Novel Urolithin Derivative Molecules as Dual MAO and Cholinesterase Inhibitor Agents

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ABSTRACT

The number of drugs used for the treatment of Alzheimer's disease is quite limited. These drugs (e.g., cholinesterase inhibitors and N-methyl-D-aspartate receptor antagonist) provide symptomatic treatment rather than a total cure. Therefore, there has been great scientific interest worldwide for the design and development of novel drug candidates for the treatment of this neurodegenerative disease.

Previous studies indicated that the urolithin (hydroxy substituted benzo[c]chromen - 6-one) scaffold can be employed to design molecules promising to act as multi target ligands against Alzheimer's disease. Within the scope of this research study, we have aimed to synthesis 3-subsitiuted benzo[c]chromen-6-one, and 3-subsistuted 7,8,9,10-tetrahydrobenzo[c]chromen-6-one derivatives connected to an amide function with a spacer. The enzyme inhibitor activities (i.e., acetylcholinesterase, butyrylcholinesterase, and monoamine oxidase B) concomitant to antioxidant and amyloid beta aggregation inhibitor characteristics of the title molecules have been planned to be screened.

The results pointed out several drug candidate molecules possessing multi target ligand feature in terms of inhibiting the aforementioned enzymes with anti-oxidant properties in ORAC assay conditions. Moreover, the computer aided docking studies revealed out the predicted binding modes of the most active compounds displaying the possible interactions with the receptors.

Keywords: Urolithin derivatives, Cholinesterase inhibitors, Monoamine oxidase B, Antioxidant.

ÖZ

Alzheimer hastalığının tedavisi için kullanılan ilaç sayısı oldukça sınırlıdır. Bu ilaçlar (kolinesteraz inhibitörleri ve N-metil-D-aspartat reseptörü antagonistleri) tam bir tedavi yerine semptomatik tedavi sağlar. Bu nedenle, bu nörodejeneratif hastalığın tedavisinde kullanılabilecek orijinal ilaç adayı bileşiklerin keşfi için tüm dünyada büyük bir bilimsel merak söz konusudur.

Önceki çalışmalarda ürolitin olarak tanımlanan hidroksi sübstitüe benzo[c] kromen-6-on türevi bileşiklerin Alzheimer Hastalığına karşı çoklu hedefe etki edebilecek bileşiklerin tasarımında kullanılabileceği gösterilmiştir. Bu araştırma çalışması kapsamında, amid grubununa bir ara grup ile bağlanmış 3-sübstitüe benzo [c] kromen-6-on ve 3-sübstitüe 7,8,9,10- tetrahidrobenzo [c] kromen-6-on türevi bileşikleri sentez etmeyi amaçladık. Bu bileşiklerin enzim inhibitör aktiviteleri (asetilkolinesteraz, butirilkolinesteraz ve monoamin oksidaz B), antioksidan ve amiloid beta agregasyon inhibitörü özelliklerini de incelemeyi planladık.

Sonuçlar, birkaç ilaç adayı molekülün çoklu hedefe yönelik ligand özelliği ile yukarıdaki enzimleri inhibe ettiğini ve ORAC test koşullarında antioksidan özelliğe sahip olduğunu gösterdi. Ayrıca bilgisayar destekli dokloma çalışmalarında en aktif bileşiklerin reseptörlerle olası etkileşimleri gösterilmiş ve öngörülen bağlanma durumları ortaya konmuştur.

Anahtar Kelimeler: Urolithin türevleri, Kolinesteraz inhibitörü, Monoamin oksidaz B, Antioksidan.

DEDICATION

To my parents

Who have been my source of inspiration, and gave me strength when I thought of giving up, who continuously provide their moral, spiritual, emotional, and financial support.

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I was blessed to have a father and mother that recognize the value of education. Your words and virtues will always be part of the deeper voice in me, giving me the guidance I need. I love you with all my heart, thanks *Mother* and *Father* for everything.

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‘I can no other answer make, but, thanks, and thanks and ever thanks.’ - Shakespeare.

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LIST OF SYMBOLS AND ABBREVIATIONS

Aβ	Amyloid Beta
AChE	Acetyl Cholin Esterase
ACh	Acetyl Choline
AD	Alzheimer’s Disease
AH	Alzheimer Hastalığı
Anal	Analysis
BuChE	Butyryl Cholin Esterase
C	Concentration
CAS	Catalytic Active Site
Calc	Calculated
Ch	Choline
CNS	Central Nervous System
Dmf	Dimethyl formamide
EA	Ellagic Acid
ETs	Ellagitannins
FAD	Flavin-Adenine Dinucleotide
IR	Infrared Spectroscopy
MAO	Mono Amine Oxidase
MTLD	Multi-Target Ligand Design
Mp	Melting point
NFT	Neuro Fibrillary Tangles
NMDA	N-Methyl-D-Aspartate
NMR	Nuclear Magnetic Resonance

OS	Oxidative Stress
ORAC	Oxygen Radical Absorbance Capacity
PDB	Protein Data Bank
R_f	Retention factor
ROS	Reactive Oxygen Species
RMSD	Root-Mean-Square Deviation
RNS	Reactive Nitrogen Species
SP	Standard Precision
TLC	Thin Layer Chromatography
THU	3-Hydroxy-7,8,9,10-tetrahydro-benzo[c]chromen-6-one
TMS	Tetra Methyl Silane
URO	3-Hydroxy-6H-benzo[c]chromen-6-one

Chapter 1

INTRODUCTION

1.1 Alzheimer disease

Alzheimer’s disease (AD) is one of those disease states having the curiosity of scientists to discover a novel cure on. This might be attributed to the devastating effects of the disease, particularly considering the dementia symptom associated with the disease. Dementia is described in two aspects: the effect on the patient, and the resulting effects on the care givers or relatives of the patient. Currently, there are more than 30 million people have been diagnosed with AD in all over the world, and it is expected to double up in each 20 years. 5% of AD cases come out with genetic component from their families that have history with the disease, whereas the rest are sporadic cases that are happening randomly in the population. The people who are 60 years old or over have higher risk of getting dementia, and it seems that the increasing of the lifespan leads to an increase of risk [1], [2].

AD gives damages to certain parts in brain and generates loses in the weight and volume of brain [3]. This outcome results in cognition and memory related failures. Because of the net devastating effect on both the patients and the family members it requires mental aid [4]. Although dementia can be seen in different parts of a regular life, AD related dementia is very specific yielding out behavioral changes and functional problems [5].

1.2 Diagnosis of Alzheimer's disease

The development of AD is categorized in three groups: Mild, moderate, and the advanced stages of the disease [6]. Unfortunately, there is no early diagnosis technique. Generally, patients with older ages apply to the clinics with dementia complaining. Following the examination by physicians and some imaging techniques employed, patients who have AD are diagnosed. The diagnosis includes the behavioral and neurophysiological evaluations concomitant to brain morphology and functional imaging investigations. Studies still indicate that there is a huge gap between the presence of the first symptoms of cognitive and memory decay and with the time for diagnosis [7]. Memory lose is one of the important diagnosis of AD, yielding out challenges on remembering own things. Difficulties on familiar tasks, problems with language and even remembering the simple words, disorientation to a place and time, and the difficulties of getting home back, impaired decisions, changes on mood and behavioral characteristics, absence of appetite, depression have all been recorded in the diagnosis step [8]. The American institute of neurological and communicative disorders association published the first diagnosis criteria for Alzheimer's disease, and later on these have been updated and revised by the national institute on aging/Alzheimer's Association and the recent guideline is formed known as the NIA-AA [9]. The protocol follows the observation in clinic and monitoring the excess of amyloid plaques and their precipitation in central nervous system (CNS) throughout the progress of AD [10]. The monitoring of amyloid beta (Aβ) aggregates and the related tau protein hyper phosphorylation is the principle point of brain imaging studies in the diagnosis of AD. Therefore, biomarkers have been employed in imaging studies for monitoring aggregated Aβ peptides and hyper phosphorylated tau protein fibrillates [11]. From this perspective ,

electroencephalography, multimodality fusion imaging, computed tomography, magnetic resonance imaging (MRI), single photon emission computed tomography, and positron emission tomography are the applied techniques [12], [13]. Unluckily, the visualizing of amyloid β-plaques and tau protein hyper phosphorylation is not sufficient for a precise diagnosis for AD.

1.3 Available treatments of Alzheimer's disease

The treatment of AD exploits from non-pharmacological and pharmacological treatments. Non-pharmacological treatment strategies consist of memory training, social and mental stimulation, music therapy, aromatherapy, and physical exercise applications. The aims of this strategy include decelerating the progress of cognitive and functional impairments, keeping and elevating the cognitional status and other skills like functional and social skills, promoting the patient staying in the environment and social relationships. Non-pharmacological treatment have been evaluated as cost-effective and positive through improving the quality of life of patients [13].

It is important to note that there is no total cure of AD with the current pharmacological treatment options. This situation has led the scientists to come up with suggestions to decrease the risk of getting AD. Since there are some important factors thought to be associated with AD, some of them such as high blood pressure, high cholesterol, obesity and diabetes are suggested factors to be controlled to prevent the possible development of AD [14]. The precise cause is not proven yet; one of the major causes and improving the progress of this disease is the low levels of acetylcholine in the hippocampus and cortex area of the brain. Adjustments in dopaminergic, serotoninergic and monoaminergic neurotransmission provide some

symptoms of AD as well. There are other causes like oxidative stress and the aggregation of Beta-amyloid which is recently proved to play important roles in the pathogenesis of AD. Metal ion accumulations head to deadly neurologic disorders and firmly related with abnormal Beta-amyloid plaques [15].

The dementia component of AD has been the main topic of the research for too many years. For that reason, discovering the tacrine as the first drug was not surprising. A cholinesterase inhibitor, tacrine, reached to the market as a cognition enhancer in 1980s. Years later, new cholinesterase inhibitors were discovered (i.e., galantamine, donepezil, and rivastigmine). Additionally, the treatment of AD includes diarrhea, nausea, instability, vomiting, weight loss, stomach ulcers, syncope, and generalized seizures treatments, since these symptoms can also occur [16]–[18]. As it is stated, none of the pharmacological treatments provide an effect on the progressive neurodegenerative character of the disease [18].

1.4 Confirmed targets and the drugs used for Alzheimer's disease

There are huge numbers of targets that linked to the improvement of dementia. One of the greatest approved targets among them is associated with the activation of the cholinergic system. Within the development of AD, the loss of the neurons is related to the decay of neurotransmitters, including acetylcholine, serotonin, and also dopamine at advanced stages. According to the research studies that have been conducted, the interaction of acetylcholine with specific subtypes of muscarinic and nicotinic receptors is a serious part of the routine cognition-related actions. The insufficient amounts of both acetylcholine in CNS and the associated cholinergic receptors make cholinergic system a useful target to consider about. Since acetylcholine is the only and simple endogenous molecule having agonist function on

both muscarinic and nicotinic receptors, it is quite difficult to design cholinergic sub-receptors specific compounds. Although there have been some molecules designed from this perspective , none of them have been found successful in clinical trials in terms of enhancing cognition related properties. Therefore, the only validated targets in the treatment of AD employing the cholinergic system becomes the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) within the CNS. These two enzymes are hydrolytic enzymes, so they have the selectivity for the hydrolysis of acetylcholine to produce choline and acetate [18], [19]. While the BuChE has mostly peripheral distribution, the studies indicated that the expression of butyrylcholinesterase have been found high depending on age and the development of AD [20]. The structures of current drugs on the treatment of AD (including memantine) are shown in Figure 1.

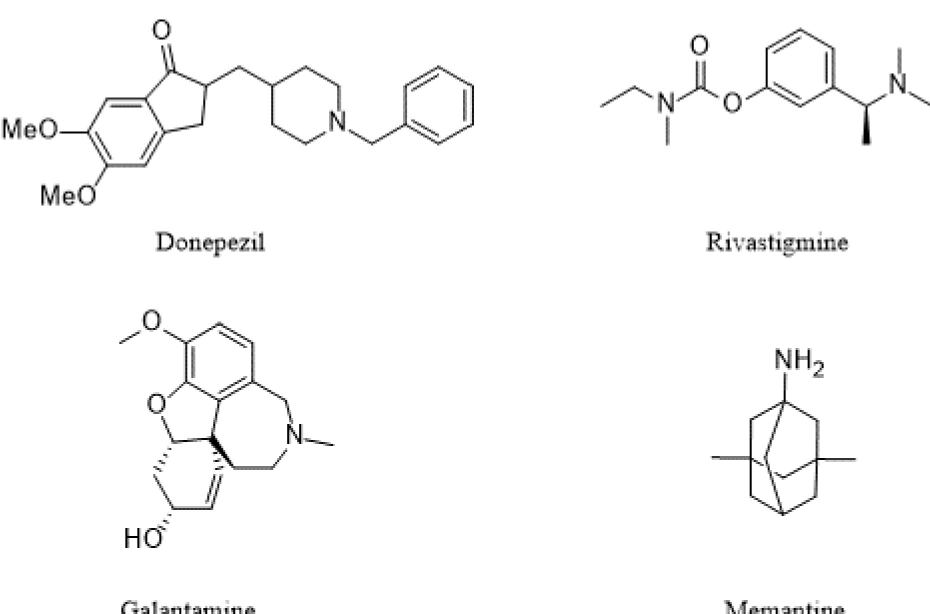


Figure 1: Drugs used in the treatment of AD [20]

Tacrine was the first approved AChE inhibitor drug licensed for the treatment of AD. With respect to its high hepatotoxicity, it’s no longer in use (Figure 2). Later on,

other AChE selective inhibitors were discovered (i.e., donepezil and galantamine). On the other hand, rivastigmine has shown to be a selective BuChE inhibitor [18], [20]. Donepezil is a synthetic compound that has a low IC_{50} for AChE inhibition [21]. Galantamine, on the other hand, is another AChE selective molecule with good bioavailability and pharmacokinetic profiles [22]. However galantamine is a natural alkaloid. The similarity between donepezil and galantamine is that both of them have interaction with the active site of AChE for preventing the binding of acetylcholine. Rivastigmine is a semi-synthetic drug derived from the natural alkaloid physostigmine and it is also a mechanism-based inhibitor. Rivastigmine is a carbamyl functionalized synthetic drug that has the ability to carbamylate the active site serine residue, which in turn makes the molecule a mechanism based inhibitor. It is important to note that rivastigmine is BuChE selective inhibitor. The carbamylated enzyme regeneration takes longer time in comparison to the reactivation of acylated cholinesterase enzymes (Figure 3) [17], [21]. This is the principle of the action of rivastigmine on cholinesterase enzymes.

Figure 2: The toxicity of tacrine [18]

Memantine is another drug used for the treatment of several stages of AD. Particularly, in severe stages of AD it is combined with one those cholinesterase inhibitor drugs [23]. Memantine displays its activity through partially antagonizing NMDA receptor antagonism. Although the exact mechanism has not been clearly proven out, it is believed in that neuronal excitotoxicity through glutamatergic neurotransmission is a part of symptoms of AD [24].

As explained, the numbers of current drugs used for the treatment of AD are quite limited. In order to stop the progressive neurodegenerative characteristics of AD alternative approaches are followed in the last few decades in the design of original molecules for the treatment of AD. Beside single targets proposed, multi-target ligand drug design is very popular to provide cognition enhancer properties concomitant to nueroprotective effects.

