



Synthesis and Docking Studies of a Novel Tetrahydroquinazoline Derivative as Promising Scaffold for Acetylcholine Esterase Inhibition

Rawan A. Alsuhamat,^a Qais Abualassal,^{b,*} Zead Helmi Abudayeh,^{b,*} Sherif S. Ebada,^{a,c,d} Amgad Albohy^{e,*}

^a Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mutah University, Al-Karak, Jordan

^b Department of Applied Pharmaceutical Sciences, Faculty of Pharmacy, Isra University, Amman, Jordan

^c Department of Pharmacognosy, Faculty of Pharmacy, Sinai University, Ismailia, Egypt

^d Department of Pharmacognosy, Faculty of Pharmacy, Ain-Shams University, Cairo, Egypt

^e Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The British University in Egypt (BUE), El-Sherouk City, Suez Desert Road, Cairo 11837, Egypt.

Abstract

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative disorders. While pathological hallmarks of this disorder are known, the exact cause of AD remains unclear. Quinazoline was found to be a promising scaffold for the design and development of Acetylcholinesterase (AChE) inhibitors. In this study, we report the synthesis of 1'-methyl-3',4'-dihydro-1'H-spiro[cyclopentane-1, 2'-quinazoline] (**4**) in 73.3% yield. The structure of compound **4** was confirmed with GC-MS, ¹H and ¹³C-NMR. Acetylcholine esterase inhibition was studied virtually with docking into AChE active site and suggests potential use of **4** as a promising scaffold for acetylcholine esterase inhibitor design which might be useful for Alzheimer's disease.

Keywords: Acetylcholine esterase inhibitor; Alzheimer disease; Quinazoline derivatives; Spiro compounds; Molecular Docking.

1. Introduction

Alzheimer's disease (AD) was named after the German neuropathologist, Alois Alzheimer, who was the first to report this disease. The term "Alzheimer's disease" was first used by psychiatrist Dr. Emil Kraepelin in 1910, but research into underlying causes, symptoms and treatments only started about 30 years ago. AD is a neurodegenerative progressive brain disorder causing irreversible form of dementia occurring among elderly people. With an estimated prevalence as high as 47 million individuals worldwide, AD represent a financial burden on human societies. In addition, the total population affected is predicted to grow up to more than 130 million by 2050 [1], [2].

Although AD is an age-related neurodegenerative disorder, other risk factors for developing AD are identified including diabetes, hypertension, cerebrovascular diseases, obesity, depression, dyslipidemia, genetics, traumatic head injury and family history [3]. Previous studies explain AD origin, which include decrease in the cholinergic transmittance through decreased cholinergic neurons and acetylcholine (ACh) levels, plaques formation caused by aggregation and accumulation of beta amyloid (A β). Other proposed causes include neurofibrillary tangles (NFTs) that is associated with abnormality and irregularity of the tau protein phosphorylation step and inflammation due to

*Corresponding author e-mail: albohy@ualberta.ca

Receive Date: 15 April 2020, Revise Date: 17 May 2020, Accept Date: 15 June 2020

DOI: 10.21608/EJCHEM.2020.27985.2588

©2020 National Information and Documentation Center (NIDOC)

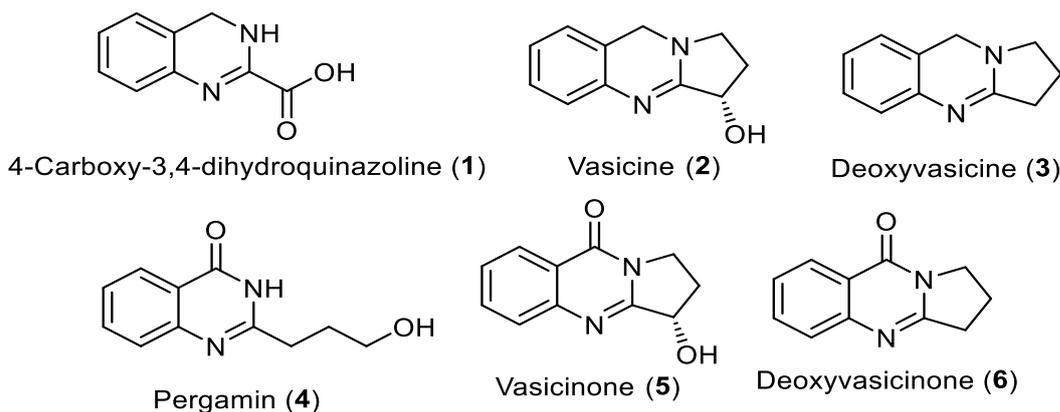


Figure 1: Structures of some 3, 4-dihydroquinazoline derivatives showing anticholinesterase activities.

