Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):1069-1072

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In-vitro evaluation of some 4-aminopyridine Schiff bases as dual acetyl/butyryl cholinesterase inhibitor

Saurabh K. Sinha

Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur, Rajasthan, India

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ABSTRACT

Schiff bases of 4-aminopyridine have been synthesized and evaluated for antiamnesic and cognition enhancing activity. In the present study to further understand the mechanism of action of these derivatives we have evaluated in-vitro acetycholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity. Enzyme kinetics studies were performed for all compounds to observe their nature of inhibition. The IC_{50} value of synthesized compounds showed maximum activity of compound 4APe compared to standard drug donepezil and rivastigmine whereas its kinetic analysis of enzyme inhibition demonstrated non-competitive inhibition for both enzymes AChE and BChE.

Keywords: 4-Aminopyridine, Antiacetycholinesterase**,** Antibutyrylcholinesterase, Enzyme kinetics

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, characterised by progressive loss of cholinergic neurons and accumulation of β-amyloid protein in the brain areas.[1] Onset starts with cognitive and short term memory destruction that slowly progresses to complete loss of cognitive function, impaired performance of activities of daily life.[2] The drug discovery concerning to AD is based either to develop an AChE inhibitor that inhibits the breakdown of acetylcholine (ACh) and improve the memory and cognition impairment[3] or to develop a β-secretase or γ-secretase inhibitor that blocks the formation of β-amyloid plaques.[4,5] It is evident that design and development of secretase inhibitors have not been achieved their true goal to treat the AD and needs to be explored as per the structure of protein. The U.S. Food and Drug Administration (FDA) have approved five medicines for the treatment of AD till date, out of which four are AChE inhibitors. It is reported that AChE could also play a vital role in accelerating senile β-amyloid peptide (Aβ) plaque deposition which is toxic to neurons.[6] BChE has also been reported to degrade ACh in healthy and AD affected brains and the role of BChE rises in the late phase of AD[7] thereby qualifying it as an additional target for the treatment.[8] Several carbamate derivatives of 4AP and Schiff bases of styrylpyridine have been synthesized and evaluated for their anticholinesterase activity.[9,10] Some 4-aminobutyric acid (GABA) and 2-indolinone derivatives of 4AP have also been reported to possess antiamnesic activity.[11] The hydrazone derivatives of dihydropyridine and indolinones have been reported as potent AChE, BChE and β-amyloid aggregation inhibitory activities.[12,13] 3-methylpyridinium and 2-thionaphthol derivatives of berberine have evaluated for AChE and BChE inhibitory activity respectively.[14]

EXPERIMENTAL SECTION

General

All reagents and solvents used in the study were of analytical grade purity and were procured from Sigma-Aldrich (India). Donepezil standard drug was obtained as a gift sample from Cipla Ltd (Maharashtra, India).

Estimation of cholinesterase activity (in-vitro)

The ability of all tested compounds to inhibit acetylcholinesterase from electric eel (E.C. 3.1.1.7) and butyrylcholinesterase from human serum (E.C. 3.1.1.8) was tested and their effectiveness in inhibition could be conclusive through their IC_{50} values. The IC50 values were determined by the Ellman's spectrophotometric method[15] which is performed by recording the rate of increase in the absorbance at 412 nm for 5 min. The stock solution of AChE was prepared by dissolving AChE in 0.1 M phosphate buffer (pH 8.0). In case of BChE stock solution was prepared by dissolving the lyophilized powder in an aqueous solution of gelatine 0.1%. The composition of final solution for assay consisted of 0.1 M phosphate buffer pH 8.0, with the addition of 340 mM 5,5'-dithio-bis (2-nitrobenzoic acid), 0.02 unit/mL of AChE, or BChE and 550 mM of substrate (acetylthiocholine iodide, ATCh or butyrylthiocholine iodide, BTCh, respectively). Five different concentrations of inhibitors between 20% and 80% (test compounds) were selected in order to obtain inhibition of the enzymatic activity. From the aliquots (50 µL), increasing concentration of the inhibitors were added to the assay solution and were preincubated for 20 min at 37 oC with the enzyme followed by the addition of substrate. Blank used in the assays consisted of all the components except AChE or BChE in order to account for the non-enzymatic reaction. Reaction rates of the assay were then compared and percent inhibition due to the presence of increasing concentrations of inhibitor was calculated. The concentration of each test compound was analyzed in triplicate, and IC50 values were determined graphically from log concentration percent inhibition curves.[16,17]

