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# Antioxidant, acetylcholinesterase and butyrylcholinesterase inhibition profiles of histamine Schiff bases

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**Abstract:** In this work, a series of histamine Schiff bases H(1-20) were assayed for antioxidant properties by using different bioanalytical methods such as DPPH-free radical scavenging assay, ABTS cation radical decolarization, cupric reducing antioxidant capacity (CUPRAC) and metal chelating methods. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition profiles were also assessed. In general, the synthesized compounds showed weak antioxidant activity against all tested methods, but some of them showed great inhibition potency against AChE and BChE enzymes. Specifically, compound H9 showed effective inhibition potency against both enzymes with percent inhibition of 97.03 and 93.64, respectively.

**Keywords:** Histamine, Schiff base, antioxidant, anticholinesterase, Alzheimer's disease.

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## INTRODUCTION

Histamine is a one of the important biogenic compound (characterized by an imidazolic core with two nitrogen atoms and aliphatic tail with an amino group) performing an important function in the pathogenesis of inflammation and regulation of capillary permeability (1). It has been also used a core pharmacophore for the designing of efficient carbonic anhydrase activators (CAAs) (2-6). It has been gaining more attention after the CAAs shown to have the role in the neurodegenerative disorder of memory and cognitive function (Alzheimer's disease) since the levels of brain carbonic anhydrases (more specifically isozenyzme VII) significantly diminish in the brain of Alzheimer's disease and older rats (7-9). On the other hand, Schiff base scaffold (R-C=N-R) is an interesting core in terms of medicinal chemistry and synthetic organic chemistry usually synthesized from the condensation of primary amines and active carbonyl groups. In the literature, some Schiff base derivatives have shown to have broad biological properties such as antimicrobial (10,11), antifungal (12), efficient carbonic anhydrase inhibitors (13-15), and antitumor activities (16,17).

Acetylcholinesterase (AChE) exists in very high concentrations over the peripheral and central nervous systems, parasympathic synapses and neuromuscular junction of human and animals (18,19). This enzyme has great importance for hydrolysis acetylcholine (ACh) to choline and acetate. Abnormal level of acetylcholinesterase (AChE) can cause various neurological disorders such as Alzheimer's diseases (AD) and Parkinson's diseases (PD) (20,21). Butyrycholinesterase (BChE), formerly named pseudocholinesterase, which is also known as nonspecific cholinesterase, is present in blood (5 µg/mL), central nervous system, glial cells, pancreas, liver and heart of vertebrates and is involved in hydrolysis and regulation of butyrylcholine. AChE and BChE sequences are

similar up to 84% and hence, their responses to a definite therapy almost yield in similar results (18-21). That's why, it is very hard to find selective inhibitors of these two similar enzymes. The AD treatment, which is one of the hardest disease to treat, involves the use of cholinesterase inhibitors such as tacrine, rivastigmine, galantamine and donepezil. AD disease's treatment has gained much attention, since this disease does not have much effective drugs (only a few). On the other hand, the main drawback with these drugs are represented by the unfavorable side effects, including nausea, vomiting and weight loss (18-21). For this reason, there is an increasing interest to design of novel and potent cholinesterase inhibitors for AD treatment.

More recently, our group showed the efficient carbonic anhydrase activation profile of histamine Schiff base derivatives (22, 23). The nanomolar potency and higher selectivity were obtained against human carbonic anhydrase VII (hCA VII) (22). Since this hCA VII involved brain metabolism, in the present study, prompted by these potent biological activities, we synthesized and examined these histamine Schiff bases as antioxidant and cholinesterase (AChE and BChE) inhibitors.

## EXPERIMENTAL SECTION

### Chemistry

General synthetic route for the preparation of structurally diverse histamine Schiff base derivatives **H(1-20)** were described in Figure 1. Firstly, histamine dihydrochloride was neutralized with potassium hydroxide (KOH) in EtOH. Then, it was reacted with different substituted aromatic, heterocyclic and aliphatic aldehyde derivatives in EtOH at room temperature to afford the corresponding histamine Schiff base derivatives **H(1-20)**. The obtained compounds were crystallized from ethyl acetate and ether to produce pure derivatives. Physicochemical and spectroscopic characterization of all compounds, **H(1-20)** have been previously described by us (22).

