Al-Nahrain Journal of Science
Al-Nahrain J. Sci., Vol. 29, Issue 2, pp. 24 – 41, June, 2026.

Design, Synthesis and In-Vitro Testing of New Sinapic Acid Derivatives as Anti-Alzheimer Disease

Ahmed A. J. Mahmood

Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq

Article's Information

Received: 15.04.2025
Accepted: 31.07.2025
Published: 15.06.2026

Keywords:
Cholinesterase,
Sinapic acid derivatives,
Docking,
Amides,
Schiff base,
ADME studies.

Abstract

In the past few decades, the cholinergic theory of Alzheimer's disease has been promoted as a crucial tool for the creation of new drugs. Cholinesterases (ChEs) play a dynamic part in the regulation of cholinergic transmission. The inhibition of ChEs is believed to be involved in the cumulative brain acetylcholine level and accordingly has been considered in the management of Alzheimer's disease. In this study, new sinapic acid derivatives were designed by docking, synthesized, and predicted for their ADME properties. They were then tested *in vitro* for their ChEs inhibitory activity. Based on the docking study, 10 new amide and Schiff base sinapic acid derivatives were synthesized and characterized using ^{1}HNMR and ^{13}CNMR. The *in vitro* testing yielded good results at 50 and 100 µM concentrations. Six new compounds showed good and potent affinity to inhibit ChEs, proving more potent than the parent compound (sinapic acid) and rivastigmine, and largely similar to that of donepezil for acetylcholinesterase (brain ChE). Compounds S4 and S9 exhibited superior activity, resembling donepezil. *In vivo* and animal testing studies will be needed to ascertain the resulting activity and safety of these new compounds.

http://doi.org/10.22401/ANJS.29.2.03
*Corresponding author: ahmedsot@uomosul.edu.iq

Alzheimer's disease (AD), the major type of neurodegenerative dementia in the elderly, is associated with a specific loss of cholinergic neurons and a reduction in acetylcholine levels [1]. The disease is characterized by memory loss and a gradual deterioration in cognitive abilities. Research indicates that approximately 35.6 million individuals around the globe are affected by dementia [2]. The cholinergic hypothesis proposes that Alzheimer's disease is activated by a reduction in acetylcholine (ACh) levels in the brain, resulting in progressive neurodegeneration. ACh plays a vital role in normal brain signaling, mainly in memory preservation and retrieval [3]. Consequently, present treatments for Alzheimer's primarily emphasize medications that boost ACh levels by inhibiting the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Although BChE is chiefly located in plasma, liver, and muscle tissues, its precise biological role remains unclear [4]. In contrast to AChE, which principally breaks down acetyl esters like acetylcholine, BChE specifically hydrolyzes butyrylcholine. Over the past two decades, substantial research efforts have focused on understanding the molecular mechanisms underlying Alzheimer's disease. The current treatment policy aims to improve central cholinergic function to boost ACh levels in the brain [5]. Consequently, several cholinergic medications, including tacrine, donepezil, rivastigmine, and galantamine, have been developed to help manage the Alzheimer's disease symptoms (see Figure 1) [6].

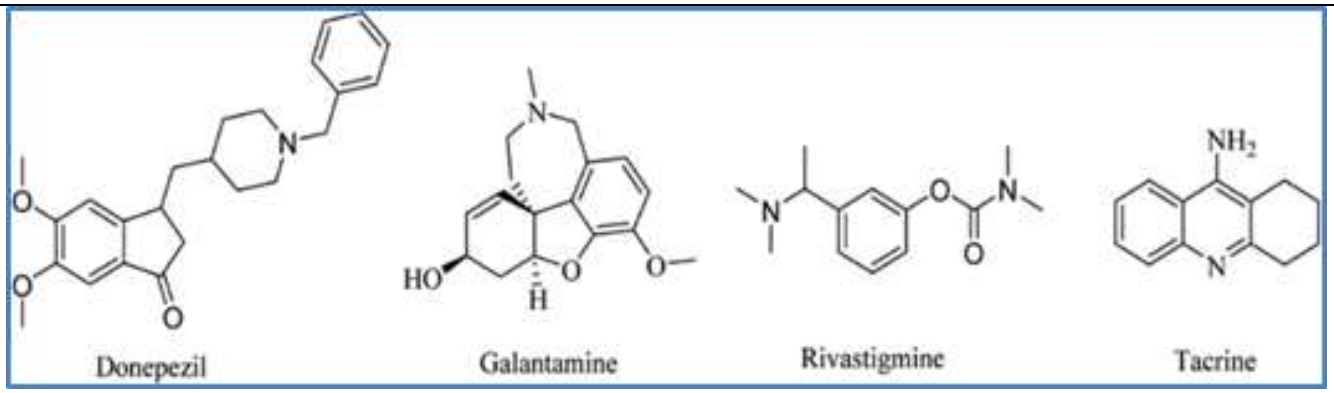


Figure 1: chemical structure of the cholinergic drugs used in AD.

However, due to adverse reactions, tacrine was made obsolete [7]. Unfortunately, the probable efficiency of these inhibitors is often compromised by the presence of central and peripheral side effects. For instance, clinical studies have revealed that tacrine has hepatotoxic liabilities [8]. The cholinergic hypothesis reveals that a loss in cholinergic action is caused by the decreased ACh level, which is hydrolyzed by ChEs in the synaptic gap [8]. The two isoenzymes (AChE and BChE) are responsible for hydrolyzing ACh. Even though AChE has additional hydrolytic action than BChE [9]. Consequently, AChE inhibitors are favored in the treatment of AD to maintain standard ACh levels in the CNS and to eradicate the symptoms of the disease [8, 9]. Sinapic acid is a derivative of cinnamic acid with numerous disease-modifying actions, particularly those concerned with oxidative stress [10]. Similarly, cinnamyl alcohol, because of its phenyl-acryl part, offers anti-inflammatory, lipid-lowering, and lipoxygenase inhibitory activity to the acids that have been esterified with it [10, 11]. Sinapic acid occurs in both free and ester forms; some esters include sinapoyl esters, sinapine (sinapoylcholine), and sinapoyl malate [11]. Sinapic acid is a phytochemical found in many edible plants such as spices, vegetables, citrus and berry fruits, cereals, and oilseed crops [12]. Sinapic acid has been verified and reported against numerous pathological conditions such as infections, inflammation, oxidative stress, cancer, diabetes, neurodegeneration, and anxiety [13-15]. Some derivatives of sinapic acid, such as sinapine, 4-vinylsyringol, and syringaldehyde, have also been considered for AChE inhibition, antimutagenicity, and antioxidant activity [16]. The structural formulas of sinapic acid and its derivatives are shown in Figure 2.

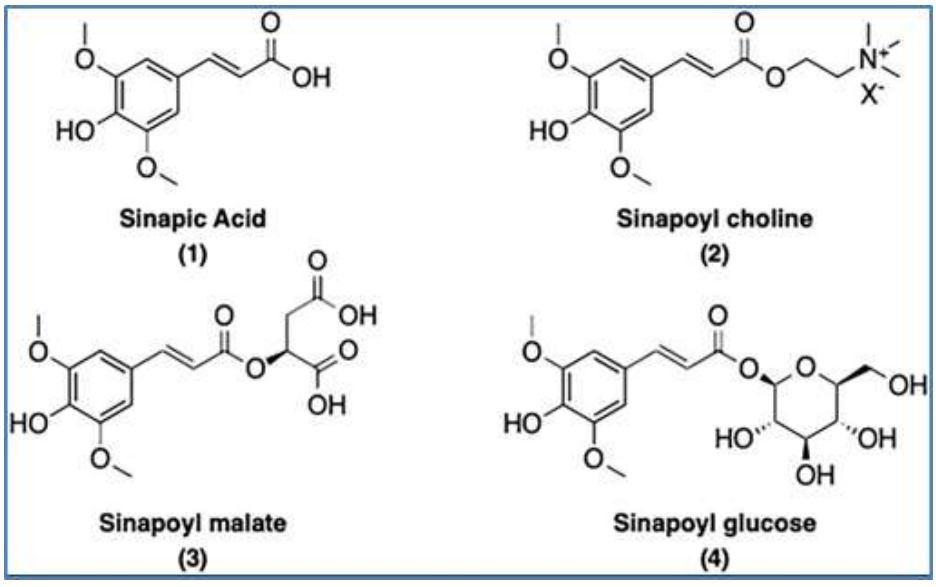


Figure 2: The chemical structure of sinapic acid derivatives.

Sinapine (sinapoyl choline) is documented as an AChE inhibitor that could offer therapeutic benefits for treating different diseases, mainly because of its antioxidative properties. There are recommendations for its probable application in food processing, cosmetics, and the pharmaceutical sector [17]. Small molecule ChE inhibitors (ChEIs) provide an effective therapeutic strategy to treat AD. Currently, the discovery of new ChEIs with multi-target effects is still of great importance [17]. In this study, we report the *in silico* design, synthesis, characterization, and *in vitro* ChE inhibition properties, in addition to an ADME study, for new amide and Schiff base derivatives of sinapic acid as potential candidates for AD treatment.

