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Some Novel Antimicrobial Therapeutic Agents for Acetylcholinesterase Inhibitors; Synthesis of Hydroxyquinoline Ester Involving Amino Acid

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ABSTRACT

The aim of this work was to investigate the new effective agents candidate for treatment of the Alzheimer's disease. So, a series of new and highly active acetylcholinesterase inhibitors derived from hydroxyquinoline ester containing amino acid were synthesized. Antibacterial activities of the molecules were studied by the well-diffusion method against *Listeria monocytogenes 4b*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi H*, *Brucella abortus*, *Staphylococcus epidermis sp.*, *Micrococcus luteus*, *Shigella dysenteria* type *10, Bacillus cereus, Pseudomonas putida* and antifungal activity against *Candida albicans*. All the synthesized compounds behave as inhibitors against acetylcholinesterase enzyme. CysEs and MetEs show more inhibition potency.

Keywords: Alzheimer's disease, acetylcholinesterase, hydroxyquinoline, amino acid, inhibition

1. INTRODUCTION

Inorganic molecules containing amino acid have been used for a long time in biological and analytical fields [1-3]. Recently there has been a considerable interest in the chemistry of amino acid-Schiff bases compounds because of their potential nuclear medicine applications [4]. Amino acid is bivalent ligand, one important strategy to improve drug potency depends upon the use of bivalent ligands. Recently, the bivalent ligand strategy was applied to the development of blood-brain barrier penetrable acetylcholinesterase-targeted therapeutic agents [5]. Bivalent ligands may be a promising drug candidate for treatment of the Alzheimer's type. Such ligands containing donor atom can act as good banding agents. Therefore, there is considerable interest in the synthesis and application of dual binding site acetylcholinesterase inhibitors with bivalent ligands.

There are reports the synthesis and evaluation of bivalent ligands such as alkylene-linked dimers of tacrine [6]. Tacrine is the prototypical cholinesterase inhibitor for the treatment of Alzheimer's disease. In vitro, heptylene-linked tacrine dimer is more potent and selective for acetylcholinesterase (AChE) inhibition than tacrine, as a result of simultaneous binding of the tacrine units to the catalytic and peripheral sites of AChE [6]. Therefore, systematic investigation of bivalent ligands is very important for AChE inhibition. So, it would be interesting to investigate the structural

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and inhibition properties involving amino acid as bivalent ligands.

Furthermore, the amyloid β-protein (Aβ) is believed to be the key mediator of Alzheimer's disease pathology. Aβ is antimicrobial peptide and made up of up to 771 amino acids. Additionally know that Alzheimer's disease whole brain homogenates have significantly higher antimicrobial activity than aged matched non-Alzheimer's disease samples and that antimicrobial peptide action correlates with tissue Aβ levels. The ability of Aβ to self-associate to form oligomeric assemblies appears to underlie the toxic events that lead to memory impairment [7]. Based on this information, it would be interesting to investigate the antimicrobial properties of derivatives involving amino acid.

Here we report the synthesis, characterization and inhibition properties for AChE (Figure1). On the other hand, ligands with amino acids are sensitive to moisture and decompose when exposed to air, hence they are synthesized as ester structure.