increased oxidative stress [4]. Among proposed causes, cholinergic hypothesis is the earliest and most investigated theory for the pathogenesis of AD. Moreover, the majority of marketed drugs are anticholinesterase inhibitors [5]. The cholinergic hypothesis of Alzheimer's disease revealed that any lack in the cholinergic neurons in the neurocortex and basal forebrain leads to major deficits in the production of the brain neurotransmitter ACh [6]. The gradual loss of ACh neurotransmitter can become fatal and cause impairment in cognitive as well as autonomic and neuromuscular functions [7], [8].

The U.S. Food and Drug Administration (FDA) has approved five medications to treat AD and its related symptoms. Four of the approved drugs (Tacrine, Rivastigmine, Donepezil, and Galantamine) are AChE inhibitors while the fifth drug, memantine, is an *N*-methyl- D-aspartate (NMDA) antagonist. These agents work through the symptomatic improvement in the patients' cognitive functions ability, leading to an improvement of the daily activities and global functions [9]. Even though these agents have enabled advances in the clinical management of AD, the research and development of new AChE inhibitors having disease-treating properties is still an ongoing challenge [10].

Quinazoline was first prepared by George Gabriel in 1903 and was isolated from the Chinese plant aseru (*Dichroafebrifuga lour*) [11]. several derivatives of this nucleus were synthesized and showed wide spectrum of biological activities that include anti-

cancer, antioxidant, anti-microbial and diuretic activities among others [12]. Moderate to strong inhibitory activities of 3, 4-dihydroquinazoline moiety towards AChE and buterylcholinesterases (BChE) were identified. Based on this, several 3, 4-dihydroquinazoline derivatives, were synthesized and evaluated for their inhibitory activity towards the AChE as shown in **Figure 1** [13], [14].

According to previously published structure activity relationship (SAR) studies conducted on quinazoline derivatives, reduction of the amide carbonyl group of quinazolinones to the corresponding quinazoline moiety, along with the presence of tertiary amine within the inhibitor structure, increases the basicity of the heterocycle core ring, which is important for high affinity towards AChE active site [15], [16]. Furthermore, a favorable hydrogen bond was formed between nitrogen atom of quinazoline moiety and backbone carbonyl oxygen of some amino acids located in gorge region of AChE including tyrosine 337, leads to a conformational change that favors binding of the inhibitor [17]. In addition, the presence of $\pi - \pi$ stacking interaction between the fused benzene ring of the quinazoline compound and the indole side chain of tryptophan 86 was found to be important for binding [18]. Based on that and through modification of galantamine, we decided to synthesize a novel spiro-quinazoline scaffold and investigate it as a new scaffold for the acetylcholine esterase inhibitors (**Figure 2**).

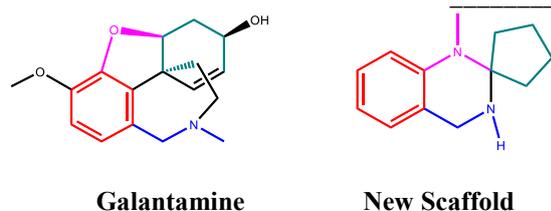


Figure 2: Structures of Galantamine and the new scaffold used in docking study showing structure similarity.

2. Experimental

2.1 Reagents and techniques

All reagents were obtained from commercial suppliers and used without any further purification. Solvents were used without any further drying. Reaction monitoring of the synthesized compounds was performed using ready-made TLC plate precoated with Kieselgel 60 F254 and visualized under ultraviolet (UV) lamp at wavelength 254 nm and with iodine. Purification of prepared compounds was done using column chromatography on silica gel pore size 63-200 μm or by crystallization from appropriate solvent. Removal of the reaction solvents was performed under reduced pressure on a rotary evaporator under normal conditions. Gas chromatography-mass spectrometry (GC-MS) was used for the separation of the mixture components and to identify their molecular weight on the same run and was done on GC-2010 plus Shimadzu with flame ionization detector (FID). Proton and carbon-13 nuclear magnetic resonance spectroscopy (^1H NMR and ^{13}C -NMR) were recorded on Bruker Avance III 500 MHz spectrometer at the University of Jordan, department of chemistry using deuterated chloroform (CDCl_3) as solvents and Tetramethylsilane (TMS) as internal standard. (See supporting Information)