1. Materials and Methods

a. Docking Study:

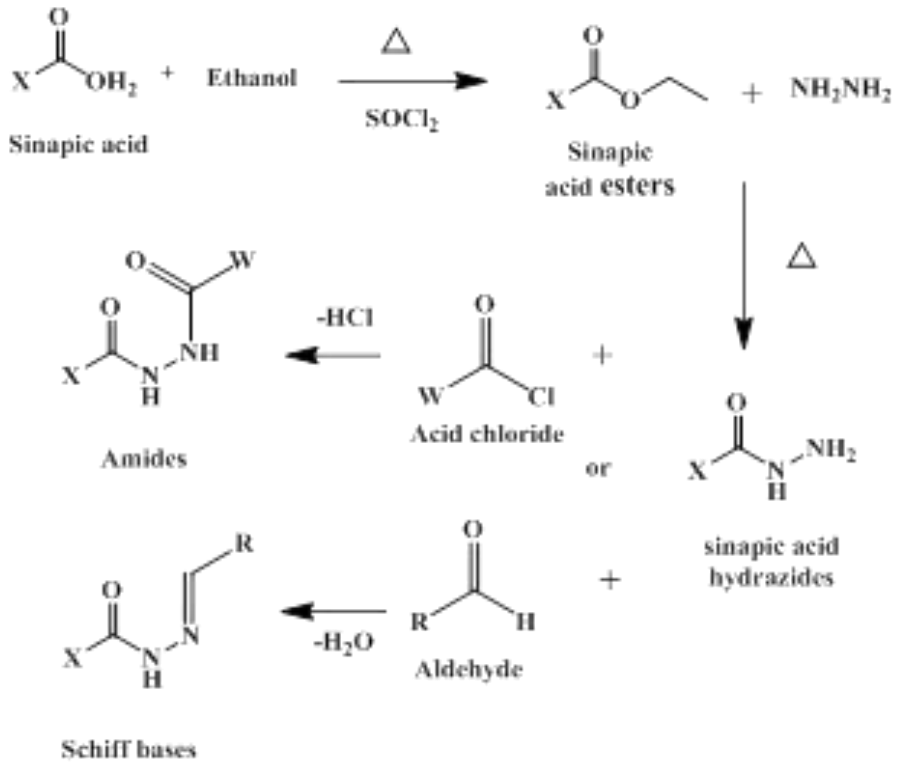
The docking procedure was performed using the online platform Mcule (https://mcule.com/apps/1-click-docking) [18]. The AChE enzyme (1E66) was utilized as a template to design an adjustable inhibitor mimicking Donepezil and Rivastigmine, aiming to create new candidates for Alzheimer's disease. To select the most effective compounds, we considered both the docking scores of the binding energies (using Donepezil and Rivastigmine as models) and an estimation of the geometric shape fitness within the active binding site of the (1E66) enzyme. The structure of the tested compounds (and all chemical compounds illustrated in this work) were drawn using ChemDraw program (PerkinElmer company), version 16.0.0.82(68). The structure of the AChE enzyme (1E66) was retrieved from the Protein Data Bank (PDB). The docking scores, expressed in Kcal/mol, were selected based on the lowest negative score, with geometric shape complementarity visualized by BIOVIA Discovery Studio Visualizer v20.1.0.19295 [18,19].

b. Chemical synthesis

Substances used in this work were acquired from international suppliers: Alpha (India), Fluka (Switzerland), Scharlau (Spain), and Merck (Germany). The open capillaries technique was employed for determining the melting points. ^{1}H NMR and ^{13}C NMR spectra in DMSO-d6 were obtained in the (university of Ankara / Turky) using a Bruker, Avance DPX 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectrometer with TMS as an internal reference.

---

i. Synthesis of the Sinapic acid hydrazides:



Scheme 1: General synthetic scheme of Sinapic acid hydrazides.

ii. Preparation of the Sinapic acid esters:

Thionyl chloride (0.01 mole) was added dropwise to an ice-cooled mixture of sinapic acid (0.01 mole) in 15 ml of absolute ethanol. The mixture was then refluxed in a hood for 2 hours. Excess $SOCl_2$ was removed via distillation under reduced pressure. After cooling, the resulting precipitate was filtered and recrystallized from ethanol as a hydrochloride salt. This was then treated with 30 ml of 5% aqueous $NaHCO_3$ solution and 30 ml of chloroform, and thoroughly mixed. The organic layer was separated and dried with magnesium sulfate, and the chloroform was evaporated to yield the free ester [20].

iii. Preparation of Sinapic acid hydrazides:

Hydrazine hydrate (80%) (35 ml) was added to a mixture of ethyl sinapate (0.01 mole) in 18 ml of absolute ethanol. The resulting mixture was heated by reflux for 5 hours. After evaporation, the product was collected and recrystallized using distilled water [21].

iv. Synthesis of the amide compounds:

- **Acid chloride preparations step:**

The carboxylic acids (2-3 mmol) were first dissolved in $SOCl_2$ (10-15 ml), and the mixture was refluxed in the hood for 40 minutes. The excess $SOCl_2$ was evaporated. Once cooled, the product was removed and used freshly in the subsequent synthesis step [22].

- **Preparation of amides products (S1-S5):**

The corresponding acid chloride (2.5×10−3 mole) dissolved in 5 ml of dichloromethane was added dropwise at 0˚C to a mixture of sinapic acid hydrazide (2.5×10−3 mole) and pyridine (2.5×10−3 mole) previously dissolved in 30 ml of dichloromethane. The resultant mixture was stirred at 25˚C for 24 hours. After solvent vaporization, the yielded product was washed 2-3 times with cold ethanol to eliminate impurities [23].

- **Synthesis of the Schiff bases compounds (S5-S10):**

The corresponding aldehydes (0.004 mole) were dissolved by heating in 20 ml of ethanol and then mixed with 20 ml of ethanolic sinapic acid hydrazide solution (0.004 mole). The resulting mixture was refluxed with stirring for 10-13 hours. After cooling, the precipitate was filtered off, dried, and washed with cold ethanol and three times with distilled water, then dried [24].

v. In vitro assay of the ChE inhibition:

Blood samples were collected from healthy subjects (male and female volunteers, aged 30±10 years) with no history of exposure to Donepezil, Rivastigmine, insecticides, or any ChEs inhibitors. The samples were collected using EDTA-test tubes. After centrifugation for 15 minutes at 3000 rpm, the erythrocytes and plasma were separated and kept in ice for the ChE assay.

vi. Electrometric assay of ChE activity:

Erythrocytes or plasma (0.2 ml) were added to a 10 ml container that previously held a combination of 3 ml of distilled water and 3 ml of barbital-phosphate buffer (pH 8.1). The pH value (pH1) was then verified with a pH meter. Afterward, 0.1 ml of an aqueous solution of acetylthiocholine (7.5%) was further added. Next, the mixture was incubated in a water bath at 37˚C for 20 minutes. The pH of the mixture in the container (pH2) was then evaluated, and the ChE enzyme activity was defined by the following equation [2, 25]:

$$ChE\ activity\ \left(\frac{pH}{20\ min.}\right) = \left(\frac{pH_1 - pH_2}{pH_{Blank}}\right) \quad \ldots(1)$$

The blank was prepared the same as above but without the enzyme source (the blood aliquot). For the preparation of the buffer solution, 1.24 g of sodium barbital, 0.163 g of potassium dihydrogen phosphate, and 35.07 g of sodium chloride were dissolved in 1 L of distilled water; the pH was adjusted to 8.1 prior to use [13,26].

vii. In-vitro ChE inhibition by the new sinapic acid derivatives:

Two distinct concentrations were prepared using distilled water for each of the new sinapic acid derivatives, Donepezil, or Rivastigmine, to achieve final concentrations of 100 and 50 µM. These were then individually added (0.1 ml) to the reaction mixture (prior to pH1 recording in the previous step) and incubated for 10 minutes at 37°C. Subsequently, the remaining ChE activity was estimated (as in the earlier step), and the percentage of enzyme inhibition was determined by the following equation [27]:

$$ChE\ inhibition\ (\%) = \frac{(ChE_1 - ChE_2)}{ChE_1} \times 100 \quad \ldots(2)$$

where ChE1 represents the enzyme activity without inhibitors (control), and ChE2 represents the activity with sinapic acid derivatives, Donepezil, or Rivastigmine (inhibitors).

viii. Statistical Analysis:

Whenever appropriate, the resulting data were subjected to analysis of variance, followed by the least significant difference test. To estimate the means of two groups, a Paired Student's t-test was utilized at a significance level of $P < 0.05$ [2,13,28].

ix. Physicochemical properties and ADMET prediction:

The drug-likeness of a molecule can be predicted from its physicochemical properties. The online tool SwissADME (http://www.swissadme.ch/) was employed for investigating and predicting the kinetic characteristics for the synthesized compounds selected from the docking procedure. First, all 2D chemical structures of newly synthesized compounds were sketched using ChemDraw software, then they were individually uploaded to the SwissADME Web Server (https://www.swissadme.ch). Finally, ADME reports for each compound were generated [29].

2. Results and Discussion

2.1. Docking results

The standard inhibitors Donepezil, Rivastigmine, and the pharmacophore (sinapic acid), along with the designed compounds, were all docked on the AChE enzyme (1E66). The docking results for sinapic acid were -7.6 Kcal/mol, for Donepezil were -9.2 Kcal/mol, and for Rivastigmine were -8.3 Kcal/mol. The structures for the substituents included in the design of the new amide and Schiff base compounds are listed in Table 1 and 3, while their docking results are listed in Table 2 and 4, respectively.

The docking scores of the standard inhibitors were considered as a control for the docking of the new compounds. Furthermore, the amino acid residues of their poses were utilized to define the active binding site of the (1E66) enzymes. The TRP 81 residue binds to all the control (standard inhibitors), while TYR 439, PHE 327, and TYR 331 amino acids attached to only two of the control inhibitors (Donepezil attached to all of them). The amino acids TRP 276, TYR 118, TRP 429, ILE 436, and GLU196 bind to only one inhibitor (either Donepezil or Rivastigmine), as shown in Figure 3 and Table 5. The active site of the AChE (1E66) enzyme is defined by these nine amino acids [30]. In addition, sinapic acid (the pharmacophore) was also docked; it appeared to interact with three of these nine amino acids that define the binding site, plus an additional three amino acids (GLY 115, TYR 127, and HIS 437) within the allosteric site (Table 5). These additional amino acids will aid the attachment of the new tested compounds with the enzyme pocket, as they are derived from sinapic acid.

Table 1: The structure for the substituents included in the designing amide derivatives.

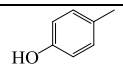
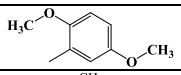
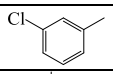
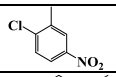
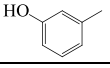
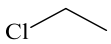
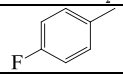
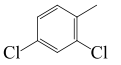
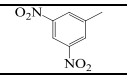
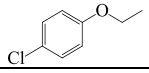
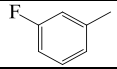
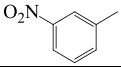
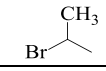
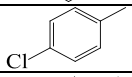
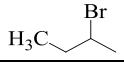
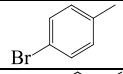
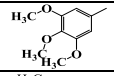
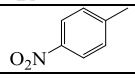
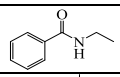
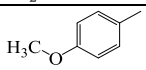
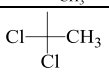
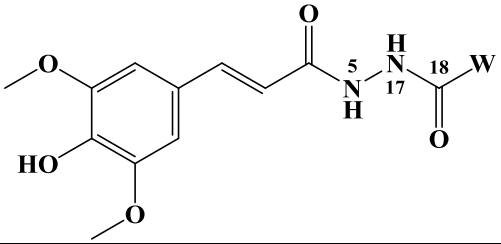
Item	W	Item	W	Item	W
W1		W11		W21	
W2		W12		W22	
W3		W13		W23	
W4		W14		W24	
W5		W15		W25	
W6		W16		W26	
W7		W17		W27	
W8		W18		W28	
W9		W19		W29	
W10		W20		W30	

Table2: Docking study results for the sinapic acid amide derivatives with 1E66 enzyme (kcal/mol).