Enzyme kinetics study

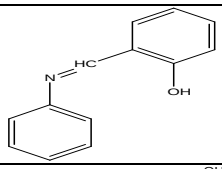
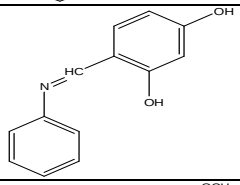
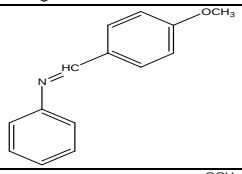
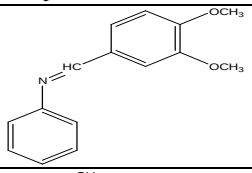
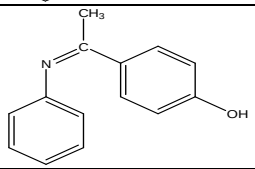
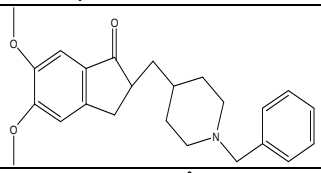
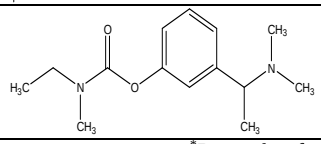
Ellman's method of spectrophotometric analysis was used to determine the type of inhibition. Acetylcholine iodide and butyrylthiocholine iodide was used as a substrate at various concentrations both below and above but near Km in a phosphate buffer at pH 8 keeping a fixed amount of cholinesterase in the absence or presence of different inhibitors. The concentration of the inhibitor was kept close to one which corresponds to 50% inhibition of the enzyme activity (IC_{50}) and their inhibitory kinetics were evaluated by the Lineweaver and Burk method.[18]

RESULTS AND DISCUSSION

The IC_{50} values of all the derivatives on AChE and BChE were determined through Ellman method and observed that compound 4APe (1.52 ± 0.66 µM) was more active than standard rivastigmine (2.25 µM) and less active than donepezil (0.04 ± 0.012 µM) with respect to their AChE inhibitory activity. In the evaluation of BChE inhibition, compound 4APe (2.16 ± 0.64) illustrated comparable activity with rivastigmine (1.66 µM) and superior activity than donepezil (15.24 ± 0.88) (Table 1).

It is worth to mention that a healthy brain contains less concentration of BChE than AChE but in AD affected brain, AChE activity decreases and BChE activity increases gradually.[19] Therefore, as AD progresses ACh regulation may become more dependent on BChE thereby paving the way for dual inhibitors to provide more persistent efficacy in early to late stage of AD than AChE selective agents.[20] The results of IC_{50} values and structure activity relationship (SAR) inferred that the activity increases in order to the number of hydroxyl and methoxy group increases on phenyl ring but more selectively the hydroxyl substituted compounds elicited better activity than methoxy substituted compounds. The substitution of the second hydrogen from imine carbon with methyl group increases in AChE inhibitory activity but the substitution by phenyl ring results a drastic increase in both AChE as well as BChE inhibitory activity. Further, enzyme kinetics study was also performed for all derivatives to gain an insight on their nature of inhibition (Table 2).

Table 1. Anticholinesterase activity of Schiff bases of 4AP, donepezil and rivastigmine

Compound Code	Compound	AChE IC_{50} (µM)	BChE IC_{50} (µM)	Selectivity for AChE[a]
4APa		56.36 ± 2.81	>1000	-
4APb		8.74 ± 0.94	422.4 ± 4.47	48.32
4APc		74.58 ± 1.35	>1000	-
4APd		15.68 ± 1.75	584.25 ± 1.26	37.26
4APe		1.52 ± 0.66	2.16 ± 0.64	5.2
Donepezil		0.04 ± 0.012	15.24 ± 0.88	381
Rivastigmine*		2.25	1.66	0.74

Data taken from Sheng et al., 2009

Table 2. Enzyme kinetics study of Schiff bases of 4AP and Donepezil

Compound	Inhibition	AChE Ki (µM) ± SEM	Inhibition	BChE Ki (µM) ± SEM
4APa	nc	52.14 ± 2.81	nc	nt
4APb	c	6.25 ± 0.94	c	415.58 ± 4.47
4APc	c	70.45 ± 1.35	nc	nt
4APd	c	12.36 ± 0.65	nc	562.58 ± 1.26
4APe	nc	1.65 ± 0.66	c	15.26 ± 0.81
Donepezil	nc	0.056 ± 0.016	nc	12.54 ± 0.76

c= competitive, nc= non-competitive, nt= not tested

The most active compound 4APe demonstrated a non-competitive inhibition for both AChE (Ki = 1.65 ± 0.66) and BChE (Ki = 15.26 ± 0.81) enzymes. Since all the hydroxyl substituted compounds showed better activity than others, hence it can concluded that hydrophobicity is a necessary parameter to cross the blood brain barrier but hydrophilicity is also important for good activity.

CONCLUSION

In conclusion we have identified a new class of potent anticholinesterase inhibitors among which benzophenone derivative 4APe deserves further study. Considering the role of BChE in the late phase of AD the dual inhibitor might also be beneficial for the treatment of AD and it can be a useful new lead to develop dual inhibitors with cognition enhancing and anti amnesic properties.

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