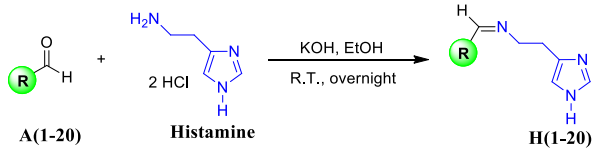


**Figure 1**. General synthetic route for the synthesis of histamine Schiff bases **H(1-20)**.

### Determination of antioxidant and anticholinesterase activity of histamine Schiff bases H(1-20)

**DPPH radical scavenging ability:** The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the synthesized compounds was determined by spectrophotometric method based on the reduction of an ethanol solution of DPPH (24). 2, 5, 10, 20 µL of 1 mM stock solution of each compound was completed to 40 µL with the DMSO and mixed with 160 µL of 0.1 mM of DPPH free radical solution. The mixture was led to stand for 30 min in the dark and the absorbance was then measured at 517 nm against a blank. Inhibition of free radical, DPPH, in percent (I %) was calculated according to the formula:

$I\ \% = (A_{control} - A_{sample})/A_{control} \times 100$;
where $A_{control}$ is the absorbance of the control reaction (containing all reagents except for the tested compounds), and $A_{sample}$ is the absorbance of the test compounds. Tests were carried out in triplicate. BHA, BHT and α-Toc were used as positive control.

**ABTS cation radical decolorization:** The percent inhibition of decolorization of ABTS (2,2`-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) cation radical is obtained as a function of time and concentration, and evaluated by comparison with the BHT, BHA and α-Toc compounds used as standard. (25, 26). The tested compounds at different concentrations are added to each well and 160 µL of 7 mM ABTS solution is added. After 6 min at room temperature, the absorbances were measured at 734 nm. ABTS cation radical decolorization activities were determined by using the equation below:

$\%Inhibition = (A_{control} - A_{sample})/A_{control}\ x100$
where A is the absorbance. Tests were carried out in triplicate. BHA, BHT and α-Toc were used as positive control.

**Metal Chelate:** The chelating ability of synthesized compounds was examined according to the method of Dinis et al. (27). The tested compounds at different concentrations were added to each well and 4 µL of 2 mM ferrous (II) chloride was added . Then 8 µL of 5 mM ferrozine was added and reaction was started. After 10 min at room temperature, the absorbance was measured at 562 nm against blank. The results were expressed as percentage of inhibition of the ferrozine-$Fe^{2+}$ complex formation. The percentage inhibition of the ferrozine -$Fe^{2+}$ complex formation was calculated using the formula given below:

$\text{Chelating ability } (\%) = (A_{control} - A_{sample})/A_{control} \times 100$

where A is the absorbance. Tests were carried out in triplicate. EDTA was used as a positive control.

**Cupric reducing antioxidant capacity (CUPRAC) method:** CUPRAC method comprises the reduction of Cu(II)-Neocuproine into its colored form Cu(I)-Neocuproine chelate in the presence of antioxidant compounds (28). The tested compounds at different concentrations were added to each well and 61 µL of $CuCl_2$,

Neocuproine and $NH_4OAc$ solutions were added. After 1 hour at room temperature, the absorbance was measured at 450 nm . The absorbance values were compared with the standard molecules BHA, BHT and α-Toc. Each sample was applied three times.

**Anticholinesterase activity of the histamine Schiff bases:** The inhibitory effect of histamine Schiff base derivatives **H(1-20)** on AChE and BChE activities was determined according to the slightly modified spectrophotometric method of Ellman et al. (29). All compounds were dissolved in DMSO to prepare stock solutions at 4 mM concentration. Aliquots of 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL AChE (or BChE) solution were mixed and incubated for 15 min at 25 °C, and DTNB (5,5'-dithio-bis(2-nitrobenzoic)acid) (10 µL) is added. The reaction was then initiated by the addition of acetylthiocholine iodide (or butyrylthiocholine iodide) (10 µL). Final concentration of the tested compounds' solution was 200 µM.