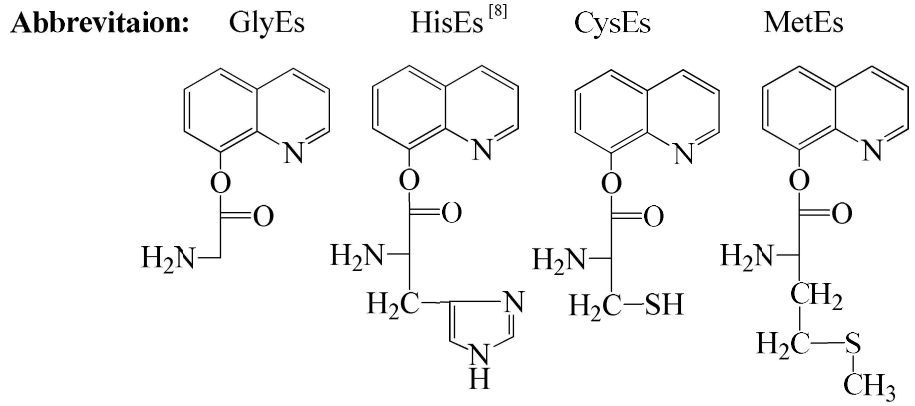


Figure 1. Studied hydroxyquinoline esters

2. EXPERIMENTAL

2.1. Equipment and Reagents

All organic solvents used in this study were purified according to standard methods. The amino acids (glycine, histidine, cysteine and methionine), hydroxyquinoline, acetylcholinesterase (EC 3.1.1.7, purified from *Electrophorus electricus* (electric eel) Type V-S, activity of 100 unit/mL) acetylthiocholine iodide (ATCh) and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. (Elemental analysis of C, H, and N were carried out on a LECO 932 elemental analyser. ^{1}H and ^{13}C-NMR spectra were recorded employing a VARIAN MERCURY 400 MHz FT spectrometer, with DMSO-d_6 as solvent. Chemical shifts (δ) are in ppm relative to TMS. IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. The LC/MS were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC, using ESI(+) method, with C-18 column. Melting points were determined with a Barnstead-Electrothermal-9200 melting point apparatus.

2.2. Synthesis of the Amino Acid Esters of 8-hydroxyquinoline (General Method)

1 ml HCl solution (37%) was added to the amino acids (10.0 mmol, 0.75 g, 1.5 g, 3.4 g and 1.5 g, glycine, histidine [8], cysteine and methionine, respectively) in 50 mL acetonitrile and stirred for 15 min at room temperature. After dissolving amino acids, 8-hydroxyquinoline (HQ), (10.0 mmol, 1.45g) solution in acetonitrile (50 mL) was added to form the amino acid-HQ esters. The mixture was refluxed for 3 h at 70-80 °C. This solution was let stand at room temperature overnight. The resulting yellow solid precipitation was filtered off, dried and recrystallized from methanol [8].

2.3. Detection of Antimicrobial Activity

The bacterial subcultures chosen were *Listeria monocytogenes 4b* ATCC19115, *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC1280, *Salmonella typhi H* NCTC-901.8394, *Brucella abortus* (A.99, UK-1995) RSKK03026, *Staphylococcus epidermis sp., Micrococcus luteus* ATCC9341, *Shigella dysenteria* type 10 NCTC 9351, *Pseudomonas putida sp., Bacillus cereus RSKK-863.* An antifungal susceptibility test was used by *Candida albicans* Y-1200-NIH, Tokyo. The ligands and the complexes were tested for their antimicrobial activity by the well-diffusion method. Each ligand and complex was kept dry at room temperature and dissolved ($1.0x10^{-4}$ M) in DMSO. DMSO was used as solvent and also for control. It was found to have no antimicrobial activity against any of the tested organisms. 1% (v/v) of 24 hours broth culture containing 10^6 CFU/ml was placed in sterile Petri dishes. Mueller- Hinton Agar (MHA) (15 ml) kept at 45 ^{o}C was then poured in to the Petri dishes and allowed to solidity. Then 6 mm diameter wells were punched carefully by using a sterile cork borer and were entirely filled with the test solutions. The plates were incubated for 24 hours at 37 ^{o}C. On completion of the incubation period, the mean value obtained for the two holes was used to calculate the zone of growth inhibition of each sample.

