ORIGINAL RESEARCH ARTICLES

PYRAZOLINE CONTAINING MOLECULES AS MULTIFUNCTIONAL AGENTS IN ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a progressive, neurodegenerative disease which is caused mainly due to accumulation of an aberrant protein known as β-amyloid in the form of senile plaques. However, over the past few years, network biology studies have indicated that classical "one drug-one target" hypothesis may not work in diseases such as AD where the biochemical disease mechanisms are intricately interconnected. Therefore, multifunctional molecules which can modulate several targets could be the key towards finding the therapeutics for this debilitating disorder. Keeping this in mind, several pyrazoline containing molecules with promising Aβ aggregation inhibition potential were explored further against key targets involved in AD, such as cholinesterases, oxidative stress and advanced glycation end products (AGE). Some potential multifunctional molecules were identified as a result of this work.

Keywords: Pyrazoline, Multifunctional, Alzheimer's disease, Cholinesterase, Oxidative stress, AGE

INTRODUCTION

Development of safe and effective treatment for Alzheimer's disease (AD) has been one of the foremost challenges in the health research. Current theory suggests that the pathology of AD begins with the aberrant accumulation of misfolded proteins within the brain. These proteins include extracellular amyloid-based plaques[1,2] along with intracellular tau-based neurofibrillary tangles (NFTs)[3]. In addition to these deposits, a higher level of DNA damage occurs in mitochondria of the frontal, parietal and temporal lobes due to oxidative stress[4]. This stress often contributes to induce apoptosis, resulting in neurodegeneration. It may also lead to synaptic degeneration via loss of synaptic proteins. The level of important neurotransmitters like acetylcholine is greatly reduced, resulting in various symptoms of AD such as memory impairment, cognitive deficits, and reduced capacity for abstract thinking[5]. Further, the formation and accumulation of advanced glycation end products (AGEs) has been implicated in the progression of age-related diseases such as AD[6]. Network biology studies have pointed out that these biochemical mechanisms occur concurrently along with many others and are proposed to be highly interconnected[7].

Owing to this complex cascade of interconnected mechanisms, the One-Disease-One-Drug approach may not be suitable when it comes to AD. Multi-Target-Directed Ligands (MTDLs) can be considered as a solution to overcome this issue since a multifunctional ligand may be more effective against this multi-factorial disease[7].

Previously, in our laboratory, several pyrazoline containing molecules, belonging to two different structural classes, were evaluated for Aβ aggregation inhibition (Fig.1). Among these, several molecules showed better activity as compared to curcumin, which was used as standard. Additionally, these molecules displayed the potential to counter Aβ induced neurotoxicity and showed good CNS penetration. Therefore, the aim of the present work was to evaluate the activities of these molecules against several key targets, namely, AChE, BuChE, AGE and oxidative stress, taking into account the complex network of molecular mechanisms in AD.

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MATERIALS AND METHODS

In vitro AChE and BuChE activity inhibition assay

A modified version of Ellman´s colorimetric assay was used for the enzymatic activity of BuChE and AChE [8-10]. For the current analyses, it was adopted to a high throughput assay for use in 384-well plates. Briefly, for the screening of the compounds, 25µL/well of a 1:450 diluted solution of a pooled human plasma and 1:768 diluted (final concentration 3.5 ng/mL) purified recombinant human AChE protein (from Sigma) were used for BuChE and AChE activity assay, respectively. These were pre-incubated with 25 µL/wells of different compounds for 10-30 minutes at room temperature. The concentration of the compounds solution was 3X to give a final concentration of 200 µM and 100 µM for BuChE and AChE, respectively, in a final volume of 75 µL in each well. The stock concentration of the compounds, when prepared in 100 % DMSO, was chosen so that the final DMSO concentration in the wells was less than 3%. Finally, 25 µL of a cocktail mix prepared in Na/K phosphate buffer, containing 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, final concentration 0.4 mM) and butyrylthiocholine iodide (BTC, final concentration 1 mM) or acetylthiocholine iodide (ATC, final concentration 0.5 mM) were added to each well using a multichannel repeated pipette, and the changes in absorbance were monitored kinetically at 412 nm wavelength for 15-20 minutes with one-minute interval, using a Tecan Infinite M1000 spectrophotometer.

