazine derivatives as innovative candidates for treatment of Alzheimer's disease

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ABSTRACT

The complex pathogenesis of Alzheimer's disease (AD) requires using multi-target ligands (MTLs) for disease management. We synthesized, characterized and evaluated a series of novel triazine analogues as MTLs for AD. The biological screening results indicated that most of our compounds displayed potent inhibitory activities against β-site APP-cleaving enzyme 1 (BACE1) using a FRET-based assay. Compounds **6c** and **6m** were found to possess significant BACE1 inhibitory properties with $IC_{50}$ values of 0.91 (±0.25) μM and 0.69 (±0.20) μM, respectively. DPPH radical scavenging activity evaluation showed that compounds with hydroxyl and pyrrole moieties had antioxidant effects. Docking evaluations provided insight into enzyme inhibitory interactions of novel synthesized compounds with the BACE1 active site involving a critical role for Gln73 and/or Phe108 alongside of Asp32. Metal chelation tests confirmed that compound **6m** is a chelator for $Fe^{2+}$, $Fe^{3+}$, $Zn^{2+}$, $Cu^{2+}$. Moreover **6m** as the most potent BACE1 inhibitor did not show any toxicity against PC12 neuronal cells. These findings demonstrate the high potential of triazine scaffolds in the design of MTLs for treatment of AD.



## 1. Introduction

About 46 million people worldwide suffer from Alzheimer's disease (AD) and this number is expected to exceed 130 million by 2050 [1,2]. The increase in the number of AD patients will directly translate into a heavier economic and societal burden and create a great demand for prevention and treatment [3]. AD is a multifactorial and highly complex process and its pathogenesis involves multiple mechanisms, some of which include changes in amyloid precursor protein (APP) metabolism, phosphorylation of tau protein, mitochondrial dysfunction, oxidative stress, etc. [4]. Among several proposed mechanisms, the amyloid cascade hypothesis is the most widely accepted [5,6]. Aβ-peptides, which exist as distinct peptides mostly between 37 and 43 amino acids long, are derived from (APP) by the sequential action of β-site APP-cleaving enzyme 1 (BACE1) and γ-secretase. Among the various Aβ isoforms, Aβ42 has a high tendency to polymerize and make fibrils [7]. Aβ aggregates into oligomers leading to the production of reactive oxygen species (ROS), oxidative stress and eventually cell death [8,9]. Several studies over the past two decades have indicated that $Cu^{+2}$, $Zn^{+2}$ $Fe^{+3}$ and $Fe^{+2}$ as biometals play a role in Aβ aggregation, changing Aβ morphology, metal dyshomeostasis, increasing the cytotoxicity of Aβ and finally generating ROS such as hydrogen peroxide and hydroxyl radicals [10,11] resulting in oxidative damage and cell death. Therefore, antioxidants with metal chelating activity or agents that reduce the toxic effects of oxidative stress could be helpful in delaying or preventing this disorder [12,13].

In addition to searching for drugs to combat AD, finding potential inhibitors of BACE1 has become one of the most pursued therapeutic strategies in the fight against AD [2]. Peptidomimetic BACE1 inhibitors including hydroxyethylene, hydroxyethylamine, hydroxymethyl carbonyl, and etc. are lacking metabolic stability and pharmacokinetic properties [14]. Nonpeptide inhibitors are developed based on their smaller size, better metabolic stability, and improved blood-brain-barrier penetration. Over the past few decades, many scaffolds such as acyl guanidine, aminoimidazole, amino/iminohydantoin, aminothiazoline, aminooxazoline and 2-aminopyridine have been developed [15,16]. The success of these small molecules suggests that BACE1 inhibitors have the potential to combat AD.

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Taken together, drugs with the potential to ameliorate multiple routes of AD pathogenesis are in demand [17]. Based on our ongoing interest to develop novel treatments for AD [18–20], we focused on multi-target properties of anti-AD agents to combat the multifactorial nature of the disease [21]. We designed a dithiophen, 1,2,4-triazine skeleton as core structure containing imide linkers bearing an appropriate aryl pendant. Fifteen derivatives were synthesized and evaluated for their multifunctional biological activities including BACE1 inhibition, antioxidant activity and metal chelating potential. Finally, molecular modeling study into BACE1 active site was performed to provide insight into the binding mode and the structure-activity relationships of the novel-(thio phene-2-yl)-1,2,4-triazine derivatives. Our results suggest that compounds with hydroxyl and indole moieties demonstrate promising BACE1 inhibitory activity.

## 2. Results and discussion

### 2.1. Designing consideration

Astex Pharmaceuticals have reported the use of fragment-based screening to identify amino pyridines with BACE1 affinity more than 1 nM (Fig. 1, compound **1**). Further modification of this structure resulted in the identification of almost 300 amino pyridine analogs with an affinity of 1 nm–100 μM [22,23]. In 2015, the critical role of the six membered 1,3,5-triazin2(1H)-one ring (Fig. 1, compound **2**) as novel cyclic guanidine BACE1 inhibitor with $IC_{50}$ = 16 μM was reported. This skeleton demonstrated good brain permeability in a pharmacokinetic assessment in the mice model with effective neuroprotective and neurogenic activities [24]. Moreover, high throughput and fragment-based screening followed by X-ray crystallography led to the discovery of efficient small molecule derivative of acylguanidine with $IC_{50}$ of 3.7 μM (compound **3**, Fig. 1). The X-ray structure of this compound with BACE1 enzyme revealed the key hydrogen bond interactions with the two catalytic aspartyl residues. The pyrrole ring in this structure forms an edge to face interaction with Tyr71 and phenyl rings oriented toward S1 and S2′ pocket [25].