2.2 Synthesis and characterization data

2.2.1 Synthesis of 2-methylaminobenzamide (2).

Compound **2** was prepared by dissolving 1-*N*-Methylisatoic anhydride (5g, 28.25 mmol, 1 equivalent (eq)) in 125 mL NH_4OH at 30 $^\circ\text{C}$ and the resulting solution was refluxed for 1.5 hours until complete consumption of the starting material. Both TLC and GC-MS were utilized to monitor the reaction,

mobile phase used was petroleum ether: ethyl acetate (1:1), retention factor (R_f) was 0.37. When starting material is consumed, the reaction was concentrated under reduced pressure; the crude mixture was purified using column chromatography using petroleum ether: ethyl acetate (8:2) as mobile phase. The reaction gave 3.7 g of the desired product (87.3 %) yield as brown crystals.

Chemical formula: $\text{C}_8\text{H}_{10}\text{N}_2\text{O}$; ^1H -NMR (CDCl_3 , 500 MHz): δ = 2.77-2.78 (3H, d, CH_3), 3.32 (1H, s, NH), 6.51-6.54 (1H, t, J = 7.4 Hz, ArH), 6.61-6.63 (1H, d, J = 8.3 Hz, ArH), 7.27-7.30 (1H, dd, J = 7.5, 7.6 Hz, ArH), 7.58-7.60 (1H, d, J = 7.8 Hz, ArH), 7.11-7.99 (br s, NH_2). GC-MS: m/z = 150.25.

2.2.2 Synthesis of 1'-methyl-1*H*-spiro[cyclopentane-1, 2'-quinazolin]-4'(3'*H*)-one (3).

A solution of cyclopentanone (3.5 mL, 40 mmol, 3 eq) in 40 mL of methanol was refluxed for around 10 min. PTSA (0.25g, 1.33 mmol, 0.1 eq) was carefully added as catalyst, and was stirred for another 10 min. Then compound **2** (2 g, 13.3 mmol, 1 eq) was added to the reaction flask. After refluxing for 2 hours, complete consumption of the starting material was observed and the reaction was quenched by cooling to room temperature. The reaction was monitored utilizing GC-MS and TLC, mobile phase used was hexane: ethyl acetate (1:1), R_f (0.36) The reaction product was crystallized from methanol to give 2.87 g of the desired pure compound (97.7% yield) as white crystals.

Chemical formula: $\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}$; ^1H NMR (CDCl_3 , 500 MHz): δ = 1.65- 2.02 (8H, m), 2.79 (3H, s), 6.73 (1H, d, J = 8.3 Hz), 6.84 (1H, t, J = 7.5 Hz), 7.37 (1H, t, J = 8.0 Hz), 7.85 (1H, s, NH), 7.92 (1H, d, J = 7.5 Hz) ; ^{13}C -NMR (CDCl_3 , 125 Hz): δ = 23.31 (NCH_3), 32.65-36.64 (4C, cyclopent), 82.06 (NCN), 114.02 (arom.), 117.70 (arom.), 118.47(arom.), 128.24(arom.), 133.86(arom.), 149.32(arom.), 164.84 (CO); GC-MS: m/z = 216.

2.2.3 Synthesis of 1'-methyl-3', 4'-dihydro-1*H*-spiro[cyclopentane-1, 2'-quinazoline] (4).

Synthesis of compound **4** was prepared by stirring 15 mL of anhydrous THF with TMSCl (5.88ml, 46.25 mmol, 5 eq). compound **3** (2g, 9.25 mmol, 1 eq) was added and refluxed for 1 hour. LiAlH_4 (4.49 g, 118.4 mmol, 12.8 eq) was added and allowed to stir for 2 hours more at room temperature. The reaction was quenched by adding 3.7 mL deionized water dropwise followed by adding the same amount of aqueous NaOH, and finally adding 11.2 mL deionized water with continuous stirring for 15 min, until no extra fizzing appeared. The resulted solution was extracted

with ethyl acetate (3X, 10ml) and the combined organic phase layer was dried over anhydrous MgSO_4 for 15 min and then filtration was done to remove the resulted salt then the filtrate evaporated under reduced pressure at temperature around 50 °C. The reaction was monitored using TLC and GC-MS, mobile phase used was hexane: ethyl acetate (1:1), R_f (0.2). The product was obtained as yellow oily liquid (73% yield) and was used with no further purification.

