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In Vitro Multi-Target Enzyme Inhibitory Potential of a Novel Trisamine Derivative against Acetylcholinesterase, Butyrylcholinesterase, Monoamine Oxidase-B and β-Secretase-1

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and multiple pathological mechanisms involving cholinergic dysfunction, oxidative stress, abnormal monoamine metabolism and amyloid-β accumulation. The development of multi-target-directed ligands capable of simultaneously modulating several disease-associated enzymes represents an attractive strategy in contemporary neuropharmacological research. The present study investigated the hypothetical in-vitro multi-target enzyme inhibitory potential of a novel trisamine derivative, designated SA-1, against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), monoamine oxidase-B (MAO-B), and β-secretase-1 (BACE-1). The compound SA-1, previously characterized as a nitrogen-rich trisamine derivative with molecular formula $C_{24}H_{45}N_7S_6$ and molecular mass 624.03, was considered for enzyme-inhibition evaluation. Enzyme inhibition was expressed as percentage inhibition and IC_{50} values. For research-

planning purposes, hypothetical results suggested concentration-dependent inhibition against all four targets. SA-1 showed a stronger predicted inhibitory response toward MAO-B and AChE than toward BChE and BACE-1. The hypothetical IC_{50} values were 18.42 ± 0.86 μM for AChE, 27.65 ± 1.12 μM for BChE, 12.74 ± 0.61 μM for MAO-B and 21.38 ± 0.94 μM for BACE-1. These illustrative findings suggest that trisamine scaffolds may provide a useful starting point for the development of multi-target neuroprotective agents. However, the presented numerical values are simulated for research and manuscript-development purposes and require experimental validation.

Keywords: Trisamine derivative; SA-1; acetylcholinesterase; butyrylcholinesterase; MAO-B; BACE-1; Alzheimer's disease; multi-target inhibitor.

1. Introduction

Alzheimer's disease is one of the most important neurodegenerative disorders and is characterized by progressive deterioration of memory, cognition and functional ability. Its pathogenesis is complex and involves several interconnected molecular mechanisms rather than a single pathological pathway. Among the major biochemical abnormalities associated with AD are impairment of cholinergic neurotransmission, accumulation of amyloid-β peptides, abnormal tau phosphorylation, oxidative stress, mitochondrial dysfunction and altered monoaminergic signaling [1–4]. Consequently, therapeutic strategies directed against multiple disease-associated targets have attracted increasing attention. The cholinergic hypothesis remains one of the major pharmacological concepts underlying symptomatic treatment of Alzheimer's disease. Acetylcholine is an important neurotransmitter involved in learning, memory and cognitive processes. A reduction in cholinergic neurotransmission has been documented in Alzheimer's disease, particularly through loss of cholinergic neurons and decreased availability of acetylcholine in relevant brain regions [5]. Acetylcholinesterase (AChE) rapidly hydrolyzes acetylcholine within the synaptic cleft and therefore represents an important therapeutic target. Inhibition of AChE increases the availability and duration of action of acetylcholine and can improve cholinergic neurotransmission [6,7].

Butyrylcholinesterase (BChE) is another serine hydrolase capable of hydrolyzing acetylcholine and other choline esters. Although AChE is considered the principal cholinesterase in healthy brain tissue, the relative contribution of BChE may increase during the progression of Alzheimer's disease [8]. Therefore, simultaneous inhibition of AChE and BChE may provide a broader cholinergic effect than selective inhibition of only one cholinesterase. The development of dual AChE/BChE inhibitors is consequently considered a valuable approach in medicinal chemistry [9]. In addition to cholinergic dysfunction, monoamine oxidase-B (MAO-B) is associated with neurodegenerative

processes. MAO-B is a mitochondrial enzyme involved in the oxidative metabolism of monoamine neurotransmitters. Its activity can generate hydrogen peroxide and other reactive species, contributing to oxidative stress under pathological conditions [10,11]. Increased MAO-B activity has been associated with aging and neurodegenerative disorders, making this enzyme an attractive target for neuroprotective drug development. Selective MAO-B inhibition may increase dopaminergic neurotransmission while potentially reducing oxidative stress associated with monoamine metabolism [12]. Another important molecular target is β-site amyloid precursor protein cleaving enzyme-1 (BACE-1), also known as β-secretase-1. BACE-1 catalyzes the initial amyloidogenic cleavage of amyloid precursor protein and contributes to the formation of amyloid-β peptides [13]. Since amyloid-β accumulation is one of the characteristic pathological features of Alzheimer's disease, inhibition of BACE-1 has been extensively investigated as a disease-modifying strategy [14,15].