AGE inhibition assay

Bovine serum albumin (10 mg/mL) was incubated with glucose (500 mM) in 0.1 M phosphate buffered saline (PBS), pH 7.4 containing 0.02% sodium azide, sample molecules and PBS was added to make up the total volume of 200 µL in 96 well plate. Aminoguanidine was used as a positive control for this study (1:1 ratio with glucose). Each solution was incubated in dark at 37°C for 21 days for formation of AGEs. After incubation fluorescent AGEs were determined by measuring fluorescence intensity (excitation wavelength of 370 nm and emission wavelength of 440 nm) using spectrofluorometer (Synergy, Epoch well plate reader). The study was carried out in triplicate.

DPPH assay

100 µL of test samples dissolved in methanol were added to 100 µL of methanolic solution of DPPH (200 µM) so that the test molecules:DPPH ratio is 1:1. Equal amount of methanol was added to the control. The samples were incubated at room temperature for 20 min and absorbance was recorded at 517 nm. The ascorbic acid was used

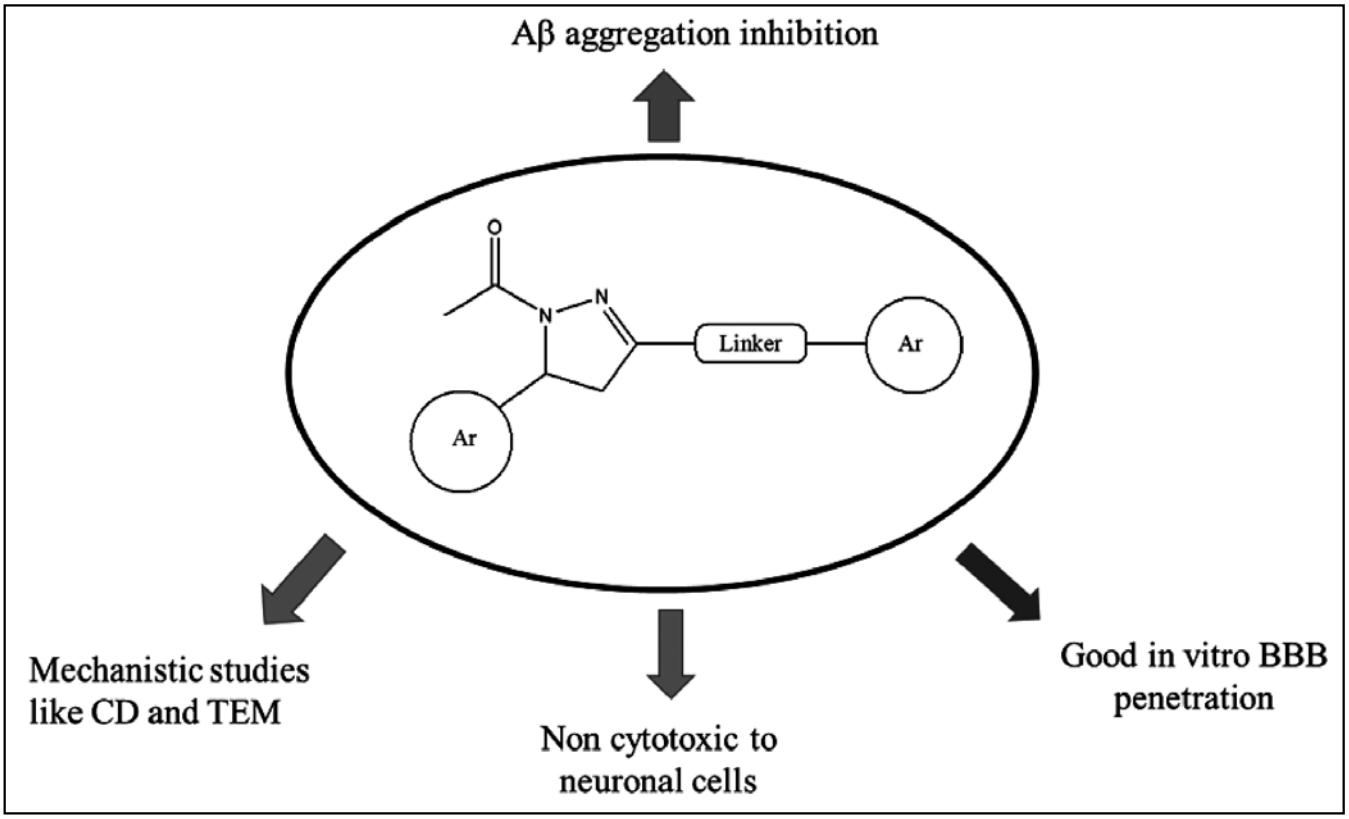
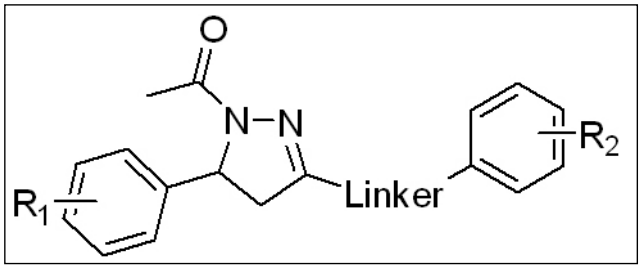