(a) 1st step: The acylation of the enzyme

E—Ser—OH → E—Ser—O—C(=O)—CH3 + Choline

Acylated enzyme

2nd step: The deacylation step (Regeneration of the enzyme)

E—ser—O—C(=O)—CH3 + H_2O → E—Ser—OH + Acetate ⇅ Acetic acid

(b) 1st step: Carbamylation of the enzyme

E—Ser—OH + Rivastigmine → E—Ser—O—C(=O)—N + HO

Carbamylated enzyme

2nd step: The decarbamylation step (Regeneration of the enzyme)

E—Ser—O—C(=O)—N + H_2O → E—Ser—OH + [OH, O, N]

Carbamylated enzyme

Regenerated enzyme

NH + CO_2

Figure 3: The mechanism of cholinesterase activity and carbamylation of cholinesterase with rivastigmine [17], [21]

Chapter 2

LITERATURE REVIEW

2.1 Acetylcholinesterase

Cholinesterases are the enzymes that catalyze the hydrolysis of the neurotransmitter acetylcholine to produce choline and acetic acid. This process is essential for permitting the cholinergic neurons to return for the latent state after getting activated. There are two cholinesterases in human biology and they are acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE has a broad tissue distribution involving central and peripheral tissues, motor and sensory fibers [25]. AChE can be in different quaternary structural organization including the assembly of more than one monomer. AChE is a serine hydrolase that exists at the intersections of neuromuscular and cholinergic brains synapses [26]. AChE plays a biological key role through turning down the impulse transmission that occurred at cholinergic synapses by quick hydrolysis of ACh to produce choline and acetate. Since AChE is a serine hydrolase that belongs to the type B carboxylesterase and has a great catalytic activity, it has the ability to degrade about 2500 molecule of acetylcholine for every second. AChE consists of two subsites, anionic and esteratic subsites, at its active sites that individually match the catalytic machinery and the choline binding pocket. The anionic subsite has lipophilic properties, uncharged, and attached to the positive quaternary amines of choline moiety for acetylcholine structure. On the other hand, esteratic subsite includes an active serine hydroxyl which is quickly

acylated through the substrate. Esteratic subsite consists of a catalytic unit, a trio of amino acids which are serine, histidine, and glutamic acid [27].

Unfortunately, alternative treatments of AD regarding the beta amyloid and tau protein hyper phosphorylation pathways have not been found successful so far in clinical trials. from this perspective, acetylcholinesterase still keeps its importance as a validated target to slow down cognitive abilities occurring throughout the development of AD and novel cholinesterase inhibitor agents retain the curiosity to be designed with additional therapeutic effects [28], [29].

2.2 Butyrylcholinesterase

Similar to AChE, BuChE is also a cholinergic enzyme belongs to the family of serine hydrolases. It possesses serine amino acid at the active site which is important for the catalytic activity through hydrolyzing acetylcholine. BuChE displays broad tissue distribution particularly in the peripher [30]. Liver, blood, heart, small intestine, kidneys, skin, muscles, white matter in the brain, lungs, stomach, and thyroid gland are typical organs that BuChE shows function [31]. BuChE is also accepted as a 'sister or cousin' enzyme of AChE, since 65% of amino acid sequence homology is present between these two cholinesterases [32]. Both enzymes have a comparable molecular forms and active center. However, there are definite perceptible differences between AChE and BuChE. AChE and BuChE are both able to hydrolyze acetylcholine. Although there is no butrylcholine neurotransmitter, BuChE has more specificity to hydrolyze synthetic butrylcholine molecule, therefore, it is named as butyrylcholinesterase [33]. Furthermore, the hydrolysis of ACh that is accomplished by AChE is faster than that by BuChE. Besides, the total expression of BuChE in human biology is higher than the one for AChE [34].

2.3 Acetylcholine

The only neurotransmitter of cholinergic system is acetylcholine, which locates in the peripheral and central nervous system. It is one of those neurotransmitters of which the existence and innervation capacity identified in the early part of 20th century [35], [36]. The stimulation of muscle shrinking is the main function of acetylcholine. Acetylcholine is present in diverse regions in the brain, including basal ganglia, hypothalamus, and cortex. This transmitter plays an important role in memory, cognition, and motor control. The activity of acetylcholine at the synapse ends after being hydrolyzed by AChE. In fact, acetylcholine has a significant function in such a way that some particular cells react only with this neurotransmitter in some parts of the brain. The communication between basal forebrain and hippocampus on controlling the memory and learning is also related to the function of acetylcholine. Even, theta waves in the brain increases by the presence of acetylcholine, leading to stronger neuron signaling [37]. Some other properties of acetylcholine are memory improvement in brain cortex, the generation of synaptogenesis, the standard improvement of synapses in brain, and supporting other neurotransmitters in communication. Moreover, acetylcholine has the ability to control the speed of nerve signals according to its functions of inhibition or excitation. The excitation function occurs in CNS and involves cognitive functions. Acetylcholine has an essential role in the engagement of sensory duties at the same time as the body wakes up to support concentration. It is perfectly possible to find this transmitter in the interneurons in CNS and makes a number of effects of arousal and reward, learning and short term memory [38]. Acetylcholine also plays a role in peripheral nervous system by acting as a neurotransmitter between skeletal muscle and motor nerve through using of neuromuscular connection. Since acetylcholine

affects the muscle movement stimulation, it is accommodated on receptors on the muscles [39]. From this point of view, the decrease in levels of acetylcholine in brain leads to lack in the neuroplasticity [40].

In the case of AD patients, a huge decrease in the levels of acetylcholine and acetylcholine receptors is characterized due to the irregularities in cholinergic system [41]. As a result, it can be clarified that AD symptoms include the impairments in the function of cholinergic signal transmission. The anticholinergic drugs cause a deficiency in memory and cognition. Finally, these reasons prove the significance of the cholinergic system and acetylcholine in memory and cognitive abilities [42].

2.4 Oxidative stress (OS)

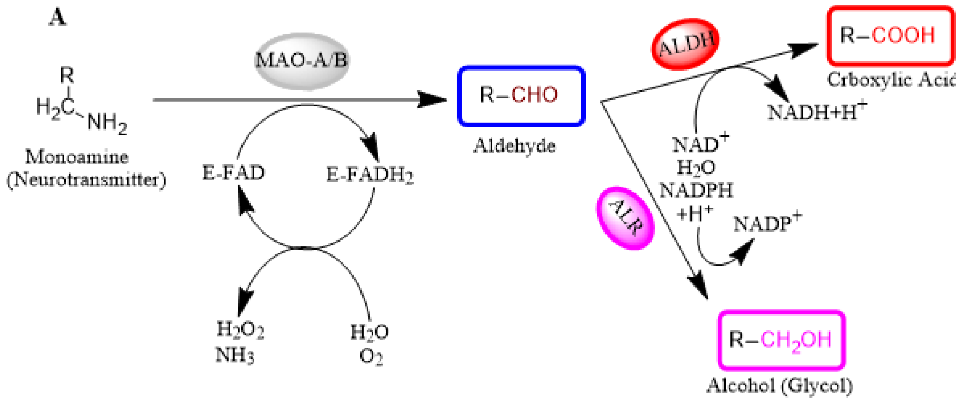
The worldwide idea of 'oxidative stress' is well-defined as the imbalance that comes to pass between the oxidants and antioxidants leading to disarrangement of redox signaling, control and damaging in the molecules. The evaluation of OS as a possible source of some CNS related disease state through redox signaling came in to mind around the last quarter of 20th century [43]. Oxidation and reduction reactions are common parts of regular physiology. Many enzymes and reactive oxygen (ROS) and nitrogen species (RNS) formation are routine in the regulation of certain biochemical cascades. In other words, OS form through the imbalance of oxidants and anti-oxidants can have devastating effects on macromolecules of cells if the capacity of the defense mechanisms is limited or consumed out. Indeed, particular cells with great metabolic activity including neurons can generate up to 10^{11} ROS/cell in a day [44]. In AD, the OS is certain. In fact, AD is classified as one of the neurodegenerative disorders. The oxidation of proteins, lipids, and nucleic acids in

neuronal cells of AD patients are typical and the pathology related to Aβ aggregate and neurofibrillary tangle formation is an oxidative stress outcome [45], [46]. These biological processes happening throughout the development of AD are thought to be the pieces of cognitive impairment, and neuropsychiatric disorders [47]. The oxidation occurring in mitochondria and, in a lower degree in nucleus of neurons has been detected in the partial cortex of AD cases [48]. Protein oxidation has been detected in common in elderly persons with and without AD, however it is more noticeable in AD patients [49]. Besides, many research studies have shown high peroxidation of lipids in the brain of AD patients, especially in the temporal lobe, where histopathologic modifications are very obvious [50].

2.5 Monoamine oxidases (MAO)

MAOs are important group of enzymes involved in the oxidative deamination of several monoamines like dopamine, noradrenaline, and serotonin. The original discovery of MAO in 1928 by Bernheim led the name of the enzyme as tyramine oxidase, since the oxidative deamination of tyramine catalyzed by MAO was observed [51]. Later on, the ability of these enzyme to oxidize different monoamines, such as catecholamines, dopamine, noradrenaline, adrenaline, and serotonin have been discovered by Blashko, Zeller, Gorkin, and Quastel [52]. These enzymes are present in the outer membrane of mitochondria. In 1968, the discovery of Johnston on the ability of clorgyline to act as inhibitor on MAO enzymes have led the distinction of two forms of MAO, MAO-A and MAO-B [53]–[55]. Serotonin, noradrenaline, and adrenaline are the preferred substrates of MAO-A, while β-phenyl ethylamine is the selective substrate of MAO-B. Both MAO-A and MAO-B oxidize dopamine, tyramine, and tryptamine [56]. MAO-B is particularly expressed in CNS and its overexpression has been identified in neurodegenerative diseases including

AD [57]. MAO-B can cover MAO-A's functions when there insufficiency in the amount of MAO-A [55], [58], [59]. MAO-A and MAO-B adjust the concentration of important neurotransmitters in the brain, such as, dopamine, adrenaline, and noradrenaline, and these are responsible for many physiological activities. It has been shown that both MAO-A and MAO-B are related to several psychiatric and neurological disorders such as AD and Parkinson's disease [60]. The first impression of employing the inhibition of MAO in the treatment of AD displays substantial diversity by comparing to its application in Parkinson's disease. Several points have been mentioned, the first one is found on the elevating levels of the activity of MAO-B in CNS during AD development. The second point is the loss of numerous kinds of activities of neurotransmitters concerning the progressive and neurodegenerative features of AD. The third point is about the oxidative stress-related effects of the products of MAO catalyzed reaction products [61]. Literally, the studies proved the insufficient levels of protection mechanisms (e.g., glutathione) inside the CNS, especially in the last stages of the disease. In addition, it is significant to mention that the hydrogen peroxide produced through the reactions catalyzed by MAO can lead to the possible formation of ROS in certain reactions such as Fenton reaction [62]. For instance, the oxidative damage on lipids to form lipid peroxides is typically an outcome of hydroxyl radical action on lipids. Therefore, the inhibition of MAO-B is considered to be a nueroprotective activity [63].



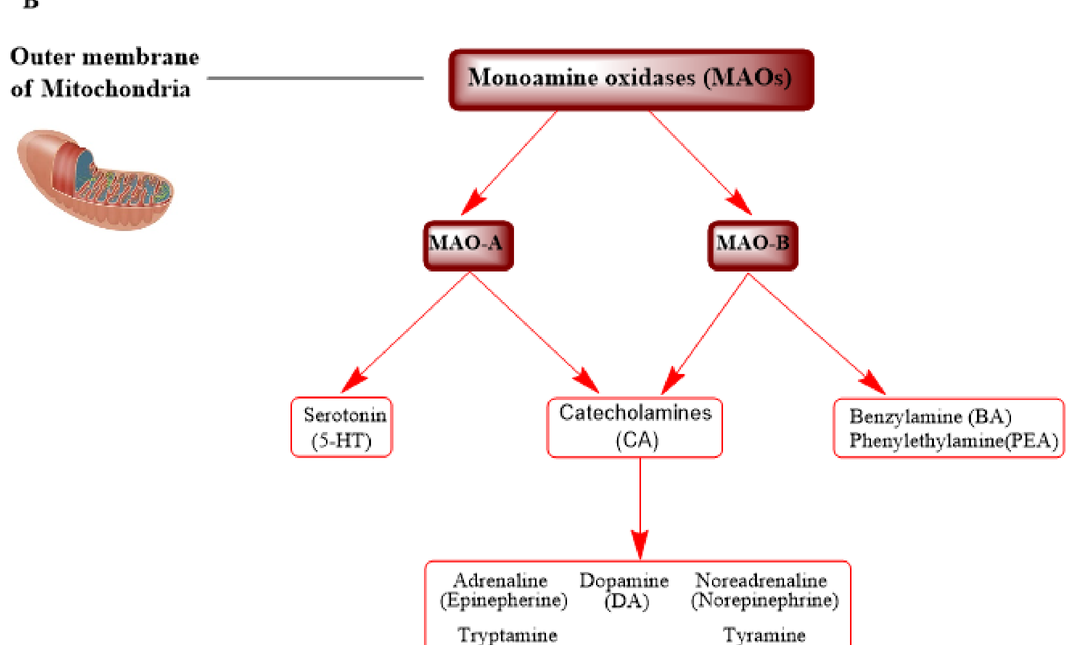


Figure 4: The process of monoamines [64]

Figure 4 shows typical oxidative deamination reactions catalyzed by MAO enzymes. As seen, the enzyme is a flavoenzyme and it employs water and oxygen molecule in order to generate the aldehyde, hydrogen peroxide, and ammonia products. The aldehyde metabolites formed are generally further subject to either aldehyde dehydrogenase (ALDH) or aldo-keto reductase (ALDR) catalyzed reactions to produce carboxylic acid, or alcohol metabolites, respectively. As also seen in part B section of Figure 4, the mitochondria membrane located MAOs are able to employ many neurotransmitters as substrates [64].

2.6 Dual cholinesterase and MAO inhibitors

The practices of the clinical trials that applied for treating AD stated that AChE inhibitors show their activity through increasing the cholinergic transmission in the synaptic cleft by preventing the degradation of ACh [65]. The present AChE inhibitors are donepezil, galantamine, and rivastigmine. Tacrine was the first drug for AD but it has been withdrawn from the market because of severe hepatotoxicity. On the other side, donepezil and rivastigmine display dose-dependent adverse effects. For that reason, it is important to focus and study on safe and alternative pharmaceuticals for the treatment of AD [66]. These drugs provide symptomatic relief however they cannot prevent the disease's progression. Henceforward, applying a single drug that turn on a specific target might not be effect enough for the therapeutic progress of AD in line for the complexity of it. The present research studies pay attention to the multi-target acting agents that can be used for the treatment of AD. These multi-target acting agents are of curiosity in order to provide both symptomatic relief related to cognitive decline and disease modifying through activities involved in the development of neurodegeneration. The strategy for multi-target acting agents includes the establishment of pharmacophore groups of diverse drugs in similar scaffold. One of the approaches in this strategy is to come up with the design of molecules possessing dual cholinesterase and MAO inhibitor characteristics. It is believed in that the addition of particularly MAO-B inhibitor properties to cholinesterase inhibitor agents may provide additional therapeutic effect for the treatment of AD [67], [68].

This idea has some standing points. First of all, MAO-B expression particularly in the CNS throughout the development of AD has been found increasing [69], [70].

This outcome in one way lowers the available amount of dopamine, which is subject to MAO-B catalyzed degradation. Moreover, the decreased dopamine amount in CNS also lowers dopaminergic system innervation. Low dopamine amounts in CNS might be also associated to the development of depression symptoms seen in many of AD patients [71]. Indeed, there are some MAO-B inhibitor drugs (e.g., rasagiline) currently used for the treatment of depressive disorders [72], [73].

On the other hand, MAO catalyze reactions are also related to the OS [74]. At first hand, dopamine metabolism catalyzed by MAO generates dopamine aldehyde metabolite. This aldehyde is subject to further metabolism reactions to yield out the corresponding carboxylic acid or alcohol metabolites. However, the dopamine itself and particularly the aldehyde metabolite can generate epoxide and quinone type reactive electrophilic metabolites of which can interact with proteins to end up with protein adducts [75], [76]. Beside the formation of electrophilic metabolites, MAO catalyze reactions end up with hydrogen peroxide, a reactive oxygen species source through many reactions including Fenton-like reaction [77].

Overall, MAO-B inhibition strategy in the pharmacotherapy of AD is thought to be a strategy that can provide neuroprotection through lowering the amounts of electrophilic reactive metabolites and hydrogen peroxide [78], [79].

From this perspective, many drug candidates that have been designed, synthesized, and evaluated for their dual cholinesterase and MAO-B inhibitor properties. Some representative molecules in this category are shown in Table 1.

