Design, Synthesis and Cholinesterase Inhibitory Potential of 6H-benzo[c] Chromen-6-one, and 7,8,9,10-tetrahydro-benzo[c] Chromen-6-one Derivatives

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ABSTRACT

Alzheimer's disease (AD) is one of the neurodegenerative diseases of the central nervous system. Although its first diagnosis by Alois Alzheimer goes back to century, its pathophysiology has not been clearly described. Therefore, there is no drug present that totally cures the disease. The main symptom of the disease, disability in cognitive symptoms, has triggered scientists to discover agents aiding in the relief of the decline in the cognition. Today, we have several agents employed in clinic to slow down the progress of the cognitive decline. These drugs are cholinesterase inhibitor molecules and they do not completely cure the disease state, but they are beneficial in the treatment of AD symptoms. The limited number of drugs and the debate on their efficiency as well as the incidence and economic burden of AD create big opportunities to medicinal chemists to design novel drug candidates that might possess better potency and efficacy for the treatment of the AD.

From this point of view; we have designed novel urolithin analogs as drug candidates for the treatment of cognitive symptoms of AD within this study. Urolithins are benzo [c] chromen analogues, available as ellagitannin metabolites. Ellagitannins are abundant in berry-fruits and various types of nuts obtained through nutrition. Although these type nutrition have been suggested to relief the symptoms of AD, their main absorbed metabolites within the biological systems, urolithins, are not potent inhibitor of cholinesterase enzymes, the validated targets of AD. Employing the drug-like properties of urolithins we have designed ten novel urolithin analogues promising to be the potent inhibitors of AD. The ten novel urolithin derivative

compounds were synthesized with appropriate methodology and their structure identification studies were achieved utilizing modern chromatography and instrumental-spectral techniques. The title compounds synthesized were evaluated in biological experiments. At one hand, their potential to inhibit cholinesterase enzymes was screened. On the other hand, the title compounds were also assayed in the cholinesterase induced amyloid beta aggregation assays.

The results indicated that, the title compounds are promising and their potency is comparable to the activity of current drugs, also employed as references in biological assays. In addition to these, the possible interaction of the title compounds with the active site of the cholinesterase enzyme was also investigated employing the molecular docking programs. The results revealed out potential functional groups present within the design crucial for the determination of the pharmacophore system.

Keywords: Cholinesterase inhibitor, Amyloid beta aggregation, Urolithin derivatives

ÖZ

Alzheimer hastalığı (AH) merkezi sinir sisteminin nörodejeneratif hastalıklarından biridir. Hastalığın Alois Alzheimer tarafından yapılan ilk tanısı, yüzyıl geriye gitse de, patofizyolojisi hala açık bir şekilde tanımlanmamıştır. Bu nedenle, hastalığı tamamen iyileştiren hiçbir ilaç mevcut değildir. Hastalığın ana semptomu, bilişsel belirtilerdeki eksiklilk, bilim insanlarını, bilişteki gerilemenin hafifletilmesine yardımcı olan yeni bileşikleri keşfetmeye yönlendirmiştir. Bugün, bilişsel gerilemenin ilerlemesini yavaşlatmak için klinikte birkaç ilacımız vardır. Bu ilaçlar kolinesteraz inhibitör molekülleridir ve hastalık durumunu tamamen iyileştirmezler, ancak AH semptomlarının tedavisinde yararlıdırlar. Sınırlı sayıda ilaç ve etkinlikleri ile birlikte AH'in insidansı ve ekonomik yükü konusundaki tartışma, farmasötik kimyacıları AH tedavisinde daha iyi potansiyel ve etkinliğe sahip olabilecek yeni ilaç adayları tasarlamak için büyük fırsatlar yaratmaktadır. Bu açıdan bakıldığında; bu çalışmamızda, AH'nin bilişsel belirtilerinin tedavisinde kullanılabilecek ilaç adayı yeni ürolithin analogları tasarladık Urolithinler, ellagitannin metabolitleri olarak bulunan benzo [c] chromen analoglarıdır. Ellagitanninler çilekli meyveler ve kuruyemişlerde bol miktarda bulunmaktadır. Bu tip beslenme, AH semptomlarını hafifletmek için önerilmiş olsa da, biyolojik sistemlerdeki ana emilen metabolitleri olan ürolithinler, AH'in onaylanmış hedefleri olan kolinesteraz enzimlerinin potent inhibitörü değildir. Ürolithinlerin ilaç- olabilme özelliklerini kullanarak, AH'in güçlü inhibitörleri olacağına inandığımız on yeni ürolitin analoğu tasarladık. On yeni ürolitin türevi bileşik, uygun metodoloji ile sentezlendi ve yapı tanımlama çalışmaları, modern kromatografi ve enstrümantal spektral teknikler kullanılarak gerçekleştirildi. Sentezlenen orijinal bileşikler biyolojik deneylerde değerlendirildi.

Bir taraftan, kolinesteraz enzimlerini inhibe etme potansiyeli tarandı. Öte yandan, orijinal bileşikleri aynı zamanda kolinesteraz ile indüklenen amiloid beta agregasyon deneylerinde aktiviteleri incelendi. Sonuçlar, bileşiklerin aktivitelerinin umut verici olduğunu ve potensiyellerinin, biyolojik çalışmalarda kullanılan referans ilaçların aktivitesiyle karşılaştırılabilir olduğunu gösterdi. Buna ek olarak, bileşikler ile kolinesteraz enziminin aktif bölgesi arasındaki olası etkileşim, moleküler yerleştirme programları kullanılarak araştırılmıştır. Sonuçlar, tasarım için kullanılan farmakofor sistemde gerekli olan önemli fonksiyonel grupları ortaya koymuştur.

Anahtar Kelimeler: Kolinesteraz inhibitörü, Amiloid beta agregasyonu, Urolithin türevleri.

To my beloved father who always supported me, whatever path I look.....

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LIST OF SYMBOLS/ABBREVIATIONS

AChE	Acetylcholinesterase
AD	Alzheimer’s disease
AH	Alzheimer hastalığı
APP	Amyloid precursor protein
ASP	Aspartyl protease
Aβ	Amyloid-β
BuChE	Butyrylcholinesterase
C	Concentration
Calcd.	Calculated
Ch	Choline
CNS	Central nervous system
CSF	Cerebrospinal fluid
DMF	Dimethylformamide
EA	Ellagic acid
ETs	Ellagitannins
FT – IR	Fourier transform infrared spectroscopy
HPLC	High-performance liquid chromatography
IR	Infrared spectrum/ spectroscopy
mp	Melting Point
NFT	Neurofibrillary tangle
NMDA	N-Methyl-D-aspartate
NMR	Nuclear Magnetic Resonance Spectroscopy
nM	nanomolar

THU	3-Hydroxy-7,8,9,10-tetrahydro-benzo[c]chromen-6-one
TLC	Thin layer Chromatography
R_f	Retention factor
URO	3-hydroxy-6H-benzo[c]chromen-6-one

Chapter 1

INTRODUCTION

Following the first recognition of Alzheimer's disease (AD) by Alois Alzheimer in 1906 AD has continued to be an interest to scientist, since it affects a big population resulting in cognition disabled people. Statistics and pharmacovigilance studies on the incidence of AD state that the disease is not specific to a country, a continent, or to a race. Even, it's common to see an incidence such as %50 of individual above 85 developing AD is quiet common in most countries [1]. Furthermore, once we remember the economic burden of the disease to patients, patient relatives and even governments, the significance of the disease becomes much more relevant. In other words, more than ten billions of dollars each year are spent for the treatment of the disease and for patient cares, and as well as for the drugs employed worldwide, therefore, it is important to understand the various aspects of AD in order to find out new answers to each of those questions that we still ask on AD. There are quite a lot review type publication on AD, available through scientific and nonscientific (i.e., magazine) sources describing the current status of AD [2].

1.1 Alzheimer's Disease

As indicated by the World Alzheimer Report 2012, AD is among the most important social, prosperity and a realistic emergency of the 21st century [3]. AD is an endless dynamic neurodegenerative illness. In fact, it is a process of cognitive decline. As known by public, dementia is the most known symptom of AD, generally recognized by patient relatives rather than the patient himself. The problem with remembering

things is quiet common to various diseases states, including AD. Therefore, dementia is not specific to AD [4]. The decline in cognitive abilities is a better statement to express dementia in AD. In fact, AD is mainly subcategorized to three steps in accordance with the cognitive decline. In first step, also referred to as mild level AD, the patient relatives recognize the cognitive disabilities of the patient. In the second level (i.e., Moderate level AD), cognitive decline worsens in such a way that communicating, speaking, understanding problems are added on the present symptoms of the mild levels. Unfortunately this contains to a final stage wherein the identification and social problems are certain. At this stage 24 hour patient care must be guaranteed [5].

AD, even in 2018, is a non-curable, life threatening disease. In fact, the above described is just the clear parts of the disease. In reality, these symptoms start with neurodegeneration and nerve loss up to 70% of the neuronal content. Science currently deals with not only improving the diagnoses techniques of the disease but also understanding the physiological and pathophysiological mechanisms of the disease [6].