No.	Docking Score	No.	Docking Score	No.	Docking Score
W1	-10.0	W11	-9.5	W21	-8.8
W2	-10.0	W12	-9.7	W22	-8.5
W3	-10.4	W13	-10.5	W23	-8.3
W4	-10.4	W14	-10.0	W24	-8.6
W5	-9.8	W15	-9.6	W25	-9.8
W6	-9.4	W16	-8.4	W26	-8.7
W7	-9.9	W17	-10.0	W27	-8.5
W8	-9.7	W18	-9.2	W28	-8.8
W9	-10.1	W19	-10.1	W29	-8.8
W10	-9.5	W20	-7.7	W30	-10.0

Table 3: The structure for the substituents included in the designing Schiff base derivatives.

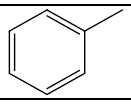
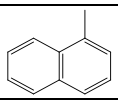
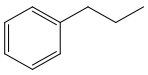
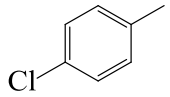
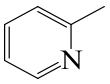
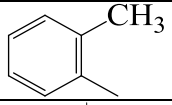
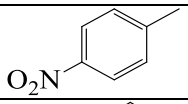
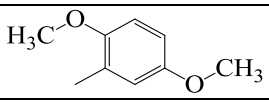
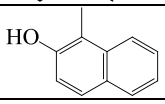
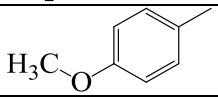
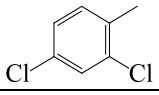
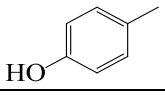
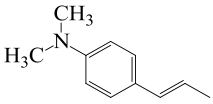
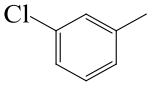
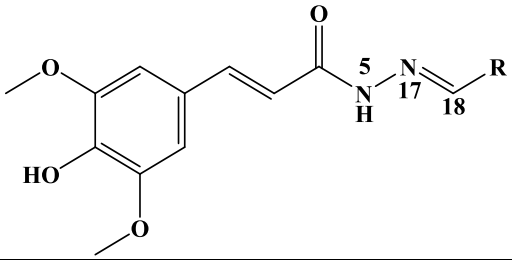
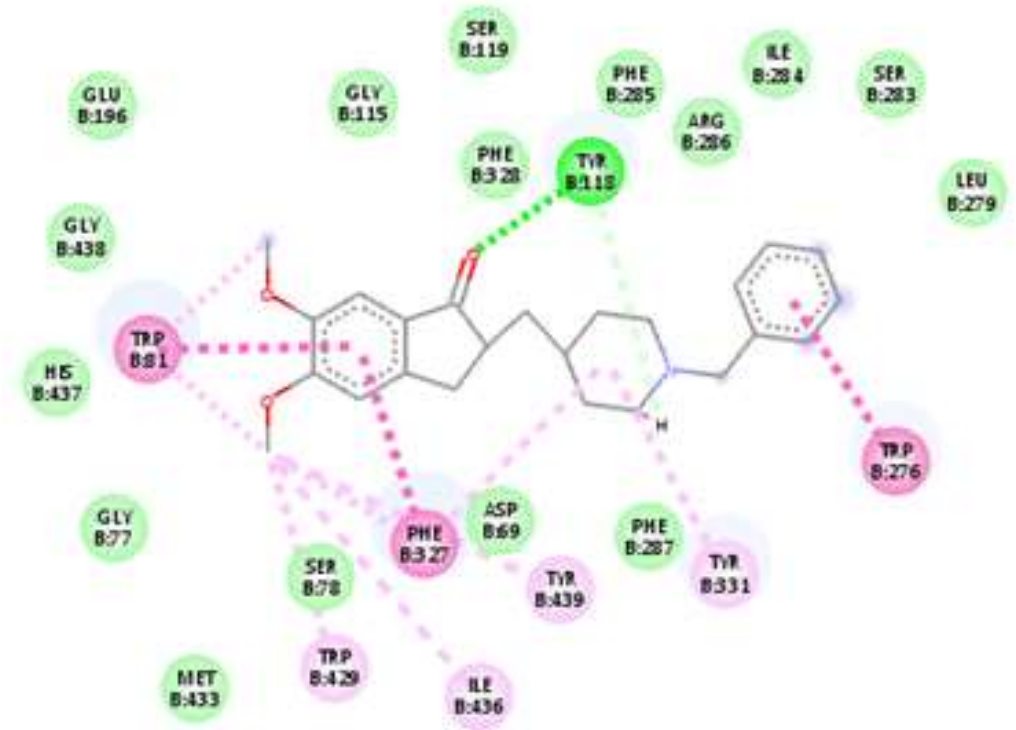
Item	R	Item	R	Item	R
R 1		R 6		R 11	
R 2		R 7		R 12	
R 3		R 8		R 13	
R 4		R 9		R 14	
R 5		R 10			

Table4: Docking study results for the sinapic acid Schiff base derivatives with 1E66 enzyme (kcal/mol).



No.	Docking Score	No.	Docking Score	No.	Docking Score
R1	-10.4	R6	-9.8	R11	-10.4
R2	-10.2	R7	-9.2	R12	-10.8
R3	-9.2	R8	-9.9	R13	-11.1
R4	-9.9	R9	-9.8	R14	-10.0
R5	-9.5	R10	-9.9		



(A)

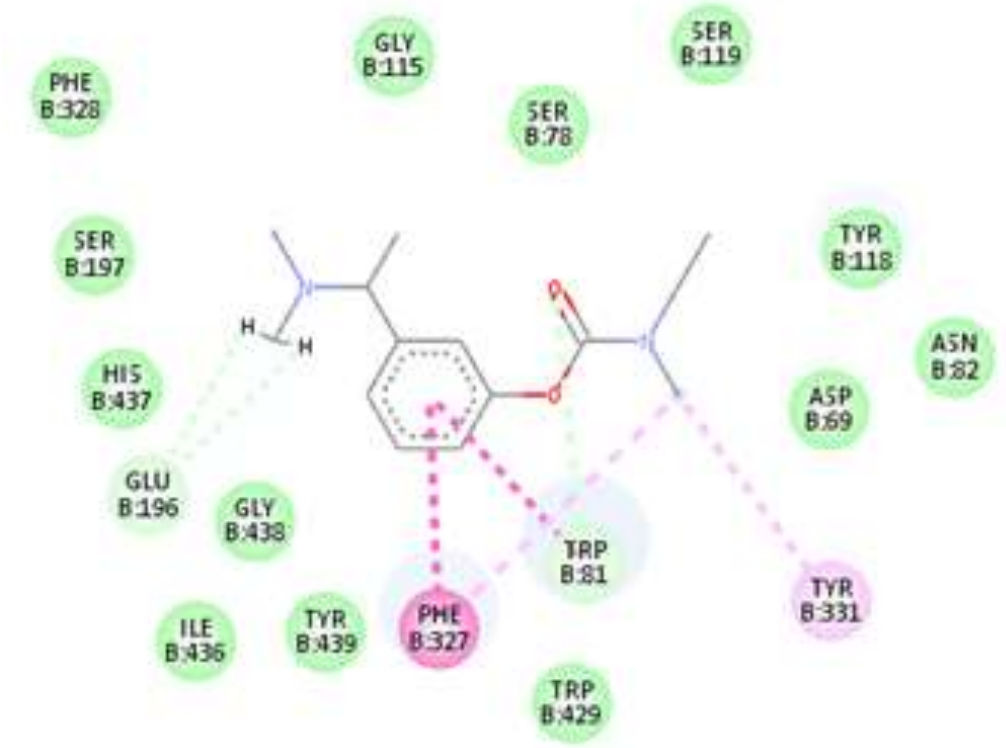
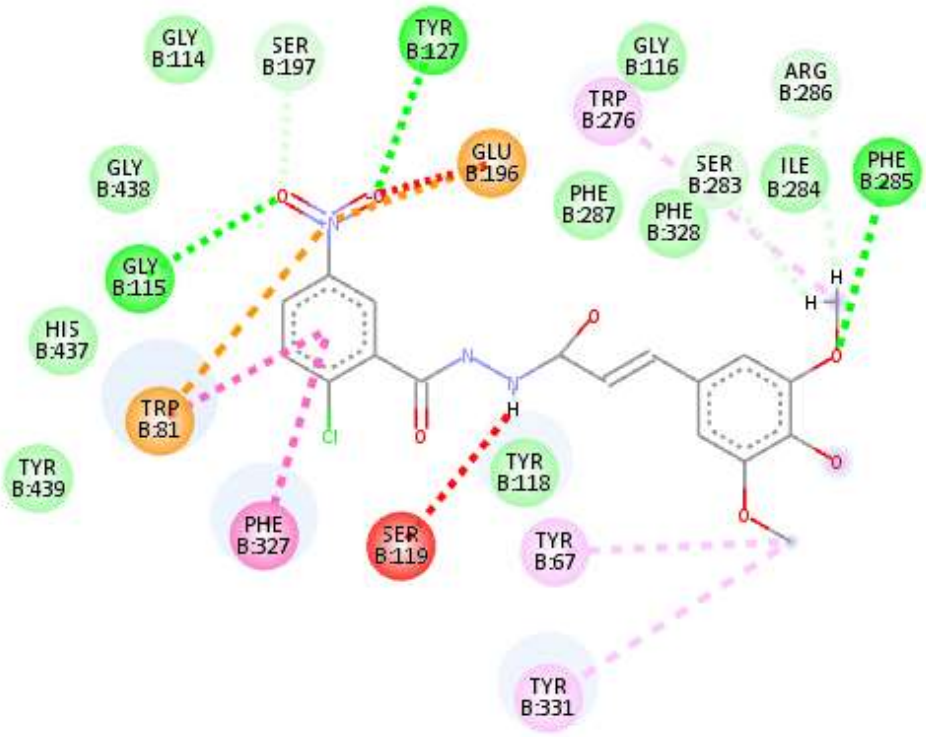
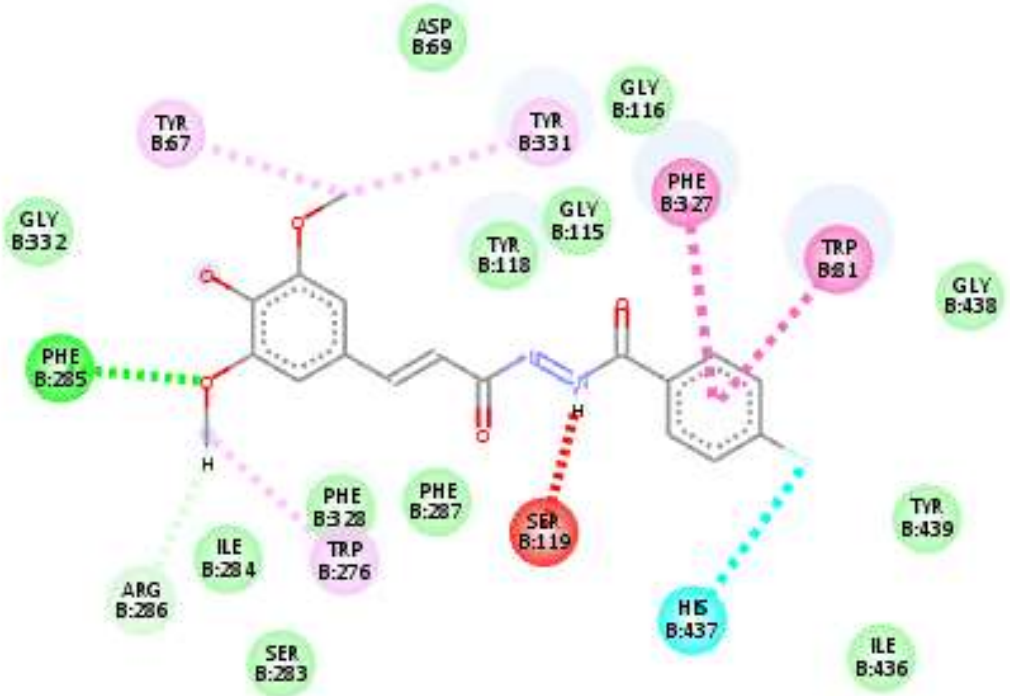