Table 1: Dual cholinesterase and MAO-B inhibitor acting agents

Dual cholinesterase and MAO-B inhibitor acting agents	Molecular Structure
Homoisoflavonoid Mannich base derivatives [80]	
MT series (novel derivatives of rasagiline and rivastigmine) [81]	
Pthalimide-alkylamine derivatives [82]	
Ladostigil [83]	
Derivations of Coumarin-dithiocarbamate hybrids [84]	

2.7 Ellagitannins

Ellagitannins exist in various numbers of dietary sources such as nuts, berries, and pomegranate. Several studies focused on the investigation of the biological actions of ellagitannins [85]. Some of these studies depended on extraction of some related plants, mainly the fruits. Ellagitannins can be defined as macromolecules that are condensed units of gallic acid derivatives through ester connection [86]. Regarding the macromolecular organization of ellagitannins, it is difficult to relate the biological actions of ellagitannin rich dietaries to different ellagitannin molecules. As seen in Figure 5, ellagitannins are subject to metabolism reactions in many living things including human kind. The studies on the metabolism of ellagitannins have shown particularly the gastrointestinal system metabolism of these compounds to breakdown the ester connections to generate ellagic acid which is further subject to some oxidation reactions [87]. Therefore, there is almost no bioavailability of ellagitannins that can trigger a systemic biological effect. It has also been stated that ellagic acid has negligible bioavailability (less than 1%) [88]. In other words, the bioavailability outcomes in clinical studies imply that none of the systemic actions of ellagitannin rich dietaries can be related to neither the ellagitannin nor the ellagic acid itself.

On the investigation of the biotransformation of ellagitannins conducted on many living things clearly established that ellagic acid is further subject to gastrointestinal system micro flora induced metabolism reactions to generate hydroxyl substituted benzo[c]chromen-6-one derivatives, also referred to as urolithins [89]. Indeed, mammalian species possess these urolithin derived metabolites in their systemic

circulation following exposure to ellagitannin rich dietaries. Even, urolithins are accepted as the biomarkers of ellagitannin exposure [90].

Figure 5: The catabolism of Ellagitannins that occurs by intestinal microbiota. Directly adapted from [87]

2.8 Urolithins and the chemistry of urolithins

Urolithins have a great attention in the last decades. They can be named as either hydroxyl substituted 6H-dibenzo[b,d]pyran-6-one derivatives or hydroxyl substituted benzo[c]chromen-6-one derivatives. Briefly, they are benzocoumarines or dibenzo-α-pyrones [91]. Urolithins could be produced in various animals after the intake of ellagitannins. Moreover, they are formed from ellagic acid and the conjugates of ellagic acid, which are frequently exist in diverse products of food that include berries, walnuts, pomegranates, and also oaking aged wines [92]–[94]. The first

compound of this natural family discovered was 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one which was named as urolithin A [95].

Further studies clearly demonstrated that ellagic acid is first subject to metabolism reactions to generate urolithin D, a tetrahydroxy substituted urolithin derivative [96]. As seen in Figure 6, further metabolism of urolithin D through dehydroxylation reactions generate urolithin C, urolithin A, and urolithin B, respectively standing for trihydroxylated, dihydroxylated, and monohydroxylated urolithins [97].

Figure 6: The chemical structures of ellagic acid and the products of urolithin metabolites [97]

The derivatives of dibenzopyran-6-one with different hydroxyl group substitutions are also identified in blood samples, as seen in the example of iso urolithin [98].

Urolithins are considered as important intermediates in the synthesis and production of different dibenzopyranone derivatives in pharmaceutical applications. Urolithin B

derivatives presented significant cholinesterase inhibitory activities in comparison to galantamine and rivastigmine [99]. Indeed, there is more interest in the development of urolithin derived agents for different pharmaceutical purposes in recent years [100]. In general, urolithin derivatives have a molecular mass less than 300 (e.g., 212 for urolithin B), therefore, this makes them eligible and improvable small molecule class agents [101].

2.9 Bioavailability & metabolism of urolithins

The studies of Doyle and Griffiths clearly demonstrated the conversion of ellagic acid to urolithins in the metabolism of mammalian species in which they have identifies major urolithins in blood samples (Figure 7) [102].

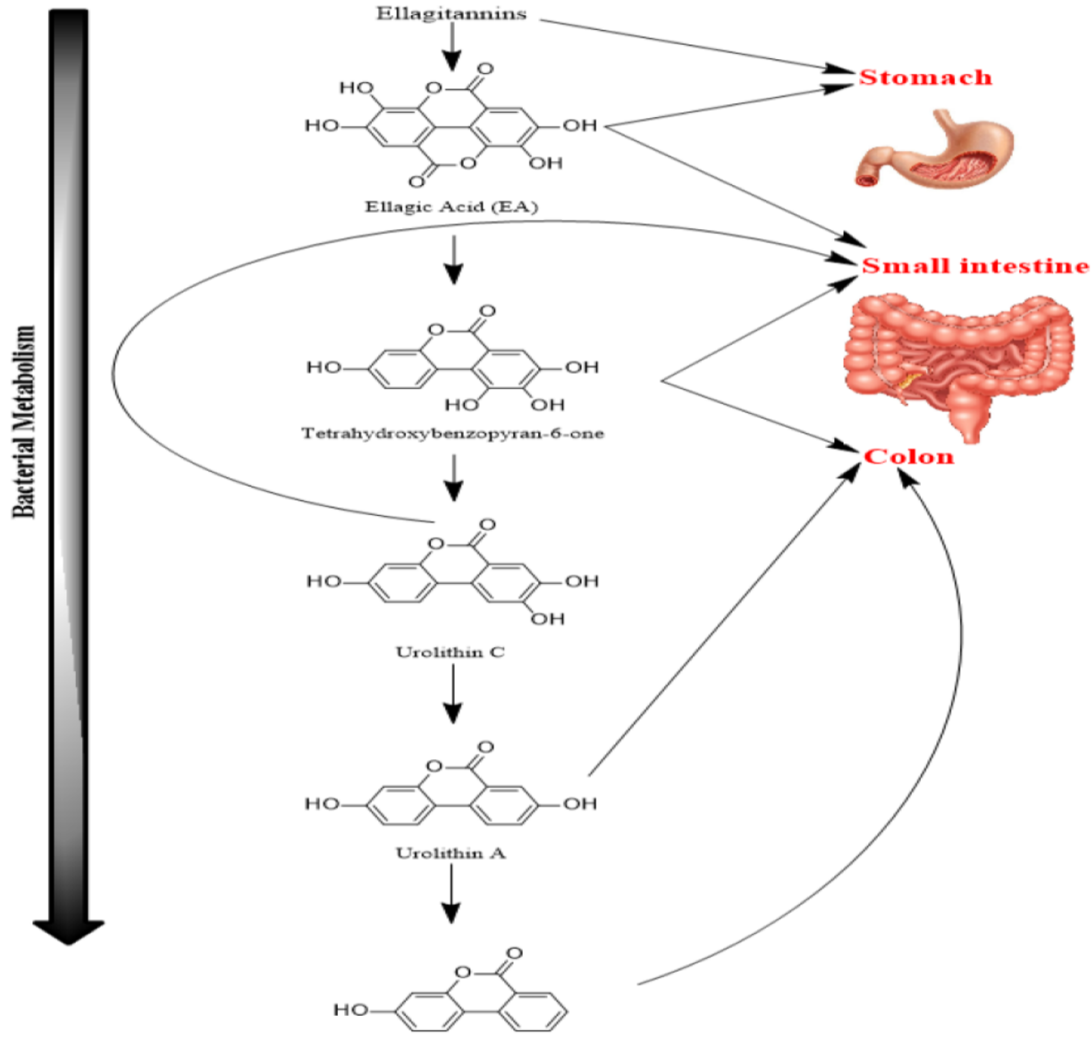


Figure 7: Productions of Urolithins [102]

The studies further clearly have shown out that the microflora within the gastrointestinal tract is particularly responsible for the conversion of ellagic acid to different urolithin compounds. Ellagic acid was not found neither in urine nor in faeces samples, however urolithins were identified as either in aglycone forms or in glucornide or sulfate conjugates [103]. Ellagic acid has only been detected in trace amounts in blood samples[104].

Other metabolism studies also displayed that urolithins can be subject to catechol-O-methyl transferace (COMT) catalyzed biotransformation reactions to generate methyl

ether metabolites [105]. Even, some of these methyl ethers concomitant to aglycone urolithins have been identified in CNS [106].

2.10 Antioxidant activity of urolithins

Following exposure to ellagitannin rich dietaries, urolithins are able to be detected in systemic circulation in few hours. Depending on the type of urolithin, the half-lives can vary, which can be expressed in day periods for some of them [107]. Following the first studies on the anticancer effect ellagitannins, the activities were later associated to the antioxidant activities of ellagitannins and their metabolites [108]. [109].

Since urolithins are phenolic hydroxylated coumarine analogues, they are able to display antioxidant activity [110]. There are many studies conducted so far in single urolithin compounds and these studies particularly have proven the antioxidant effects of urolithins particularly under ORAC assay conditions [111]. It was clearly shown that for those urolithin analogues in which higher phenolic hydroxyl substitution was present the antioxidant activity was also found higher.

2.11 Urolithins in the treatment of Alzheimer's disease

There are limited number of studies conducted so far on the investigation of pure urolithin compounds' activities on the validated and non-validated targets of AD. In a recent study Noshadi et al., displayed that urolithins A and B concomitant to their methyl ether metabolites are cholinesterase inhibitor agents comparable to the activity of galantamine [112]. In the same study, the antioxidant activities of the compound were also displayed.

In some other studies, urolithin scaffold has been employed to design novel agents against AD. In general cholinesterase, MAO-B, Cyclooxygenase, Aβ aggregation inhibitor characteristics of these compounds were investigated concomitant to screening antioxidant activities. Some representative studies in this category are displayed in Table 2.

Table 2: Some representative urolithin molecules designed against AD

Urolithin derivatives	Molecular Structure
Rivastigmine-like analogue of 3-Hydroxy-6H-benzo[c]chromen-6-one (A), and Donepezil-like analogue of 7,8,9,10-tetrahydroxybenzo[chromen]-6-one (B) [19].	A) R_1 R_2 O O; B) O N n O O
Amine derivatives of A) 3-Hydroxy-6H-benzo[c]chromen-6-one and B) 7,8,9,10-tetrahydroxybenzo[chromen]-6-one [113].	A) O N n O O; B) O N n O O
Derivatives of 3-Hydroxy-6H-benzo[c]chromen-6-one [112].	O R_1 O O R_3 R_2; OH R_1 O O R_3 R_2

Chapter 3

OBJECTIVES OF THE STUDY

The current treatment approaches for AD include cholinesterase inhibitor drugs and NMDA (N-methyl-D-aspartate) receptor antagonist memantine. These drugs provide symptomatic treatment that means they are able to slow down the decline in cognitive disabilities seen through the AD. Indeed, many studies so far indicated the importance of some muscarinic and nicotinic receptors for the regulation of cognition related functions [114]. From this perspective, the strategy using acetylcholinesterase through the inhibition of cholinesterases provides more available acetylcholine in CNS that is acting on cholinergic receptors. On the other hand, the partial antagonist function of memantine on NMDA receptors lowers the toxic effects of excitatory amino acids which are believed to be involved in the enhancement of cognitive abilities.

It is obvious that the number of drugs used for the treatment of AD is quite limited. Although these drugs provide a delay in the progression of the disease, they lack a total cure. The studies in the last couple decades indicated several significant pathophysiological cascades having function in the development of the disease. These include the Aβ aggregate formation cascades, tau protein fibrillation cascades, and some other mechanisms playing role in oxidative stress [115]. Unfortunately none of the drugs candidates targeting these mechanisms were found successful in clinical trials in terms of relieving cognition related disabilities [116]. Therefore, it is

a topic of the debate whether these mechanisms are the outcomes of the progression of the disease or they are the causative factors.

It is certain that AD is a neurodegenerative disease. Neural loss is valid throughout the development of the disease and this ends up with deficiencies in many neurotransmitter acting systems. Acetylcholine is only one of them. In reality dopaminergic, serotoninergic deficiencies are also observed in AD patients [117].
Since the current drugs do not provide alternative treatment strategies, the current approaches in the design of the molecules for the treatment of AD is based on multi target ligand design (MTLD). Based on the validated cognition enhancer activities of cholinesterase inhibitor agents, the MTLD strategy generally employs the design of candidates having concomitant to another mechanism providing neuroprotection. These neuroprotection cascades might include amyloid beta, tau protein fibrillation, MAO-B inhibition, and some other antioxidants activities.

Previous studies on urolithin derivatives indicated the ability of the urolithin scaffold to be employed as a potent enzyme inhibition and antioxidant purposes [112]. Urolithins are hydroxyl substituted benzo[c]chromen-6-one derivatives. These compounds are formed to the biotransformation of ellagitannins containing food such as nuts and berries. Through the aid of the micrflora within the gastrointestinal treat ellagitannins are converted to these hydroxylate benzo[c]chromen-6-one derivatives through stepwise oxidation and hydrolysis reactions.

Within this study, we used the basic urolithin scaffold and designed a series of compounds in which benzo[c]chromen-6-one (URO series) and 7,8,9,10-tetrahydro-

3-hydroxybenzo[c]chromen-6-one (THU series) were linked to an amide function with a spacer. The structures of the title molecules are shown in Figure 8.

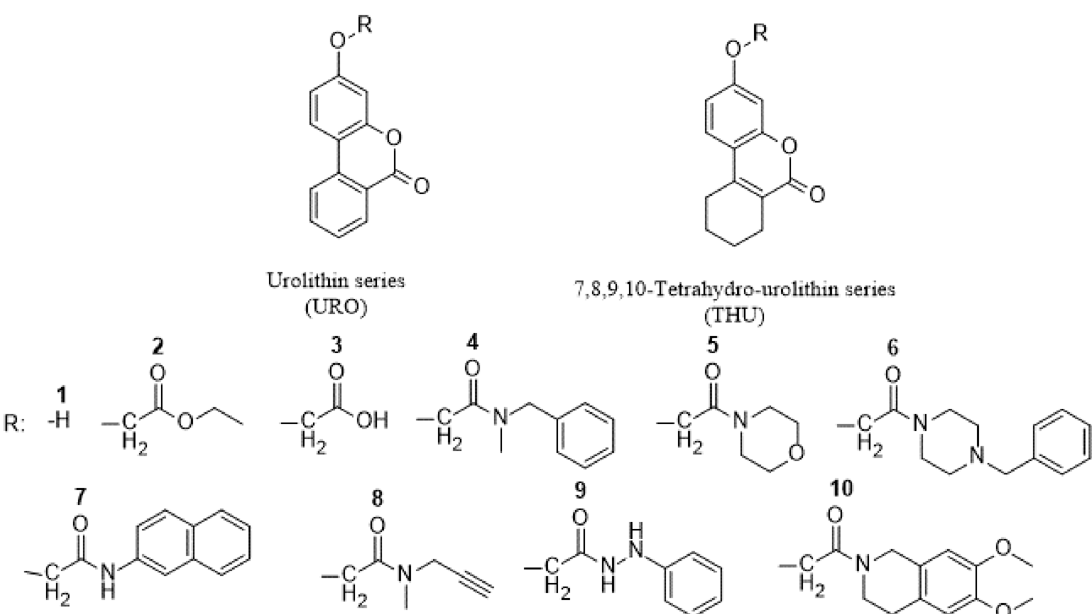


Figure 8: The structures of the title molecules

We have aimed to screen the cholinesterase (i.e., acetylcholinesterase and butyrylcholinesterase) and MAO-B inhibitory potential of the title molecules. Besides, the capacity of the molecules to prevent Aβ aggregation has also been aimed to be analyzed. Furthermore, the antioxidant properties of the compounds in ORAC (Oxygen Radical Absorbance Capacity) antioxidant assay systems have also been found important to be evaluated. Finally, it has been aimed to find out possible interactions of the title amide derivatives through docking studies.

Chapter 4

EXPERIMENTAL

4.1 Chemistry

4.1.1 General

The chemicals used in this research study were purchased through the aid of local chemical supplier companies. Thin layer chromatography (TLC) applied through Merck an aluminum-packed silica gel plate was used to monitor the reactions. Cyclohexane/acetone (3:2, v/v), toluene-methanol (9:1, v/v), and ethyl acetate- n-hexane (1:1, v/v) were used as mobile phases in TLC studies and they are named as S1, S2, and S3, respectively. Advion Expression CMS device was used to perform mass spectral analysis of the compounds. In these studies ASAP probe was employed for the direct application of the molecules to the device. Negative and positive modes were used to search the ions. The infrared spectroscopy was performed to observe functional group changes through using a Shimadzu FT-IR Prestige model spectrophotometer. Proton and carbon NMR spectroscopy studies were also included for structural identification studies. Accordingly, deuterated DMSO was used as solvent and the chemical shifts were recorded in ppm. A Bruker-400NMR spectrometer was used and tetramethylsilane was employed as internal standard. A CHNS elemental analyzer (Thermo Fisher Flash Smart model) was used for elemental analysis.