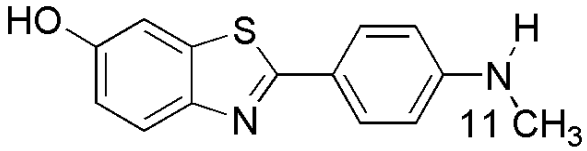
1.2 Diagnosis of Alzheimer's Disease

It is accepted that AD is a progressive disease. In other word, there is a latent period of the disease before the appearing of the symptoms [7]. Therefore, biomarkers and their identification, characteristic to AD, are very important topics of nowadays [8]. It is clinically known that excess amyloid plaque biosynthesis and their precipitation happen during the development of the disease. This accompanies intracellular neurofibrillary tangle formation related to the tau protein hyperphosphorylation [9]. From this point of view cerebrospinal fluid (CSF) amyloid beta, total tau, and

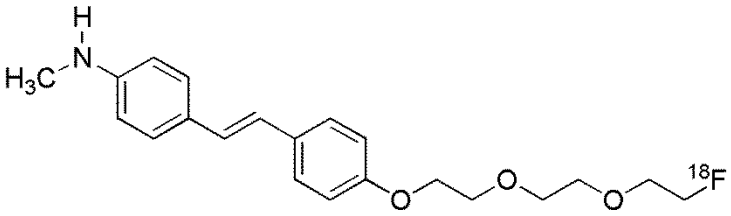
phosphorylated tau are evaluated as AD biomarkers. The major part and the component of amyloid plaques are the β-amyloid peptides consisting of 40 and 42 amino acids ($A\beta_{1-42}$ and $A\beta_{1-40}$), which are derived from the amyloid precursor protein [10]. Neurofibrillary tangles are made up of paired helical filaments consisting of hyperphosphorylated tau protein (phospho-tau). Tau protein is an intracellular protein that is released upon neuronal death. The combination of elevated $A\beta_{1-42}/A\beta_{1-40}$, and total tau and hyperphosphorylation tau in cerebrospinal fluid (CSF) are considered to be the basic components of neurodegeneration in AD [11]. In addition to these, C-reactive protein, homocysteine, α-synuclein index, dehydroepiandrosterone sulphate, lymphocyte microRNA are some other items detected and quantified to monitor the development of this disease [12].

Unfortunately, the imagining of amyloid β-plaques and tau protein hyperphosphorylation is not enough for an absolute diagnosis of the disease, since these are also accompanying clinical outcomes throughout the aging process. Electroencephalography, multimodality fusion imaging, computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), and positron emission tomography (PET) are the computational techniques employed to monitor AD. There is also research interest in finding out biocompatible drugs available to interact the biomarkers during the computational monitoring applications [13]. Some of these compounds are shown in Figure 1. It is obvious that the exact mechanism for the generation of AD is unknown that, in turn, emphasizes the correct diagnosis of the disease, including by an expert physician. This includes memory and cognition related complaints of the patient.

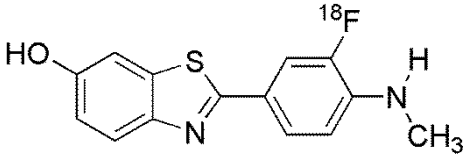
Besides, regarding the basic role of the cholinergic system, within the progress of AD, some techniques are also developed. For instance, the catalytic potential of cholinesterase action can be assessed by radioactive compounds, and nicotinic receptor binding can be evaluated employing ^{11}C- nicotine [14].



2-[4-(methylamino) phenyl]-1, 3-benzothiazol-6-ol
(Trade name, Pittsburgh compound B)



4-[(E)-2-[4-[2-[2-(2-fluoranylethoxy) ethoxy]ethoxy]phenyl]ethenyl]-N-methylaniline
(Trade name, Neuraceq)



2-[3-fluoranyl-4-(methylamino) phenyl]-1,3-benzothiazol-6-ol
(Trade name, Vizamyl)

Figure 1: Structure of drugs used in the diagnosis of AD [15]

1.3 Physiological and Pathophysiological Mechanisms of Alzheimer's Disease

Although more than a century passed following the first diagnosis of AD, the exact physiological and pathophysiological of AD still remains unclear [16]. However,

scientists agree upon that AD is a complex neurodegenerative disease. First of all, it involves neurodegeneration leading to neuronal loss. This accompanies a gradual decrease in the levels of neurotransmitters. Indeed, acetylcholine, dopamine, serotonin are typical examples to those neurotransmitters. Particularly, the decline in the levels of acetylcholine is associated with the cognitive disability symptoms of AD. It is clearly pointed out that M1 and M3 type muscarinic receptors are involved for keeping the cognation in a regular healthy person. Therefore, lower acetylcholine results in lower muscarinic receptor activation that, in turn, triggers cognitive disabilities [17].

As described previously, amyloid-β hypothesis, tau protein trigged neurofibrillary tangle formation are linked to the neurodegeneration in the brain. However the exact mechanisms of hyperphosphorylation and amyloid-β plaque formation have not been totally understood yet. So far, researches have tried to target Aβ biosynthesizing enzymes and kinases responsible for tau protein phosphorylation [18, 19]. α-Secretase, β-secretase, γ-secretase, GSK-3kinase are typical examples to that. Although scientists have been successful to target these enzymes with specific drug candidate agents, the symptoms of AD couldn't be treated. In other words, those drug candidates were shown to efficiently lower the amyloid-β plaque formation and tau protein hyperphosphorylation, but they were not able to relive the symptoms of AD, particularly the cognitive decline [20].

Some other mechanisms were also shown within the generation of AD. Oxidative stress is one of them. In reality oxidative stress is always shown as a component of neurodegenerative diseases, including AD, schizophrenia, and Parkinson disease [21]. The metal catalyzed Fenton Reaction leads to the generation of reactive oxygen

species. Since defence mechanisms are particularly inactivated with respect to both neurodegeneration and inflammation, oxidative stress accelerates the development of AD. Therefore, metal binding agents were also tried for the treatment of disease [22].

1.4 Targets Employed in the Treatment of Alzheimer's Disease

There are various biological targets offered for the treatment of AD. Unfortunately, most of them are clinically nonevalidated targets. Among them, cholinergic system is the main clinically approved biological target [23]. Indeed, currently utilized AD prescribed drugs (i.e., rivastigmine, galantamine, and donepezil) are either acetylcholinesterase or butyrylcholinesterase inhibitor molecules [24]. These drugs increase the acetylcholine levels through the inhibition of cholinesterase enzymes, responsible for the degradation of acetylcholine to acetate and choline (Figure 2) [25]. It is pretty well established that acetylcholine aids in cognitive functions via acting on M1 and M3 type muscarinic receptors [26]. The studies on finding out selective M1 or M3 agonist agents have not revealed out successful results so far. The main reason for the failure is the structural resemblance of muscarinic receptors. Most agents developed for this aim have shown poor selectivity for muscarinic receptors resulting in side effects, beside the benefit obtained for cognitive improvement [27].

Figure 2: Acetylcholinesterase catalyzed degradation of acetylcholine [28]

Memantine is the only alternative agent to cholinesterase inhibitors in the current clinic. It is an NMDA receptor antagonist. NMDA receptor is a kind of glutamatergic receptor involved in the excitation of central nervous system (CNS). When it is

remembered that glutamate, the natural agonist of NMDA receptor, is the main excitatory neurotransmitter of CNS, blocking its activity contributes to the relieving of the symptoms of AD [29]. However, this drug is not approved in every country. The basic reason is the debate ongoing for the clinical benefit of NMDA receptor antagonism for the treatment of AD. Besides, the side effects of memantine are also serious [29-30].

It is impossible to state AD without discussing amyloid-β plaques. As stated previously, AD developed patients generate excessive amyloid-β plaques in CNS. These amyloid-β plaques were shown to be involved in various neurotoxic mechanisms leading to neuronal cell death. Therefore, the relation between the enhancement of cognition and the lowering the levels of amyloid-β plaques has been investigated in detail [31]. From this point of view Aβ cascade has been investigated. Particularly, β-secretase and γ-secretase enzyme inhibitor agents were developed to decrease Aβ peptides from the precursor Aβ protein [32]. Even, vaccines were developed to prevent Aβ aggregation. Unfortunately, those agents were found poor in the gaining of cognitive functions, although some of them were able to successfully clean out Aβ plaques. Oxidative stress, as it is involved in a variety of CNS diseases, has also been investigated for its function in the development of AD. Although there are certain mechanisms pointing out oxidative stress leading neurodegeneration in AD, neither vitamin C nor vitamin E or any other antioxidant agent were found useful for relieving the symptoms of AD [33].

Tau-protein hyperphosphorylation and its aggregation are also important for neurodegeneration. This cascade is also related to Aβ plaque formation. Specific kinases are involved within this hyperphosphorylation process. The studies to find

out kinase inhibitor molecules revealed out poor agents for the treatment of AD, mainly because of selectivity problems [34].

The presence of iron at high amounts is neurotoxic to CNS in various ways. The Fenton Reaction, catalyzed by iron, results in oxygen radical. In addition, iron has been shown to accelerate Aβ plaque formation. Therefore, iron chelating agents were investigated for their aid in the treatment of AD. Unfortunately, these agents were also found out far away for a benefit for the treatment of AD [35].

1.5 Current Drugs Used for the Treatment of Alzheimer's Disease

There are four drugs approved for the treatment of AD worldwide. As indicated, three of these agents are donepezil, rivastigmine, and galantamine, the cholinesterase inhibitor molecules. Among them, donepezil is the most potent one, since it is able to inhibit AChE with an IC_{50} of 8nM [36]. Galantamine, although not potent as donepezil, is another strong AChE inhibitor agent with an IC_{50} around 800 nM. Both donepezil and galantamine are AChE selective agents, since they inhibit BuChE with higher corresponding IC_{50} values [37].

The interpretation of the potential of rivastigmine to inhibit cholinesterase enzymes is quiet difficult. Rivastigmine, different than donepezil and galantamine, is a BuChE selective agent. However, its IC_{50}s for AChE and BuChE are pretty much lower (i.e., around 15μM and 35 μM, respectively for BuChE and AChE) [38].

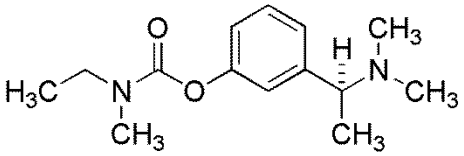
The literature indicates that rivastigmine, with respect to its carbamylation potential, is a long acting cholinesterase inhibitor molecule that might be observed in vivo assays rather than in vitro experiments [39].

The origin of the employment of cholinergic system starts with the introduction of tacrine, another cholinesterase inhibitor molecule, to clinic around 1980s. Tacrine is not an approved drug today. The reason is that tacrine undergoes excessive hepatic metabolism yielding out electrophilic metabolites toxic to hepatic cells. Therefore, tacrine was withdrawn from the market couple years after its approval [40].