Figure 3: poses for (A) donepezil and (B) rivastigmine.

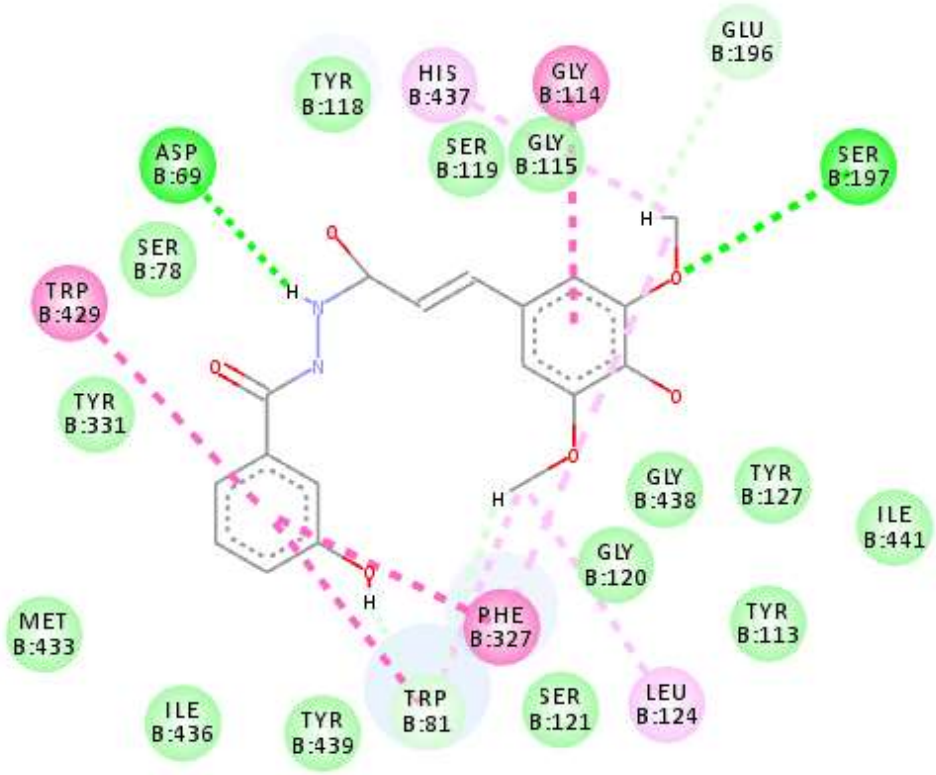
Considering the collaborative docking of the new designated compounds with the (1E66) enzyme (Tables 2 and 4), the results demonstrated that 5 amido compounds with the substituents (W3, W4, W9, W13, and W19) and 5 Schiff bases with the substituents (R1, R2, R11, R12, and R13) exhibited the greatest binding score. All 10 compounds were attached to 5-7 out of the 9 residues that define the enzyme's active site (mostly those binding 3 or 2 of the control inhibitors). Additionally, 3-7 further residues for each of the tested new compounds appeared to enhance enzyme binding (Figure 4 and Table 5). Furthermore, 4-7 of the designated compounds occupied the 3 amino acids (GLY 115, TYR 127, and HIS 437) within the allosteric site that were attached to the pharmacophore sinapic acid (Table 5). From the above binding scores and the screening of the structure-activity relationship (SAR) of the binding powers, we observed that compounds with W3 (with 2-chloro-5-nitro moiety) and W19 (with 3,5-dimethylbenzin moiety) substituents had the most powerful attachment ability. To a lesser extent, compounds with W9 (with 4-chlorobenz moiety) and R2 (with 4-nitrobenzo moiety) substituents were believed to be competitive reversible inhibitors for the AChE enzyme, similar to Donepezil and potentially better than and Rivastigmine (Figure 4).


GLY B:114
SER B:197
TYR B:127
GLY B:116
ARG B:286
TRP B:276
GLU B:196
GLY B:438
SER B:283
ILE B:284
PHE B:285
PHE B:287
PHE B:328
GLY B:115
HIS B:437
TRP B:81
TYR B:439
TYR B:118
PHE B:327
SER B:119
TYR B:67
TYR B:331

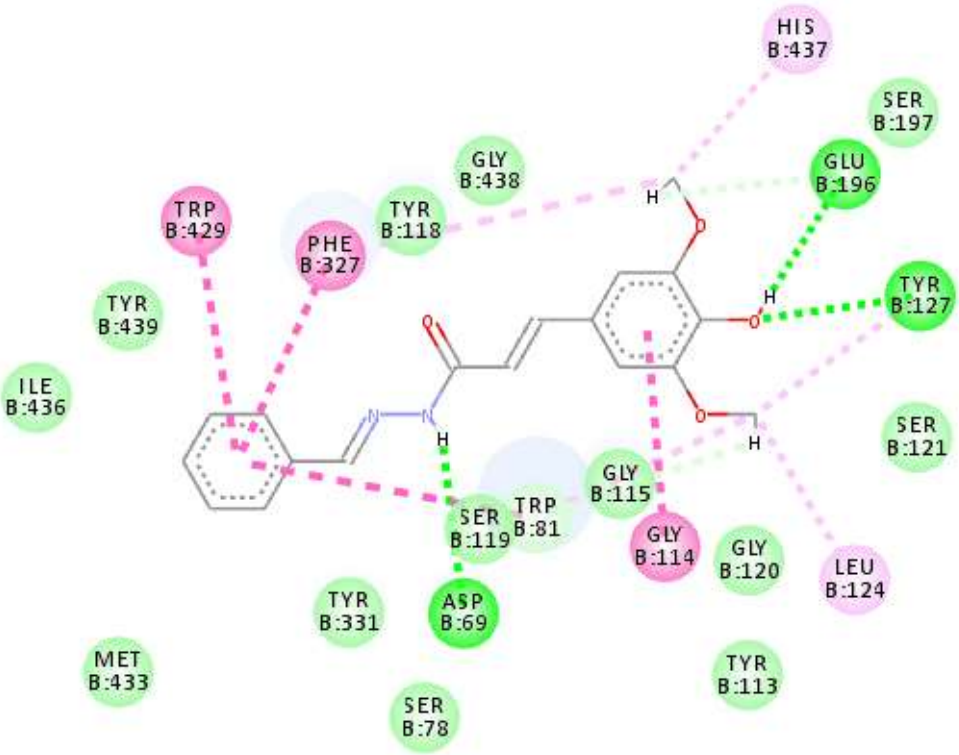
(A)


ASP B:69
GLY B:116
TYR B:67
TYR B:331
PHE B:327
GLY B:332
GLY B:115
TYR B:118
TRP B:81
GLY B:438
PHE B:285
PHE B:328
PHE B:287
SER B:119
TYR B:439
ILE B:284
TRP B:276
ARG B:286
HIS B:437
SER B:283
ILE B:436