2.4. AChE Activitiy Assay

AChE activity measurements were performed at 30 ºC according to the spectrophotometric assay of Ellman [9]. ATCh was used as substrate for all experiments. Stock inhibitor solutions were prepared in DMSO and diluated with $1.0x10^{-4}$ M pH 7.0 phosphate buffer. The reaction took place in a final volume of 3.0 mL of phosphate-buffered solution pH 8.0, containing 0.042 unit/mL of AChE, $1.67x10^{-4}$ M DTNB, $1.0x10^{-4}$ M ATCh and inhibitor (varying from $2.5x10^{-4}$ M to $1.0x10^{-7}$ M) solution used to produce the yellow anion of 5-thio-2-nitrobenzoic acid. After 30 minute incubation, the absorbance of mixture was monitored with the spectrophotometer at 412 nm. One sample without inhibitor was always present to yield the 100% of AChE activity. IC_{50} and K_i values calculated with GraphPad Prism 6 (GraphPad Software). Inhibition types were determined in the absence and presence of inhibitor ($1.0x10^{-5}$, $5.0x10^{-6}$ and $1.0x10^{-6}$ M) with using $5.0x10^{-6}$ - $1.0x10^{-3}$ M ATCh from Lineweaver-Burke plots. Each determination was repeated three times and the values obtained as average.

3. RESULTS AND DISCUSSION

Analytical data and some of the physical properties of the amino acid esters of 8-hydroxyquinoline are summarized in Table 1. The amino acid esters are soluble in DMF and DMSO and H_2O.

Table 1. Analytical data of amino acid esters of 8-hydroxyquinoline

Abbreviation of compounds	Empiric formula % Yield	Formula Weight Mp (℃)	Analysis % found (Calcd) C	H	N	S
GlyEs	$C_{11}H_{10}O_2N_2$. $4.5H_2O$ 70	283.21 155-160	45.94 (46.65)	4.96 (6.71)	10.82 (9.89)	-
HisEs	$C_{15}H_{15}N_4O_2Cl$. $4.5H_2O$ 60	417.76 187	43.16 (42.77)	6.23 (5.21)	13.42 (13.85)	-
CysEs	$C_{12}H_{12}O_2N_2S$.$4.5H_2O$ 50	329.38 160	43.22 (43.75)	4.48 (6.37)	8.34 (8.5)	8.99 (9.73)
MetEs	$C_{14}H_{16}O_2N_2S$.$5H_2O$ 40	366.42 175	45.73 (45.89)	5.36 (7.15)	7.99 (7.64)	10.17 (8.75)

3.1. IR, UV- VIS and NMR Spectra of Amino Acid Esters of 8-Hydroxyquinoline

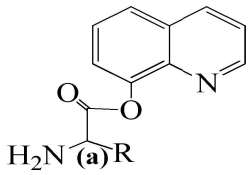
Table 2 summarizes the main IR, fragmants and molecular ion peaks of amino acid esters. In the IR spectra of the amino acid esters, the strong absorptions at 1614-1626 cm^{-1} and 1717-1738 cm^{-1} are attributed to the $\nu(C=N)_{Quinoline}$ and $\nu(C=O)_{Ester}$ bands. The aromatic stretching bands are observed in the range 1581- 1600 cm^{-1}. The observation of strong bands 1224-1298 and 1095-1126, 2767-2772 cm^{-1} may be attributed to the $\nu(C\text{-}O\text{-}C)_{Asymmetric}$ and $\nu(C\text{-}O\text{-}C)_{Symmetric}$, respectively [10,11].

Table 2. Important IR vibration frequencies (cm^{-1}) and fragmants and molecular ions peaks of amino acid esters of 8-hydroxyquinoline