4.1.2 Preparation of synthesis intermediates

4.1.2.1 3-Hydroxy-6H-benzo[c]chromen-6-one (URO 1)

The solution of 15 mmole 2-iodobenzoic acid, 45 mmole resorcinol, and 55 mmole sodium hydroxide in 30 mL of distilled water was prepared and it was refluxed for 1 h. 10 mL of 20% CuSO4 solution in water was dropwise added to the reaction mixture at the end of this period. Following the additional stirring and refluxing for 10 minutes, the precipitate formed in the reaction vessel was filtered off after cooling to room temperature. The product obtained was washed with acidified water. White-yellow powder, yield obtained 80%. TLC: S1 R_f 0.5, S2 R_f 0.2, S3 R_f 0.4. Mp: 198 °C. IR: 1702 cm^{-1} (lactone carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 10.30 (s, 1 H); 8.15–8.12(m, 3 H); 7.83 (t, 1 H, J=6.1); 7.50 (t, 1 H, J=6.1); 6.77–6.74 (m, 2 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 162 ppm for lactone carbonyl. MS: 212.9 ($M-H^+$). Anal. calc. for $C_{13}H_8O_3$: C 73.58, H 3.80; found C 73.69, H 3.76.

4.1.2.2 Ethyl 2-(6-oxo-6H-benzo[c]chromen-3-yloxy)acetate (URO 2)

To the solution of 10 mmole URO 1 in 30 mL DMF, 15 mmole of sodium hydride was added and the mixture was stirred for 5 minutes in room temperature. 30 mmole of ethyl 2-chloroactetate was added to the mixture and the reaction was ran for 30 minute at room temperature. At the end of this period, the mixture was poured into cold water and the product precipitated was filtered off and washed with cold water. Light brown powder, yield obtained 80%. TLC: S1 R_f 0.7, S2 R_f 0.3, S3 R_f 0.6. Mp: 220 °C. IR: 1743 cm^{-1} (aliphatic ester carbonyl), 1704 cm^{-1} (lactone carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 8.37–8.20 (m, 3 H); 7.80 (t, 1 H, J=6.0); 7.66 (t, 1 H, J=6.0); 7.07–6.93 (m, 2 H), 4.89 (s, 2H), 4.19 (q, 2H, J=7.1Hz), 1.26 (t, 3H, J=7.1Hz). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 169 and 165 ppm for

carbonyl carbons. MS: 299.5 (MH^+). Anal. calc. for $C_{17}H_{14}O_5$: C 68.45, H 7.73; found C 68.11, H 7.68.

4.1.2.3 2-(6-oxo-6H-benzo[c]chromen-3-yloxy)acetic acid (URO 3)

In to the 1 g potassium hydroxide dissolved solution of 30 mL methanol, 10 mmole of URO 2 was added. The mixture was refluxed for 2 h. at the end of the time, the methanol was evaporated and the residue left was added 50 mL of ice cold distilled water. The product precipitated was filtered off. White to light brown powder, yield obtained 89%. TLC: S1 R_f 0.2, S2 R_f 0.06, S3 R_f 0.1. Mp: 240 °C. IR: 1727 cm^{-1} (carboxylic acid carbonyl), 1700 cm^{-1} (lactone carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 13.1 (bs, 1 H); 8.32–8.17 (m, 3 H); 7.89 (t, 1 H, J=6.0); 7.60 (t, 1 H, J=6.0); 7.10–6.90 (m, 2 H), 4.75 (s, 2H). ^{13}C-NMR (DMSOd_6, 125 MHz): major rotamer: 178 and 161 ppm for carbonyl carbons. MS: 269.4 ($M\text{-}H^-$). Anal. calc. for $C_{15}H_{10}O_5$: C 66.67, H 3.73; found C 67.02, H 3.71.

4.1.2.4 7,8,9,10-tetrahydro-3-hydroxybenzo[c]chromen-6-one (THU 1)

Following the heating of 90 mmole resorcinol, 99 mmole 2-oxocyclohexanecarboxylate, and 20 mmole ZrCl4 for 50 minutes under neat conditions THU 1 formed and precipitated was filtered off and washed with ice cold water. Yellow powder, yield obtained 87%. TLC: S1 R_f 0.4, S2 R_f 0.3, S3 R_f 0.6. Mp: 190 °C. IR: 1737 cm^{-1} (lactone carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 10.31 (bs, 1 H); 7.50 (d, 1 H, J=8.8); 6.75 (d, 1 H, J=8.8); 6.65 (s, 1 H); 2.71 (t, 2 H, J=5.6); 2.48 (t, 2 H, J=6.0); 1.73-1.67 (m, 4 H). ^{13}C-NMR (DMSOd_6, 125 MHz): major rotamer: 161 ppm for lactone carbonyl. MS: 216.8 ($M\text{-}H^+$). Anal. calc. for $C_{13}H_{12}O_3$: C 72.21, H 5.59; found C 71.77, H 5.70.

4.1.2.5 Ethyl 2-(7,8,9,10-tetrahydro-6-oxo-6H-benzo[c]chromen-3-yloxy)acetate (THU 2)

The protocol and the stoichiometry of the synthesis of THU 2 from THU 1 is totally the same as the protocol described for the synthesis of URO 2 under the section 4.1.2.2, beside the employment of THU 1 as the synthesis reactant. White powder, yield obtained 79%. TLC: S1 R_f 0.6, S2 R_f 0.5, S3 R_f 0.7. Mp: 217 °C. IR: 1764 cm^{-1} (aliphatic ester carbonyl), 1702 cm^{-1} (lactone carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 7.60 (d, 1 H, J=9,6); 6.94 (m, 2 H); 4.88 (s, 2H); 4.15 (q, 2H, J=7.2Hz); 2.74 (t, 2H, J=6.0Hz); 2.37 (t, 2H, J=5.2Hz); 1.71 (m, 4H), 1.19 (t, 3H, J=7.2). ^{13}C-NMR (DMSOd_6, 125 MHz): major rotamer: 168 and 161 ppm for carbonyl carbons. MS: 303.6 (MH^+). Anal. calc. for $C_{17}H_{18}O_5$: C 67.54, H 6.00; found C 67.44, H 6.09.

4.1.2.6 2-(7,8,9,10-tetrahydro-6-oxo-6H-benzo[c]chromen-3- yloxy) acetic acid (THU 3)

The protocol and the stoichiometry of the synthesis of THU 3 from THU 2 is totally the same as the protocol described for the synthesis of URO 3 from URO 2 as described under the section of 4.1.2.3, beside the employment of THU 2 as the synthesis reactant. White powder, yield obtained 87%. TLC: S1 R_f 0.2, S2 R_f 0.06, S3 R_f 0.08. Mp: 235 °C. IR: 1751 cm^{-1} (aliphatic ester carbonyl), 1705 cm^{-1} (lactone carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 7.63 (d, 1 H, J=8,8); 6.95 (m, 2 H); 4.80 (s, 2H); 2.77 (t, 2H, J=5.6Hz); 2.40 (t, 2H, J=5.2Hz); 1.75 (m, 4H). ^{13}C-NMR (DMSOd_6, 125 MHz): major rotamer: 170 and 161 ppm for carbonyl carbons. MS: 273.5 (M^+). Anal. calc. for $C_{15}H_{14}O_5$: C 65.69, H 5.15; found C 66.01, H 4.99.

4.1.3 General procedure for the synthesis of urolithin amides

The title URO and THU amide derivatives (THU 4-10, URO 4-10) were synthesized using THU 3 and URO 3 as the starting materials, respectively. Accordingly, 2.3

mmole of either URO 3 or THU 3 solution in 25 mL of dichloromethane was added 2.3 mmole of thionyl chloride. The mixture was refluxed for 6 h. following the reaction time, the organic solvent was evaporated to get rid of the excess hydrochloric acid formed. To the residue left, 20 mL of fresh dichloromethane was added. The new solution prepared was placed in an ice bath and 2.3 mmole of an appropriate amine in 20 mL of dichloromethane was dropwise added to the solution. The title amide compounds were obtained through the evaporation of the organic solvent and applying column chromatography work up using ethyl acetate- n-hexane (3:1, v/v) as the mobile phase.

4.1.3.1 2- (6-oxo-6H-benzo[c]chromen-3-yloxy)- N- benzyl- N- methylacetamide (URO 4)

Yellow powder, yield obtained 63%. TLC: S1 R_f 0.5, S2 R_f 0.3, S3 R_f 0.4. Mp: 248 °C. IR: 1727 cm^{-1} (lactone carbonyl), 1643 cm^{-1} (amide carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 8.36-8.21 (m, 3 H); 7.92 (t, 1 H, J=7.6); 7.61 (t, 1 H, J=6.8); 7.42 (t, 1 H, J=6.8); 7.34-7.23 (m, 4H), 7.04 (t, 1 H, J=7.2); 5.10 (s, 2 H); 4.54 (s, 2 H); 2.98 (s, 3 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 169 and 161 ppm for carbonyl carbons. MS: 274.6 (MH^+). Anal. calc. for $C_{23}H_{19}NO_4$: C 73.98, H 5.13, N 3.75; found C 73.99, H 4.99, N 3.81.

4.1.3.2 3-(2-morpholino-2-oxoethoxy)-6H-benzo[c]chromen-6-one (URO 5)

Light yellow powder, yield obtained 58%. IR: 1727 cm^{-1} (lactone carbonyl), 1654 cm^{-1} (amide carbonyl). TLC: S1 R_f 0.4, S2 R_f 0.2, S3 R_f 0.2. Mp: 254 °C. ^{1}HNMR ($DMSOd_6$, 400 MHz): 8.35 (d, 1 H, J=8.6); 8.01-7.95 (m, 2 H); 7.80 (t, 1 H, J=6.8); 7.52 (t, 1 H, J=6.8); 7.01 (d, 1 H, J=8.8), 6.89 (d, 1 H, J=2.4); 4.78 (s, 2 H); 3.71-3.57 (m, 4 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 166 and 159 ppm for

carbonyl carbons. MS: 340.6 (MH^+). Anal. calc. for $C_{19}H_{17}NO_5$: C 67.25, H 5.05, N 4.13; found C 67.61, H 4.88, N 4.11.

4.1.3.3 3- (2-(4-benzylpiperazin-1-yl)- 2- oxoethoxy)-6H-benzo[c]chromen-6-one (URO 6)

White powder, yield obtained 55%. TLC: S1 R_f 0.7, S2 R_f 0.6, S3 R_f 0.7. Mp: 263 °C. IR: 1721 cm^{-1} (lactone carbonyl), 1649 cm^{-1} (amide carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 8.46-8.24 (m, 3 H); 7.88 (t, 1 H, J=7.6); 7.65 (t, 1 H, J=6.8); 7.37-7.24 (m, 5H), 7.02 (t, 1 H, J=7.2); 4.97 (s, 2 H); 3.44 (s, 2 H); 2.70-2.59 (M, 8 H). ^{13}C-NMR (DMSOd_6, 125 MHz): major rotamer: 166 and 161 ppm for carbonyl carbons. MS: 429.2 (MH^+). Anal. calc. for $C_{26}H_{24}N_2O_4$: C 72.88, H 5.65, N 6.54; found C 73.41, H 5.90, N 6.49.

4.1.3.4 2- (6-oxo-6H-benzo[c]chromen-3-yloxy)- N- (naphthalen-1-yl) acetamide (URO 7)

Yellowish powder, yield obtained 60%. TLC: S1 R_f 0.6, S2 R_f 0.5, S3 R_f 0.6. Mp: 270 °C. IR: 1718 cm^{-1} (lactone carbonyl), 1630 cm^{-1} (amide carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 10.05 (s, 1 H); 8.30-8.18 (m, 3 H); 7.85-7.34 (m, 8 H); 6.82-6.75 (m, 3 H); 5.06 (s, 2 H). ^{13}C-NMR (DMSOd_6, 125 MHz): major rotamer: 167 and 161 ppm for carbonyl carbons. MS: 396.10 (MH^+). Anal. calc. for $C_{25}H_{17}NO_4$: C 75.94, H 4.33, N 3.54; found C 80.12, H 4.36, N 3.47.

4.1.3.5 2- (6-oxo-6H-benzo[c]chromen-3-yloxy)- N-methyl-N- (prop-2-ynyl)acet-amide (URO 8)

Brown powder, yield obtained 51%. TLC: S1 R_f 0.8, S2 R_f 0.8, S3 R_f 0.7. Mp: 240 °C. IR: 1719 cm^{-1} (lactone carbonyl), 1668 cm^{-1} (amide carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 8.33-8.18 (m, 3 H); 7.89 (t, 1 H, J=7.2); 7.59 (t, 1H, J=7.2); 7.00-6.91 (m, 2 H); 5.00 (s, 2 H); 3.02 (s, 2 H); 2.89 (s, 3H); 2.08 (s, 1H). ^{13}C-NMR

(DMSOd$_6$, 125 MHz): major rotamer: 167 and 160 ppm for carbonyl carbons. MS: 322.0 (MH^+). Anal. calc. for $C_{19}H_{15}NO_4$: C 71.02, H 4.71, N 4.36; found C 71.28, H 4.70, N 4.33.

4.1.3.6 2-(6-oxo-6H-benzo[c]chromen-3-yloxy)-N'-phenylacetohydrazide (URO 9)

Yellow powder, yield obtained 48%. TLC: S1 R_f 0.3, S2 R_f 0.2, S3 R_f 0.3. Mp: 255 °C. IR: 1719 cm^{-1} (lactone carbonyl), 1641 cm^{-1} (amide carbonyl). ^{1}HNMR (DMSOd$_6$, 400 MHz): 11.01 (s, 1 H); 10.05 (s, 1 H); 8.30-8.22 (m, 3 H); 7.89 (t, 1 H, J=7.2); 7.59 (t, 1H, J=7.2); 7.11-6.91 (m, 7 H); 5.07 (s, 2 H). ^{13}C-NMR (DMSOd$_6$, 125 MHz): major rotamer: 169 and 159 ppm for carbonyl carbons. MS: 361.0 (MH^+). Anal. calc. for $C_{21}H_{16}N_2O_4$: C 69.99, H 4.48, N 7.77; found C 70.48, H 4.74, N 7.60.

4.1.3.7 3- (2- (3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)-2-oxoethoxy)-6H-benzo[c]chromen-6-one (URO 10)

Brown-yellow powder, yield obtained 58%. TLC: S1 R_f 0.2, S2 R_f 0.2, S3 R_f 0.2. Mp: 299 °C. IR: 1724 cm^{-1} (lactone carbonyl), 1665 cm^{-1} (amide carbonyl). ^{1}HNMR (DMSOd$_6$, 400 MHz): 8.31-8.17 (m, 3 H); 7.89 (t, 1 H, J=7.2); 7.58 (t, 1H, J=7.6); 7.03-6.99 (m, 2 H); 6.80-6.72 (m, 2 H); 5.04 (s, 2 H); 3.72-3.63 (m, 10H), 3.27-3.17 (m, 2 H). ^{13}C-NMR (DMSOd$_6$, 125 MHz): major rotamer: 167 and 161 ppm for carbonyl carbons. MS: 446.01 (MH^+). Anal. calc. for $C_{26}H_{23}NO_6$: C 70.10, H 5.20, N 3.14; found C 70.14, H 5.12, N 3.21.

4.1.3.8 2- (7,8,9,10-tetrahydro-6-oxo-6H-benzo[c]chromen-3-yloxy)-N-benzyl-N-methylacetamide (THU 4)

White powder, yield obtained 68%. TLC: S1 R_f 0.5, S2 R_f 0.3, S3 R_f 0.4. Mp: 245 °C. IR: 1700 cm^{-1} (lactone carbonyl), 1676c m^{-1} (amide carbonyl). ^{1}HNMR

($DMSOd_6$, 400 MHz): 7.48-7.18 (m, 6 H); 6.85 (d, 1 H, J=9); 6.67 (d, 1 H, J=9); 4.81 (s, 2 H); 4.60 (s, 2 H); 2.99 (s, 3 H); 2.74 (t, 2 H, J=5.8); 2.56 (t, 2 H, J=5.8); 1.86-1.78 (m, 4 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 167 and 159 ppm for carbonyl carbons. MS: 378.1 (MH^+). Anal. calc. for $C_{23}H_{23}NO_4$: C 73.19, H 6.14, N 3.71; found C 72.80, H 6.22, N 3.56.