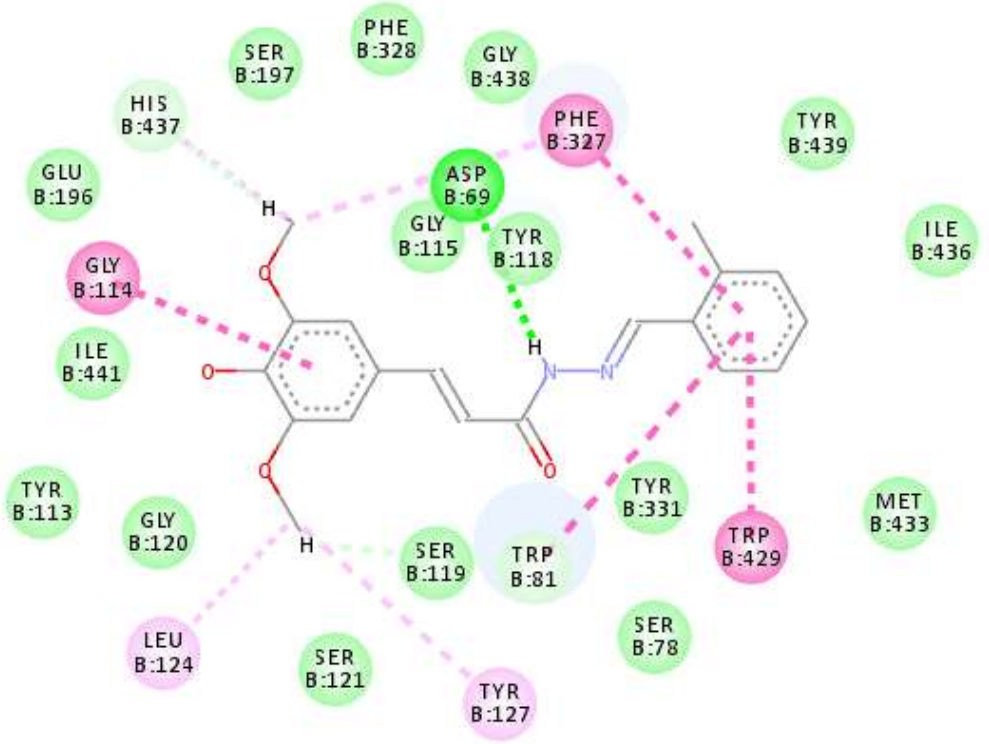
(B)


GLU B:196
TYR B:118
HIS B:437
GLY B:114
SER B:119
GLY B:115
ASP B:69
SER B:197
SER B:78
TRP B:429
TYR B:331
GLY B:438
TYR B:127
ILE B:441
GLY B:120
MET B:433
PHE B:327
TYR B:113
TRP B:81
SER B:121
LEU B:124
ILE B:436
TYR B:439

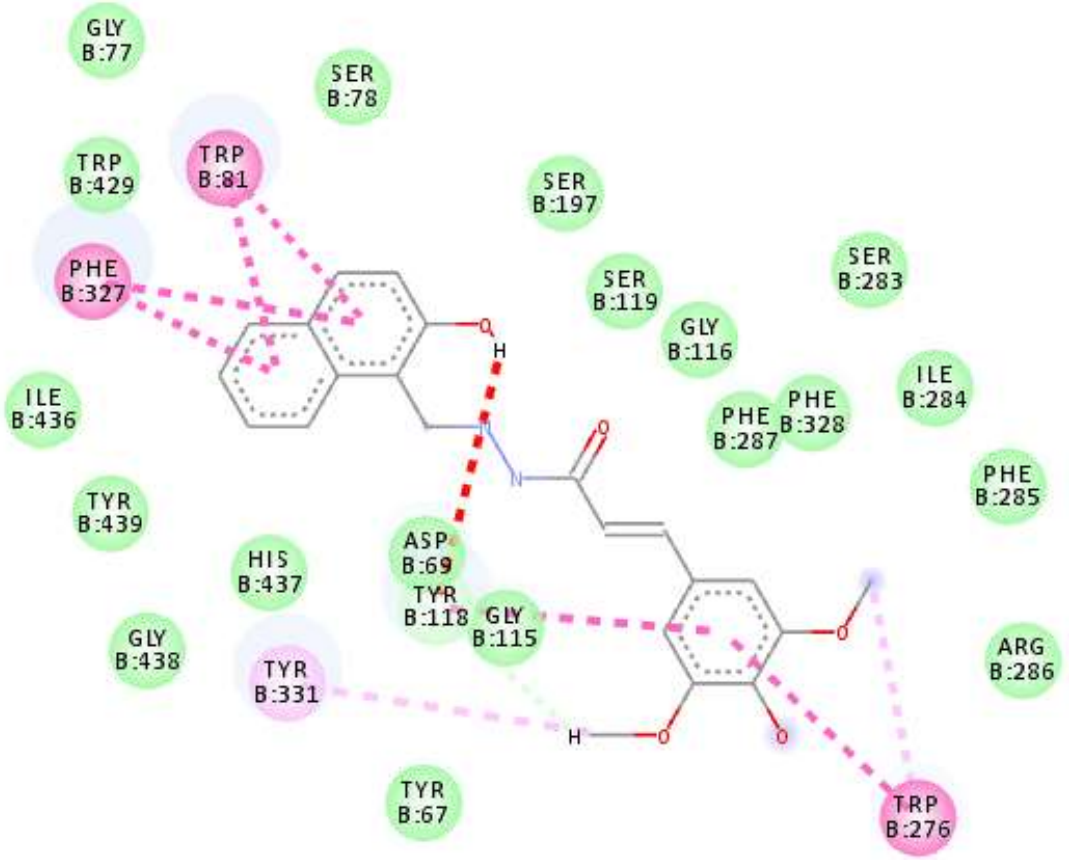
(C)


HIS B:437
SER B:197
GLY B:438
GLU B:196
TRP B:429
TYR B:118
PHE B:327
TYR B:127
TYR B:439
ILE B:436
SER B:121
GLY B:115
SER B:119
TRP B:81
GLY B:114
GLY B:120
LEU B:124
TYR B:331
ASP B:69
MET B:433
TYR B:113
SER B:78

(D)



(E)



(F)

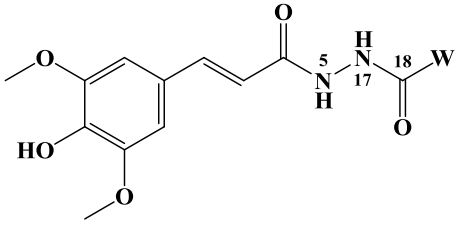
Figure 4: poses for (A) sinapic acid+W3, (B) sinapic acid+W4, (C) sinapic acid+W13, (D) sinapic acid+R1, (E) sinapic acid+R2, (F) sinapic acid+R13.

Table 5: Docking results and the amino acids interactions of the best compounds with 1E66 enzyme.

Comp.	Scores Kcal/mol.	TRP 81	TYR 439	PHE 327	TYR 331	TRP 276	TYR 118	TRP 429	ILE 436	GLU 196	GLY 115	TYR 127	HIS 437	SER 119	PHE 285	ARG 286	LEU 124	TYR 67	SER 197	GLY 114
Donep.	-9.2	×	×	×	×	×	×	×	×	×										
Rivast.	-8.3	×		×	×															
Sinapic acid	-7.6	×	×	×					×	×	×	×	×							
W3	-10.4	×		×	×	×				×	×	×		×	×	×	×	×	×	
W4	-10.4	×		×	×	×							×	×	×	×		×		
W9	-10.1	×		×	×					×	×		×					×	×	×
W13	-10.5	×		×	×		×			×	×	×	×		×			×	×	
W19	-10.1	×		×				×		×	×		×					×	×	
R1	-10.4	×		×				×		×	×	×	×				×	×		×
R2	-10.2	×	×	×				×	×		×	×	×	×			×			×
R11	-10.4	×		×	×		×	×						×				×		
R12	-10.8	×		×				×			×	×	×				×	×		×
R13	-11.1	×		×	×	×	×													

2.2. Chemical study results:

Both the final 5 amide and the 5 Schiff base sinapic acid synthesized derivatives were characterized, and their chemical structures were identified by ^{1}H NMR and ^{13}C NMR, in addition to recording their names and physical properties.



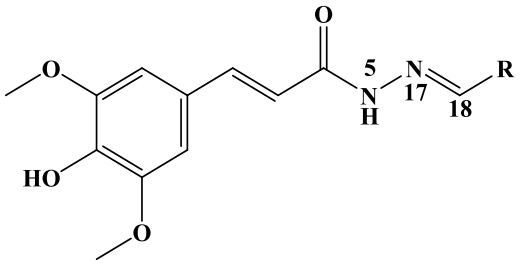
S1(Sinap+W3)[(E)-2-chloro-N'-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)-5-nitrobenzohydrazide].**yield (81%). color (faint gray). Mwt. (421.79 g/mol)**. **The ^{1}H NMR of S1** (δ, ppm) DMSO/d6: 9.99 (s, 1H, N 17), 9.71 (d, 1H, N 5), 8.37 (d, 1H, C 21), 8.26 (m, 2H, O 16, C 23), 7.75 (d, 1H, C 24), 7.56 (d, 1H, C 3), 6.92 (s, 2H, C 7,11), 6.78 (d, 1H, C5), 3.87 (s, 6H, C 13,15). **The ^{13}C NMR of S1** (δ, ppm) DMSO/d6: 167.86 (C1), 163.59 (C18), 148.08 (C8,10), 147.31 (C22), 142.41 (C3), 138.09 (C9), 134.92 (C19), 131.79 (C24), 126.93 (C23), 126.30 (C6), 123.35 (C21), 116.69 (C2), 107.07 (C7,11), 56.27 (C13,15).

S2(Sinap+W4)[(E)-4-fluoro-N'-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)benzohydrazide]. **yield (78%). color (gray). Mwt. (360.34 g/mol). The ^{1}H NMR of S2** (δ, ppm) DMSO/d6: 9.82 (d, 1H, N 17), 9.62 (d, 1H, N 5), 8.24 (s, 1H, O 16), 7.96 (m, 2H, C 21,25), 7.55 (d, 1H, C 3), 7.26 (m, 2H, C 22,24), 6.95 (s, 2H, C 7,11), 6.68 (d, 1H, C2), 3.83 (s, 6H, C 13,15). **The ^{13}C NMR of S2** (δ, ppm) DMSO/d6: 166.56 (C1), 165.93 (C18), 164.50 (C23), 148.08 (C8,10), 144.45 (C3), 137.99 (C9), 130.45 (C19), 130.45 (C21,25), 127.30 (C6), 116.68 (C2), 115.92 (C22,24), 107.07 (C7,11), 56.27 (C13,15).