$\%Inhibition = (A_{control}-A_{sample})/A_{control}$ x100
where A is the absorbance. Tests were carried out in triplicate. Galantamine was used as positive control.

$IC_{50}$ values were calculated from the equation of the curve obtained from the concentration-inhibition graph. The IC50 value is the value that obtained from the concentration curve of an inhibitor where the response is reduced by half.

**Statistical analysis:** The results of the antioxidant and anticholinesterase activity assays are expressed as the mean ± SD of three parallel measurements. The statistical significance was estimated using a Student's t-test, where p-values < 0.05 were considered significant.

## RESULTS AND DISCUSSION

Here we report the synthesis, antioxidant, acetylcholinesterase and butyrylcholinesterase inhibition activities of a large series of histamine Schiff base derivatives obtained from histamine as a lead molecule. A large series of histamine Schiff base derivatives **H(1-20)** was synthesized by the reaction of histamine with substituted aldehydes **A(1-20)** as described by us previously (22).

The antioxidant capacities of synthesized histamine Schiff base derivatives are determined by using several antioxidant methods, including DPPH free radical scavenging, ABTS cation radical decolorization, cupric reducing (CUPRAC) and metal chelating methods. Acetylcholinesterase and butyrylcholinesterase inhibition activities were also tested.

The results revealed that histamine Schiff base derivatives **H(1-20)** displayed no significant efficiencies in case of DPPH free radical scavenging method. It was considered that compound **H14** (2,3,4,5,6-F substituted) was the least active compound with >1000 µM activity. The compounds **H9** (2-OH, 3-Br), **H10** (2-OH, 5-Br), **H18** (4-iPr) and **H19** (4-CN) were also shown weak antioxidant activity with $IC_{50}$ values of 833.41, 858.21, 945.23 and 846.00 µM, respectively. The weak to moderate antioxidant activity were observed for remaining compounds with $IC_{50}$ values from 249.63 µM to 685.68 µM. In the current series, all compounds were less active than the standard **BHA** (61.72 µM), **BHT** (232.11 µM), and **α-Toc** (56.86 µM) (Table 1) in case of DPPH free radical scavenging method.

The ABTS cation radical scavenging activities of synthesized histamine Schiff base derivatives and controls BHT, BHA, and α-Toc were also analyzed and $IC_{50}$ values of compounds were summarized in Table 1. The weak to moderate antioxidant activity were obtained from the series with $IC_{50}$ values ranging from 89.18 µM to 868.89 µM. The compounds **H1** (valeraldehyde), **H3** (4-Me), **H7** (2-OH, 3-Me), **H18** (4-iPr) and **H19** (4-CN) had no activity in ABTS cation radical scavenging assay with $IC_{50}$ values >1000 µM. Only four compounds showed moderate activity which are **H9** (2-OH, 3-Br), **H12** (2-OH, 3,5-diBr), **H16** (2-MeO, 5-Br), and **H20** (2-Me, 5-Br) with $IC_{50}$ values of 89.18, 129.24, 123.23, and 127.99 µM, respectively. Interestingly, these most active four compounds in the ABTS cation radical scavenging assay have brom in their structure at 3 and/or 5 positions of the phenyl ring.

**Table 1.** DPPH radical scavenging, ABTS cation radical decolorization and metal chelating activities of histamine Schiff base derivatives **H(1-20)** and controls BHA, BHT, α-Toc, and EDTA.