4.1.3.9 3- (2-morpholino-2-oxoethoxy)- 7,8,9,10-tetrahydrobenzo [c] chromen-6-one (THU 5)

White powder, yield obtained 71%. TLC: S1 R_f 0.4, S2 R_f 0.2, S3 R_f 0.2. Mp: 250 °C. IR: 1694 cm^{-1} (lactone carbonyl), 1651 cm^{-1} (amide carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 7.48 (d, 1 H, J=8.8); 6.92 (d, 1 H, J=8,8); 6.82 (d, 1 H, J=2.8); 4.76 (s, 2 H); 3.70-3.56 (m, 4 H); 2.74 (t, 2 H, J=9.6); 2.56 (t, 2 H, J=8.0); 1.87-1.78 (m, 4 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 167 and 162 ppm for carbonyl carbons. MS: 344.1 (MH^+). Anal. calc. for $C_{19}H_{21}NO_5$: C 66.46, H 6.16, N 4.08; found C 66.74, H 6.14, N 4.01.

4.1.3.10 3- (2-(4-benzylpiperazin-1-yl)- 2-oxoethoxy) -7,8,9,10- tetrahydrobenzo-[c]chromen-6-one (THU 6)

White powder, yield obtained 63%. TLC: S1 R_f 0.3, S2 R_f 0.2, S3 R_f 0.2. Mp: 260 °C. IR: 1700 cm^{-1} (lactone carbonyl), 1654 cm^{-1} (amide carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 7.43 (d, 1 H, J=8.8); 7.30-7.25 (m, 5 H); 6.89 (d, 1 H, J=9.0); 6.77 (d, 1 H, J=2.4); 4.75 (s, 2 H); 3.51 (s, 2 H); 2.71 (t, 2 H, J=9.6); 2.52 (t, 2 H, J=8.0); 2.44-2.40 (m, 4 H); 1.83-1.77 (m, 4 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 166 and 159 ppm for carbonyl carbons. MS: 432.2 (M^+). Anal. calc. for $C_{26}H_{28}N_2O_4$: C 72.20, H 6.53, N 6.48; found C 71.88, H 6.39, N 6.57.

4.1.3.11 2-(7,8,9,10-tetrahydro-6-oxo-6H-benzo[c]chromen-3-yloxy)-N-(naphthalen-1-yl)acetamide (THU 7)

Light yellow powder, yield obtained 58%. TLC: S1 R_f 0.7, S2 R_f 0.6, S3 R_f 0.7. Mp: 268 °C. IR: 1705 cm^{-1} (lactone carbonyl), 1662 cm^{-1} (amide carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 10.33 (s, 1 H); 7.50-7.14 (m, 8 H); 6.95 (d, 1 H, J=9,0); 6.73 (d, 1 H, J=2.8); 5.01 (s, 2 H); 2.73 (t, 2 H, J=5.6); 2.44 (t, 2 H, J=5.6); 1.71-1.66 (m, 4 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 167 and 160 ppm for carbonyl carbons. MS: 400.2 (MH^+). Anal. calc. for $C_{25}H_{21}NO_4$: C 75.17, H 5.30, N 3.51; found C 75.41, H 5.20, N 3.39.

4.1.3.12 2- (7,8,9,10-tetrahydro-6-oxo-6H-benzo[c]chromen-3-yloxy)- N-methyl-N-(prop-2-ynyl)acetamide (THU 8)

White powder, yield obtained 58%. TLC: S1 R_f 0.4, S2 R_f 0.3, S3 R_f 0.6. Mp: 237 °C. IR: 1695 cm^{-1} (lactone carbonyl), 1670 cm^{-1} (amide carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 7.47 (d, 1 H, J=8.8); 6.92 (d, 1 H, J=9,0); 6.78 (d, 1 H, J=2.4); 4.76 (s, 2 H); 3.21 (s, 2 H); 3.04 (s, 3H); 2.74 (t, 2 H, J=5.2); 2.55 (t, 2 H, J=5.2); 2.37 (s, 1 H); 1.85-1.60 (m, 4 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 166 and 160 ppm for carbonyl carbons. MS: 326.1 (MH^+). Anal. calc. for $C_{19}H_{19}NO_4$: C 70.14, H 5.89, N 4.31; found C 70.01, H 5.88, N 4.39.

4.1.3.13 2- (7,8,9,10-tetrahydro-6-oxo-6H-benzo[c]chromen-3-yloxy)- N'-phenyl-acetohydrazide (THU 9)

White powder, yield obtained 58%. TLC: S1 R_f 0.3, S2 R_f 0.2, S3 R_f 0.3. Mp: 250 °C. IR: 1695 cm^{-1} (lactone carbonyl), 1670 cm^{-1} (amide carbonyl). ^{1}HNMR ($DMSOd_6$, 400 MHz): 7.53 (d, 1 H, J=8.8); 7.26-7.19 (m, 2 H); 6.95-6.84 (m, 5 H); 4.69 (s, 2 H); 2.75 (t, 2 H, J=5.6); 2.54 (t, 2 H, J=5.6); 1.88-1.63 (m, 4 H). ^{13}C-NMR ($DMSOd_6$, 125 MHz): major rotamer: 168 and 159 ppm for carbonyl carbons. MS:

365.2 (MH^+). Anal. calc. for $C_{21}H_{20}N_2O_4$: C 69.22, H 5.53, N 7.69; found C 69.58, H 5.58, N 7.80.

4.1.3.14 3- (2-(3,4-dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)- 2-oxoethoxy)- 7, 8,9,10-tetrahydrobenzo[c]chromen-6-one (THU 10)

Light yellow powder, yield obtained 71%. TLC: S1 R_f 0.3, S2 R_f 0.3, S3 R_f 0.2. Mp: 290 °C. IR: 1695 cm^{-1} (lactone carbonyl), 1673 cm^{-1} (amide carbonyl). ^{1}HNMR (DMSOd_6, 400 MHz): 7.46 (d, 1 H, J=8.8); 6.95 (d, 1 H, J=8.8); 6.83 (s, 1 H); 6.63-6.58 (m, 2H); 4.83 (s, 2 H); 4.67 (s, 2H); 3.86 (s, 3 H), 3.80 (s, 3H); 3.74 (t, 2 H, J=6); 2.85 (t, 2 H, J=6); 2.72 (t, 2 H, J=5.6); 2.54 (t, 2 H, J=5.6); 1.85-1.77 (m, 4 H). ^{13}C-NMR (DMSOd_6, 125 MHz): major rotamer: 166 and 159 ppm for carbonyl carbons. MS: 450.2 (MH^+). Anal. calc. for $C_{26}H_{27}NO_6$: C 69.47, H 6.05, N 3.12; found C 69.75, H 5.99, N 3.20.

4.2 Biological evaluation

4.2.1 Cholinesterase inhibition assays

Modified Ellmann's method was followed to measure the potential of the synthesize intermediates and final amide compounds to inhibit both AChE and BuChE [118]. Each enzyme assay was performed in 50 mM of Tris HCl buffer (pH 8.0), containing 6.8 mM DTNB solution, 20 mM $MgCl_2$, 100 mM NaCl, and 10 µL of AChE or BuChE solution (0.4 U/mL from Human recombinant AChE or 1.64 U/mL from Human recombinant BuChE, from Sigma Aldrich, Dorset, England), and 2 µL of each sample solution in a total volume of 190 µL. Depending on the enzyme used, the either 10 µL of 10 mM acetylthiocholine iodide solution or 10 µL of 1.5 mM butyrylthiocholine iodide solution was added to initiate the enzyme catalyzed reaction. In control group, representing the full activity, no inhibitor was used. UV measurements were performed at 412 nm employing a 96-well microplate reader

(i.e., Varioskan Flash, Thermo Scientific, USA) immediately after the incubation time (15 min) at 27 °C. Percent inhibition of test compounds was calculated via the formula (FA-IA)/FA×100, where FA represents the full activity obtained in the absence of inhibitor and IA is the activity obtained in the presence of an inhibitor (i.e., test or reference compound). The IC_{50}s for AChE and BuChE were calculated through plotting the percent inhibition against the concentration of test materials. Each experiment was run in triplicates and the results were represented as mean ± standard deviations. Galantamine and donepezil were used as standard inhibitors for both cholinesterases.

4.2.2 MAO-B inhibition assays

A MAO assay kit (i.e., Sigma–Aldrich Monoamine Oxidase Assay Kit, Catalog number MAK-136, Dorset, England) was employed to measure the potential of the title amide derivatives and synthesis intermediates to inhibit MAO-B [119]. Accordingly, p-tyramine was employed as the substrate of the reaction and the MAO-B catalyzed formation of hydrogen peroxide was measured employing a dye reagent through a fluorescence assay in which excitation and emission wavelengths were set to 530 nm and 585 nm, respectively. In full activity tests, no inhibitor was employed. Assays were performed in triplicates and the percent inhibition was plotted against concentration to obtain IC_{50}s. Pargyline was employed as the reference inhibitor as provided within the kit. The mean ± standard deviation of IC_{50}s obtained was represented.

4.2.3 Inhibition of A*β* aggregation

Thioflavin T fluorescence spectroscopy method was employed to screen the potential of the molecules to inhibit amyloid beta ($A\beta_{1-42}$ from Sigma Aldrich, USA) self-aggregation [120]. Briefly, for the preparation of amyloid beta, 1 mg was dissolved

in 0.5 mL of HFIP (Hexafluoroisopropanol). Following that HFIP was evaporated and 2.3 mM A*β* stock solution was prepared in DMSO. 2 μL of this solution was transferred to the each well of 24-well multidish microplate. Each well was further added 18 μL of 0.2 M sodium phosphate buffer (pH 8.0) containing either title molecules or reference compounds (i.e., resveratrol). The final solutions were incubated for 36 h at RT. Following the incubation time 1.5 μM thioflavin T solution in 50 mM glycine NaOH buffer (pH 8.5) was added to generate a total volume of 2 mL. Fluorescence analysis through a Varioskan Flash Thermo Scientific instrument was performed with excitation and emissions wavelengths set to 446, and 490 nm, respectively. Each experiment was run in triplicates and percent inhibition results were presented as mean ± standard deviation. In control experiments no reference or test compounds were employed. Both the test compounds and standards were employed at 100 μM final concentration. The formula 100-(IFi/IFo x 100) was used to calculate the potential of the test compounds to prevent Aβ aggregation. In the formula, IFi; and IFo represent the fluorescence intensities obtained in the presence and absence of test/reference compounds used.

4.2.4 Antioxidant assays

Oxygen radical absorbance capacity (ORAC) test was employed to determine the antioxidant activity of the compounds [121]. Accordingly, assays were performed in total of 200 μL volume in 75 mM phosphate buffer pH 7,4. 20 μL of test substances (final 10 μM concentration) and 120 μL of fluorescein (150 nM final concentrations) were incubated for 20 min at 37 ºC. Following the incubation time, 60 μL of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (12 mM final concentration) was added to each solution well. Measurements were performed through recording fluorescence readings (i.e., excitation at 485 nm and emission at 535 nm in a Thermo

Scientific Varioskan Flash Multimode Reader) at 5 min intervals up 90 min. 0.5–8 μM final concentrations of trolox were used as standards. Each assay was done in triplicates. The ORAC values, calculated as difference of the areas under the quenching curves of fluorescein between the blank and the sample, were expressed as μmol trolox equivalents per μmol of compounds.

4.3 Docking studies

Docking studies were carried out with Schrödinger suite 2018-1 using the same protocol implemented in our previous study [112], [122]. In particular, the crystal structures of hAChE (PDB ID: 6O4W), hBuChE (PDB IDs: 6F7Q) and hMAO-B (PDB ID: 6FVZ) were downloaded from the Protein Data Bank (PDB) and prepared with Schrödinger's Protein Preparation Wizard tool [123]. Water molecules and residues defined as hetero atoms in PDB were removed, except the FAD in the crystal structure of MAO-B (PDB ID: 6FVZ). During the protein preparation step, hydrogen atoms and missing side chain residues were added; then residues protonation states were predicted by PROPKA at pH 7.0 and the hydrogen bonding network was optimized. As final step, the proteins were subjected to a restrained energy minimization using the OPLS3 force field with default settings. The ligand structures under investigation were prepared by means of Schrödinger's LigPrep tool with following settings: stereoisomers generated at pH 7.0 ± 2.0 with Epik, possible tautomers generation, OPLS3 as force field [124]. Afterwards, a maximum of 25 conformers were generated for each ligand using ConfGen and the outputs were minimized with OLPS_2005 force field (default force field) [125], [126]. In order to dock our inhibitors at the relative binding pocket, grids boxes were generated using the Receptor Grid Generation tool. The co-crystallized inhibitor of each protein was selected as the center of the grid. Molecular docking studies were performed with

Glide Standard Precision (SP) mode from the Schrödinger suite. The option "sample ring conformation" was turned on and a maximum of three docking poses were output for each ligand [127]. Subsequentially, the first poses were refined and minimized using protein-ligand complex refinement allowing flexibility for the residues within 2 Å from the ligand; VSGB and OPLS3 were used as solvation model and force field [128]. The implemented protocol was first tested via re-docking studies in order to check the ability to reproduce the co-crystallized inhibitor structures. In all three cases, low root-mean-square deviation (RMSD) values were obtained: 6O4W = 0.836 Å, 6F7Q = 0.631 Å, 6FVZ = 0.109 Å (RMSD of heavy atoms).

Chapter 5

RESULTS AND DISCUSSION

5.1 Chemistry

The synthesis of the title molecules are shown in Figure 9. Resorcinol was treated with 2-idobenzoic acid in the presence of aqueous base to obtain URO 1. On the other hand, the reaction between ethyl 2-oxocyclohexanecarboxylate and resorcinol in the present of ZrCl4 yielded out THU 1, as shown in previous studies [112], [129]–[134]. These two compounds were employed as starting materials for the synthesis of the title URO and THU series (i.e., 6H-benzo[c]chromen-6-one, and 7,8,9,10-tetrahydro-benzo[c]chromen-6-one derivatives, respectively). The treatment of URO 1 and THU 1 with ethyl 2-chloroacetate under ether synthesis conditions generated URO 2 and THU 2 intermediated. The ester derivatives (i.e., URO 2 and THU 2) were hydrolyzed to their corresponding carboxylic acid derivatives (i.e., URO 3 and THU 3) in the presence of potassium hydroxide and methanol.

The URO series

The THU series

Reagents and conditions: (a) 2-Iodobenzoic acid, NaOH, CuSO4, H2O, reflux, 1 h.(b) Ethyl 2-oxocyclohexanecarboxylate, ZrCl4,70 °C, 1 h; (c) Ethyl 2-chloroacetate, NaH, DMF, RT, 1h ; (d) KOH, MeOH, reflux, 2h; (e) Two steps in-situ; at first, thionyl chloride, DCM, 6h reflux, then appropriate amine, 30 min, 0 ºC.

Figure 9: The synthetic protocol followed

Schotten Baumann amide synthesis protocol was followed to obtain the final amide derivatives starting from the corresponding carboxylic acid synthesis intermediates [135], [136]. Accordingly, URO 3 and THU 3 were treated with thionyl chloride to in-situ obtain the acyl chlorides intermediates. Without the purification of the acyl halide intermediates, the intermediates were treated with appropriate amines.

Infrared, proton NMR, and carbon 13 NMR spectroscopic techniques were employed for the spectral characterization of both synthesis intermediates and the final molecules. In addition, mass spectroscopic and elemental analysis were conducted for structure identification studies. The yields obtained for intermediate synthesis were found good. Final step yields were assessed moderate in general.

5.2 Enzyme inhibition

Table 3 shows the inhibitor potential of URO 1 to 10 and THU 1 to 10 against acetylcholinesterase, butyrylcholinesterase, and MAO-B.