Memantine, as stated, an NMDA receptor antagonist, is also utilized for the treatment of AD in some countries. However its effect for the treatment of AD and the benefit of NMDA receptor antagonism are still questioned topics [41]. The structures of these four cholinesterase inhibitor used drugs for the treatment of AD are shown in figure 3.

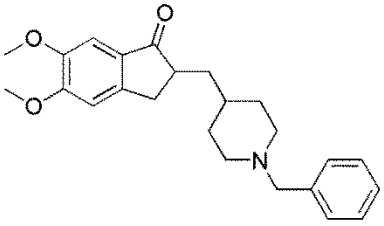
The cholinesterase inhibitors drugs are currently prescribed for the mild and moderate levels of AD. The clinical and pharmacovigilance studies indicate that these drugs are only able to slow down the cognitive disabilities within the progress of AD. In other words, they do not care the disease or provide perfect cognitive improvement. Beside, each of these drugs also displays their known side effects. Particularly, these side effects are related to the peripheral effects [42].

Rivastigmine



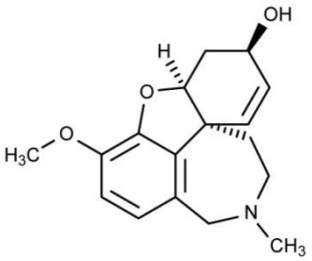
[3-[(1S)-1-(dimethylamino)ethyl]phenyl] N-ethyl-N-methylcarbamate

Donepezile



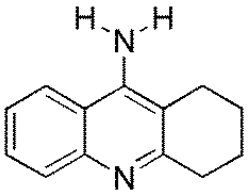
2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one

Galantamine



(4aS,6R,8aS)- 5,6,9,10,11,12- hexahydro- 3-methoxy- 11-methyl- 4aH-[1]benzofuro[3a,3,2-ef] [2] benzazepin- 6-ol

Tacrine



1,2,3,4-tetrahydroacridin-9-amine

Figure 3: Structure of current drugs used for AD [43]

1.6 Objectives of the Study

With respect to the current targets offered for treatment of AD, it is obvious that cholinergic system is the only one approved by the scientific authority [44]. The amyloid-β plaque formation and the consequent tau protein hyperphosphorylation appear to be the outcomes within the progress of AD. Even phase studies for the development of novel molecules for the treatment of AD reveal out that prevention of amyloid-β formation or its total clearance do not stop the progress of the disease or it improves cognitive function [45].

Currently there are three cholinesterase inhibitor drugs (i.e., rivastigmine, galantamine, donepezil) used for the treatment of cognitive disabilities of AD. Unfortunately, these drugs offer benefit for mild to moderate stages of AD. Furthermore, their potential is also quite variable. Particularly considering the limited number of drugs, novel cholinesterase inhibitor agents with better pharmacological properties are definitely needed [46].

In a previous study, it was shown that urolithin (i.e., benzo[c]chromene) derivatives can be converted to potent cholinesterase inhibitor agents. The basic pharmacophore groups within the structures of donepezil, galantamine, rivastigmine were efficiently utilized for the derivatization of those urolithin derivatives in the same study [47]. Within this research our particular aim was to find out alternative derivatization on urolithin compounds. The most abundant technique applied within the design of cholinesterase inhibitor agents is the employment of Aryl-Spacer- Tertiary amine pharmacophore. Within this study the first question we asked was the flexibility of the tertiary amine within this pharmacophore. From this point of view we have decided to utilize 1,2,3,4- tetrahydroisoquinoline structure for the tertiary amine portion of the pharmacophore group embedded on urolithin derivatives . In the next part, we have also questioned the presence of benzyl group on the tertiary amine, as it is present on each cholinesterase inhibitor drugs.

Since the optimum spacer length was found out to be three carbon atoms within the pervious study, our drug design has been shaped to possess urolithin- propylene- tertiary amine organization without a benzyl group. The title compounds designed employing this strategic is shown in Figure 4.

The synthesis of the title compounds, their structure identification studies, pharmacological activity screening studies (i.e., the determination of IC_{50}s for both cholinesterase enzymes, and inhibition of amyloid-β aggregation), structure activity relationships, and molecular docking studies were evaluated as basic assays to be conducted to find answers to the questions we asked and also to achieve the objectives of this study.

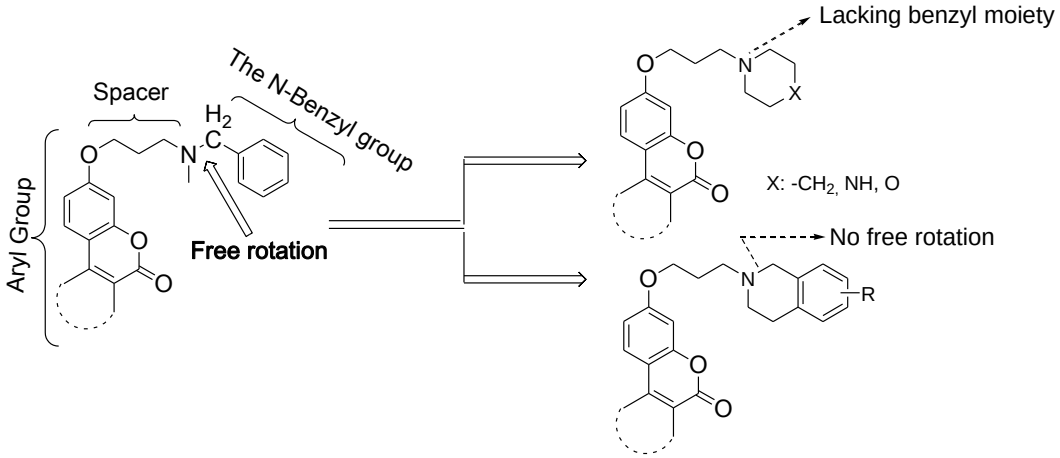


Figure 4: The desigin and general structure of the title compounds

Chapter 2

LITERATURE REVIEW

2.1 Acetylcholinesterase Enzyme

Various tissues in body such as nerves and muscles, motor and sensory fibers, cholinergic and non-cholinergic fibers, central and peripheral tissues release acetylcholinesterase (AChE, acetylcholine acetyl hydrolase, E.C. 3.1.1.7). AChE, has lower activity in sensory neurons in comparison to motor neurons [48]. Although this enzyme can be found in different molecular forms, it displaces similar catalectic properties. It can be found in monomeric and *tetrameric* forms regarding the quaternary structure of the protein [49].

AChE is a kind of serine hydrolyses enzyme. Its main function is to hydrolyze the neurotransmitter acetylcholine yielding acetate and choline. This terminates the biological actions of acetylcholine. AChE is one of the fast enzymes in the body in terms of its catalytic turn over. With the respect to the function of Acetylcholine, Acetylcholinesterase inhibition is one of the key strategies for some neurological disorders including Alzheimer's disease (AD), ataxia, senile dementia, and myasthenia gravis [50].

It has long been known that memory and cognation of the organism can be plaused by blocking the release of acetylcholine. This is parallel to the knowledge that choline acetyl transfers, the acetylcholine producing enzyme, function is the key for

the cognation skills. Furthermore, its concentrations are also low in AD patients. In 1980s, it was shown that there was a fall in the number of cholinergic neurons in AD patients that was concomitant to the reduction of acetylcholine receptors. [51].

Based on the information aforementioned above acetylcholinesterase inhibition is a target to treat AD. The acetylcholinesterase inhibitors, however, provide symptomatic treatment, since cholinergic innervation also goes down in time with respect to the loss in neuronal cells. Hence, the acetylcholinesterase inhibitors slow down the disease developments through keeping the level of acetylcholine high. On the other hand fibrrelari Beta amyloid pluck formation, a characteristic pathophysiological outcome of the diseases, is proportional to cholinergic neurons loss [52]. This is significant once it is remembered that acetylcholinesterase induces amyloid Beta aggregation. Indeed, the beta amyloid aggregate analysis indicate the presence of ACHE stick to amyloid beta plucks (52-53) [53].

2.2 Butyrylcholinesterase Enzyme

There is a secondary acetylcholine esterase (i.e., also referred to as plasma cholinesterase, Pseudo-cholinesterase, human butyryl-cholinesterase (BuChE, EC 3.1.1.8), butyryl-cholinesterase or acyl-choline acyl-hydrolase) which is mostly found in the liver. However it is also found in amyloid pluck aggregates and neurofibrillary tangles in AD patients [54]. BuChE has higher catalytic turnover to hydrolase butyrel choline, therefore its name is originated with the butyryl-suffixed. However, it is important to state that there is no butyrel choline as a neurotransmitter within the body, and butyrylcholinesterase hydrolyses acetylcholine within the system. BChE is biosynthesized in the liver and possesses high tissue distribution, including the central nervous system [55]. According to Perry et al., there is an

increase in the butyryl-cholinesterase activity in the brains of the elderly (60-90 years) [56]. However, in AD patients, AChE activity declines with the age [55-56]. Suggestions are made in several studies that BChE might perform a role in the process when beta-amyloid (Aβ) is changed to cut peptide analogues starting from the precursor protein. Darvesh et al., 2011; Guillozet et al., 1997 suggest an association between this malignant form and neurotic tissue degeneration and clinical dementia. Other researchers (e.g., Diamant et al., 2006; Podoly et al., 2010, shared their findings on BChE and how it eliminates the formation of amyloid fibril, so when it is present in amyloid plaques, it indicates the incorporation of this enzyme into Aβ fibrils at a late phase [57]. BChE in serum is found in four quaternary structures: G1 (the monomer unit); G1-ALB (the albumin bound monomer); G2 (the dimer unit), and G4 (the tetrameric unit which is the abundant form) [58-61]. Of over 65 variants for BChE gene, the K variant (BChE-K; 1615A; rs1803274) is of more interest to researchers as a risk factor for AD since it was associated with 33% decline of BChE molecules in plasma in the first place [61]. It is of importance to note that BChE is mainly found in the central and peripheral nervous systems and it is known to be the most dominant enzyme in plasma to hydrolyze ACh which is virtually an AChE-free zone of BChE levels beat AChE in all the body tissues except muscle and brain [62]. It is known that there are ten times more BChE molecules in body than AChE. With Alzheimer's, 85% of acetylcholinesterase is lost in particular regions of the brain while butyryl-cholinesterase levels (specifically the G1 form) increase as the disease develops [63].