Chemical formula: $\text{C}_{13}\text{H}_{18}\text{N}_2$; ^1H NMR (CDCl_3 , 500 MHz): δ = 1.3 - 1.9 (m, 8H, cyclopent), 2.86 (s, 3H), 3.12 (m, NH), 3.78 (s, 2H), 6.62 - 6.65 (m, 2H, arom.), 7.02-7.23 (m, 2 H, arom.); ^{13}C -NMR (CDCl_3 , 125 Hz): δ = 24.02 (NCH₃), 30.29-33.20 (4 C, cyclopent), 52.30 (NCN), 59.74 (CH₂ N), 109.71 (arom.), 116.16 (arom.), 124.40 (arom.), 128.58(arom.), 129.31(arom.), 149.56 (arom.) ; MS, m/z: 204, (R_t = 9.5min)

2.3 Molecular modelling

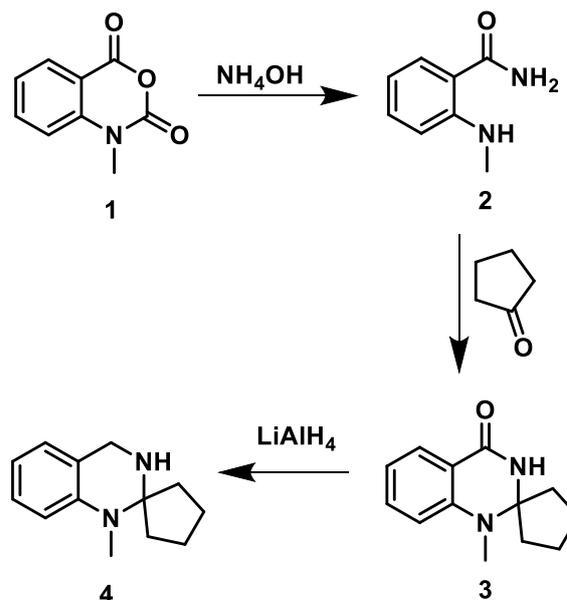
Docking of the newly synthesized target compound 1'-methyl-3', 4'-dihydro-1'H-spiro [cyclopentane-1, 2'-quinazoline] (**4**) was performed using Autodock Vina software [19]. The 3D crystallographic structure of acetylcholine esterase was downloaded from protein data bank (www.rcsb.org) (PDB ID: 4EY6). This PDB was chosen because it has co-crystallized galantamine which is structurally similar to our compound. The protein was prepared by removing waters, adding hydrogens and adding Gasteiger charges using autodock tools. Tested ligands (Galantamine, protonated galantamine and protonated test compound) were prepared by minimization with 1000 steps of steepest descent algorithm using MMFF94 force field. Compound **4** was protonated at pH 7.4. ADME properties were calculated using SwissADME server [20] and toxicity of compound **4** was predicted using pkCSM server [21] (*see supporting information*)

3. Results and Discussion

3.1. Chemistry

As shown in **Scheme 1**, compound **4** (1'-methyl-3', 4'-dihydro-1'H-spiro[cyclopentane-1, 2'-quinazoline]) was prepared by the action of ammonia aqueous solution (NH_4OH) on 1-N-Methylisatoic anhydride to give 2-methylaminobenzamide (**2**). Reacting **2** with cyclopentanone was the key step in this scheme with formation of the spiro compound **3** using Toluene-4-sulfonic acid as a catalyst. Our novel product compound **4** was formed through activation of carbonyl carbon of the amide group with trimethylsilyl

chloride (TMSCl) followed by a reduction step with lithium aluminum hydride (LiAlH_4) ending with 73.3% yield. The structure of synthesized compounds was thoroughly analyzed by ^1H -NMR, ^{13}C -NMR and GC-MS analytical techniques. Compound **4** was separated and analyzed by ^1H -NMR spectroscopy, which showed characteristic multiplet of one proton at δ 3.12 due to reduction of carbonyl and new singlet peak for two protons at δ 3.78 (N-CH₂-), indicates the formations of Compound **4**.