Table 3: The potential of urolithin derivative to inhibit cholinesterases and MAO-B

Title molecules	**IC_{50} (µM)**		
	AChE	**BuChE**	**MAO-B**
URO 1	> 50	> 50	42.4 ± 1.6
URO 2	35.9 ± 1.1	> 50	41.8 ± 0.1
URO 3	> 50	> 50	37.0 ± 0.9
URO 4	2.5 ± 0.2	4.4 ± 0.1	27.4 ± 1.0
URO 5	13.8 ± 0.7	17.0± 0.4	34.8 ± 0.9
URO 6	2.0 ± 0.1	4.1 ± 0.3	30.1 ± 0.2
URO 7	8.3 ± 0.1	13.6 ± 1.1	28.6 ± 0.5
URO 8	11.9 ± 0.6	14.6± 0.3	15.0 ± 0.4
URO 9	7.2 ± 0.4	9.9 ± 0.7	18.3 ± 0.7
URO 10	7.9 ± 0.5	9.3± 0.1	27.5 ± 0.2
THU 1	29.6 ± 0.4	31.1 ± 0.6	35.3 ± 1.1
THU 2	28.2 ± 0.6	35.8 ± 0.5	37.5 ± 0.4
THU 3	30.1 ± 0.2	33.7 ± 0.4	36.1 ± 0.8
THU 4	1.3 ± 0.1	1.9 ± 0.2	22.5 ± 0.4
THU 5	10.5 ± 0.3	14.3± 0.7	27.6 ± 0.1
THU 6	1.7 ± 0.2	3.0 ± 0.2	24.3 ± 1.1
THU 7	7.0 ± 0.3	12.1 ± 0.1	23.5 ± 0.3
THU 8	6.5 ± 0.3	8.9 ± 0.3	12.1 ± 0.8
THU 9	6.0 ± 0.1	10.4 ± 0.2	17.9 ± 0.4
THU 10	8.3 ± 0.4	10.5± 0.4	22.8 ± 1.2
Reference molecules			
Donepezil	0,3 ± 0.01	9,0 ± 0.1	NT
Rivastigmine	28.1 ± 0.2	11.4 ± 0.1	NT
Pargyline	NT	NT	2.2 ± 0.3

In general, the title amide derivatives were all evaluated as cholinesterase inhibitor agents within the IC_{50} range of 1-35 μM. comparing the activities of the corresponding THU and URO derivatives, the THU analogues displayed higher cholinesterase inhibitor potential. For almost all amide derivatives, the selectivity was higher towards AChE. The most active compounds for both AChE and BuChE inhibition were THU 4, THU 6, URO 4, and URO 6 in the series. These compounds possess a methylene group with a free rotation bridging the amide nitrogen and the phenyl ring that is considered to be important for their higher inhibitory potential.

Once the activities were compared with the reference molecules employed, it was observed that none of the compounds were found superior to the activity of donepezil on acetylcholinesterase. On the other hand, many molecules in the series were found to possess comparable or superior activity against butyrylcholinesterase in comparison to the activities of donepezil and galantamine on this enzyme. Similarly many compounds in the series appeared to show higher activity against acetylcholinesterase in comparison to the activity of rivastigmine on this enzyme.

The potential of the synthesis intermediates to inhibit cholinesterase enzymes were found poor. This observation was evaluated significant to display the design of the molecules for the inhibition of cholinesterases.

Coumarines and flavonoids are known to have monoamine oxidase inhibitory potential in general. Regarding this point the MAO-B inhibitory potential of the title compounds were assessed and the results are shown in Table 3. Accordingly, all the compounds in the URO and THU series, including the synthesis intermediates, were shown to possess MAO-B inhibitory potential. The final amide derivatives displayed

higher activity in comparison to the activities of synthesis intermediates. 12-30 µM IC_{50} range was obtained for the MAO-B inhibition characteristics of the title amide analogues. The reference molecule pargyline were found to display the highest activity.

In overall evaluation of the results for the inhibition of MAO-B, the coumarine-spacer-amide scaffold employed in the design of molecules was again assessed as significant since almost all the title amide molecules displayed higher activity in comparison to the activities of synthesis intermediates. Similar to the results obtained cholinesterase inhibition, the activities of THU derivatives were found slightly higher in comparison to the activities of their corresponding URO analogues against MAO-B. THU 8 and URO 8 displayed the highest activities in the series. Both of these compound possess propargyl group in their structure, similar to the propargyl substituted MAO-B inhibitor drugs pargyline, selegiline, and rasagiline. Therefore it is reasonable to state that propargyl group, as seen in the design of MAO-B inhibitor drugs, plays an important role for the high MAO-B inhibitor characteristics of the title compounds THU 8 and URO 8 [137]. Some MAO inhibitor drugs such as isocarboxazid possess a carbohydrazide scaffold thought to be important in the inhibitory action against MAO [138]. The carbohydrazide analogues, THU 9 and URO 9, also were found to possess MAO-B inhibitor characteristics. From this perspective, the carbohydrazide moiety in these structures might be critical for their potential to inhibit MAO-B.

5.3 Docking studies

Docking studies were employed to predict the possible binding interactions of the most active compounds to the corresponding enzymes. The interaction of THU 4

with acetylcholinesterase and butyrylcholinesterase obtained according to the docking studies are shown in Figure 10 A and Figure 10 B, respectively. Accordingly, THU 4 accommodates both to the peripheral and active sites of the cholinesterase enzyme. Mainly, the tetrahydrourolithin moiety is stabilized in the peripheral site, while the benzyl amide portion of the molecule gets in to the catalytic active site. In a detail description, the lactone carbonyl generates hydrogen bonding with Phe295 and there is also π–π interaction with Tyr341. On the other hand, van der Waals interactions were established in the active site Trp86 of the enzyme and the benzyl group of THU 4. Regarding interactions between THU 4 and butyrylcholinesterase, hydrogen bonds were observed with Ser198 and His438 amino acid residues.in addition, π–π interactions were also predicted present with the Phe329 and Trp82

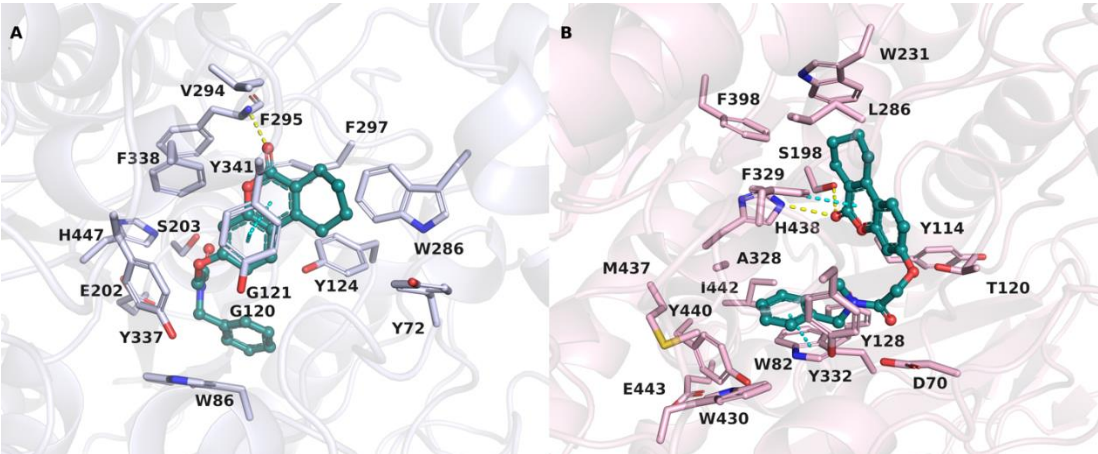


Figure 10: Docking poses of compound THU 4 at the binding site of hAChE **(A)** and hBuChE **(B)**.

PDB IDs: 6O4W and 6F7Q. The side chains of the amino acids surrounding the ligand are shown as white sticks (hAChE) and pink sticks (hBuChE); for residue F295 (hAChE), the (hAChE), the main chain is displayed. Hydrogen bon interactions are illustrated with dashed yellow lines, whereas π–π stacking interactions with

dashed cyan lines. The docking poses are represented as ball and stick and colored in dark cyan.

The predicted interaction of THU 8 with MAO-B is shown in Figure 11. Accordingly, a hydrogen bond with Tyr435 residue was observed. Moreover hydrophobic interactions were observed with the I198, I316, and L167 residues.

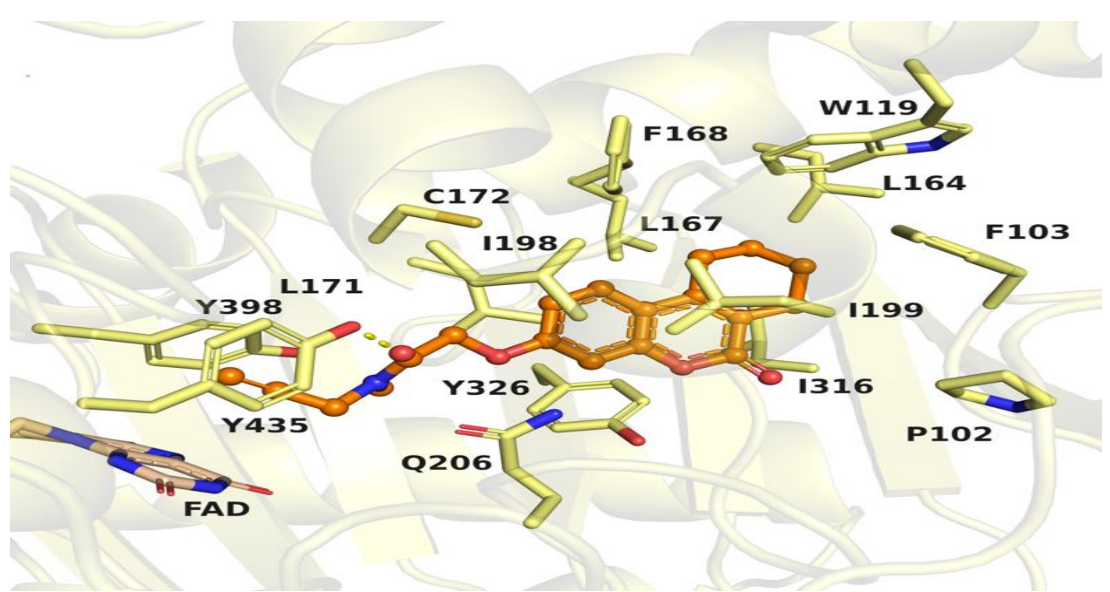


Figure 11: Docking pose of compound THU 8 at the hMAO-B.

Active site; PDB ID: 6FVZ. The side chains of the amino acids surrounding the ligand are shown as yellow sticks, whereas FAD as beige stick. Hydrogen bond interaction is illustrated with a dashed yellow line. The docking pose is represented as ball and stick and colored in orange.

5.4 The potential of the compounds to inhibit Aβ aggregation

AD pathogenesis also includes the excess aggregation of amyloid beta plaques in the CNS and this is thought to be a process in the neurodegenerative characteristics of AD. From this perspective, amyloid beta aggregate formation inhibitor design is

considered to be one of the important strategies in MTLD for the treatment of AD [139], [140].

Table 4 shows the potential of the title compounds to inhibit amyloid beta aggregation. As seen, majority of the compounds displayed moderate activity. The percent inhibition range of the title molecules were found to be 25-50% in general. Different than enzyme inhibition assays, the activities of URO series were found slightly higher. The most active compound in the series was naphthyl substituted derivative, URO 7. None of the compounds displayed superior activity in comparison to the activity of resveratrol, the reference molecule used. On the other hand, the activities of both URO and THU series were found comparable or superior to the activity of currently used cholinesterase inhibitor drug donepezil. The inhibitory potentials of synthesis intermediates against amyloid beta aggregation were weaker in comparison to the activities of the majority of title amide derivatives.

Table 4: The potential of the compounds to inhibit amyloid beta aggregation

URO series	**% Inhibition**	**THU series**	**% Inhibition**
URO 1	20.8 ± 1.2	THU 1	17.2 ± 1.4
URO 2	18.8 ± 1.6	THU 2	18.5 ± 2.9
URO 3	18.9 ± 1.4	THU 3	15.4 ± 1.1
URO 4	44.2 ± 0.9	THU 4	28.1 ± 1.9
URO 5	28.1 ± 2.9	THU 5	22.8 ± 1.4
URO 6	27.9 ± 0.8	THU 6	19.8 ± 3.1
URO 7	51.9 ± 1.4	THU 7	30.9 ± 1.8
URO 8	30.1 ± 1.0	THU 8	24.7 ± 0.5
URO 9	25.8 ± 1.5	THU 9	23.8 ± 1.7
URO 10	36.1 ± 2.0	THU 10	23.9 ± 2.0
Resveratrol	70.9 ± 0.55	Donepezil	26.1 ± 1.1

The overall evaluation on the basis of the results obtained for amyloid beta aggregation assays pointed out the improvable characteristics of the molecules to

increase their amyloid beta aggregation preventive characteristics, since coumarine derivatives in general also possess potential to inhibit amyloid beta aggregation [141]. From this perspective, it might be considered that the coumarine scaffold in the structures of the molecules plays the major role in the inhibition of amyloid beta aggregation, since the majority of title molecules displayed similar inhibitory characteristics.

5.5 The potential of the compounds to act as antioxidants

The Oxygen Radical Absorbance Capacity (ORAC) assay was used to screen the antioxidant potential of the title compounds. Trolox was used as standard and the results are presented as micromole trolox equivalence, as seen in Table 5.

Table 5: ORAC assay results of the title molecules

Title molecules URO series	**ORAC (µmolTrolox equivalent / µmol of test compound)**	**Title molecules THU series**	**ORAC (µmolTrolox equivalent / µmol of test compound)**
URO 1	4.2 ± 0.02	**THU 1**	4.7 ± 0.21
URO 2	4.0 ± 0.14	**THU 2**	4.4 ± 0.07
URO 3	4.7 ± 0.27	**THU 3**	4.3 ± 0.04
URO 4	3.3 ± 0.07	**THU 4**	3.7 ± 0.28
URO 5	3.7 ± 0.10	**THU 5**	3.2 ± 0.15
URO 6	4.1 ± 0.21	**THU 6**	3.5 ± 0.24
URO 7	3.4 ± 0.11	**THU 7**	3.5 ± 0.04
URO 8	2.9 ± 0.16	**THU 8**	3.2 ± 0.19
URO 9	2.9 ± 0.32	**THU 9**	3.3 ± 0.07
URO 10	3.0 ± 0.08	**THU 10**	3.3 ± 0.28

According to the results, all the title molecules, including the synthesis intermediates, displayed 3-4 μM trolox equivalence at their 1 μM concentration. In general, the title amides were found to possess higher activity in comparison to the activity of synthesis intermediates. Coumarine base compounds are known to be active under ORAC assay conditions [122]. Since URO and THU series are coumarine based compounds, the overall activities obtained for the title molecules might be relevant to the coumarine scaffold in their structures. The similar antioxidant activities obtained might be also attributed to this feature.

5.6 Conclusion

14 amide functionalized 3-substituted benzo[c]chromen-6-one (URO 4-10) and 7,8,9,10-tetrahydrobenzo[c]chromen (THU 4-10) have been designed and synthesized concomitant to synthesis intermediates (URO 1-3 and THU 1-3 series).

Among the molecules THU 4 has been formed as the most potent AChE and BuChE inhibitor molecule. Although the activity of THU 4 against AChE were lower in comparison to the activity of donepezil, the reference drug molecule, it’s activity were higher with respect to the activities of galantamine, another AChE reference, on both cholinesterases and the activity of donepezil against BuChE. The docking studies revealed out the predicted binding modes of THU 4 with cholinesterases. Accordingly, the lactone carbonyl and the benzyl group have been found critical to dock and interact with the active site of the enzymes.

THU 8, a propargyl substituted derivative displayed the highest activity against MAO-B enzymes. The docking studies pointed out the significance of several hydrogen bonding and hydrogen interactions. Since its urolithin analogue URO 8 also displaying high potential to inhibit MAO-B, the results pointed out the importance of propargyl similar to propargyl bearing drug molecules such as pargyline and rasagiline. However, these compounds were not as potent as the reference molecule pargyline employed.

The anti-oxidant assay results under ORAC assay conditions showed that each test material possessed antioxidant activity. Therefore, these results were related to the general scaffold of the title molecules (i.e., the chromen ring and the spacer unit).

Overall, the results obtained under the experimental conditions utilized displayed that the title molecules have multi-target ligand acting capacity against some of the validated and non-validated targets of AD.

REFERENCES

[1] Yiannopoulou, Konstantina G., and Sokratis G. Papageorgiou. "Current and future treatments in alzheimer disease: an update." *Journal of central nervous system disease* 12 (2020): 1179573520907397.