S3(Sinap+W9)[(E)-N'-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)-4-nitrobenzohydrazide]. **yield (85%). color (brown). Mwt.(387.35 g/mol). The ^{1}H NMR of S3** (δ, ppm) DMSO/d6: 9.88 (d, 1H, N 17), 9.65 (d, 1H, N 5), 8.32 (m, 2H, C 22,24), 8.24 (s, 1H, O 16), 8.11 (dt, 2H, C 21,25), 7.55 (d, 1H, C 3), 6.88 (s, 2H, C 7,11), 6.75 (d, 1H, C2), 3.77 (s, 6H, C 13,15). **The ^{13}C NMR of S3** (δ, ppm) DMSO/d6: 167.86 (C1), 166.69 (C18), 149.48 (C23), 147.48 (C8,10), 142.41 (C3), 138.09 (C9), 136.98 (C19), 128.10 (C21,25), 126.33 (C6), 124.03 (C22,24), 116.68 (C2), 106.45 (C7,11), 52.55 (C13,15).

S4(Sinap+W13)[(E)-3-hydroxy-N'-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)benzohydrazide]. **yield (90%). color (dark brown). Mwt.(385.61 /mol)**. **The ^{1}H NMR of S4** (δ, ppm) DMSO/d6: 9.83 (d, 1H, N 17), 9.62 (d, 1H, N 5), 8.85 (s, 1H, O 26), 8.54 (s, 1H, O 16), 7.53 (m, 2H, C 3,25), 7.30 (m, 2H, C 21,24), 6.91 (m, 3H, C 7,11,23), 6.77 (d, 1H, C 2), 3.87 (s, 6H, C 13,15). **The ^{13}C NMR of S4** (δ, ppm) DMSO/d6: 166.96 (C1), 167.07 (C18), 157.09 (C22), 148.08

(C8,10), 144.47 (C3), 136.79 (C9), 132.54 (C19), 130.27 (C24), 125.33 (C6), 119.85 (C23), 118.77 (C25), 116.68 (C2), 112.65 (C21), 107.07 (C7,11), 56.27 (C13,15).



S5(Sinap+W19)[(E)-N'-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)-3,5-dimethylbenzohydrazide]. **yield (79%). color (gray). Mwt.(370.41 g/mol)**. **The ^{1}H NMR of S5** (δ, ppm) DMSO/d6: 9.61 (d, 1H, N 5), 9.26 (d, 1H, N 17), 8.24 (s, 1H, O 16), 7.66 (d, 2H, C 21,25), 7.44 (d, 1H, C 3), 7.09 (m, 1H, C 23), 6.85 (s, 2H, C 7,11), 6.67 (d, 1H, C 2), 3.98 (s, 6H, C 13,15), 2.29 (s, 6H, C 26,27). **The ^{13}C NMR of S5** (δ, ppm) DMSO/d6: 167.86 (C1), 166.83 (C18), 148.08 (C8,10), 142.41 (C3), 138.09 (C9), 137.11 (C22,24), 133.14 (C23), 131.96 (C19), 126.87 (C21,25), 125.02 (C6), 116.56 (C2), 107.45 (C7,11), 55.35 (C13,15), 20.91 (C26,27).

S6(Sinap+R1)[(E)-N'-((E)-benzylidene)-3-(4-hydroxy-3,5-dimethoxyphenyl)acrylohydrazide]. **yield (87%). color (dark yellow). Mwt.(326.35 g/mol)**. **The ^{1}H NMR of S6** (δ, ppm) DMSO/d6: 9.73 (s, 1H, N 5), 8.24 (s, 1H, O 16), 8.13 (s, 1H, C 18), 7.67 (m, 2H, C 20,24), 7.55 (d, 1H, C 3), 7.36-7.45 (m, 3H, C 21,22,23,), 6.92 (s, 2H, C 7,11), 6.88 (d, 1H, C 2), 3.77 (s, 6H, C 13,15). **The ^{13}C NMR of S6** (δ, ppm) DMSO/d6: 165.81 (C1), 148.08 (C8,10), 146.79 (C18), 143.39 (C3), 135.45 (C9), 132.78 (C19), 130.30 (C22), 128.75 (C21,23), 128.10 (C20,24), 126.16 (C6), 118.32 (C2), 107.08 (C7,11), 56.27 (C13,15).

S7(Sinap+R2)[(E)-N'-((E)-4-chlorobenzylidene)-3-(4-hydroxy-3,5-dimethoxyphenyl)acrylohydrazide].**yield (90%). color (dark yellow). Mwt.(360.79 g/mol)**. **The ^{1}H NMR of S7** (δ, ppm) DMSO/d6: 9.77 (s, 1H, N 5), 8.64 (s, 1H, O 16), 8.23 (s, 1H, C 18), 7.55-7.66 (m, 4H, C 20,21,23,24), 7.51 (d, 1H, C 3), 6.83 (s, 2H, C 7,11), 6.83 (d, 1H, C 2), 3.87 (s, 6H, C 13,15). **The ^{13}C NMR of S7** (δ, ppm) DMSO/d6: 167.71 (C1), 147.58 (C8,10), 147.26 (C18), 142.34 (C3), 138.09 (C9), 135.08 (C22), 132.89 (C19), 127.67 (C21,23), 127.88 (C20,24), 125.56 (C6), 117.54 (C2), 106.71 (C7,11), 58.65 (C13,15).

S8(Sinap+R11)[(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-N'-((E)-3-phenylpropylidene)acrylohydrazide]. **yield (67%). color (yellow). Mwt.(354.41 g/mol)**. **The ^{1}H NMR of S8** (δ, ppm) DMSO/d6: 9.83 (s, 1H, N 5), 8.25 (s, 1H, O 16), 7.54 (d, 1H, C 3), 7.22-7.55 (m, 5H, C 22,23,24,25,26), 7.13 (t, 1H, C 18), 6.92 (s, 2H, C 7,11), 6.75 (d, 1H, C 2), 3.95 (s, 6H, C 13,15), 2.86 (tt, 2H, C 20) 2.51 (m, 2H, C 19). **The ^{13}C NMR of S8** (δ, ppm) DMSO/d6: 166.01 (C1), 149.38 (C18), 148.18 (C8,10), 143.39 (C3), 141.13 (C21), 138.09 (C9), 128.78 (C22,26), 128.53 (C23,25), 126.55 (C24), 126.16 (C6), 118.38 (C2), 107.08 (C7,11), 56.27 (C13,15), 31.35 (C19,20).

S9(Sinap+R12)[(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-N'-((E)-2-methylbenzylidene)acrylohydrazide]. **yield (88%). color (off-white). Mwt.(340.38 g/mol)**. **The ^{1}H NMR of S9** (δ, ppm) DMSO/d6: 9.78 (s, 1H, N 5), 8.28 (s, 1H, C 18), 8.10 (s, 1H, O 16), 7.64 (dd, 1H, C 24), 7.53 (d, 1H, C 3), 7.21-7.30 (m, 3H, C 21,22,23), 6.92 (s, 2H, C 7,11), 6.84 (s, 1H, C 2), 3.87 (s, 6H, C 13,15), 2.43 (s, 3H, C 25). **The ^{13}C NMR of S9** (δ, ppm) DMSO/d6: 165.81 (C1), 148.08 (C8,10), 145.29 (C18), 142.49 (C3), 138.09 (C9), 137.46 (C20), 133.58 (C19), 130.99 (C22), 130.02 (C21), 128.02 (C24), 127.50 (C23), 126.16 (C6), 118.32 (C2), 107.08 (C7,11), 56.27 (C13,15), 19.77 (C25).

S10(Sinap+R13)[(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-N'-((E)-(2-hydroxynaphthalen-1-yl)methylene) acrylohydrazide].**yield (76%). color (yellow). Mwt.(392.41 g/mol)**. **The ^{1}H NMR of S10** (δ, ppm) DMSO/d6: 9.77 (s, 1H, N 5), 9.06 (s, 1H, C 18), 8.84 (s, 1H, C 29), 8.24 (s, 1H, O16), 8.11 (dd, 1H, C 28), 7.87 (dt, 1H, C 25), 7.83 (d, 1H, C 22), 7.49-7.56 (m, 3H, C 3,26,27), 7.24 (d, 1H, C 21), 6.92 (s, 2H, C 7,11), 6.83 (d, 1H, C 2), 3.87 (s, 6H, C 13,15). **The ^{13}C NMR of S10** (δ, ppm) DMSO/d6: 166.89 (C1), 156.06 (C20), 148.08 (C8,10), 143.51 (C18), 142.39 (C3), 138.09 (C9), 132.92 (C24), 131.40 (C22), 128.23 (C25), 127.91 (C27), 127.50 (C26), 126.83 (C23), 126.16 (C6), 125.77 (C28), 118.32 (C2), 116.60 (C21), 113.58 (C19), 107.08 (C7,11), 56.27 (C13,15).

1H NMR characterizes the occurrence of the amide bonds: a single proton for both H-N5 at 9.61-9.99 ppm and H-N17 at 9.26-9.88 ppm, confirming the reaction of the sinapic acid hydrazide amine with the carboxylic acids to form the amido compounds (S1-S5). Conversely, the appearance of a single imine proton for both H-N5 (of the first amine group) at 9.73-9.83 ppm and H-C18 at 7.13-9.06 ppm confirms the reaction of the sinapic acid hydrazide amine with aldehydes, forming the azomethine group (-N=CH-) of the Schiff bases (S6-S10). However, in the 13C-NMR, the emergence of

both carbonyl C1 at 166.65-167.86 ppm and C18 at 163.59-167.07 ppm confirms the reaction of the sinapic acid hydrazide amine with the carboxylic acids to form the amide compounds (S1-S5). Similarly, the presence of both ca -149.38 ppm confirms the reaction of the sinapic acid hydrazide amine with aldehydes, forming the Schiff bases (S6-S10).

2.3. In-vitro ChE inhibition results:

The electrometric method was utilized to assess the *in vitro* inhibition of plasma and erythrocyte ChE activities by sinapic acid derivatives, Donepezil, or Rivastigmine. The results are presented in Table 6.