2.3 Acetylcholine

It is the principle neurotransmitter of the cholinergic system. It could be found in both peripheral and central nervous systems. It was in 1914 when Henry Hallett Dale

discovered this neurotransmitter and later on Loewi confirmed its existence [64, 65]. Acetylcholine acts at all neuromuscular (nerve-to-skeletal muscle) connections. Its main duty is to stimulate muscle contractions causing all the observed muscle behavior. This transmitter can be found in different regions of the brain such as basal ganglia, hypothalamus and cortex as well as working for parasympathetic half of the autonomic nervous system. Its most important effects are proper memory and cognition and motor control. When acetylcholine's work is over at a synapse, its action is stopped when it is broken down by enzyme acetylcholinesterase. Another important feature of acetylcholine is the fact that some particular cells only react to this transmitter in dissimilar parts of the brain. Communication between two regions of the brain, the basal forebrain and the hippocampus which control memory and learning respectively, is enabled by activated receptors. The presence of Acetylcholine in the brain increases theta waves, thus resulting in better quality and stronger neuron signaling [66].

There are other important features to be mentioned about acetylcholine. One is that it improves memory encoding in brain cortex. The other is its ability to generate synaptogenesis, the normal development of synapses in the brain, resulting in advancement of memory encoding. Meanwhile, it also helps other transmitters in communicating the messages they carry. In AD, lower acetyl choline levels, therefore, results in the lack of neruplastisities [67].

In addition, acetylcholine can increase or decrease the speed of nerve signals due to excitatory and inhibitory functions. Excitatory function mainly works in the central nervous system involving learning, memory, arousal, and neuroplasticity. Acetyl choline acts a substantial role in the engagement of sensory functions at the time the

body wakes up to assist concentration. It is quite possible to find acetylcholine in interneurons in the central nervous system where it leaves several effects of arousal and reward, learning and short-term memory [68]. AD is a firm reason why cholinoceptive pathways degenerate. Another key role of acetylcholine in peripheral nervous system is that it plays as a neurotransmitter between skeletal muscle and motor nerve via a neuromuscular connection. Its effective impact is shown as muscle movement stimulation. Acetylcholine is accepted by receptors on the muscles which eventually lead to the contraction of skeletal muscles [69].

For Alzheimer's there is no definite treatment or a certain prophylactic agent since it is a type of neurodegenerative disease which brings dementia along with it. Pathologic characteristics of Alzheimer's include two abnormal deposits, namely senile plaques and neurofibrillary tangles as well as extensive neuronal loss. Due to the observation of cholinergic abnormalities in AD, a considerable reduction in acetylcholine receptors is identified [70]. Thus, AD symptoms can be explained by dysfunction of cholinergic signal transmission.it is also clear that anticholinergic drugs cause deficit in memory and cognation. This also guaranties the importance of the cholinergic system and acetylcholine in memory and cognitive abilities [71].

2.4 β-Amyloid Pathway

The amyloid precursor protein, which is substrate for Aβ production (APP), is formed and provided by various specified enzymes. One pathway is an amyloidogenic that is produced toward the formation of amyloid plaque in brain. Another one is not amyloidogenic. Ratios will be different and might change according to mutations, environmental factors and age of the individual. According to amyloidogenic pathway, APP is spilt by two different enzymes; a transmembrane

aspartic protease which accounts as β-secretase to make a soluble N-terminal fragment and a membrane-bound C-terminal fragment [72].

Aβ can be accumulated by levels into microscopic plaques. Plaques are formed by a multi-step polymerization mechanism which congregate together to form fibrils with a regular β-sheet structure. These fibrils stick together and organize with other substances as a result to form plaques. Although they can be accumulated in the extracellular space of the cerebrum where they aggregate to form amyloid plaque, Aβ plaques intercept and cut off brain cells with blocking spots of cell to cell connection and bring down and damage an activity and activating immune cells that stimulate inflammation known to be dangerous and deadly to cells as well as causing oxidative damage to cell [73].

2.4.1 β-Secretase Inhibitors

β-site amyloid cleaving enzyme-1 or precursor protein cleaving enzyme (BACE1,menapsin or Asp2) and BACE2(Asp1) are the two isoforms that are defined to this date as amyloid β-Secretase which is necessary for the generation of the neurotoxic Aβ peptide and has an active role in the behavior of AD. These two isoforms have certain roles for etiology of AD but BACE1 is the main and major isoform in the brain that has a place with the aspartyl protease (ASP) family and BACE2 takes an insignificant part in AD. According to a function of BACE1 in reducing the levels of Aβ in the AD brain, it is appeared as a first drug target for AD patient .there are quiet available BACE inhibitors designed and synthesized so far. However none of these BACE inhibitor molecules have been able to complete phase studies and reached to the market yet. Beside strong adverse reactions are also reported concomitant to BACE inhibitor application [74-75].The structure of a BACE inhibitor is shown in figure 5.

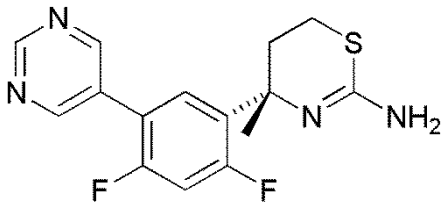
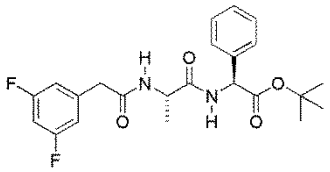


Figure 5: Structure of a β-Secretase (BACE1) inhibitor (i.e., LY2811376)

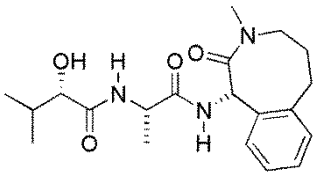
2.4.2 γ-Secretase Inhibitors

The other important and major enzyme target for the progress of AD therapy is γ-secretase [76]. γ-Secretase is a multi-subunit aspartyl protease according to pharmacological and convergence of genetic studies. γ-Secretase can cleave APP and transmembrane proteins within their transmembrane domains [77]. The three proteins help and keep the stability and maturation of the γ-Secretase complex [78].

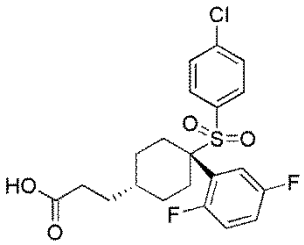
The γ-Secretase complex displays a high degree of heterogeneity. Regarding this point, the ideal drug design to target the enzyme would be selective targeting of certain subunits rather than the whole protein [79]. γ-Secretase displays a diverse hydrolytic activity towards a big range of physiologically important proteins. It was also considered to be an alternative target within the concept of the treatment of AD, I case of the design of bioavailable and CNS-penetrable inhibitor molecules [80]. Obviously potent inhibitors of this compound are expected to lower the production of amyloid beta, resulting in lower plaque formation. LY450139, as shown in Figure 6, was one of the molecules within this series, also tried in phase trials with no satisfactory results. However, the results absolutely indicated the lower formation of amyloid beta production, and consequent lower plaque formation with respect to the employment of this molecule [81-82].



DAPT (GSI-IX)



LY450139



MK-0752

Figure 6: Structure of some γ-Secretase inhibitors

2.4.3 Tau-Protein

The most generally investigated and displayed neurofibrillary lesion is the neurofibrillary tangle (NFT), stored in cell bodies. It shows up inside neuronal pathways as dystrophic neurites and neuropil strings [83]. The generous increment in mass tau levels that goes with lesion arrangement comes about fundamentally from amassing of insoluble tau totals in all probability since they or their misfielded forerunners sidestep endogenous freedom systems [84]. Notwithstanding filling in as markers for a differential conclusion and arranging of infection, tau totals can encourage illness proliferation and fill in as immediate wellsprings of harmfulness [85]. Despite the fact that the tau species that intercede lethality and the instruments through which they act are not built up, work in model frameworks proposes a few conceivable outcomes. To begin with, numerical demonstrating tests anticipate that mass collection of cytoplasmic totals in cell bodies can discourage neuronal vitality digestion through atomic crowding impacts once a Border crisis level is surpassed. In spite of the fact that loss of ordinary tau protein is all around endured in creature models investigated in [85], concurrent consumption of various classes of

microtubule related proteins has extreme results [86, 87]. At long last, assured tau aggregate can straightforwardly upset layer trustworthiness. Thus, and on the grounds that tau collection is a simply neurotic process disconnected to ordinary tau work, differing techniques for repressing tau misfolding and accumulation are being explored as potential treatments against neurofibrillary lesion arrangement and infection movement. Tau accumulation inhibitors perceived to date fall into two wide automated classes. The first rate looks at administrators that either covalently alter tau particularly or develop the improvement of covalent securities inside or between tau proteins to yield aggregation maladroit things in an aggregation method. Covalent inhibitors can assault any or all species by all accounts, to be particularly viable modifiers of tau monomer, from which every aggregated species is eventually determined [88]. Secondary expansive class of collection inhibitors cooperates with tau species non-covalently at different focuses in the aggregation pathway. These inhibitors are basically asserted by themselves and seem to act through various instruments that show discouragement in the aggregation accumulation penchant of tau-ligand complexes. For instance, little atoms can associate squarely with tau monomers. It has been recommended that even transient associations could discourage passage into conglomeration pathways by adjusting the rate at which locally unfurled polypeptides receive collection able compliances [89]. The last are specified partially by dissolvable uncovered patches of hydrophobic deposits. Hypothetically, a fast rate of interconversion between conglomeration equipped and clumsy adaptations (i.e., the reconfiguration rate) ought to discourage development of stable intermolecular collaborations with accomplice peptides by limiting dissolvable introduction of hydrophobic buildups that support intermolecular affiliation [90].