[2] A. Association, "Alzheimer's disease facts and figures," *Alzheimer's Dement.*, vol. 13, no. 4, pp. 325–373, 2017.

[3] T. Gómez-Isla, J. L. Price, D. W. McKeel Jr, J. C. Morris, J. H. Growdon, and B. T. Hyman, "' Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease': Correction.," 1997.

[4] Y. Huang and L. Mucke, "Review Alzheimer Mechanisms and Therapeutic Strategies," *Cell*, vol. 148, no. 6, pp. 1204–1222, 2012.

[5] D. M. Castro, C. Dillon, G. Machnicki, and R. F. Allegri, "The economic cost of Alzheimer ' s disease Family or public-health burden ?," vol. 4, no. 4, pp. 262–267, 2010.

[6] W. H. Suh, K. S. Suslick, and Y. Suh, "Therapeutic Agents for Alzheimer ' s Disease," pp. 259–269, 2005.

[7] P. Werner, "Knowledge about symptoms of Alzheimer's disease: correlates and relationship to help-seeking behavior," *Int. J. Geriatr. Psychiatry*, vol. 18, no. 11, pp. 1029–1036, 2003.

[8] L. Altweck, T. C. Marshall, N. Ferenczi, and K. Lefringhausen, “Mental health literacy: a cross-cultural approach to knowledge and beliefs about depression, schizophrenia and generalized anxiety disorder,” *Front. Psychol.*, vol. 6, p. 1272, 2015.

[9] G. M. McKhann *et al.*, “The diagnosis of dementia due to Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease,” *Alzheimer’s Dement.*, vol. 7, no. 3, pp. 263–269, 2011.

[10] N. Mattsson *et al.*, “Erratum: CSF biomarker variability in the Alzheimer’s Association quality control program (Alzheimer’s and Dementia (2013) 9 (251-261)),” *Alzheimer’s Dement.*, vol. 11, no. 2, p. 237, 2015.

[11] M. Herrera-Rivero, “Late-onset Alzheimer’s disease: risk factors, clinical diagnosis and the search for biomarkers,” *Neurodegener. Dis.*, p. 35, 2013.

[12] S. Sharma and W. Lippincott, “Emerging biomarkers in Alzheimer’s disease (Recent Update),” *Curr. Alzheimer Res.*, 2018.

[13] C. Ballard, Z. Khan, H. Clack, and A. Corbett, “Nonpharmacological Treatment of Alzheimer Disease,” *Can. J. Psychiatry*, vol. 56, no. 10, pp. 589–595, Oct. 2011.

[14] A. Martínez, *Emerging Drugs and Targets for Alzheimer’s Disease: Volume 1: Beta-Amyloid, Tau Protein and Glucose Metabolism*. Royal Society of

Chemistry, 2010.

[15] A. Mohsin, “Donepezil : A review of the recent structural modifications and their impact on anti-Alzheimer activity,” pp. 1–16, 2012.

[16] A. Clegg *et al.*, “Clinical and cost-effectiveness of donepezil rivastigmine and galantamine for Alzheimer’s disease: a rapid and systematic review,” in *Database of Abstracts of Reviews of Effects (DARE): Quality-assessed Reviews [Internet]*, Centre for Reviews and Dissemination (UK), 2001.

[17] R. A. Hansen, G. Gartlehner, A. P. Webb, L. C. Morgan, C. G. Moore, and D. E. Jonas, “Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer’s disease: a systematic review and meta-analysis,” *Clin. Interv. Aging*, vol. 3, no. 2, p. 211, 2008.

[18] H. O. Gülcan and I. E. Orhan, “The Neurodegenerative Characteristics of Alzheimer’s Disease and Related Multi-Target Drug Design Studies,” in *Pharmaceutical Biocatalysis*, Jenny Stanford Publishing, 2019, pp. 473–504.

[19] ozan gulcan Sahin, Mustafa Fethi, “Bioorganic & Medicinal Chemistry Design , synthesis and biological evaluation of novel 6 H -benzo [c] chromen-6-one derivatives as potential cholinesterase inhibitors,” vol. 22, pp. 5141–5154, 2014.

[20] A. Chatonnet and O. Lockridge, “Comparison of butyrylcholinesterase and acetylcholinesterase,” *Biochem. J.*, vol. 260, no. 3, pp. 625–634, 1989.

[21] H. Sugimoto, "Donepezil hydrochloride: a treatment drug for Alzheimer's disease," *Chem. Rec.*, vol. 1, no. 1, pp. 63–73, 2001.

[22] S. Dall'Acqua, "Plant-derived acetylcholinesterase inhibitory alkaloids for the treatment of Alzheimer's disease," *Bot. Targets Ther.*, vol. 3, pp. 19–28, 2013.

[23] D. M. Robinson and G. M. Keating, "Memantine," *Drugs*, vol. 66, no. 11, pp. 1515–1534, 2006.

[24] R. Howard *et al.*, "Donepezil and memantine for moderate-to-severe Alzheimer's disease," *N. Engl. J. Med.*, vol. 366, no. 10, pp. 893–903, 2012.

[25] R. M. Lane, S. G. Potkin, and A. Enz, "Targeting acetylcholinesterase and butyrylcholinesterase in dementia," *Int. J. Neuropsychopharmacol.*, vol. 9, no. 1, pp. 101–124, 2006.

[26] W. H. Suh, K. S. Suslick, and Y.-H. Suh, "Therapeutic agents for Alzheimer's disease," *Curr. Med. Chem. Nerv. Syst. Agents*, vol. 5, no. 4, pp. 259–269, 2005.

[27] M. B. Colovic, D. Z. Krstic, T. D. Lazarevic-Pasti, A. M. Bondzic, and V. M. Vasic, "Acetylcholinesterase inhibitors: pharmacology and toxicology," *Curr. Neuropharmacol.*, vol. 11, no. 3, pp. 315–335, 2013.

[28] Y. Madav, S. Wairkar, and B. Prabhakar, "Recent therapeutic strategies targeting beta amyloid and tauopathies in Alzheimer's disease," *Brain Res.*

Bull., vol. 146, pp. 171–184, 2019.

[29] A. Haake, K. Nguyen, L. Friedman, B. Chakkamparambil, and G. T. Grossberg, "An update on the utility and safety of cholinesterase inhibitors for the treatment of Alzheimer's disease," *Expert Opin. Drug Saf.*, vol. 19, no. 2, pp. 147–157, 2020.

[30] S. Darvesh, D. A. Hopkins, and C. Geula, "Neurobiology of butyrylcholinesterase," *Nat. Rev. Neurosci.*, vol. 4, no. 2, pp. 131–138, 2003.

[31] J. Patočka, K. Kuča, and D. Jun, "Acetylcholinesterase and butyrylcholinesterase–important enzymes of human body," *Acta Medica (Hradec Kral.*, vol. 47, no. 4, pp. 215–228, 2004.

[32] G. Mushtaq, N. H Greig, J. A Khan, and M. A Kamal, "Status of acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease and type 2 diabetes mellitus," *CNS Neurol. Disord. Targets (Formerly Curr. Drug Targets-CNS Neurol. Disord.*, vol. 13, no. 8, pp. 1432–1439, 2014.

[33] A. Asghar *et al.*, "Synthesis, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities, and molecular docking studies of a novel compound based on combination of flurbiprofen and isoniazide," *RSC Adv.*, vol. 10, no. 33, pp. 19346–19352, 2020.

[34] O. Lockridge, "Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses,"

Pharmacol. Ther., vol. 148, pp. 34–46, 2015.

[35] E. S. Valenstein, "The discovery of chemical neurotransmitters," *Brain Cogn.*, vol. 49, no. 1, pp. 73–95, 2002.

[36] G. R. VandenBos, *APA dictionary of psychology.* American Psychological Association, 2007.

[37] D. B. Drachman, "Trophic functions of the neuron. 3. Mechanisms of neurotrophic interactions. The role of acetylcholine as a neurotropic transmitter.," *Ann. N. Y. Acad. Sci.*, vol. 228, pp. 160–176, 1974.

[38] C. G. Coleman, H. A. Baghdoyan, and R. Lydic, "Dialysis delivery of an adenosine A2A agonist into the pontine reticular formation of C57BL/6J mouse increases pontine acetylcholine release and sleep," *J. Neurochem.*, vol. 96, no. 6, pp. 1750–1759, 2006.

[39] M. S. Buckwalter *et al.*, "Increased T cell recruitment to the CNS after amyloid β1–42 immunization in Alzheimer's mice overproducing transforming growth factor-β1," *J. Neurosci.*, vol. 26, no. 44, pp. 11437–11441, 2006.

[40] S. Tuccaron, "The synthesis of acetylcholine: twenty years of progress," in *Progress in brain research*, vol. 84, Elsevier, 1990, pp. 467–477.

[41] G. Ehrenstein, Z. Galdzicki, and G. D. Lange, "The choline-leakage

hypothesis for the loss of acetylcholine in Alzheimer's disease," *Biophys. J.*, vol. 73, no. 3, pp. 1276–1280, 1997.

[42] A. Nordberg, "Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications," *Biol. Psychiatry*, vol. 49, no. 3, pp. 200–210, 2001.

[43] H. Sies, "Oxidative Stress: Concept and Some Practical Aspects," *Antioxidants*, vol. 9, no. 9, p. 852, 2020.

[44] D. J. Bonda *et al.*, "Oxidative stress in Alzheimer disease: a possibility for prevention," *Neuropharmacology*, vol. 59, no. 4–5, pp. 290–294, 2010.

[45] R. J. Castellani *et al.*, "Active glycation in neurofibrillary pathology of Alzheimer disease: Nε-(carboxymethyl) lysine and hexitol-lysine," *Free Radic. Biol. Med.*, vol. 31, no. 2, pp. 175–180, 2001.

[46] A. Nunomura *et al.*, "RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease," *J. Neurosci.*, vol. 19, no. 6, pp. 1959–1964, 1999.

[47] M. Ruthirakuhan *et al.*, "Agitation, oxidative stress, and cytokines in Alzheimer disease: biomarker analyses from a clinical trial with nabilone for agitation," *J. Geriatr. Psychiatry Neurol.*, vol. 33, no. 4, pp. 175–184, 2020.

[48] G. Aliev, M. A. Smith, M. E. Obrenovich, C. Jack, and G. Perry, "Role of vascular hypoperfusion-induced oxidative stress and mitochondria failure in

the pathogenesis of Alzheimer disease," *Neurotox. Res.*, vol. 5, no. 7, pp. 491–504, 2003.

[49] S. Aldred, S. Bennett, and P. Mecocci, "Increased low-density lipoprotein oxidation, but not total plasma protein oxidation, in Alzheimer's disease," *Clin. Biochem.*, vol. 43, no. 3, pp. 267–271, 2010.

[50] Y. Christen, "Oxidative stress and Alzheimer disease," *Am. J. Clin. Nutr.*, vol. 71, no. 2, pp. 621S-629S, 2000.

[51] G. Jo *et al.*, "Discovery of monoamine oxidase A inhibitors derived from in silico docking," *Bull. Korean. Chem. Soc*, vol. 33, pp. 3841–3844, 2012.

[52] J. H. Quastel, "Amine oxidases," in *Control Mechanisms in the Nervous System*, Springer, 1970, pp. 285–312.

[53] Ö. D. Can *et al.*, "MAO enzymes inhibitory activity of new benzimidazole derivatives including hydrazone and propargyl side chains," *Eur. J. Med. Chem.*, vol. 131, pp. 92–106, 2017.

[54] M. B. H. Youdim, "Monoamine oxidase inhibitors, and iron chelators in depressive illness and neurodegenerative diseases," *J. Neural Transm.*, vol. 125, no. 11, pp. 1719–1733, 2018.

[55] T. Nagatsu, "Progress in monoamine oxidase (MAO) research in relation to genetic engineering," *Neurotoxicology*, vol. 25, no. 1–2, pp. 11–20, 2004.

[56] V. Glover, M. Sandler, F. Owen, and G. J. Riley, "Dopamine is a monoamine oxidase B substrate in man," *Nature*, vol. 265, no. 5589, pp. 80–81, 1977.

[57] J. P. M. Finberg, "Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release," *Pharmacol. Ther.*, vol. 143, no. 2, pp. 133–152, 2014.

[58] M. D. Berry, A. V Juorio, and I. A. Paterson, "The functional role of monoamine oxidases A and B in the mammalian central nervous system," *Prog. Neurobiol.*, vol. 42, no. 3, pp. 375–391, 1994.

[59] K. F. Tipton, M. B. H. Youdim, C. J. Barwell, B. A. Callingham, and G. A. Lyles, "Amine oxidases: function and dysfunction," *J. Neural Trans*, vol. 41, pp. 1–57, 1992.

[60] J. P. M. Finberg, M. B. H. Youdim, P. Riederer, and K. F. Tipton, *MAO-the mother of all amine oxidases*, vol. 52. Springer Science & Business Media, 2013.

[61] S. Manzoor and N. Hoda, "A Comprehensive Review of Monoamine Oxidase Inhibitors as Anti-Alzheimer's Disease Agents: A Review," *Eur. J. Med. Chem.*, p. 112787, 2020.

[62] M. Abdel-Daim, "Md. Sahab Uddin, Abdullah Al Mamun, Md. Tanvir Kabir, Ghulam Md Ashraf, May N. Bin-Jumah & Mohamed."

[63] T. Yoshikawa and Y. Naito, “What is oxidative stress?,” *Japan Med. Assoc. J.*, vol. 45, no. 7, pp. 271–276, 2002.

[64] A. W. K. Yeung, M. G. Georgieva, A. G. Atanasov, and N. T. Tzvetkov, “Monoamine oxidases (MAOs) as privileged molecular targets in neuroscience: Research literature analysis,” *Front. Mol. Neurosci.*, vol. 12, p. 143, 2019.

[65] B. Kumar *et al.*, “4, 6-Diphenylpyrimidine derivatives as dual inhibitors of monoamine oxidase and acetylcholinesterase for the treatment of Alzheimer’s disease,” *ACS Chem. Neurosci.*, vol. 10, no. 1, pp. 252–265, 2018.

[66] C. Yamali *et al.*, “Phenothiazine-based Chalcones as Potential Dual-Target Inhibitors Towards Cholinesterases (AChE, BuChE) and Monoamine Oxidases (MAO-A, MAO-B),” *J. Heterocycl. Chem.*, 2020.

[67] J. T. Coyle, D. L. Price, and M. R. Delong, “Alzheimer’s disease: a disorder of cortical cholinergic innervation,” *Science (80-.).*, vol. 219, no. 4589, pp. 1184–1190, 1983.

[68] S. C. Newman, “The prevalence of depression in Alzheimer’s disease and vascular dementia in a population sample,” *J. Affect. Disord.*, vol. 52, no. 1–3, pp. 169–176, 1999.

[69] A. M. Palmer, G. C. Stratmann, A. W. Procter, and D. M. Bowen, “Possible neurotransmitter basis of behavioral changes in Alzheimer’s disease,” *Ann.*

Neurol. Off. J. Am. Neurol. Assoc. Child Neurol. Soc., vol. 23, no. 6, pp. 616–620, 1988.

[70] P. F. Good, P. Werner, A. Hsu, C. W. Olanow, and D. P. Perl, "Evidence of neuronal oxidative damage in Alzheimer's disease.," *Am. J. Pathol.*, vol. 149, no. 1, p. 21, 1996.

[71] J. Saura *et al.*, "Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography," *Neuroscience*, vol. 62, no. 1, pp. 15–30, 1994.

[72] M. Weinstock, "Selectivity of cholinesterase inhibition," *CNS Drugs*, vol. 12, no. 4, pp. 307–323, 1999.

[73] M. Sano *et al.*, "A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease," *N. Engl. J. Med.*, vol. 336, no. 17, pp. 1216–1222, 1997.

[74] S. Abu-Raya, E. Blaugrund, V. Trembovler, E. Shilderman-Bloch, E. Shohami, and P. Lazarovici, "Rasagiline, a monoamine oxidase-B inhibitor, protects NGF-differentiated PC12 cells against oxygen-glucose deprivation," *J. Neurosci. Res.*, vol. 58, no. 3, pp. 456–463, 1999.

[75] I. A. Paterson and W. G. Tatton, "Antiapoptotic actions of monoamine oxidase B inhibitors," in *Advances in Pharmacology*, vol. 42, Elsevier, 1997, pp. 312–315.