On the other hand, accumulation of Aβ to form aggregates can be accelerated by metal ions. In this context, compounds containing electron donor groups can form complexes with biometals and reduce the formation of fibrillary Aβ plaques [13]. It has been reported that the triazine moiety (compound **4**, Fig. 1) could serve as the metal chelator backbone to overcome AD [26–28].

Considering the above mentioned factors and based on our previous efforts for the treatment of AD [29,30], we exploited a molecular hybridization strategy to design novel di-(thiophene-2-yl)-1,2,4-triazinederivatives bearing different aryl hydrazone moieties (Fig. 1, structure **A**). Apart from the metal chelating potential of the 1,2,4-triazine moiety, a cyclic guanidine was used to provide favorable H-bond interactions with the key catalytic aspartates (Asp32 and 228) of BACE1 active site. The phenyl groups of compound **3** were bio isoestericaly replaced with thiophen moieties to modulate the lipophilic characteristics of the designed backbone and enable access to the S2 and S2′ pockets of BACE1 active site. Derivatization of the hybridized backbone was performed using the involvement of different aryl pendants with effective antioxidant and radical scavenging potential.

### 2.2. Chemistry

In an attempt to provide the structural features for BACE1 inhibitory activity, a series of 1,2,4 triazine derivatives were synthesized according to the previously reported method [31]. A schematic of the synthetic pathway is depicted in Fig. 2. First, commercially available thenil **1** was reacted with thiosemicarbazide **2** refluxing in ethanol to afford compound **3** (yield 80%). Methylation of thiol derivative **3** was performed via the reaction with methyl iodide in basic ethanol. Compound **4** in Fig. 2 was added to an excess of hydrazine hydrate in ethanol at reflux conditions. The crude product **4** was purified by recrystallization in ethanol. Finally, target compounds were prepared in 70–90% yield by adding selected aldehyde to warm solution of compound **5** in ethanol and treated with a few drops of glacial acetic acid. The structures of purified products were confirmed by IR, $^{1}H$ NMR, $^{13}C$ NMR and mass spectroscopy.

### 2.3. Evaluation of BACE1 inhibitory activity and structure–activity relationship

The BACE1 inhibitory activity of 15 arylidene hydrazone derivatives of di-(thiophene-2-yl)- 1,2,4-triazine scaffolds was

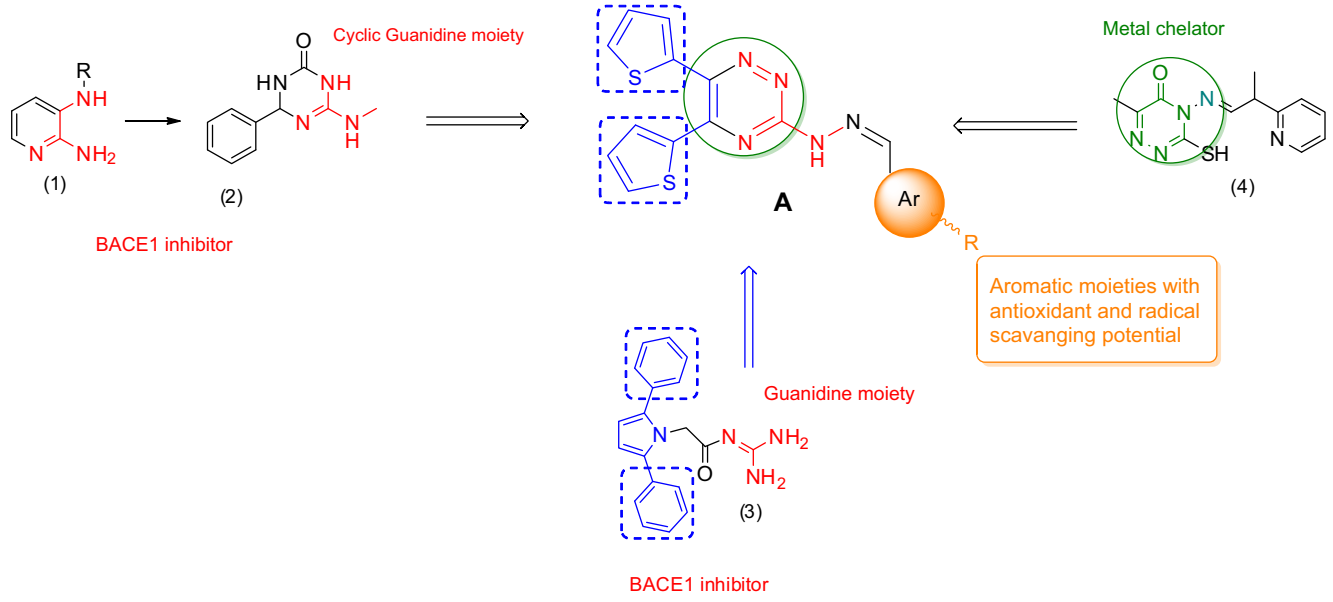


**Fig. 1.** Designing of a novel series of 1,2,4-triazines linked to the different arylhydrazone moieties as dual metal chelator and BACE1 inhibitor with antioxidant potential.