2.5 Urolithins and the Chemistry of Urolithin

Urolithins are formed by a group of metabolites delivered from ellagic acid (EA) and ellagitannins (ETs) by gut microbiota [91]. ETs are frequently found in various nourishment items including berries (raspberries ,strawberries, blackberries), pomegranate, tropical fruits(Camu-Camu), nuts (almonds ,walnuts, chestnuts, oak. Mainly punicalagins, punicalins and ellagic acid, the basic chemical components of berries, nuts, and pomegranate, are intensively investigated for their biological activities (Figure 7). Punicalagins are subject to extensive metabolism in different segments of the gastraointestinal tract, indicating the process is also pH specific [93].

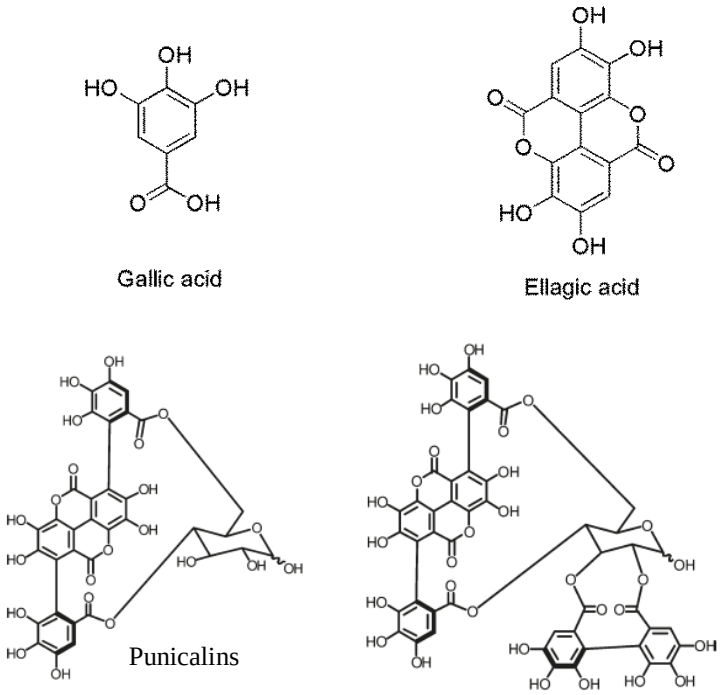


Figure 7: Chemical structures of pomegranate polyphenols

Ellagic acid and its derivatives remained after hydrolysis reactions also display poor bioavailability, however, they become substrates to GI tract flora (bacterial) enzymes. It is known that these microbial enzymes show critical contribution to the digestion of various xenobiotics. This can end up in some cases the digestion process

of quite important chemical structures from diverse sources. Gastrointestinal biotransformations acting on the short chain fatty acids release through the action of probiotic bacteria, for instance, is one of those critical examples. However, in certain cases, these reactions can trigger toxic bioactivation to yield up toxic materials [94]. One of the most encountered intestinal bacteria catalyzed reactions are polyphenol compounds. Since diverse chemicals possess these polyphenol structures, their pharmacokinetic and pharmacodynamic features are dramatically affected trhough the action of these gut related microorganisms. [95]. Urolithin analogues are typical examples, since they are not the main sources of diet. These compounds are simply formed by the catalytic activity of microorganism related enzymes on ellagic acid derivatives (Figure 8). This is not specific to human, since other mammalians can display this feature as well. The literature right now has quite distinctive proofs for these biotransformations resulting in the formation of urolithins from ellagic acid analogues [96]. Even, these studies are not limited to human, since animal model studies are also present [97].

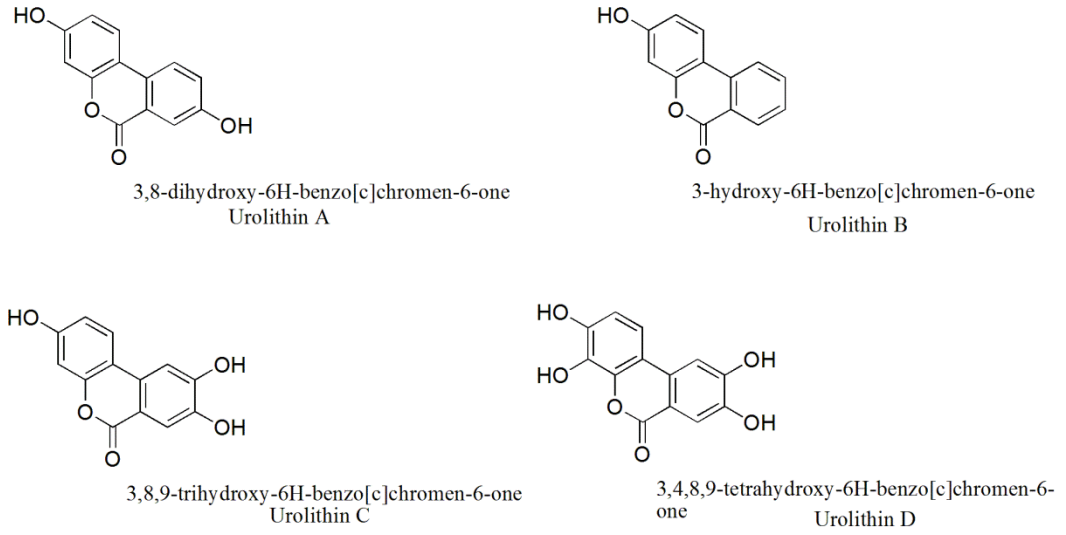


Figure 8: Chemical structure of urolithins, (Compound structures of ellagitannin gut microbiota metabolites)

Previous studies have demonstrated the feasibility of ellagitannin. In vivo research demonstrates that ellagitannin-containing nourishment items can be particularly compelling in balancing intestinal aggravation. The organization of pomegranate, almond, raspberry, and strawberry arrangements appeared to enhance the histological indications of synthetically initiated aggravation in gut mucosa, which was generally joined by the diminished invasion of insusceptible cells, decreased overexpression of proinflammatory elements, and the restraint of irritation related atomic pathways [98]. Potentilla erecta rhizome in one interventional clinical review, separate controlled to patients with ulcerative colitis appeared to enhance the clinical movement list, and additionally to decline levels of C-receptive protein [99].

2.5.1 Bioavailability, Metabolism of Urolithins

A speedy maintenance and assimilation of EA was represented by Doyle and Griffiths in rats. These makers recognized UroA and another metabolite (most probably UroB) in fertilizer and pee (Figure 8 and 9) both were shown to be of microfloral cause since none were found in sans germ creatures. Unaltered EA was not recognized in pee or excrement. These urolithins are, as they were, ingested and

glucuronidated by the intestinal cells. For this situation, no methyl ethers are created since UroA and UroB don't have ortho-dihydroxyl groups. On account of UroB, an extra hydroxyl can be presented by CYP450 (Cytochrome P450 is a group of isozymes in charge of the biotransformation of a few medications) and this builds the conceivable outcomes of glucuronidation and upgrades the discharge of the metabolite [100]. A both free EA and a few conjugates were recognized in mice pee, bile and blood found by Teal and Martin which a sorption of ^{3}H-EA happened generally inside two hours of oral organization. They were low levels in blood; tissues and bile that ingested compounds were discharged in urine [101].

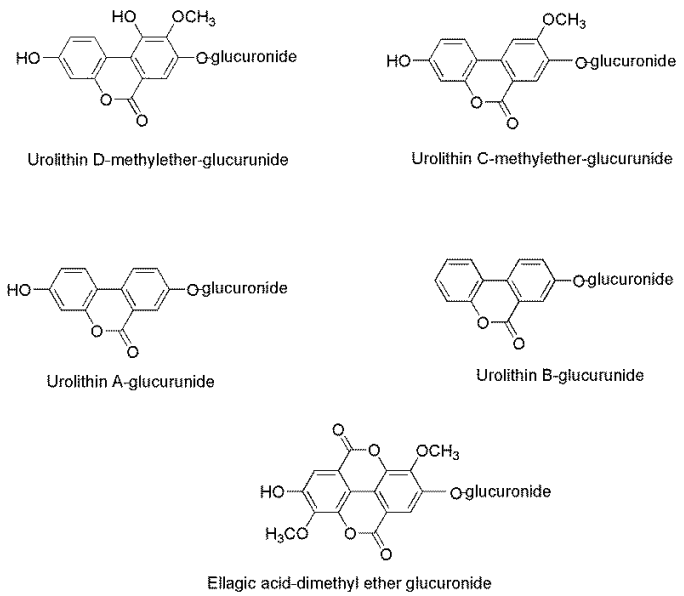


Figure 9: Ellagic acid metabolites found in plasma and urine after Ets intake

Urolithin A and B conjugates are the primary metabolites distinguished in plasma and pee albeit some trihydroxy subordinates (hydroxyl-UroA) or EA-dimethyl ether glucuronide have likewise been recognized in littler quantity. The tetrahydroxy-urolithins, trihydroxy-urolithins and EA subordinates are not recognized in fringe plasma, but rather they are invested in the small digestive tract and they are transported to the liver where they are additionally processed and discharged with

bile to the small digestive tract setting up an entero-hepatic dissemination that is in charge of the long existence of urolithins in plasma and pee. To the extent we know, these metabolites do not amass in organ tissues, except for gall-bladder and pee bladder where they are aggregated with the discharge liquids [102].

2.5.2 Antioxidant Activity and functions of Urolithins

Urolithins show up in human systemic dissemination inside a couple of hours of utilization of pomegranate items. Their half-life, including their metabolites, can reach to periods which might be expressed by days [103]. Past examinations announced urolithins as irrelevant cancer prevention agents (antioxidants) contrasted with the first ellagitannins [104]. In recent times, a cell test was connected interestingly to thoroughly assess the cancer prevention agent intensity of some of these compounds. Among the compounds tested some of them were referred to as also the microbial metabolites of dietary consumption [105]. Consequently, these compounds were shown to possess antioxidant activity with varying potentials [106]. The antiaoxidant activities were tried to relate to anticancer activities of these compounds as well. Among those main bioavailable metabolites, mainly Urolithin C and Urolithin D was found to possess high antioxidant activity[107]. This is an expected result once remembered that the C and D analogues have the highest phenolic hydroxyl groups among the main urolithin compounds exposed. They, in addition, indicated higher activity in comparison to the potential of vitamin C. It is known that urolithin compounds further undergo metabolism reactions yielding out the methylated derivatives. The same studies also indicated that methylation reactions end up with lower active compounds in terms of antioxidant activity. The most abundant metabolite Urolithin A, which is present in tissues, plasma, and stomach related track was shown to have a weak antioxidant activity [108]. Although

Urolithin A has a half-life close to 3 days, its biological activity appears to be weaker than higher phenol analogues [109-110].