Table 6: In-vitro inhibition of human plasma and erythrocyte ChE activities by sinapic acid derivatives, Donepezil or Rivastigmine

Comp.	µM	Plasma ChE		Erythrocyte ChE	
		Δ pH/20 min	% Inhib.	Δ pH/20 min	% Inhib.
Control	0	1.02 ± 0.012	0	1.12 ± 0.013	0
S1	50	0.69±0.004❋	38.83	0.09±0.004❋	90.77
	100	0.41± 0.012❋	63.65	0.08± 0.003❋	92.35
S2	50	0.66± 0.014❋	40.88	0.10± 0.022❋	89.76
	100	0.36± 0.043❋	67.66	0.06± 0.002❋	94.38
S3	50	0.67± 0.004❋	40.03	0.18± 0.014❋	82.68
	100	0.47± 0.011❋	57.79	0.10± 0.003❋	90.33
S4	50	0.62± 0.021❋	44.34	0.05± 0.033❋	94.82
	100	0.33± 0.02❋	70.50	0.02± 0.002❋	98.04
S5	50	0.70± 0.002❋	37.54	0.22± 0.031❋	78.68
	100	0.51± 0.033❋	54.79	0.08±0.004❋	92.33
S6	50	0.66±0.001❋	40.84	0.12± 0.041❋	88.72
	100	0.44± 0.003❋	60.63	0.06± 0.003❋	94.37
S7	50	0.66± 0.001❋	41.40	0.21±0.002❋	79.65
	100	0.55±0.004❋	50.78	0.12± 0.005❋	88.31
S8	50	0.61±0.001❋	45.84	0.17± 0.005❋	83.76
	100	0.35± 0.032❋	68.67	0.09± 0.032❋	91.32
S9	50	0.62± 0.013❋	44.64	0.06± 0.023❋	94.42
	100	0.31±0.002❋	72.58	0.02± 0.001❋	98.34
S10	50	0.68± 0.004❋	39.37	0.15±0.003❋	85.68
	100	0.44± 0.003❋	60.56	0.07±0.003❋	93.35
Donepezil	50	0.65±0.002❋	41.81	0.06± 0.002❋	93.74
	100	0.37± 0.004❋	66.61	0.02± 0.002❋	98.38
Rivastigmine	50	0.71± 0.022❋	37.05	0.20± 0.014❋	80.64
	100	0.50± 0.032❋	55.72	0.09± 0.016❋	91.37
Sinapic acid	50	0.72± 0.012❋	35.30	0.18± 0.004❋	82.07
	100	0.56± 0.011❋	50.34	0.12± 0.023❋	88.64

N=2-3 / concentration groups.
ChE values are means ± SE
❋ Significantly different from the respective control (0) group, P<0.05

Based on the established correlation between ChE inhibition *in vivo* and *in vitro* [31], this work aimed to test the hypothesis of modifying and enhancing the sinapic acid scaffold, which resembles indigenous neurotransmitters acting on ChE enzymes. The goal was to create a more powerful inhibitor for these enzymes by altering its chemical structure with various amide and Schiff base substituents. The incorporation of new derivatives, resulting from naturally occurring, well-known safe nutrient compounds, may enhance their affinity and minimize side effects, as they would undergo the same metabolic pathways as the parent compound. This would produce metabolites already known to be safe for humans. The *in vitro* affinity testing of the new compounds to conjugate and inhibit ChE

enzymes yielded good results at both 50 and 100 µM concentrations. The newly synthesized compounds exhibited varying percentages of ChE activity inhibition, which depended on their selectivity for the enzyme and the affinity of the modifying chemical group. All new compounds demonstrated reasonable selectivity for plasma ChE (BChE) while showing a stronger affinity for erythrocyte or brain ChE (AChE). This might be attributed to the similar selectivity of their parent compound. The most important aspect here is the brain AChE, as the new derivatives are intended for treating and managing Alzheimer's disease. The *in vitro* affinity testing outcomes indicate that only six of the ten synthesized compounds (those selected based on the docking study) showed good and potent affinity to inhibit AChEs (Table 6, Figure 5). These compounds were more potent than the parent compound (sinapic acid) and rivastigmine, and largely similar to donepezil, with compounds S4 and S9 showing superior activity resembling donepezil. Concerning BChE, the results were similar to both rivastigmine and donepezil; they all exhibited moderate affinity, which is preferable for reducing unwanted peripheral side effects resulting from BChE inhibition. Three of these six active derivatives were amides, and the other three were Schiff base derivatives, suggesting that both amide and Schiff base moieties with their substituents interact with ChE in a similar manner. It's worth noting that three of these six active derivatives had superior and excellent docking results (S1, S4, and S9), providing a positive indication that the docking process is reliable and its results can be depended upon in designing and synthesizing new biologically active compounds.

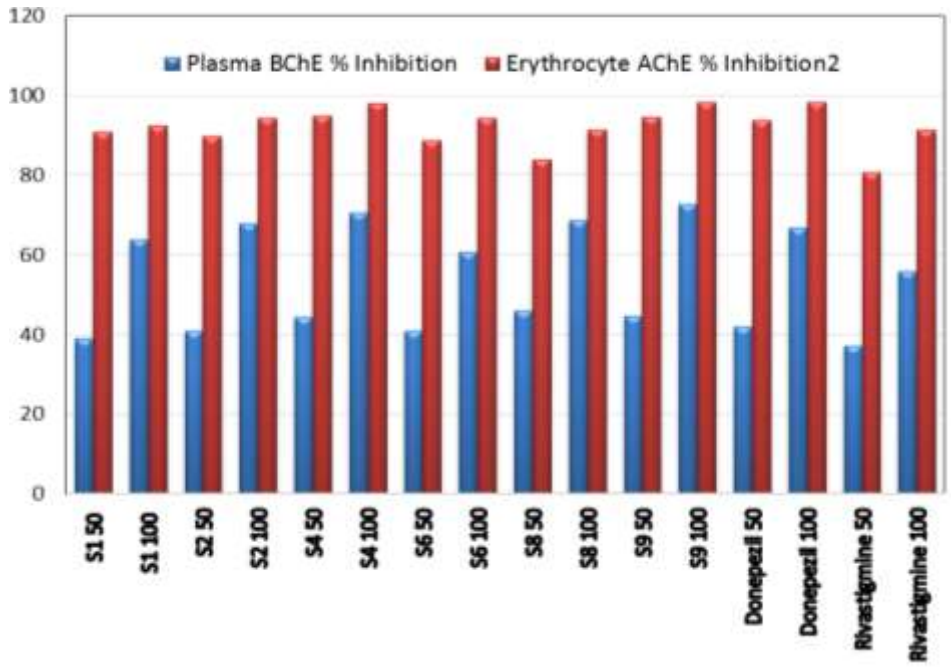


Figure 5 : In vitro inhibition of human plasma and erythrocyte ChE activities by best sinapic acid derivatives, Donepezil or Rivastigmine

The six active derivatives were: S1 (2-chloro-5-nitro phenyl with an amide moiety), S2 (4-fluorophenyl with an amide moiety), S4 (3-hydroxyphenyl with an amide moiety), S6 (phenyl with a -HC=N- moiety), S8 (3-phenylpropylidene with a -HC=N- moiety), and S9 (2-methyl phenyl with a -HC=N- moiety). These are thought to be new potential competitive reversible inhibitors for the AChE enzyme, resembling donepezil and somewhat better than rivastigmine. All six of these active derivatives contain an aromatic benzene ring in their substitutions. The three amide derivatives have aromatic hydrophilic substituents with either Cl and NO_2, F, or OH electronegative moieties. These atoms or groups produce more powerful potentiation with the active pocket of the ChEs than their parent compound does. The remaining three Schiff base compounds have hydrophobic moieties that may intertwine and bind to other sites in the active pocket, enhancing the interaction of their parent

compound with the AChE enzyme. Despite the powerful action of these six new compounds, there's still a need for further *in vivo* and animal testing studies to confirm their activity and safety before they can be csonsidered as potential candidates for treating Alzheimer's disease.

2.4. The ADME study outcomes:

The 10 newly synthesized compounds that performed best in the docking phase were further evaluated for their pharmacokinetic properties and drug-likeness. According to Lipinski's Rule of Five, an orally active, drug-like compound should have no more than one violation of the following criteria: hydrogen bond acceptors (HBA) ≤10, hydrogen bond donors (HBD) ≤5, an octanol-water partition coefficient (LogP) ≤5, and a molecular weight (MW) <500 Da, with an ideal log S value of ≥−4 [29,32]. Based on the data in Table 7, all 10 tested compounds met the criteria for hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and LogP. The water solubility (LogS) was also within adequate limits, with the exception of compounds S7 and S10. This suggests enhanced solubility and blood-brain barrier (BBB) penetration, as increased hydrophobicity improves penetration through the gastrointestinal (GI) tract and BBB. Consequently, these compounds are likely to have good BBB penetration and very good GI crossing abilities (with the exception of S1 and S3). This indicates a strong possibility that these compounds, if approved as drugs in future clinical trials, could be available in the central nervous system [33]. Additionally, the molecular weight (MW) for all 10 new compounds appears to be within standard ranges. Nearly all of them show no inhibiting capacity for the liver's CYP450 enzymes (except S7). Lastly, according to Lipinski's rule (rule of fives), all the synthesized compounds are within acceptable values.

Table 7: The predicted pharmacokinetics of the new compounds predicted by SwissADME.

Compound No.	Physicochemical Properties			Lipophilicity	Water Solubility	Pharmacokinetics			Drug likeness
	Molecular weight g/mol	No. H- bond acceptors	No. H-bond donors	Log Po/w	Log S	GI absorption	BBB permeant	CYP450	Lipinski
S1	421.79	7	3	1.76	-3.96	Low	Yes	No	Yes
S2	360.34	6	3	2.41	-3.45	High	Yes	No	Yes
S3	367.34	7	3	1.33	-3.36	Low	Yes	No	Yes
S4	358.35	6	4	1.68	-3.15	High	Yes	No	Yes
S5	370.40	5	3	2.77	-3.90	High	Yes	No	Yes
S6	326.35	5	2	2.55	-3.56	High	Yes	No	Yes
S7	360.79	5	2	3.08	-4.16	High	Yes	Yes	Yes
S8	354.40	5	2	3.01	-3.73	High	Yes	No	Yes
S9	340.37	5	2	2.88	-3.87	High	Yes	No	Yes
S10	392.40	6	3	3.30	-5.10	High	Yes	No	Yes

3. Conclusions

In this work, we tested the hypothesis of modifying and enhancing the sinapic acid scaffold, which resembles indigenous neurotransmitters acting on ChE enzymes. The *in vitro* affinity testing for the newly synthesized compounds to attach the enzyme pocket yielded acceptable outcomes at both concentrations used (50 and 100 μM). Six new compounds showed good and potent affinity to inhibit the ChEs, proving more potent than the parent compound (sinapic acid) and rivastigmine, and largely similar to that of donepezil for AChE. Specifically, compounds S4 and S9 exhibited superior activity, resembling donepezil. Three of these six compounds were amides and three were Schiff bases. The three amides have aromatic hydrophilic substituents with either Cl and NO_2, F, or OH electronegative moieties. These atoms or groups produced more powerful potentiation with the active pocket of the ChEs than the parent compound. The remaining three Schiff base compounds possessed hydrophobic moieties. Further in vivo and animal testing studies will be needed to ascertain the activity and safety of these new compounds.