Scheme 1: Synthesis of target tetrahydroquinazoline derivative **4**.

3.2 Molecular modelling

Docking procedure was validated by redocking of galantamine (co-crystallized ligand) and comparing its pose to the crystal structure. The docking process was able to reproduce the crystal structure with high accuracy (RMSD of 0.376) as shown in **Figure 3**. To test the effect of protonation, protonated and unprotonated galantamine were docked in the active site and we found that protonation (which will naturally happen at normal pH) shows slight improvement in the docking score (**Table 1**). Based on that we decided to use the protonated form of compound **4**.

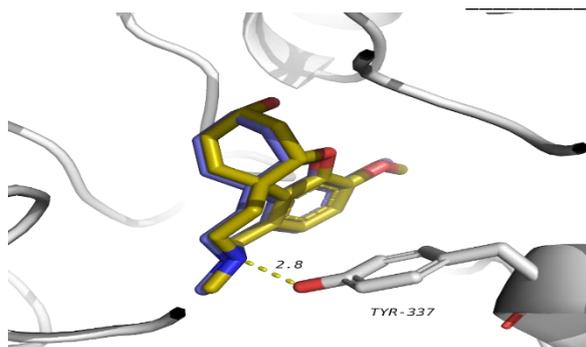


Figure 3: Overlapping of the docked galantamine (blue) with galantamine crystal structure (yellow) in the active site of acetylcholine esterase (PDB ID: 4EY6). Interaction with TYR337 is shown as yellow dotted line (2.8 Å)

Next step was to try to dock compound **4** in the active site of acetylcholine esterase in order to propose its potential binding mode. Protonated compound **4** was found to bind in the same spot similar to galantamine but with smaller docking energy (**Table 1**). Protonated compound **4** has docking score of -8.0 kcal/mol compared with the docking score of -10.1 kcal/mol for galantamine and -10.6 kcal/mol for protonated galantamine. Compound **4** is intended to be a lead compound for AChE inhibitors that will go through cycles of lead modification and improvement. Based on that, docking score suggest that this new scaffold will be a promising start for the design of novel AChE inhibitors

Table 1: Results of docking study of compound **4** in the active site of AChE

<i>Compound</i>	Docking score (kcal/mol)
Unprotonated Galantamine	-10.1
Protonated Galantamine	-10.6
Protonated compound 4	-8.0

The binding modes of galantamine and compound **4** were very close showing similar interactions as illustrated in **Figure 4**. For example, interaction with TYR337 is known to be important for binding of acetylcholine esterase inhibitors. The distance between phenolic hydroxyl group of TYR337 and interacting N in galantamine and compound **4** was found to be 2.8 and 2.9 Å, respectively. In addition, TRP86 was found to form a hydrophobic interaction with both galantamine and Compound **4**. On the other

hand, hydrogen bond with GLU202 can take place in Galantamine but not with target compound. These results suggest that tetrahydroquinazoline derivative **4** could be a potential scaffold for the design of new acetylcholine esterase inhibitors. Some modification might be suggested based on the docking studies. This includes adding a hydroxy group at different positions on the aromatic ring which might be useful to form hydrogen bond with GLU202 similar to that in galantamine.

Since Compound **4** was found to possess a promising scaffold for the design of AChEs inhibitors, we decided to study its ADME properties as well as its toxicity profile virtually. SwissADME server [20] was used to predict the ADME profile. Compound **4** was predicted to be orally absorbed and to cross blood brain barrier (BBB) which is important for drugs used for the treatment of Alzheimer's disease. This is clearly seen in the Brain Or IntestinaL EstimatedD permeation method known as BOILED-Egg diagram [22] based on the compound physicochemical properties (**Figure 5**). The compound was also found to obey Lipinski's rule of five with no violation (*see supporting information*). The compound did not show any alerts regarding pan-assay interference compounds (PAINS) or Brenk's alerts [23] which suggest it is suitable as a medicinal chemistry lead. Toxicity profile was also predicted using pkCSM server [21] (*see supporting information*). Compound **4** was predicted to show no toxicity in the AMES test and hepatotoxicity. Compound **4** was also expected not to cause toxicity related to the inhibition of potassium channels encoded by hERG which is the main cause of QT prolongation that lead to fatal arrhythmias. On the other hand, compound **4** was expected to cause skin sensitization, which is a side effect of some drugs.