| Comp. | R | $IC_{50}$ (μM)[a] | | |
|---|---|---|---|---|
| | | **DPPH Free Radical Scavenging Activity** | **ABTS Cation Radical Scavenging Activity** | **Metal Chelating Activity** |
| **H1** | -Valeryl | 428.41±1.33 | >1000 | 125.45±1.43 |
| **H2** | -Furyl | 471.66±0.79 | 593.67±1.16 | 150.38±0.97 |
| **H3** | -4-Me-Phenyl | 685.68±1.01 | >1000 | 101.38±0.80 |
| **H4** | -4-MeO-Phenyl | 368.16±1.57 | 585.67±1.16 | 147.31±0.97 |
| **H5** | -4-$N(Me)_2$-Phenyl | 581.67±0.59 | 601.07±0.96 | 200.58±0.87 |
| **H6** | -2-OH-Phenyl | 630.43±1.39 | 255.71±0.39 | 296.25±0.63 |
| **H7** | -2-OH-3Me-Phenyl | 623.83±1.45 | >1000 | 203.37±1.12 |
| **H8** | -2-OH-3MeO-Phenyl | 249.63±0.28 | 220.92±1.11 | 116.62±1.28 |
| **H9** | -2-OH-3Br-Phenyl | 832.41±1.43 | 89.18±0.02 | 82.33±0.58 |
| **H10** | -2-OH-5Br-Phenyl | 858.21±1.67 | 448.01±1.02 | 115.66±1.28 |
| **H11** | -2-OH-5Cl-Phenyl | 389.76±1.29 | 511.38±1.01 | 86.65±1.11 |
| **H12** | -2-OH-3,5-diBr-Phenyl | 472.79±0.87 | 129.24±0.32 | 219.34±1.05 |
| **H13** | -2-OH-3,5-diCl-Phenyl | 541.52±0.89 | 577.82±0.51 | 127.59±0.85 |
| **H14** | -2,3,4,5,6-PentaF-Phenyl | >1000 | 749.89±1.57 | 209.31±0.44 |
| **H15** | -2-Br-Phenyl | 539.42±1.28 | 868.89±1.88 | 146.53±0.27 |
| **H16** | -2-MeO-5-Br-Phenyl | 625.23±1.26 | 123.23±0.63 | 70.34±0.81 |
| **H17** | -2-COOH-Phenyl | 579.38±0.89 | 508.46±0.57 | 110.26±0.98 |
| **H18** | -4-iPr-Phenyl | 945.23±0.75 | >1000 | 132.22±0.92 |
| **H19** | -4-CN-Phenyl | 846±0.96 | >1000 | 208.38±0.86 |
| **H20** | -2-Me-5-Br-Phenyl | 631.17±1.43 | 127.99±0.65 | 183.09±0.21 |
| **BHA[b]** | - | 61.72±0.85 | 45.40±1.08 | - |
| **BHT[b]** | - | 232.11±3.01 | 26.54±0.18 | - |
| **α-TOC[b]** | - | 56.86±0.77 | 34.12±0.41 | - |
| **EDTA[b]** | - | - | - | 52.35±1.15 |

[a] $IC_{50}$ values represent the means (standard deviation of three parallel measurements ($p < 0.05$).
[b] Reference compounds.

The metal chelating effect of the histamine Schiff base derivatives on ferrous ions was summarized in Table 1 and compared with the standard EDTA. In the current series, all compounds showed weak chelating activity except the compounds **H9** (2-OH, 3-Br), **H11** (2-OH, 5-Cl), and **H16** (2-MeO,5-Br) which are exhibited good chelating activity with $IC_{50}$ values of 82.33, 86.65, and 70.34 μM, respectively. All compounds showed less activity than standard EDTA ($IC_{50}$= 52.35 μM).

The cupric reducing antioxidant capacity (CUPRAC) method was also applied to identify the antioxidant activity of the synthesized histamine Schiff base derivatives. The activity of the compounds increased with increasing concentration as shown in Table 2. The results of the CUPRAC test of the synthesized compounds at 10, 25, 50 and 100 μM were compared with standards BHT, BHA and α-Toc. In the current study, all of the tested histamine Schiff base derivatives exhibited weak activity in CUPRAC assay.