Conflict of Interest: The author declares that there are no conflicts of interest.

Funding: The author declares that there are no funders for this work.

References:

[1] Toka, F.; Koçyiğit-Kaymakçıoğlu, B.; Sağlık, B.N.; Levent, S.; Özkay, Y. and Kaplancıklı, Z.A.; "Synthesis and biological evaluation of new pyrazolone Schiff bases as monoamine oxidase and cholinesterase inhibitors". Bioorg. Chem., 84: 41–50, 2019.

[2] Mahmood, A.A.J.; Zainal, A.A. and Khalaf, M.M.; "Cholinesterase activity in non-alcoholic-fatty liver disease in diabetic patients taking oral antidiabetic drugs". Curr. Top. Pharmacol., 25: 43–51, 2021.

[3] Zheng, Q.; Sun, W.; Qu, M. and Bais, S.; "Synergistic Effect of Selected Hydroxy Cinnamic Acid Derivative (HCA) With Rivastigmine in Dementia". Pharm. Lett., 9(11): 54-62, 2017.

[4] Pourtaher, H.; Hasaninejad, A. and Iraji, A.; "Design, synthesis, in silico and biological evaluations of novel polysubstituted pyrroles as selective acetylcholinesterase inhibitors against Alzheimer's disease". Sci. Rep., 12:15236, 2022.

[5] aheri, M.; Aslani, S.; Ghafouri, H.; Mohammadi, A.; Akbary Moghaddam, V.; Moradi, N. and Naeimi, H.; "Synthesis, in vitro biological evaluation and molecular modelling of new 2-chloro-3-hydrazinopyrazine derivatives as potent acetylcholinesterase inhibitors on PC12 cells". BMC Chem., 16:7, 2022.

[6] Chen, Y.; Zhu, J.; Mo, J.; Yang, H.; Jiang, X.; Lin, H.; Gu, K.; Pei, Y.; Wu, L.; Tan, R.; Hou, J.; Chen, J.; Lv, Y.; Bian, Y. and Sun, H.; "Synthesis and bioevaluation of new tacrine-cinnamic acid hybrids as cholinesterase inhibitors against Alzheimer's disease". J. Enzyme Inhib. Med. Chem., 33(1): 290-302, 2018.

[7] Mahmood, A.A.J.; "Synthesis, antioxidant and antimicrobial activities for new 4,4'-methylenedianiline amide compounds". Egypt. J. Chem., 64(12): 6999–7005, 2021.

[8] Szwajgier, D.; "Anticholinesterase activity of selected phenolic acids and flavonoids - interaction testing in model solutions". Ann. Agric. Environ. Med., 22(4): 690-4, 2015.

[9] Haji Ali, S.; Osmaniye, D.; Sağlık, B.N.; Levent, S.; Özkay, Y. and Kaplancıklı, Z.A.; "Design, Synthesis, and Evaluation of Novel 2H-Benzo[b][1,4]thiazin-3(4H)-one Derivatives as New Acetylcholinesterase Inhibitors". Molecules, 27(7):2121, 2022.

[10] Motebennur, S.L.; Nandeshwarappa, B.P. and Katagi, M.S.; "Drug Candidates for the Treatment of Alzheimer's Disease: New Findings from 2021 and 2022". Drugs Drug Candidates, 2(3):571-590, 2023.

[11] Hussein, W.; Sağlık, B.N.; Levent, S.; Korkut, B.; Ilgın, S.; Özkay, Y. and Kaplancıklı, Z.A.; "Synthesis and Biological Evaluation of New Cholinesterase Inhibitors for Alzheimer's Disease". Molecules, 23(8):2033, 2018.

[12] Nguyen, V.P.T.; Stewart, J.D.; Ioannou, I. and Allais, F.; "Sinapic Acid and Sinapate Esters in Brassica: Innate Accumulation, Biosynthesis, Accessibility via Chemical Synthesis or Recovery From Biomass, and Biological Activities". Front. Chem., 9:664602, 2021.

[13] Mahmood, A.A.J.; Khalaf, M.M. and Zainal, A.A.; "Cholinesterases activities in diabetic and hyperlipidemic patient". Indian J. Public Health Res. Dev., 10(10): 2812–2816, 2019.

[14] Chen, C.; "Sinapic Acid and Its Derivatives as Medicine in Oxidative Stress-Induced Diseases and Aging". Oxid. Med. Cell Longev., 3571614, 2016.

[15] Guo, Y.; Yang, H.; Huang, Z.; Tian, S.; Li, Q.; Du, C.; Chen, T.; Liu, Y.; Sun, H. and Liu, Z.;

"Design, Synthesis, and Evaluation of Acetylcholinesterase and Butyrylcholinesterase Dual-Target Inhibitors against Alzheimer's Diseases". Molecules, 25(3):489, 2020.

[16] Dgachi, Y.; Bautista-Aguilera, O.M.; Benchekroun, M.; Martin, H.; Bonet, A.; Knez, D.; Godyń, J.; Malawska, B.; Gobec, S.; Chioua, M.; Janockova, J.; Soukup, O.; Chabchoub, F.; Marco-Contelles, J. and Ismaili, L.; "Synthesis and Biological Evaluation of Benzochromenopyrimidinones as Cholinesterase Inhibitors and Potent Antioxidant, Non-Hepatotoxic Agents for Alzheimer's Disease". Molecules, 21(5):634, 2016.

[17] Nićiforović, N. and Abramovič, H.; "Sinapic Acid and Its Derivatives: Natural Sources and Bioactivity". Compr. Rev. Food Sci. Food Saf., 13(1):34-51, 2014.

[18] Saleh, A. and Mahmood, A.; "Docking Study for New 7-Aminocephalosporinic Acid Derivatives as Potential Inhibitors for ß-Lactamases". Iraqi J. Pharm., 20: 23-32, 2023.

[19] Saleh, A.A. and Mahmood, A.A.J.; "New P-Aminodiphenylamine Amide Compounds: Design, Synthesis And Anti B-Lactamases Activity Evaluation". Chem. Probl., 1(22), 20-32, 2024.

[20] Srivastava, K.A.; Verma, S. and Shubhra, S.; "Synthesis and Biological activity of some Novel Chalcone derivatives containing [1, 3, 4] oxadiazole-2(3H)-thione". Res. J. Recent Sci., 4: 74-79, 2015.

[21] Galge, R.; Raju, A. and Degani, M.S.; "Synthesis and In Vitro Antimicrobial Activity of 1,3,4-Oxadiazole-2-thiol and its Analogs". J. Heterocycl. Chem., 52(2), 352-357, 014.

[22] Mahmood, A.A.J. and Bahnam, S.P.; "Docking, synthesis and ß-lactamase inhibitory activity evaluation for new amide compounds" Kimya Problemleri, 22(2): 139-149, 2024.

[23] Mahmood, A.A.J.; "Synthesis, antimicrobial and antioxidant activities evaluation for new amide compounds". In: *AIP Conference Proceedings*, Proceedings of the 5th International Conference on Buildings, Construction and Environmental Engineering, Amman, Jordan, November 21–23, 2023; Fattah, M.Y., Al-Mukhtar, M., Al-Dahawai, A. and Al-Barrak, A., Eds.; AIP Publishing: Melville, NY, 2024.

[24] Mahmood, A.A.J.; Al-Iraqi, M.A. and Abachi, F.T.; "New 4,4'-Methylenedianiline Monobactame Compounds: Synthesis, Antioxidant and Antimicrobial Activities Evaluation". In: *AIP Conference Proceedings*, Proceedings of the 4th International Conference on Materials Engineering & Science, Duhok, Kurdistan Region, Iraq, October 6–7, 2021; Dahham, O.S., Ed.; AIP Publishing: Melville, NY, 2022.

[25] Mahmood A AJ. and Ahmed O. A.H. "In vitro Inhibition of Human Plasma and Erythrocyte Cholinesterases by a Selected Drugs (Enzymes Inhibitors)". Tikrit J. of Pharm. Sci., 7(2): 12-19, 2011.

[26] Mahmood, A.A.J.; "In vitro inhibition of human blood cholinesterases and protection against chlorpyriphos (organophosphate) by weak anticholinesterase drugs". Iraqi J. Pharm., 11(1),18-29, 2011.

[27] [27] Mustafa, Y.F.; Mahmood, A.A.J. and Qasim, I.T.; "Synthesis of new metoclopramide derivatives and in vitro evaluation of their human cholinesterases protection against Chlorpyrifos". Iraqi J. Pharm., 11(2), 58-69, 2011.

[28] Ahmed, O.A. and Mohammad, F.K.; "A simplified eleclromelric technique for rapid measurement of human blood cholinesterase activity". Internet J. Toxicol., 2:55-58, 2005.

[29] Bahnam, S.; Jasim, M. and Mahmood, A.A.J.; "In silico study of new schiff base-and amide-boronic acid derivatives as potential inhibitors of ß-lactamases". Indian Drugs, 9, 28, 37-48 2023.

[30] Artunç, T.; Cetinkaya, Y. and Menzek, A.; "Investigation of cholinesterase and α-glucosidase enzyme activities, and molecular docking and dft studies for 1,2-disubstituted cyclopentane derivatives with phenyl and benzyl units". Mol. Diversity, 29, 1305–1321, 2025.

[31] Mohammad, F.K.; Alias, A.S. and Ahmed, O.A.; "Electrometric measurement of plasma, erythrocyte and whole blood cholinesterase activities in healthy human volunteers". J. Med. Toxicol., 3:25-30, 2007.

[32] Bashir, M.; Oglah, M. and Mahmood, A.; "In silico study of nsaids conjugated with coumarins as new anti-inflammatory compounds". Indian Drugs, 61: 20-29, 2024.

[33] Ertl, P. and Schuffenhauer, A.; "Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions". J. Cheminform., 1(1):8, 2009.