[76] M. B. H. Youdim, A. Wadia, W. Tatton, and M. Weinstock, "The anti-Parkinson drug rasagiline and its cholinesterase inhibitor derivatives exert neuroprotection unrelated to MAO inhibition in cell culture and in vivo," *Ann. N. Y. Acad. Sci.*, vol. 939, no. 1, pp. 450–458, 2001.

[77] C. W. Olanow *et al.*, "A double-blind, delayed-start trial of rasagiline in Parkinson's disease," *N. Engl. J. Med.*, vol. 361, no. 13, pp. 1268–1278, 2009.

[78] M. B. H. Youdim, "The path from anti Parkinson drug selegiline and rasagiline to multifunctional neuroprotective anti Alzheimer drugs ladostigil and m30," *Curr. Alzheimer Res.*, vol. 3, no. 5, pp. 541–550, 2006.

[79] J. Knoll, "History of deprenyl--the first selective inhibitor of monoamine oxidase type B," *Vopr. Med. Khim.*, vol. 43, no. 6, pp. 482–493, 1997.

[80] Y. Li *et al.*, "Multitarget drug design strategy against Alzheimer's disease: Homoisoflavonoid Mannich base derivatives serve as acetylcholinesterase and monoamine oxidase B dual inhibitors with multifunctional properties," *Bioorg. Med. Chem.*, vol. 25, no. 2, pp. 714–726, 2017.

[81] W. Liu *et al.*, "Design, synthesis and evaluation of novel dual monoamine-cholinesterase inhibitors as potential treatment for Alzheimer's disease," *Neuropharmacology*, vol. 109, pp. 376–385, 2016.

[82] Z. Sang *et al.*, "Design, synthesis and biological evaluation of phthalimide-alkylamine derivatives as balanced multifunctional cholinesterase and

monoamine oxidase-B inhibitors for the treatment of Alzheimer's disease," *Bioorg. Med. Chem. Lett.*, vol. 27, no. 22, pp. 5053–5059, 2017.

[83] O. Bar-Am, O. Weinreb, T. Amit, and M. B. H. Youdim, "The novel Cholinesterase–Monoamine Oxidase inhibitor and Antioxidant, Ladostigil, confers Neuroprotection in neuroblastoma cells and aged rats," *J. Mol. Neurosci.*, vol. 37, no. 2, pp. 135–145, 2009.

[84] Q. He *et al.*, "Coumarin-dithiocarbamate hybrids as novel multitarget AChE and MAO-B inhibitors against Alzheimer's disease: Design, synthesis and biological evaluation," *Bioorg. Chem.*, vol. 81, pp. 512–528, 2018.

[85] S. Tejada *et al.*, "Neuroprotective effects of Ellagitannins: A brief review," *Curr. Drug Targets*, vol. 18, no. 13, pp. 1518–1528, 2017.

[86] J. M. Lorenzo, P. E. Munekata, P. Putnik, D. B. Kovačević, V. Muchenje, and F. J. Barba, "Sources, Chemistry, and Biological Potential of Ellagitannins and Ellagic Acid Derivatives," in *Studies in Natural Products Chemistry*, vol. 60, Elsevier, 2019, pp. 189–221.

[87] J. C. Espín, M. Larrosa, M. T. García-Conesa, and F. Tomás-Barberán, "Biological significance of urolithins, the gut microbial ellagic acid-derived metabolites: the evidence so far," *Evidence-Based Complement. Altern. Med.*, vol. 2013, 2013.

[88] T. Yoshida, Y. Amakura, and M. Yoshimura, "Structural features and

biological properties of ellagitannins in some plant families of the order Myrtales," *Int. J. Mol. Sci.*, vol. 11, no. 1, pp. 79–106, 2010.

[89] J. Yuzugulen, B. Noshadi, K. Shukur, M. F. Sahin, and H. O. Gulcan, "The metabolites of ellagitannin metabolism urolithins display various biological activities," *EMU J. Pharm. Sci.*, vol. 2, no. 2, pp. 102–110, 2019.

[90] R. García-Villalba, J. A. Giménez-Bastida, M. A. Ávila-Gálvez, F. A. Tomás-Barberán, J. C. Espín, and A. González-Sarrías, "Ellagitannins and Their Gut Microbiota-Derived Metabolites: Urolithins," *Diet. Polyphenols Their Metab. Heal. Eff.*, pp. 319–364, 2020.

[91] R. Gonzalez-Barrio, P. Truchado, H. Ito, J. C. Espin, and F. A. Tomas-Barberan, "UV and MS identification of urolithins and nasutins, the bioavailable metabolites of ellagitannins and ellagic acid in different mammals," *J. Agric. Food Chem.*, vol. 59, no. 4, pp. 1152–1162, 2011.

[92] B. Cerdá, R. Llorach, J. J. Cerón, J. C. Espín, and F. A. Tomás-Barberán, "Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice," *Eur. J. Nutr.*, vol. 42, no. 1, pp. 18–28, 2003.

[93] B. Cerdá, F. A. Tomás-Barberán, and J. C. Espín, "Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability," *J. Agric. Food Chem.*, vol. 53, no. 2, pp. 227–235, 2005.

[94] B. Doyle and L. A. Griffiths, “The metabolism of ellagic acid in the rat,” *Xenobiotica*, vol. 10, no. 4, pp. 247–256, 1980.

[95] E. Lederer, “Chemistry and biochemistry of some mammalian secretions and excretions,” *J. Chem. Soc.*, pp. 2115–2125, 1949.

[96] M. Romo-Vaquero *et al.*, “Interindividual variability in the human metabolism of ellagic acid: Contribution of Gordonibacter to urolithin production,” *J. Funct. Foods*, vol. 17, pp. 785–791, 2015.

[97] J. A. Giménez-Bastida *et al.*, “Urolithins, ellagitannin metabolites produced by colon microbiota, inhibit quorum sensing in Yersinia enterocolitica: phenotypic response and associated molecular changes,” *Food Chem.*, vol. 132, no. 3, pp. 1465–1474, 2012.

[98] S. Alfei, B. Marengo, and G. Zuccari, “Oxidative Stress, Antioxidant Capabilities, and Bioavailability: Ellagic Acid or Urolithins?,” *Antioxidants*, vol. 9, no. 8, p. 707, 2020.

[99] L. Mele *et al.*, “Antiatherogenic effects of ellagic acid and urolithins in vitro,” *Arch. Biochem. Biophys.*, vol. 599, pp. 42–50, 2016.

[100] C. Sánchez-González, C. J. Ciudad, V. Noe, and M. Izquierdo-Pulido, “Health benefits of walnut polyphenols: An exploration beyond their lipid profile,” *Crit. Rev. Food Sci. Nutr.*, vol. 57, no. 16, pp. 3373–3383, 2017.

[101] J. Djedjibegovic, A. Marjanovic, E. Panieri, and L. Saso, “Ellagic Acid-Derived Urolithins as Modulators of Oxidative Stress,” *Oxid. Med. Cell. Longev.*, vol. 2020, 2020.

[102] C. Usta, S. Ozdemir, M. Schiariti, and P. E. Puddu, “The pharmacological use of ellagic acid-rich pomegranate fruit,” *Int. J. Food Sci. Nutr.*, vol. 64, no. 7, pp. 907–913, 2013.

[103] A. Crozier, D. Del Rio, and M. N. Clifford, “Bioavailability of dietary flavonoids and phenolic compounds,” *Mol. Aspects Med.*, vol. 31, no. 6, pp. 446–467, 2010.

[104] F. A. Tomás-Barberan, J. C. Espín, and M. T. García-Conesa, “Bioavailability and metabolism of ellagic acid and ellagitannins,” in *Chemistry and biology of ellagitannins: an underestimated class of Bioactive plant polyphenols*, World Scientific, 2009, pp. 273–297.

[105] M. Monagas *et al.*, “Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites,” *Food Funct.*, vol. 1, no. 3, pp. 233–253, 2010.

[106] S. Alfei *et al.*, “Ellagic acid a multi-target bioactive compound for drug discovery in CNS? A narrative review,” *Eur. J. Med. Chem.*, vol. 183, p. 111724, 2019.

[107] L. Wang, “Pomegranate Juice and Pomegranate Derived Natural Products as

Alternative Treatment for Cancer Progression and Metastasis." UC Riverside, 2013.

[108] M. Larrosa *et al.*, "Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model," *J. Agric. Food Chem.*, vol. 57, no. 6, pp. 2211–2220, 2009.

[109] D. Bialonska, S. G. Kasimsetty, S. I. Khan, and D. Ferreira, "Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay," *J. Agric. Food Chem.*, vol. 57, no. 21, pp. 10181–10186, 2009.

[110] Z. Mao, W. Sun, L. Fu, H. Luo, D. Lai, and L. Zhou, "Natural dibenzo-α-pyrones and their bioactivities," *Molecules*, vol. 19, no. 4, pp. 5088–5108, 2014.

[111] T. Kallio, J. Kallio, M. Jaakkola, M. Mäki, P. Kilpeläinen, and V. Virtanen, "Urolithins display both antioxidant and pro-oxidant activities depending on assay system and conditions," *J. Agric. Food Chem.*, vol. 61, no. 45, pp. 10720–10729, 2013.

[112] B. Noshadi *et al.*, "Synthesis, Characterization, Molecular Docking, and Biological Activities of Some Natural and Synthetic Urolithin Analogs," *Chem. Biodivers.*, vol. 17, no. 8, p. e2000197, 2020.

[113] M. Norouzbahari *et al.*, "Design, synthesis and characterization of novel

urolithin derivatives as cholinesterase inhibitor agents," *Lett. Drug Des. Discov.*, vol. 15, no. 11, pp. 1131–1140, 2018.

[114] C. P. Hiwase, N. S. Bhandekar, M. J. Umekar, and K. R. Gupta, "Therapeutic approaches in alzheimer's disease: β-amyloid peptide inhibitors."

[115] A. Kumar, J. Sidhu, A. Goyal, and J. W. Tsao, *Alzheimer Disease*. StatPearls Publishing, Treasure Island (FL), 2020.

[116] S.-Y. Hung and W.-M. Fu, "Drug candidates in clinical trials for Alzheimer's disease," *J. Biomed. Sci.*, vol. 24, no. 1, p. 47, 2017.

[117] F.-M. Werner and R. Covenas, "Serotonergic drugs: agonists/antagonists at specific serotonergic subreceptors for the treatment of cognitive, depressant and psychotic symptoms in Alzheimer's disease," *Curr. Pharm. Des.*, vol. 22, no. 14, pp. 2064–2071, 2016.

[118] O. Holas, K. Musilek, M. Pohanka, and K. Kuca, "The progress in the cholinesterase quantification methods," *Expert Opin. Drug Discov.*, vol. 7, no. 12, pp. 1207–1223, 2012.

[119] M. Çeçen *et al.*, "Design, Synthesis, and Biological Evaluation of Pyridazinones Containing the (2-Fluorophenyl) Piperazine Moiety as Selective MAO-B Inhibitors," *Molecules*, vol. 25, no. 22, p. 5371, 2020.

[120] K. Gade Malmos *et al.*, "ThT 101: a primer on the use of thioflavin T to

investigate amyloid formation," *Amyloid*, vol. 24, no. 1, pp. 1–16, 2017.

[121] G. Cao, H. M. Alessio, and R. G. Cutler, "Oxygen-radical absorbance capacity assay for antioxidants," *Free Radic. Biol. Med.*, vol. 14, no. 3, pp. 303–311, 1993.

[122] R. F. Guíñez *et al.*, "Synthesis and evaluation of antioxidant and trypanocidal properties of a selected series of coumarin derivatives," *Future Med. Chem.*, vol. 5, no. 16, pp. 1911–1922, 2013.

[123] S. Release, "2: Maestro, Schrödinger, LLC, New York, NY, 2017," *Receiv. Febr.*, vol. 21, p. 2018, 2016.

[124] S. Release, "2: LigPrep, Schrödinger, LLC, New York, NY, 2017," *New York, NY*, 2017.

[125] S. Release, "2: Maestro, version 11.8. Schrödinger, LLC, New York." 2018.

[126] K. S. Watts, P. Dalal, R. B. Murphy, W. Sherman, R. A. Friesner, and J. C. Shelley, "ConfGen: a conformational search method for efficient generation of bioactive conformers," *J. Chem. Inf. Model.*, vol. 50, no. 4, pp. 534–546, 2010.

[127] S. Release, "4: Glide," *Schrödinger, LLC, New York, NY*, 2018.

[128] S. Prime, "LLC: New York," *NY, USA*, 2018.

[129] A. Samadi *et al.*, “Synthesis, biological assessment and molecular modeling of new multipotent MAO and cholinesterase inhibitors as potential drugs for the treatment of Alzheimer’s disease,” *Eur. J. Med. Chem.*, vol. 46, no. 9, pp. 4665–4668, 2011.

[130] J. Sterling *et al.*, “Novel dual inhibitors of AChE and MAO derived from hydroxy aminoindan and phenethylamine as potential treatment for Alzheimer’s disease,” *J. Med. Chem.*, vol. 45, no. 24, pp. 5260–5279, 2002.

[131] L. Emilsson, P. Saetre, J. Balciuniene, A. Castensson, N. Cairns, and E. E. Jazin, “Increased monoamine oxidase messenger RNA expression levels in frontal cortex of Alzheimer’s disease patients,” *Neurosci. Lett.*, vol. 326, no. 1, pp. 56–60, 2002.

[132] E. Borroni *et al.*, “Sembragiline: a novel, selective monoamine oxidase type B inhibitor for the treatment of Alzheimer’s disease,” *J. Pharmacol. Exp. Ther.*, vol. 362, no. 3, pp. 413–423, 2017.

[133] M. S. Uddin, M. T. Kabir, M. M. Rahman, B. Mathew, M. A. Shah, and G. M. Ashraf, “TV 3326 for Alzheimer’s dementia: a novel multimodal ChE and MAO inhibitors to mitigate Alzheimer’s-like neuropathology,” *J. Pharm. Pharmacol.*, 2020.

[134] M. S. Manhas, S. N. Ganguly, S. Mukherjee, A. K. Jain, and A. K. Bose, “Microwave initiated reactions: Pechmann coumarin synthesis, Biginelli reaction, and acylation,” *Tetrahedron Lett.*, vol. 47, no. 14, pp. 2423–2425,

2006.

[135] H. O. Gulcan, E. Kupeli, S. Unlu, E. Yesilada, and M. F. Sahin, “4-(5-Chloro-2 (3H)-benzoxazolon-3-yl) Butanoic Acid Derivatives: Synthesis, Antinociceptive and Anti-inflammatory Properties,” *Arch. der Pharm. An Int. J. Pharm. Med. Chem.*, vol. 336, no. 10, pp. 477–482, 2003.

[136] H. O. GÜLCAN, S. ÜNLÜ, E. BANOĞLU, M. F. ŞAHİN, E. KÜPELİ, and E. YEŞİLADA, “Synthesis of new 4-(5-chloro-2-oxo-3H-benzoxazol-3-yl) butanamide derivatives and their analgesic and anti-inflammatory properties,” *Turkish J. Chem.*, vol. 27, no. 4, pp. 467–476, 2003.

[137] I. Bolea, A. Gella, and M. Unzeta, “Propargylamine-derived multitarget-directed ligands: fighting Alzheimer’s disease with monoamine oxidase inhibitors,” *J. Neural Transm.*, vol. 120, no. 6, pp. 893–902, 2013.

[138] S. Zhou, G. Chen, and G. Huang, “Design, synthesis and biological evaluation of lazabemide derivatives as inhibitors of monoamine oxidase,” *Bioorg. Med. Chem.*, vol. 26, no. 17, pp. 4863–4870, 2018.

[139] P. Grunwald, *Pharmaceutical Biocatalysis: Fundamentals, Enzyme Inhibitors, and Enzymes in Health and Diseases*. CRC Press, 2019.

[140] B. Kilic *et al.*, “Design and synthesis of some new carboxamide and propanamide derivatives bearing phenylpyridazine as a core ring and the investigation of their inhibitory potential on in-vitro acetylcholinesterase and

butyrylcholinesterase," *Bioorg. Chem.*, vol. 79, pp. 235–249, 2018.

[141] D. D. Soto-Ortega *et al.*, "Inhibition of amyloid-β aggregation by coumarin analogs can be manipulated by functionalization of the aromatic center," *Bioorg. Med. Chem.*, vol. 19, no. 8, pp. 2596–2602, 2011.