Abbreviation of compounds	$\nu(C=N)_{Quinoline}$ $\nu(C=O)_{Ester}$	$\nu(C\text{-}O\text{-}C)_{Asym./Sym}$ $\nu(C=C)_{Aromatic.}$	Fragmants and molecular ions peaks			
GlyEs	1614 / 1717	1248 / 1128 1581	$[HQ]^+$ 146	$[HQ+H_2O]^+$ 163	$[GlyEs\text{-}CO_2]^+$ 158	$[GlyEs+1/2H_2O]^+$ 212
HisEs	1618 / 1737	1224 / 1126 1600	$[HQ]^+$ 146	$[His]^+$ 155.8	$[HisEs\text{-}H_2O]^+$ 299.1	-
CysEs	1627 / 1738	1298 / 1095 1596	$[HQ]^+$ 146	$[HQ+H_2O]^+$ 163	$[CysEs\text{-}CO_2]^+$ 204	$[CysEs+H_2O]^+$ 265.3
MetEs	1626 / 1735	1296 / 1095 1595	$[HQ]^+$ 146	$[Met+H_2O]^+$ 166	$[MetEs+H_2O]^+$ 294	$[MetEs+2H_2O]^+$ 313

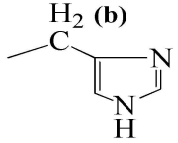
Table 3. ^1H NMR chemical (ppm) of amino acid esters of 8-hydroxyquinoline

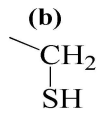
Compounds	-CH imidazole	N-CH quinoline	-CH aromatic	-CH**(a)**	-CH_2**(b)**	-CH_3**(c)**	-NH/ -SH
GlyEs	-	9.02 (d, J=1.3)	7.20-7.72 (m)	4.27 (t, J=6.3)	3.59-3.12 (m)	---	
HisEs	8.99 (dd, J=4.7,1.5) 8.77 (dd, J=8.4, 1.3)	9.11(d, J=1.2)	7.81-7.38 (m)	4.38 (t, J=7.1)	3.42-3.23(m)	---	11.05-9.14(b)/ -
CysEs	-	9.09 (d, J=1.4)	8.65-7.70 (m)	4.13(d, J=5.8)	3.31-3.33 (m)	---	-/ 1.04
MetEs	-	9.10 (d, J=1.2)	8.89-8.00 (m)	4.03 (d, J=6.7)	2.60-2.65 (m)	2.05(s)	-

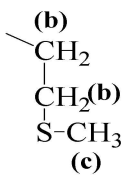


GlyEs HisEs CysEs MetEs

R: -







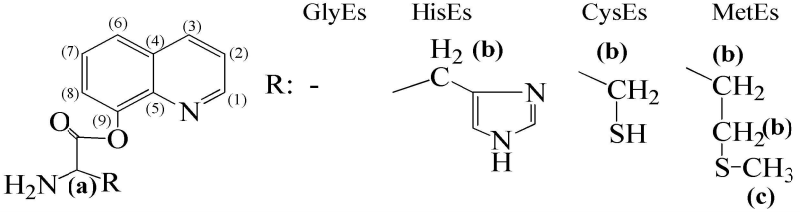
Mass spectra provide evidence for the molecular formula of the synthesized amino acid esters. LC-mass spectra for the amino acid esters are [GlyEs+1/2H_2O]$^+$212 (m/z: 97.4), [HisEs-H_2O]$^+$299.1 (m/z: 91.3), [CysEs+H_2O]$^+$265.3(m/z: 67.4),[MetEs+2H_2O]$^+$313 (m/z: 97.4).

The ^{1}H NMR spectrum of amino acid esters, recorded in DMSO-d_6 showed the following signals: imidazole proton (-CH) at 8.99 ppm and 8.77 ppm, quinoline N-CH protons at 9.02-9.11 ppm (1H), aromatic-CH proton at 7.20-8.89 ppm and aliphatic -CH/-CH_2 and -CH_3 at 4.03-4.38 ppm (1H) / 2.60-3.59 ppm (2H) and 2.05 ppm (3H), respectively (Table 3).

More detailed information about the structure of amino acid esters were provided by the ^{13}C NMR spectra data. The ^{13}C-NMR spectra data of amino acid esters (Table 4) are in accord with the proposed structures. Imidazole, aromatic and carboxyl C atoms are observed at 25.57-118.33, 39.41-151.92 and 169.31-170.99 ppm, respectively.