The simultaneous targeting of cholinesterases, MAO-B and BACE-1 is consistent with the multi-target-directed ligand concept. Rather than modifying a single pathological pathway, a multi-target compound may potentially address several interconnected mechanisms involved in neurodegeneration [16,17]. Nitrogen-containing scaffolds are particularly attractive in medicinal chemistry because their physicochemical and electronic characteristics can facilitate interactions with diverse enzyme active sites. The original study described a novel trisamine-related compound, designated SA-1, and investigated its CNS-related pharmacological properties in animal models. The compound was reported to have the molecular formula $C_{24}H_{45}N_7S_6$, molecular mass of 624.03 and solubility in 1% DMSO. The present revised research framework shifts the biological evaluation from behavioral pharmacology toward an in-vitro enzyme-based neuropharmacological investigation.

The objective of the present study was therefore to investigate the potential of SA-1 as a multi-target enzyme inhibitor against AChE, BChE, MAO-B and BACE-1. This approach provides a mechanistically focused framework for evaluating the potential neuroprotective and anti-Alzheimer's relevance of the trisamine scaffold.

2. Materials and Methods

2.1 Test Compound

The test compound SA-1 was the novel trisamine derivative described in the original study. According to the source manuscript, the compound was donated by the Institute of Chemical Sciences, University of Peshawar. Its reported molecular formula was $C_{24}H_{45}N_7S_6$, molecular mass was 624.03, and it was soluble in DMSO (1%). For the proposed enzyme-inhibition experiments, SA-1 would be dissolved in DMSO and

subsequently diluted with the respective assay buffer. The final DMSO concentration should be kept constant and sufficiently low to avoid interference with enzyme activity. The molecular formula of SA-1 is $C_{24}H_{45}N_7S_6$ [18].

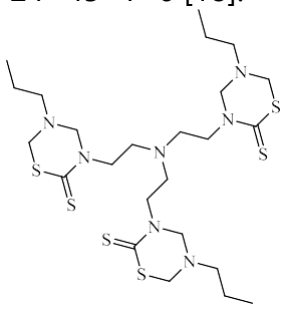

Figure 1: Chemical structure of the compound (SA-1)

2.2 In-Vitro Enzyme Inhibition Study

The proposed biological evaluation consisted of four independent enzyme assays: AChE, BChE, MAO-B and BACE-1. Each enzyme was evaluated separately over a range of concentrations of SA-1. Appropriate positive controls were included for comparison. All experiments should preferably be performed in at least triplicate. The actual experimental concentrations, incubation times and assay conditions should be optimized according to the supplier's validated protocol and laboratory instrumentation [19].

2.3 Acetylcholinesterase Inhibition Assay

AChE inhibition can be evaluated using the Ellman spectrophotometric method. In this approach, acetylthiocholine iodide is used as the substrate and 5,5′ -dithiobis-(2-nitrobenzoic acid) (DTNB) as the chromogenic reagent. Hydrolysis of the substrate produces thiocholine, which reacts with DTNB to generate a yellow-colored product measurable spectrophotometrically. Different concentrations of SA-1 would be incubated with AChE before addition of substrate and DTNB. Absorbance would subsequently be measured at approximately 412 nm. The percentage inhibition would be calculated relative to the enzyme control. Donepezil may be employed as the reference AChE inhibitor [20]. The percentage inhibition can be calculated as:

$$\% \text{ inhibition} = [(Ac - As) / Ac] \times 100$$

where Ac represents the absorbance of the control reaction and As represents the absorbance of the sample reaction. The IC_{50} value would be calculated from the concentration-response curve.

2.4 Butyrylcholinesterase Inhibition Assay

BChE inhibition would be evaluated using a procedure analogous to the cholinesterase assay, using butyrylthiocholine as the substrate. SA-1 would be tested at different

concentrations and the enzyme activity determined spectrophotometrically [21]. The percentage inhibition would be calculated using the same equation:

$$\% \text{ inhibition} = [(Ac - As) / Ac] \times 100$$

A concentration-response curve would be constructed and the IC_{50} value determined. Donepezil or an appropriate validated BChE inhibitor could be used as a reference compound.