**Table 2**. Absorbance values for the cupric ion reducing antioxidant capacity (CUPRAC), of the histamine Schiff base derivatives **H(1-20)** and controls BHA, BHT, and α-Toc.

| Comp. | | Absorbance Values[a] | | | |
|---|---|---|---|---|---|
| | R | 10 µM | 25 µM | 50 µM | 100 µM |
| **H1** | -Valeryl | 0.082±0.003 | 0.085±0.002 | 0.093±0.006 | 0.121±0.002 |
| **H2** | -Furyl | 0.065±0.002 | 0.071±0.003 | 0.082±0.004 | 0.111±0.002 |
| **H3** | -4-Me-Phenyl | 0.068±0.004 | 0.070±0.003 | 0.073±0.003 | 0.081±0.001 |
| **H4** | -4-MeO-Phenyl | 0.072±0.006 | 0.073±0.001 | 0.081±0.001 | 0.131±0.004 |
| **H5** | -4-$N(Me)_2$-Phenyl | 0.083±0.001 | 0.097±0.002 | 0.135±0.008 | 0.191±0.001 |
| **H6** | -2-OH-Phenyl | 0.074±0.002 | 0.082±0.001 | 0.096±0.005 | 0.118±0.002 |
| **H7** | -2-OH-3-Me-Phenyl | 0.074±0.003 | 0.092±0.005 | 0.112±0.006 | 0.141±0.002 |
| **H8** | -2-OH-3-MeO-Phenyl | 0.089±0.006 | 0.101±0.002 | 0.125±0.001 | 0.174±0.001 |
| **H9** | -2-OH-3-Br-Phenyl | 0.093±0.001 | 0.094±0.002 | 0.154±0.004 | 0.228±0.007 |
| **H10** | -2-OH-5-Br-Phenyl | 0.092±0.002 | 0.101±0.001 | 0.109±0.007 | 0.177±0.005 |
| **H11** | -2-OH-5-Cl-Phenyl | 0.081±0.001 | 0.085±0.004 | 0.101±0.004 | 0.109±0.005 |
| **H12** | -2-OH-3,5-diBr-Phenyl | 0.099±0.002 | 0.121±0.003 | 0.140±0.001 | 0.212±0.001 |
| **H13** | -2-OH-3,5-diCl-Phenyl | 0.079±0.007 | 0.091±0.005 | 0.102±0.005 | 0.199±0.009 |
| **H14** | -2,3,4,5,6-PentaF-Phenyl | 0.099±0.003 | 0.100±0.002 | 0.130±0.001 | 0.192±0.005 |
| **H15** | -2-Br-Phenyl | 0.084±0.006 | 0.086±0.003 | 0.091±0.006 | 0.095±0.001 |
| **H16** | -2-MeO-5-Br-Phenyl | 0.085±0.001 | 0.087±0.008 | 0.095±0.001 | 0.101±0.006 |
| **H17** | -2-COOH-Phenyl | 0.088±0.009 | 0.097±0.002 | 0.101±0.005 | 0.109±0.006 |
| **H18** | -4-iPr-Phenyl | 0.079±0.013 | 0.084±0.002 | 0.093±0.008 | 0.099±0.006 |
| **H19** | -4-CN-Phenyl | 0.082±0.001 | 0.100±0.003 | 0.102±0.008 | 0.142±0.001 |
| **H20** | -2-Me-5-Br-Phenyl | 0.085±0.003 | 0.095±0.001 | 0.105±0.007 | 0.133±0.003 |
| **BHA[b]** | - | 0.288±0.015 | 0.572±0.046 | 1.026±0.013 | 1.984±0.035 |
| **BHT[b]** | - | 0.303±0.010 | 0.610±0.010 | 1.167±0.024 | 2.000±0.173 |
| **α-TOC[b]** | - | 0.179**±**0.001 | 0.296**±**0.012 | 0.482**±**0.017 | 0.912**±**0.065 |

[a]Values expressed are means ± SD of three parallel absorbance measurements ($p<0.05$)
[b] Reference compounds