Fig. 1: General structure of previously explored in-house pyrazoline derivatives as Aβ aggregation inhibitors

Table I: Biological evaluation N-acetylpyrazolines with/without ethenyl linker



Code	R^1	R^2	Linker (L)	% AChE inhibition	% BuChE inhibition	% AGE inhibition	DPPH assay
1.	H	H	-	18.12	28.75	57.8	24.53
2.	4-F	H	-	1.9	28.3	63.3	23.8
3.	4-Cl	H	-	20.7	54.7	77.9	24.9
4.	4-CH_3	H	-	9.5	75.4	39.9	24.4
5.	4-OCH_3	H	-	16.9	69.3	56.4	23.1
6.	3,4-dimethoxy	H	-	9.5	47.2	43.9	24.8
7.	H	4-F	-	14.4	40.5	24.8	25.2
8.	H	4-Cl	-	26.3	21.3	55.1	25.3
9.	H	4-CH_3	-	11.4	47.1	79.6	27.2
10.	H	4-OCH_3	-	9.8	18.0	45.4	26.8
11.	H	3,4-dimethoxy	-	15.0	41.3	73.1	25.4
12.	H	H	HC=CH	No inhibition	43.32	38.8	23.8
13.	2-Cl	2-Cl	HC=CH	18.7	16.2	3.8	27.2
14.	3-Cl	3-Cl	HC=CH	26.1	6.2	52.4	24.1
15.	4-Cl	4-Cl	HC=CH	79.8	28.7	64.3	26.5
16.	3-F	3-F	HC=CH	No inhibition	30.5	53.6	23.1
17.	4-F	4-F	HC=CH	32	No inhibition	67.3	26.15
18.	4-CH_3	4-CH_3	HC=CH	16.4	20.6	4.0	24.8
19.	4-CF_3	4-CF_3	HC=CH	76.2	16.6	61.0	25.3
20.	4-OCH_3	4-OCH_3	HC=CH	71.4	22.9	70.5	27.2
21.	3,4-dimethoxy	3,4-dimethoxy	HC=CH	20.1	8.8	61	24.9
22.	2-thienyl	2-thienyl	HC=CH	9.5	28.9	0.5	26.8

**All the assays were carried out in triplicates. The percentage error ranged from 0.5-4.3%. The inhibitory activities for standards are as follows: Donepezil (For AChE): 99.7%, Tacrine (For BuChE): 99.7%, Aminoguanidine (For AGE): 44.5%, Ascorbic acid (For DPPH): 98%*

as standard and was prepared in same way as that of test molecules. % DPPH inhibition calculated using the following formula:-

$$\% \text{ DPPH inhibition} = \left[\frac{\text{Abs (Control)} - \text{Abs (sample)}}{\text{Abs (Control)}} \right] \times 100$$

where, Abs = Absorbance at 517 nm.

RESULTS

The summary of activities of pyrazoline derivatives has been depicted in Table 1.