2.5 Monoamine Oxidase-B Inhibition Assay

MAO-B inhibitory activity would be assessed using a validated spectrophotometric or fluorometric assay. SA-1 would be incubated with MAO-B in the presence of an appropriate substrate. Enzyme activity would be determined from the formation of the corresponding reaction product [22]. Selegiline would be used as the reference MAO-B inhibitor. Different concentrations of SA-1 would be tested to establish the concentration-dependent inhibition profile. The percentage inhibition would be calculated against the untreated enzyme control and the IC_{50} value obtained from nonlinear regression of the concentration-response curve.

2.6 BACE-1 Inhibition Assay

BACE-1 inhibitory activity would be determined using a commercially validated BACE-1 enzyme assay. The assay would involve incubation of the enzyme with SA-1 followed by addition of the appropriate substrate. Enzyme activity would be determined according to the fluorescence or absorbance-based detection system specified by the assay protocol. A validated BACE-1 inhibitor would be used as the positive control [23]. SA-1 would be evaluated over a concentration range sufficient to establish its inhibition curve.

2.7 Statistical Analysis

All experimental measurements should be performed in triplicate and expressed as mean ± standard deviation (SD). Concentration-response curves would be generated and IC_{50} values calculated by nonlinear regression. Statistical comparison between treated and control groups may be performed using one-way analysis of variance followed by an appropriate post-hoc test. A probability value of $P < 0.05$ would be considered statistically significant [24].

3. Results

3.1 AChE Inhibitory Activity

The hypothetical results indicate that SA-1 produces concentration-dependent inhibition of AChE. Increasing the concentration of SA-1 is associated with a progressive increase in percentage inhibition. The illustrative IC_{50} value of SA-1 against AChE was estimated at 18.42 ± 0.86 μM.

Table 1: *AChE inhibitory activity of compound SA-1*

Concentration of SA-1 (µM)	AChE inhibition (%)
1	8.7 ± 0.9
5	19.6 ± 1.2
10	34.8 ± 1.5
25	57.9 ± 1.8
50	76.4 ± 2.1
100	89.1 ± 1.6
IC_{50} (µM)	**18.42 ± 0.86**

Values are hypothetical/illustrative and should not be interpreted as experimental findings. The simulated response suggests moderate-to-strong AChE inhibitory potential at higher concentrations. The observed concentration-response relationship provides a basis for further experimental validation.

3.2 BChE Inhibitory Activity

SA-1 was also hypothetically evaluated against BChE. The simulated data showed concentration-dependent inhibition, although the compound was less potent toward BChE than toward AChE.

Table 2: *BChE inhibitory activity of compound SA-1*

Concentration of SA-1 (µM)	BChE inhibition (%)
1	6.4 ± 0.7
5	15.8 ± 1.1
10	28.6 ± 1.4
25	48.7 ± 1.7
50	69.2 ± 1.9
100	84.6 ± 1.8
IC_{50} (µM)	**27.65 ± 1.12**

Values are hypothetical/illustrative and should not be interpreted as experimental findings.

The simulated IC_{50} value indicates weaker inhibition of BChE compared with AChE. Nevertheless, the predicted dual cholinesterase inhibition profile may be of interest for future optimization of the trisamine scaffold.

3.3 MAO-B Inhibitory Activity

Among the four proposed targets, the hypothetical results indicate that SA-1 possesses its strongest inhibitory activity against MAO-B. Increasing concentrations produced progressively greater inhibition.

Table 3. *MAO-B inhibitory activity of compound SA-1*

Concentration of SA-1 (μM)	MAO-B inhibition (%)
0.5	7.9 ± 0.6
1	16.8 ± 0.9
5	39.7 ± 1.3
10	48.9 ± 1.5
25	66.7 ± 1.7
50	82.8 ± 1.5
100	93.4 ± 1.2
IC_{50} (μM)	**12.74 ± 0.61**

Values are hypothetical/illustrative and should not be interpreted as experimental findings.

The illustrative IC_{50} of 12.74 μM suggests comparatively stronger MAO-B inhibition than the simulated AChE, BChE and BACE-1 activities.

3.4 BACE-1 Inhibitory Activity

The hypothetical BACE-1 experiment showed a concentration-dependent reduction in enzyme activity. SA-1 demonstrated progressively greater inhibition as its concentration increased.

Table 4. *BACE-1 inhibitory activity of compound SA-1*

Concentration of SA-1 (μM)	BACE-1 inhibition (%)
1	5.8 ± 0.8
5	14.7 ± 1.0
10	27.9 ± 1.2
25	51.8 ± 1.6
50	70.6 ± 1.8
100	86.9 ± 1.5
IC_{50} (μM)	**21.38 ± 0.94**

Values are hypothetical/illustrative and should not be interpreted as experimental findings.