Table 4. ^{13}C NMR chemical (ppm) of amino acid esters of 8-Hydroxyquinoline

Compounds	-CH imidazole	-CH (1-9)	-C=O	-C(a)	-CH_2(b)	-CH_3(c)
GlyEs	-	148.57, 136.62, 127.96, 122.29, 118.18, 111.81, 40.80, 40.53, 40.25	169.31	39.97	-	-
HisEs	25.57, 140.79, 129.67, 118.33	151.92, 147.05, 129.18, 127.58, 122.48, 118.43, 113.76, 40.81, 39.41	169.31	51.47	-	-
CysEs	-	149.36, 146.15, 144.94, 130.68, 130.14, 129.81, 122.74, 118.70, 116.30	169.37	54.39	24.68	-
MetEs	-	150.39, 145.80, 143.90, 131.99, 130.05, 129.95, 122.63, 118.60, 115.24	170.99	51.33	29.91, 29.05	14.71



3.2. Biological Activity

All the synthesized compounds were screened for antimicrobial activity in DMSO solvent as a control substance. The compounds were tested with the same concentrations in DMSO solution ($1.0x10^{-4}$ M). All the synthesized compounds and antibiotic exhibited varying degree of inhibitory effects on the growth of different tested strains (Table 5, Figure 2).

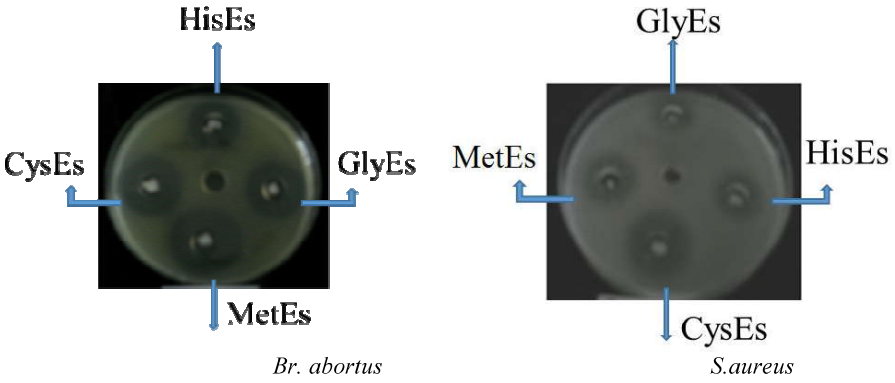
Table 5. Antimicrobial activity of studied molecules ($1.0x10^{-4}$ M) and standard reagents (diameter of zone inhibition (mm))

		GlyEs	MetEs	HisEs	CysEs	Control (DMSO)
Gram (+)	*Sh.dys. typ 7*	-	15	14	20	-
	P.putida	11	13	12	15	-
	E.aerogenes	12	17	12	11	-
	Br. Abortus	24	25	23	24	-
	L.monocytogenes 4b	-	23	19	20	-
	B.cereus	-	15	16	12	-
Gram (-)	*S.aureus*	11	15	14	16	-
	S.epidermis	12	16	14	22	-
	M.luteus	15	10	12	20	-
	E.coli	-	13	11	15	-
Antifungal	*C. albicans*	16	24	20	21	-

Positive control	*S.aureus*	*P.putida*	*E.coli*	*Br. abortus*	*C.albicans*
K30	25	14	25	-	-
SXT25	24	18	18	-	-
AMP10	30	8	10	-	-
AMC30	30	15	14	-	-
NYS100	-	-	-	-	-

SXT25, Sulphamethoxazol 25µg; AMP10, Ampicillin 10µg; NYS100, Nystatin 100µg; SCF, Sulbactam (30 µg)