In this study, most active results were observed against cholinesterase (AChE and BChE) activity which some of the compounds are comparable with standard drug galantamine (Table 3). Specifically compound **H9** (2-OH,3-Br) was determined to have highest activity against acetylcholinesterase enzyme at 200 µM with 97.03% inhibition. Other potent AChE inhibitors from the series were the compounds **H2**, **H10** (2-OH,5-Br), **H12** (2-OH, 3,5-diBr), and **H13** (2-OH, 3,5-diCl) with % inhibitions 80.36, 83.63, 84.18, and 79.34, respectively. The remaining compounds showed no activity (NA) against AChE, except the compound **H18** (4-iPr) which is a moderate active inhibitor with percent inhibition of 49.57. Another cholinesterase enzyme, butyrylcholinesterase, was also potently inhibited with some of the compounds from the series. The compound **H9** were also most potent compound against BChE enzyme with percent inhibition of 93.64 which is more active than standard drug galantamine (percent inhibition of 87.86). Another potent compound was **H12** with percent inhibition of 84.24, which is comparable with the standard drug. The moderate activity was observed for some of the compounds with % inhibition ranging from 21.43 to 66.62. The remaining compounds **H1**, **H3**, **H4**, **H5**, **H6**, **H7**, **H8**, and **H14** had no activity against BChE enzyme. As a result of cholinesterase activities, the compound **H9** (2-OH,3-Br) showed great inhibition potency against both AChE and BChE which is more active than standard drug galantamine (Table 3).

**Table 3**. Anticholinesterase activity of the histamine Schiff base derivatives **H(1-20)** at 200 µM and standard drug galantamine.

| Comp. | R | AChE (Inhibition %)[a] | BChE (Inhibition %)[a] |
|---|---|---|---|
| **H1** | -Valeryl | NA | NA |
| **H2** | -Furyl | 80.36±1.65 | 41.58±0.83 |
| **H3** | -4-Me-Phenyl | NA | NA |
| **H4** | -4-MeO-Phenyl | NA | NA |
| **H5** | -4-N(Me)$_2$-Phenyl | NA | NA |
| **H6** | -2-OH-Phenyl | NA | NA |
| **H7** | -2-OH-3-Me-Phenyl | NA | NA |
| **H8** | -2-OH-3-MeO-Phenyl | NA | NA |
| **H9** | -2-OH-3-Br-Phenyl | 97.03±2.30 | 93.64±1.36 |
| **H10** | -2-OH-5-Br-Phenyl | 83.63±0.16 | 66.62±1.44 |
| **H11** | -2-OH-5-Cl-Phenyl | NA | 21.43±0.26 |
| **H12** | -2-OH-3,5-diBr-Phenyl | 84.18±1.39 | 84.24±0.12 |
| **H13** | -2-OH-3,5-diCl-Phenyl | 79.34±0.31 | 37.28±0.48 |
| **H14** | -2,3,4,5,6-PentaF-Phenyl | NA | NA |
| **H15** | -2-Br-Phenyl | NA | 31.20±0.45 |
| **H16** | -2-MeO-5-Br-Phenyl | NA | 49.19±1.21 |
| **H17** | -2-COOH-Phenyl | NA | 40.31±0.56 |
| **H18** | -4-iPr-Phenyl | 49.57±0.76 | 38.37±0.23 |
| **H19** | -4-CN-Phenyl | NA | 59.45±1.55 |
| **H20** | -2-Me-5-Br-Phenyl | NA | 38.88±1.03 |
| **Galantamine[b]** | | 84.20±0.74 | 87.86±0.24 |

[a] 200 µM
[b] Standard compound
NA: Not Active

## CONCLUSIONS

In the present study, histamine Schiff base derivatives **H(1-20)** were synthesized from the condensation reaction of histamine and substituted aldehydes **A(1-20)**. The antioxidant activities of the synthesized compounds were investigated by DPPH, ABTS, metal chelating and CUPRAC methods. The antioxidant activity of the histamine Schiff base derivatives was obtained as a weak to moderately active compounds against tested methods. The cholinesterase activity of the compounds was also tested against AChE and BChE enzymes and the compound **H9** showed excellent percentage inhibition for both enzymes and exhibited better inhibition than standard drug galantamine. Since the AChE and BChE enzymes are related to neurodegenerative disorders and their inhibition is important for these types of diseases, these histamine Schiff base derivatives may be considered of interest as tools for the development of new drug candidates for the AD and PD.

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