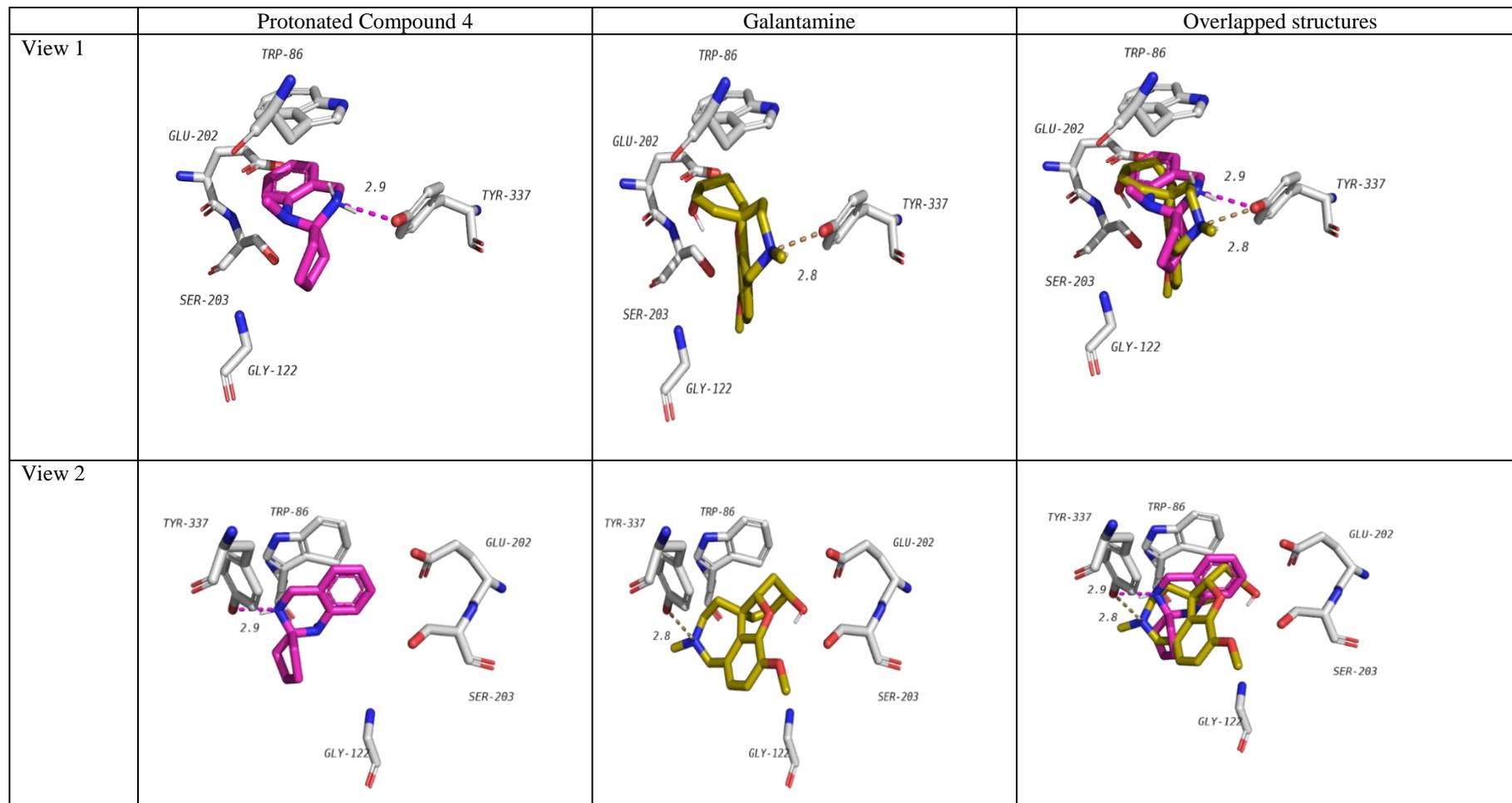


Figure 4: Galantamine interactions compared with compound 4 binding mode.