2.5.3 Urolithins in the Treatment of Alzheimer's Disease

Pomegranate concentrate and juice have been accounted to demonstrate neuroprotective impacts against AD pathogenesis in a few transgenic creature models. However, the capable bioactive compound/s have not been characterized [111].

After the utilization of pomegranate juice and pomegranate concentrates, digestion and the bioavailability of ellagitannins in human subjects are well established. The significant pomegranate ellagitannins, PA, and others are not discovered in place available for use, but instead, are hydrolyzed to discharge EA and after that following this biotransformed by gut microbiota to yield urolithins (6H-dibenzo[b, d]pyran-6-one subordinates) [112].

Silico computational reviews, used to anticipate blood-mind obstruction porousness, uncovered that none of the chemical structures of compounds recognized in the pomegranate separate, yet the urolithins, satisfied criteria required for infiltration. Methyl-urolithin B (8-methoxy-6H-dibenzo[b, d]pyran-6-one), and urolithins prohibited β-amyloid fibrillation in vitro and however not chemical structures of compounds recognized in the pomegranate concentrate or its dominating ellagitannins, had a defensive impact in Caenorhabditis elegans post acceptance of amyloid β_{1-42} instigated neurotoxicity and loss of motion. Consequently, urolithins are the conceivable cerebrum absorbable mixes which add to pomegranate's anti-AD impacts justifying further in vivo studies applied for these compounds [113].

According to a previous research by Gulcan H.et al, the efficient conversions of urolithin (i.e., benzo[c]chromene) derivatives to potent cholinesterase inhibitor agents were shown. The basic pharmacophore groups within the structures of donepezil, galantamine, rivastigmine were efficiently utilized for the derivatization of those urolithin derivatives in the same study [47].

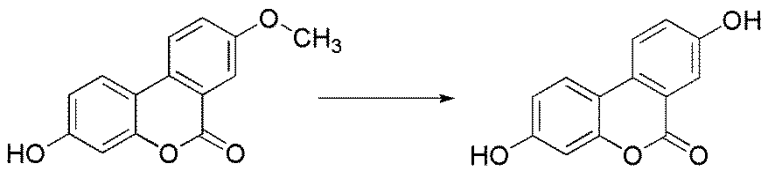
2.6 Synthetic Approaches on Urolithins

Urolithin is a natural dibenzopyranone extracted from the exudation of shilajit but is quite having great value; it is also a metabolite of ellagic acid. Urolithins are benzo[c]chromene analogue metabolites of ellagitannins, abundantly found in pomegranate and berry fruits.

In medical literature four isoforms of urolithin have been introduced, urolithin A, urolithin B, urolithin C and urolithin D which were synthesized by the either resorcinol or pyrogallol with the appropriately substituted benzoic acids [114].

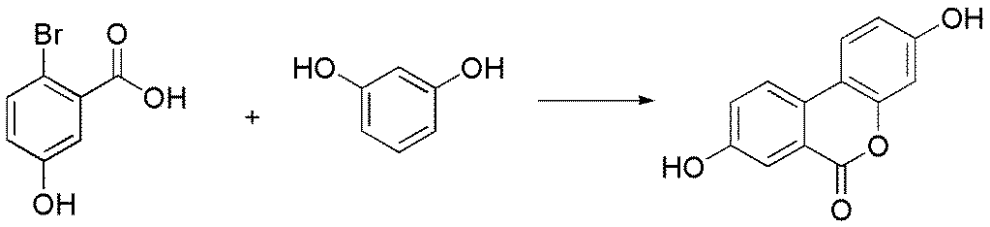
2.6.1 Synthetic Approach of Urolithin A (3,8-dihydroxybenzo[c]chromen-6-one)

Urolithin A was synthesized by three different methods. The first approach synthesized as bridging scaffold linking ellagic acid and coumarin analogs in CK2 inhibitors design by Cozza, et al through the hydrogen bromide in presence of Acetic acid as single step [115].

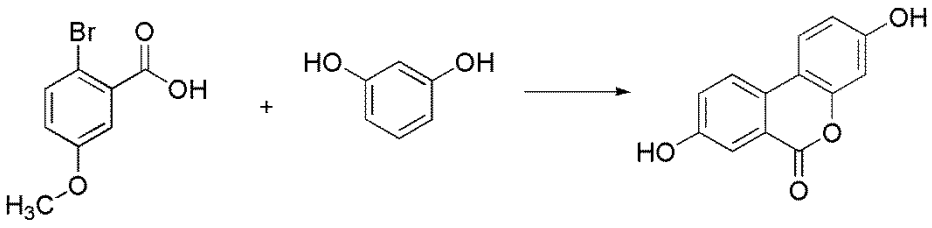


3-Hydroxy-8-methoxy-6H-dibenzo[b,d]pyran-6-one 3,8-dihydroxybenzo[c]chromen-6-one

The second method to synthesize of urolithin A is esterification of Carboxylic acids which was done by Sarkar, et al and they obtained 66% yield. In their structure-activity relationship studies indicated that the planar conjugated lactone moiety of EA was essential for macrophage migration inhibitory factor inhibition [116].

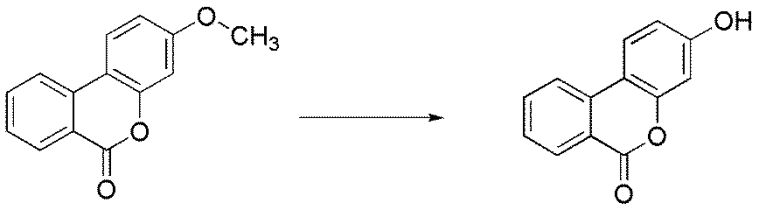


The third method applied By Nealmongkol et al. employing esterification of carboxylic acids and formation of alkyl halides/ alcohols from ethers resulted in urolithins display both Antioxidant and Pro-oxidant Activities depending on assay system and Conditions [117].

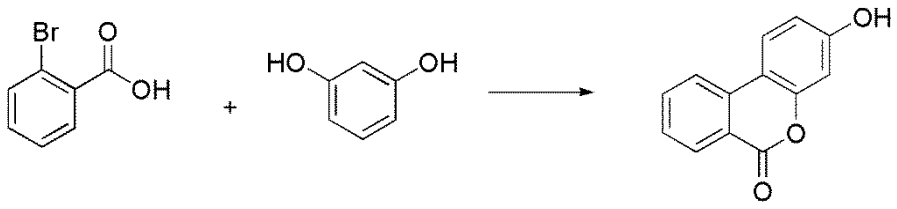


2.6.2 Synthetic Approach of Urolithin B (3-hydroxybenzo[c]chromen-6-one)

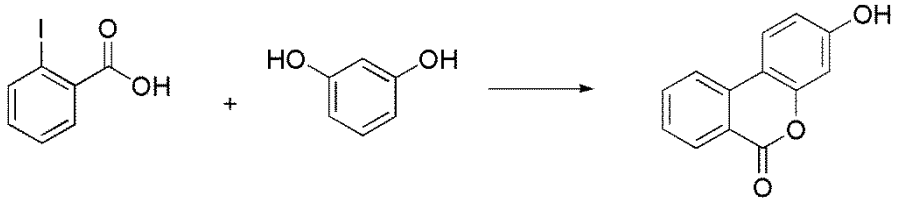
Urolithin B (3-Hydroxy-6H-dibenzo[b,d]pyran-6-one), synthesized by Nealmongkol,et al as single step which were found to exhibit potent antioxidant activity [118].



Another method for the preparation of 3-hydroxy-6H-benzo[c]chromen-6-one have shown by Kudou, Kazuhiro, et al through the esterification of the corresponding carboxylic acids [119].

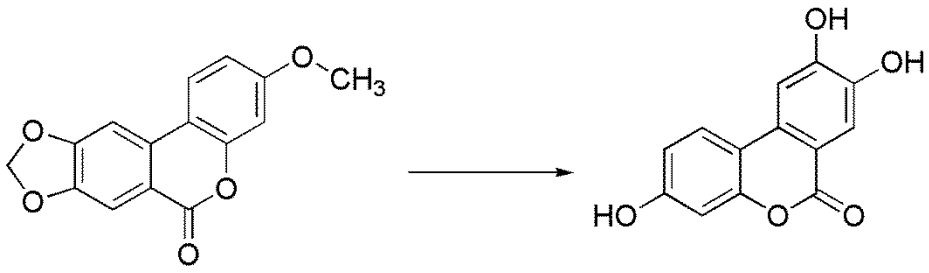


The technique shown above was improved by Gulcan H et al. In their study, the utilized 2-Iodo benzoic acid as starting molecule promoting higher yield and purity [47].