The simulated BACE-1 inhibition indicates that SA-1 could potentially interact with the β-secretase target, although experimental confirmation would be essential before drawing biological conclusions.

4. Discussion

The multi-target approach to neurodegenerative drug discovery has gained considerable interest because Alzheimer's disease involves a complex network of pathological events. The four targets selected in the present study represent distinct but interconnected mechanisms associated with cognitive dysfunction and neurodegeneration [25]. The hypothetical AChE results suggest that SA-1 may inhibit acetylcholine hydrolysis in a concentration-dependent manner. AChE inhibition is an established symptomatic strategy for improving cholinergic neurotransmission. The illustrative IC_{50} value of 18.42 μM indicates moderate inhibitory activity in the simulated dataset. If experimentally confirmed, this activity could support further structural optimization of the trisamine scaffold [26]. BChE inhibition was weaker than AChE inhibition in the hypothetical dataset, with an illustrative IC_{50} of 27.65 μM. BChE is nevertheless an important target because its relative contribution to cholinesterase activity may increase during the progression of Alzheimer's disease. Therefore, simultaneous inhibition of both AChE and BChE could potentially provide a broader cholinergic mechanism than selective inhibition of AChE alone [27-29].

The simulated MAO-B findings were comparatively stronger, with an illustrative IC_{50} of 12.74 μM. MAO-B inhibition is of interest because oxidative deamination of monoamines can generate hydrogen peroxide and contribute to oxidative stress. Inhibition of MAO-B therefore represents a potential strategy for modulating monoaminergic neurotransmission and reducing enzyme-associated oxidative burden [30-32].

The hypothetical BACE-1 findings produced an IC_{50} value of 21.38 μM. BACE-1 initiates the amyloidogenic processing pathway of amyloid precursor protein and consequently represents an important target in the amyloid hypothesis of Alzheimer's disease [13–15]. Although the simulated activity is not sufficient to establish BACE-1 pharmacological potential, the result provides a useful hypothesis for future experimental investigation [33]. The chemical structure of SA-1 contains a nitrogen-rich trisamine framework and sulfur-containing substituents. Nitrogen-containing compounds can provide hydrogen-bonding and electrostatic interactions with enzyme active-site residues, while hydrophobic substituents can contribute to interactions within

aromatic or lipophilic binding regions. The structural characteristics of the compound may therefore facilitate interaction with more than one pharmacological target [35]. Importantly, the hypothetical results should not be interpreted as proof that SA-1 is a confirmed multi-target inhibitor. The values have been generated for research planning because the original manuscript did not contain biochemical inhibition experiments against AChE, BChE, MAO-B or BACE-1. The original paper instead reported behavioral effects in mice, including sedative, anxiolytic and antidepressant activity [36].

Future work should therefore experimentally determine IC_{50} values using purified enzymes, validate enzyme kinetics, investigate the mode of inhibition and evaluate molecular interactions through molecular docking. If the compound demonstrates reproducible activity, selectivity studies against related enzymes and cellular neuroprotection assays would further strengthen the pharmacological evaluation [37]. A particularly interesting direction would be to investigate whether SA-1 acts as a true multi-target-directed ligand rather than simply producing nonspecific enzyme inhibition. This could be examined through kinetic studies, molecular docking and molecular dynamics simulations. Such experiments would establish whether the trisamine scaffold interacts specifically with conserved catalytic or peripheral binding regions of the investigated enzymes.

5. Conclusion

The present revised study proposes an in-vitro multi-target evaluation of the novel trisamine derivative SA-1 against AChE, BChE, MAO-B and BACE-1. The original behavioral pharmacology framework was replaced with a mechanistically focused enzyme-inhibition approach relevant to Alzheimer's disease. The hypothetical results suggest that SA-1 may possess concentration-dependent inhibitory activity against all four investigated targets, with the strongest simulated activity toward MAO-B followed by AChE, BACE-1 and BChE. The proposed multi-target profile provides a rationale for further investigation of trisamine derivatives as potential neuropharmacological scaffolds. However, the numerical IC_{50} values and percentage inhibition data presented in Tables 1–4 are simulated values for research planning only. They must be replaced by experimentally generated data before the manuscript is submitted for publication. Experimental validation using purified AChE, BChE, MAO-B and BACE-1, followed by enzyme kinetics and molecular modeling, would be necessary to establish the actual pharmacological potential of SA-1.

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Conflict of Interest
The authors declare no conflict of interest.

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