Discussion

Structure activity relationships were studied taking into consideration the AChE inhibition, BuChE inhibition, AGE inhibition and antioxidant activities separately.

For AChE inhibition, all the molecules without the linker (1-11) displayed low inhibitory activity. For the molecules with ethenyl linker, electron withdrawing groups such as 4-Cl (15) and 4–CF3 (19) resulted in molecules with more than 75% inhibition. Decreasing the halogen size from –Cl to –F resulted in lowering of activity. 4-Methoxy substituted molecule (20) showed 71% inhibition while 3,4-dimethoxy substitution resulted in molecule with 20% inhibition (21). Replacement of phenyl ring with thiophene (22) rendered the molecule inactive.

In contrast to AChE inhibition, for BuChE inhibition, all the molecules with ethenyl linker displayed lower activity except molecule 12. Among the molecules without the linker, molecule 4, with 4-methyl substitution, was found to be the most active molecule with more than 75% inhibition.

With respect to AGE inhibition, most of the molecules showed comparable or more inhibition than standard aminoguanidine. For molecules without the linker, 9, 15 and 17 were found to be highly active. In molecules with ethenyl linker, methoxy substituted molecule 25 was found to display highest inhibition. Addition of thiophene ring rendered molecule inactive with respect to AGE inhibition.

In case of antioxidant activity, all the molecules displayed low inhibitory activity in the range of 20-30%. Hence, several promising molecules such as 15, 19 and 20 were identified as potential, multifunctional molecules (highlighted bold in Table I).

Thus, in the present work, several pyrazoline containing molecules were evaluated for their activity on key targets involved in AD such as AChE, BuChE, AGE and oxidative stress. Combined with their Aβ aggregation inhibitory activity, these molecules could be considered as potential multifunctional agents in AD.

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REFERENCES

1. A. K. Das, A. Rawat, D. Bhowmik, R. Pandit, D. Huster, S. Maiti, An early folding contact between Phe19 and Leu34 is critical for amyloid-β oligomer toxicity, **ACS Chem. Neuro.** 2015, *6*, 1290-1295.
2. D. Bhowmik, A. K. Das, S. Maiti, Rapid, cell-free assay for membrane-active forms of amyloid-β, **Langmuir**, 2015, 31 (14), 4049-4053
3. L. M. Refolo, H. M. Fillit, Drug discovery for Alzheimer's disease: the end of the beginning., **J. Mol. Neurosci.** 2004, 24, 1-8.
4. L. Huang, C. Lu, Y. Sun, F. Mao, Z. Luo, T. Su, et al., Multitarget-Directed Benzylideneindanone Derivatives: Anti-β-Amyloid (Aβ) Aggregation, Antioxidant, Metal Chelation, and Monoamine Oxidase B (MAO-B) Inhibition Properties against Alzheimer's Disease, **J. Med. Chem**. 2012, 55, 8483-8492.
5. H. S. Kundaikar, M. S. Degani, Insights into the Interaction Mechanism of Ligands with Aβ42 Based on Molecular Dynamics Simulations and Mechanics: Implications of Role of Common Binding Site in Drug Design for Alzheimer's Disease, **Chem. Biol. Drug Des.** 2015, 86, 805-812.
6. V. Srikanth, A. Maczurek, T. Phan, M. Steele, B. Westcott, D. Juskiw, et al., Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. **Neurobiol. Aging** 2011, 32, 763-777.
7. A. Cavalli, M. L. Bolognesi, A. Mìnarini, M. Rosini, V. Tumiatti, M. Recanatini, *et al.*, Multi-target-Directed Ligands To Combat Neurodegenerative Diseases, **J. Med. Chem. 2008**, 51, 347-372.
8. G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, **Biochem. Pharmacol**. 1961, *7*, 88-95.
9. R. Kumar, A. Kumar, B. Långström, T. Darreh-Shori, Discovery of novel choline acetyltransferase inhibitors using structure-based virtual screening, **Scientific Reports** 2017, 7, 16287, DOI:10.1038/s41598-017-16033-w.
10. A. Kumar, T. Darreh-Shori, DMSO: a mixed-competitive inhibitor of human acetylcholinesterase, **ACS Chem. Neuro.** 2017, 8, 2618-2625.