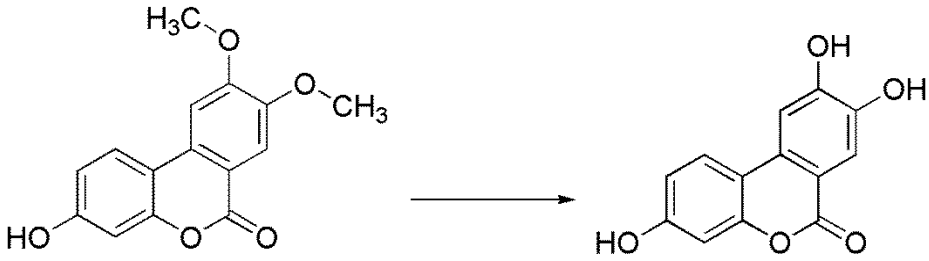


2.6.3 Synthetic Approach of Urolithin C (3,8,9-trihydroxybenzo[c]chromen-6-one)

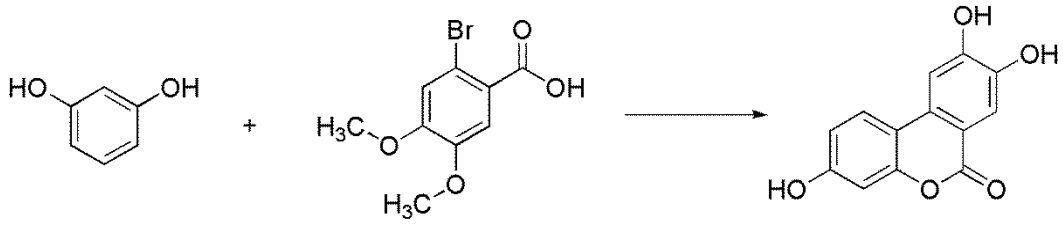
The urolithin C designed and synthesized by Nealmongkol, Prattya et al, in single step. As shown below [120].



Rinsch et.al, pointed out a demethylation synthetic methodology for the synthesis of this compound as shown below [121].

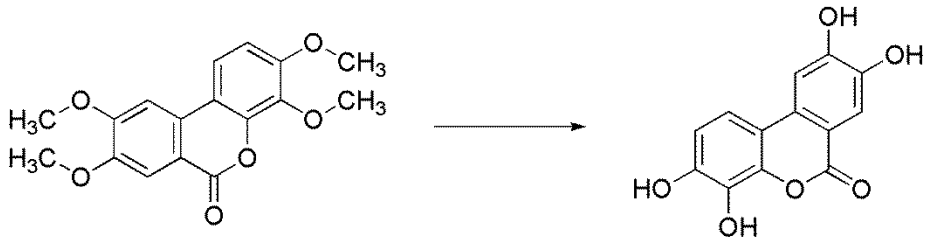


Another method carried out in the same study employed resorcinol and 4,5-dihydroxy -2-bromo benzoic acid as starting materials [122].

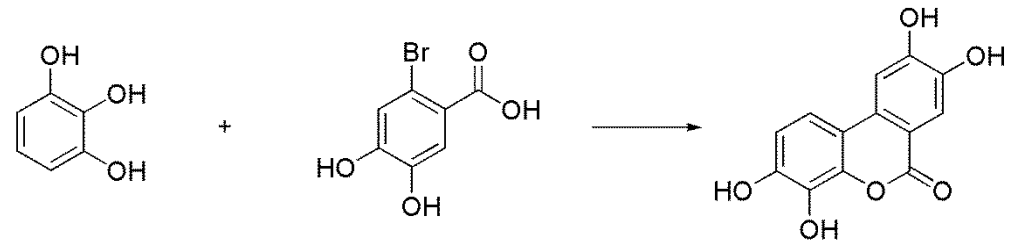


2.6.4 Synthetic Approach of Urolithin D (3,4,8,9-tetrahydroxybenzo[c]chromen-6-one)

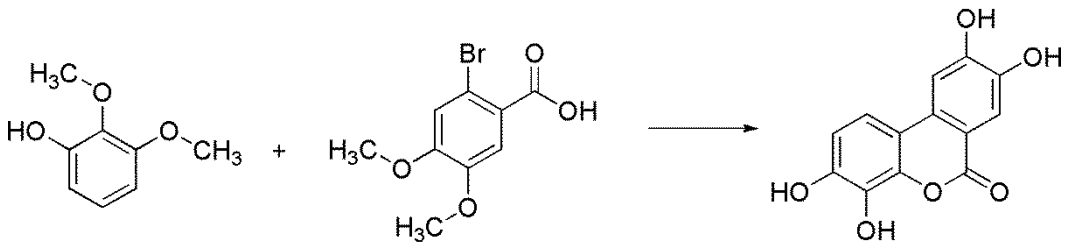
Rinsch et al used a method to prepare urolithin D in presence of BBr_3 [122].



Another pathway to synthesize urolithin D was shown by Kasimsetty et al as shown below [123].



A final methodology to synthesize urolithin D was done by Rinsch Christopher L, et al, as shown below [124].



Chapter 3

EXPERIMENTAL

3.1 Materials

Chemicals used were purchased unless otherwise stated and directly employed in the experiments without any purification. Resorcinol, Ethyl-2-oxocyclohexanecarboxylate, Zirconium(IV) chloride,1-Bromo-3-chloropropane, Sodium hydride,2-Iodobenzoic acid, Copper(II) sulfate,1,2,3,4-Tetrahydroisoquinoline, Morpholine, Piperidine, 6,7-Dimethoxy-1,2,3,tetrahydro isoquinoline, Piperazine, Potassium carbonate, Sodium iodide, Acetonitril, Ethylacetate, Methanol, Chloroform were obtained from Acros organic, Merk and Sigma Aldrich. Cholinesterase enzymes, β-Amyloid secretase, Acetylthiocholine Iodide, Butyrylthiocholine Iodide, 5,5'-Dithiobis (2-nitrobenzoic acid),Tris (hydroxymethyl) aminomethane, Galanthamine hydrobromide are all from Sigma Aldrich.

3.2 Instruments

Infrared spectra

FT-IR Spectra were determined on a Shimadzu FT-IR Prestige 21, L1600300 Spectrum TWO LiTa, and serial number 92106. For each experiment, 30 mg of dry powder compound was utilized without employing a KBr disc. The IR spectrums obtained for each compound was evaluated with respect to the functional groups.

Thin layer Chromatography (TLC)

TLC was performed on Merck aluminum-packed silica gel plates (silica gel 60F-254) to monitor the reactions. Furthermore, the purity of the final product was also evaluated employing the TLC technique. For these purposes the following mobile phase, are utilized.

System 1: Ethyl acetate - n-Hexane -Methanol (70:20:10)

System 2: Ethyl acetate - n-Hexane (80:20)

System 3: Ethyl acetate - n-Hexane - Methanol- Chloroform (60:20:10:10)

Then, the R_f value were determined and reported.

Mass Spectroscopic Analysis

A Waters TOF-MS was utilized. Formic acid was added to make the compounds gain quaternary amine structure available for positive mode detection. The capillary was arranged to 2500 V. The sample cone Vs was 100 V. 150°C was set for desolvation temperature. The source temperature was at 100 °C.

Microwave Instrument

A CEM DISCOVER-SP W/ACTIVENT DC8 152 model Single Mode was used to achieve the final step of the synthetic pathway of the title compounds.

Melting point

Melting points were determined using an IA9200×6 MK2 model Electrothermal Apparatus and the data are uncorrected.

In order to analyze the melting points, 3 mg of each compound was filled in a melting point capillary tube. The capillary tube was sealed from one end and inserted inside the instrument.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H NMR and ^{13}C NMR spectra of the title compounds were obtained employing a Bruker-400 Model NMR instrument. Tetramethylsilane (TMS) was used as internal standard. Deuterated dimethyl sulfate was used as solvent. For each experiment 10-15 mg of each compound was utilized.

High-performance liquid chromatography (HPLC)

HPLC studies were performed using an Agilent Technologies 1260 model instrument with PDA detector equipment. Purities of all final compounds were confirmed as greater than 99%. The column used was Eclipse XDB-C18 (5µm, 4.6 ×150mm) with a temperature of 25°C and a flow rate of 0.5 mL/min. The ratio of mobile phase A (acetonitrile) and B (phosphate buffer, pH 7) was 50:50. Retention time of each compound was measured and recorded, concomitant to the purity level identified.

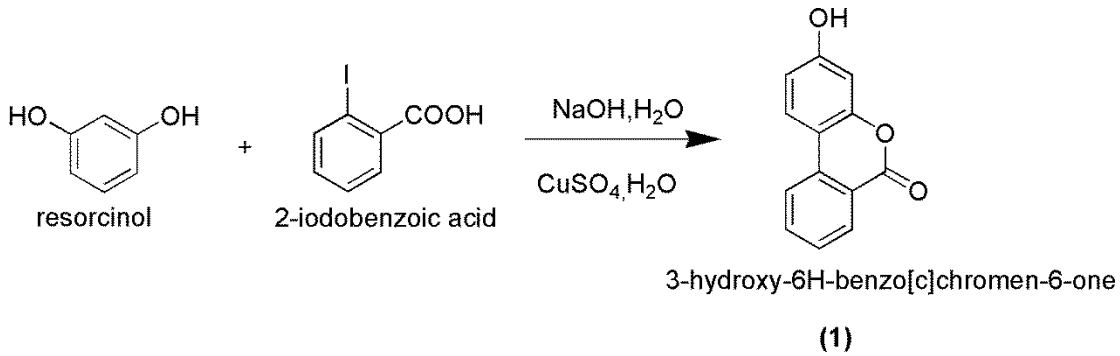
3.3 Synthetic Methodologies

3-hydroxy-6H-benzo[c]chromen-6-one **(1)** 3-Hydroxy-7,8,9,10-tetrahydro-benzo[c]chromen-6-one **(2)**, 3-(3-Chloropropoxy)-6H-benzo[c]chromen-6-one **(3)**, 3-(3-Chloropropoxy)-7,8,9,10-tetrahydro-6Hbenzo[c]chromen-6-one **(4)** were synthesized and purified according to the literature [47].

3.3.1 Synthesis of 3-Hydroxy-6H-benzo[c]chromen-6-one (1)

2-iodobenzoic acid (12 mmol), resorcinol (36 mmol), and aqueous NaOH solution (44 mmol) (30 mL) was refluxed for 1 h in an oil bath. Following that, $CuSO_4$ (28%, 25 mL) solution in water was added to the mixture. The resulting mixture was further refluxed for 10 min. The precipitate formed was filtered, and washed with water. The compound was obtained as white powder. Yield obtained: 72%. 1H NMR (DMSO-

d6, 400 MHz): δ = 10.33 (s, 1H, OH), 8.17-8.13 (m, 3H), 7.87-7.85 (m, 1H), 7.52 (s, 1H), 6.78-6.76 (m, 2H), Synthetic scheme is given in Scheme 1.