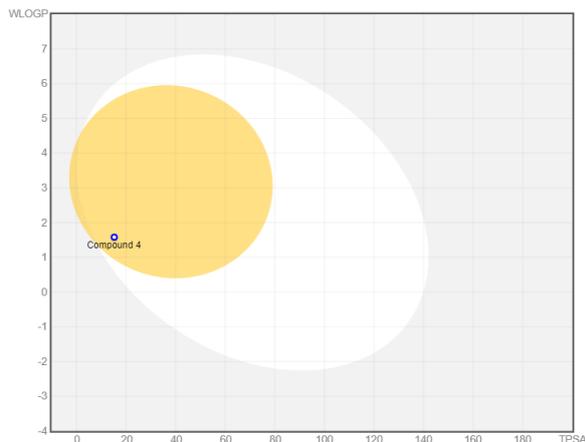


Figure 5: BOILED-Egg diagram of compound 4 suggests that the compound will be orally active and will cross the blood brain barrier (BBB)

4. Conclusions

In summary, design and synthesis of a novel quinazoline derivative compound **4** (1'-methyl-3', 4'-dihydro-1'H-spiro[cyclopentane-1, 2'-quinazoline]) which might be used as cholinesterase inhibitor was achieved from 1-N-methylisatoic anhydride in a good yield (73.3%) as yellow oily liquid. This compound was characterized using GC-MS, ^1H NMR and ^{13}C NMR. Mass spectra of the synthesized compound showed molecular ion peaks at its respective molecular weight, MS, m/z : 204, ($R_t = 9.5$ min). NMR spectra of the synthesized compound correlates well with its proposed structure. The synthesized compound (**4**) was screened virtually in docking study for cholinesterase inhibitory activity. The *in silico* molecular binding interactions demonstrated that **4** has potential affinity towards AchE binding site and could be a promising scaffold for antiacetylcholinesterase activity.

5. Conflicts of interest

There are no conflicts to declare.

6. Abbreviations

Ach, Acetylcholine; AChE, Acetylcholinesterase; AD, Alzheimer's disease; A β , β -amyloid; BChEs, Buterylcholinesterases; CAS, Catalytic active site; CATs, Choline acetyltransferase; CDC, Centers for

Disease Control and Prevention; CDCl_3 , Deuterated chloroform; CNS, Central nervous system; COX-2, Cyclooxygenase-2; DCC, N,N' -Dicyclohexylcarbodiimide; DCM, Dichloromethane; DMF, N,N -Dimethylformamide; Eq, Equivalent; FDA, Food and Drug Administration; FID, Flame ionization detection; NMDA, N -methyl-D-aspartate; NMR, Nuclear magnetic resonance spectroscopy; NOS, Nitric oxide Synthase; PAS, Peripheral anionic site; PTSA, Toluene-4-sulfonic acid monohydrate; R_f , Retention factor; ROS, Reactive oxygen species; SAR, Structure activity relationship; SOCl_2 , Thionylchloride; SPs, Senile plaques; TEA, Triethylamine; THF, Tetrahydrofuran; TLC, Thin layer chromatography; TMS, Tetramethylsilan; TMSCl , Trimethylsilyl chloride; R_t , Retention time; UV, Ultraviolet.

7. Acknowledgements

The authors would like to thank Faculty of Pharmacy, Isra University for financial support of this research project.

8. REFERENCES

- [1] Ramirez-Bermudez J., Alzheimer's disease: critical notes on the history of a medical concept. *Archives of medical research*, 43(8), 595-599 (2012)
- [2] Unzeta, M., Esteban G., Bolea I., Fogel W., Ramsay R., Youdim M., Tipton K., and Contelles, J., Multi-Target Directed Donepezil-Like Ligands for Alzheimer's Disease, *Frontiers in Neuroscience*. 10, 205 (2016)
- [3] Pike K. E., Savage G., Villemagne V. L., Ng S., Moss S. A., Maruff P., and Rowe C. C., β -amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. *Brain*, 130(11), 2837-2844 (2007)
- [4] Pithadia A. S., and Lim M. H., Metal-associated amyloid- β species in Alzheimer's disease. *Current Opinion in Chemical Biology*, 16(1-2), 67-73 (2012)
- [5] Martinez A. and Castro A., Novel cholinesterase inhibitors as future effective drugs for the treatment of Alzheimer's disease, *NeuroPharma*, 15, 1-12(2006)
- [6] Hampel, H., Mesulam, M. M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T. and