MetEs, HisEs, CysEs compounds were active against studied all of gram (+), gram (-) and antifungal. But GlyEs was inactive against *Sh.dys. typ 7, L.monocytogenes, B.cereus* and *E.coli.* All the compounds were active against *Br. abortus* except. *Br. abortus* is a gram-negative bacterium that causes premature abortion of a cattle fetus. What makes this bacterium so dangerous is that it can be transferred from an animal to a human host. In humans, this disease causes both acute and chronic symptoms, but can be treated with antibiotics. In general, the CysEs is more potent bactericides than other amino acid derivatives. Furthermore, the antibacterial activity of these compounds was also compared with five commercial antibiotics, namely Kanamycin, Sulfamethoxazol, Ampicillin, Amoxycillin and Nystatin. It was seen that the synthesized compounds were effective as the antibiotics mentioned.



Br. abortus *S.aureus*

Figure 2. Imaging of antimicrobial affectivities of hydroxyquinoline esters against *Br. abortus* and *S.aureus*

3.3. AChE activitiy results

In this study, our aim was to determine the inhibitory effects of new amino acid esters of 8-Hydroxyquinoline. IC_{50} (IC_{50} represents the molarity of inhibiting a 50% decrease of enzyme activity) K_i (inhibitor-enzyme dissociation constant) values and inhibiton types were given in Table 6. As seen in Table 6, all the compounds behave as inhibitors against AChE. The inhibition potency of the compounds indicates an increasing inhibitory effect on AChE: MetEs>CysEs>GlyEs>HisEs. When the inhibitory prosess of compounds is compared with respect to K_i values, same affinity can be observed. Inbibition type was determined as noncompetitive for HisEs, GlyEs and uncompetitive for MetEs, CysEs because of studied molecule binds to an enzyme somewhere other than the active site [12].

Table 6. The result of inhibition studies for all compounds on AChE

Compounds	IC_{50} (μM)	K_i (mM)	Inhibition type
HisEs	10.3	24.31	Noncompetitive
GlyEs	8.64	19.32	Noncompetitive
CysEs	6.70	8.43	Uncompetitive
MetEs	6.68	7.73	Uncompetitive

The reason of MetEs and CysEs in better inhibitory potency can be explained depending upon molecules structures. Due to the sulphur atom in soft base, sulphur atom-containing compounds may have prevented the substrate locating at enzyme active site with making hydrogen bond. Moreover, when the linearity of molecule structures getting longer, it can be easier to interact with enzyme active sites for both MetEs and CysEs. In literature, it is seen that suitable length bis-tacrines have greater inhibitory potency and selectivity than tacrine itself [6]. Less inhibition potency of HisEs can be explained depending upon steric hindrance.

Despite IC_{50} values of CysEs and MetEs are very close to each other, K_i values are different. Due to K_i of MetEs is smaller than CysEs, MetEs is more advantageous (Figure 3).

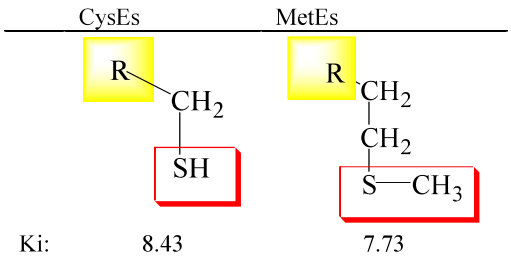


Figure 3. The structures that have highest inhibitory effect

This case can be explained with property electron-donating of methyl group in MetEs. Electron pairs on sulphur atom may allow making hydrogen bonds with enzyme [13, 14].

4. CONCLUSIONS

In summary, a range of amino acid derivatives have been prepared for preliminary screening as antimicrobial agents and inhibitors against AChE enzyme. Novel amino acid esters of 8-hydroxyquinoline were prepared. The structural characterizations of synthesized compounds were made by using the elemental analyses and different spectroscopic methods. All the synthesized compounds behave as inhibitors against AChE enzyme. CysEs and MetEs show more inhibition potency. The antimicrobial data shows that the CysEs is superior to the other synthesized compounds.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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