- Khachaturian, Z. S. ., The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*, 141(7), 1917-1933 (2018).
- [7] Wright C. I., Geula C. and Mesulam, M. M., Neuroglial cholinesterases in the normal brain and in Alzheimer's disease: relationship to plaques, tangles, and patterns of selective vulnerability, *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 34(3), 373-384 (1993).
- [8] Wang, X. C., Xu, Y. M., Li, H. Y., Wu, C. Y., Xu, T. T., Luo, N. C. and Quan, S. J. (2018). Jiao-Tai-Wan Improves Cognitive Dysfunctions through Cholinergic Pathway in Scopolamine-Treated Mice. *BioMed research international*, (2018).
- [9] Mohamed T. and Rao P. P., 2, 4-Disubstituted quinazolines as amyloid- β aggregation inhibitors with dual cholinesterase inhibition and antioxidant properties: Development and structure-activity relationship (SAR) studies. *European journal of medicinal chemistry*, 126, 823-843 (2017)
- [10] Gill S. S., Anderson G. M., Fischer H. D., Bell C. M., Li P., Normand S. L. T. and Rochon P. A. Syncope and its consequences in patients with dementia receiving cholinesterase inhibitors: a population-based cohort study. *Archives of Internal Medicine*, 169(9), 867-873 (2009).
- [11] Ram V. J., Sethi A., Nath M. and Pratap R., The Chemistry of Heterocycles: Chemistry of Six to Eight Membered N, O, S, P and Se Heterocycles. Elsevier, (2019).
- [12] Selvam T. P. and Kumar P. V., Quinazoline marketed drugs. *Research in Pharmacy*, 1(1). (2011).
- [13] Seo H. N., Choi J. Y., Choe Y. J., Kim Y., Rhim H., Lee, S. H. and Lee, J. Y. Discovery of potent T-type calcium channel blocker. *Bioorganic & medicinal chemistry letters*, 17(21), 5740-5743 (2007).
- [14] Kang H. B., Rim H. K., Park J. Y., Choi H. W., Choi D. L., Seo J. H. and Lee, K. T., In vivo evaluation of oral anti-tumoral effect of 3, 4-dihydroquinazoline derivative on solid tumor. *Bioorganic & medicinal chemistry letters*, 22(2), 1198-1201 (2012).
- [15] Darras F. H., Kling B., Sawatzky E., Heilmann J., and Decker, M., Cyclic acyl guanidines bearing carbamate moieties allow potent and dirigible cholinesterase inhibition of either acetyl- or butyrylcholinesterase. *Bioorganic & medicinal chemistry*, 22(17), 5020-5034 (2014).
- [16] Darras F. H., Pockes S., Huang G., Wehle S., Strasser A., Wittmann H. J. and Decker, M., Synthesis, biological evaluation, and computational studies of Tri- and tetracyclic nitrogen-bridgehead compounds as potent dual-acting AChE inhibitors and h H3 receptor antagonists. *ACS chemical neuroscience*, 5(3), 225-242. (2014).
- [17] Park B., Nam J. H., Kim J. H., Kim H. J., Onnis V., Balboni G. and Lee, J. Y., 3, 4-Dihydroquinazoline derivatives inhibit the activities of cholinesterase enzymes. *Bioorganic & medicinal chemistry letters*, 27(5), 1179-1185 (2017).
- [18] Darras F. H., Wehle S., Huang G., Sotriffer C. A. and Decker, M., Amine substitution of quinazolinones leads to selective nanomolar AChE inhibitors with 'inverted' binding mode. *Bioorganic & medicinal chemistry*, 22(17), 4867-4881 (2014).
- [19] Trott O. and Olson A.J., AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31, 455-461 (2010).
- [20] Daina, A.; Michielin, O.; Zoete, V., SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7, 42717 (2017).
- [21] Pires, D. E.; Blundell, T. L.; Ascher, D. B., pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *Journal of Medicinal Chemistry*, 58 (9), 4066-4072 (2015).
- [22] Daina, A.; Zoete, V., A BOILED-Egg: To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. *ChemMedChem*, 11 (11), 1117-1121 (2016).
- [23] Brenk, R. et al. Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. *ChemMedChem*, 3, 435-444 (2008).