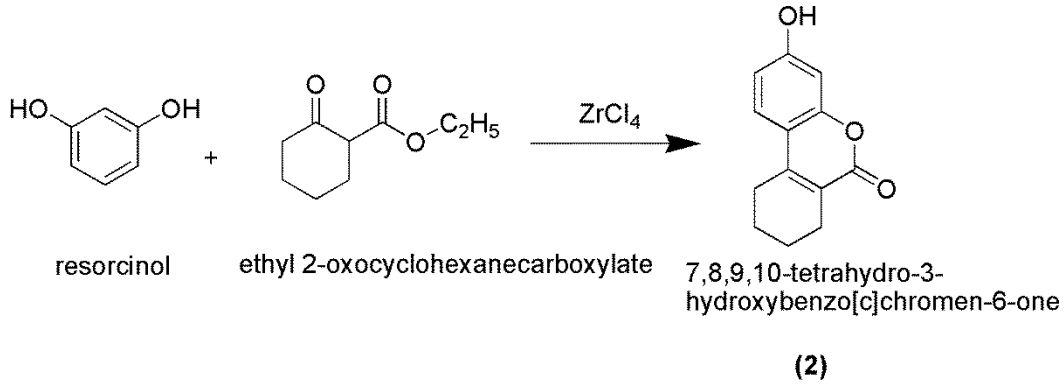


(1)

Scheme 1: Synthesis of 3-Hydroxy-6H-benzo[c]chromen-6-one

3.3.2 Synthesis of 3-Hydroxy-7,8,9,10-tetrahydro-benzo[c]chromen-6-one (2)

Resorcinol (13.6 mmol) and ethyl 2-oxocyclohexanecarboxylate (15.8 mmol) were mixed neat and heated at 75°C in the presence of zirconium (IV) chloride (0.106 g) for 25 min. At the end of the time, the reaction mixture was set to room temperature and it was added to 30 mL of cold water. The product precipitated was filtered. Yield obtained: 80 %, 1H NMR (DMSO-d6, 400 MHz): δ = 10.31 (s, 1H, OH), 7.46 (d, 1H), 6.74 (d, 1H), 6.65 (s, 1H), 2.68-2.65 (m, 2H), 2.34-2.31 (m, 2H), 1.70-1.64 (m, 4H), Synthetic scheme is given in Scheme 2.

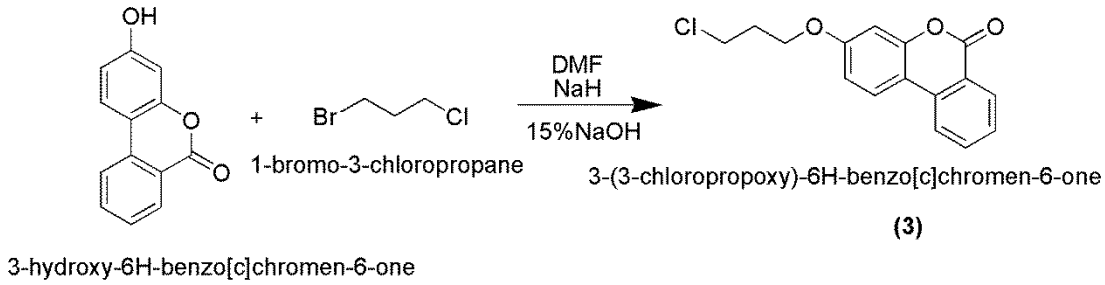


(2)

Scheme 2: Synthesis of 3-Hydroxy-7,8,9,10-tetrahydro-benzo[c]chromen-6-one

3.3.3 Synthesis of 3-(3-Chloropropoxy)-6H-benzo[c]chromen-6-one (3)

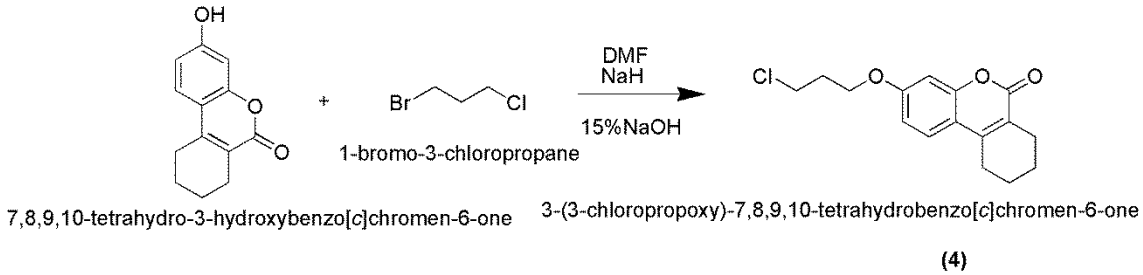
3-Hydroxy-6H-benzo[c]chromen-6-one **(1)** (46.2 mmol) and NaH (69 mmol) were mixed in DMF (40 mL). 1,3-Dichloropropane (115.5 mmol) was added to this mixture and the reaction was stirred at ambient temperature for 6 h. Following that, the reaction was decanted into 200 mL cold aqueous 10% NaOH. 50 ML of n-hexane was also added to make two phases. The precipitate formed was obtained through filtration. Yield obtained: 78 %; 1H NMR (DMSO-d6, 400 MHz) δ = 8.22–8.13 (m, 3H), 7.85 (s, 1H), 7.55 (s, 1H), 6.95-6.90 (m, 2H), 4.17-4.12 (m, 2H), 3.80-3.76 (m, 2H), 2.18-2.14 (m, 2H), Synthetic scheme is given in Scheme 3.



Scheme 3: Synthesis of 3-(3-Chloropropoxy)-6H-benzo[c]chromen-6-one

3.3.4 Synthesis of 3 -(3-Chloropropoxy) -7,8,9,10- tetrahydro - 6Hbenzo [c]chromen- 6- one (4)

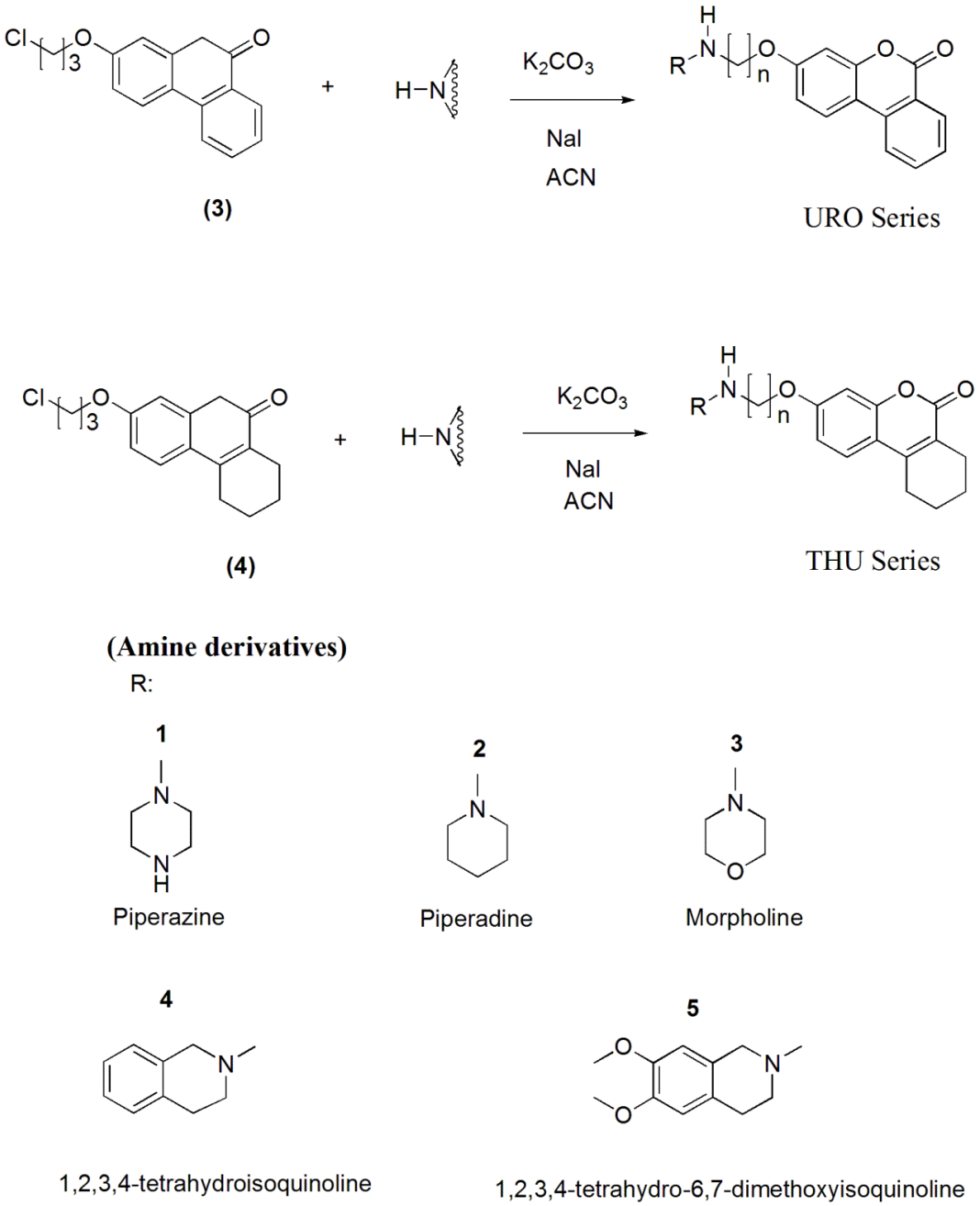
3-(3-Chloropropoxy)-7,8,9,10-tetrahydro-6Hbenzo[c]chromen-6-one was synthesized according to procedure given for compound **3** using 3-Hydroxy-7,8,9,10-tetrahydro-benzo[c]chromen-6-one (**2**) as starting material. Yield obtained: 82 %. ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 7.56 (d, J = 7.0 Hz, 1H), 6.91-6.88 (m, 2H), 4.16-4.12 (m, 2H), 3.79-3.74 (m, 2H), 2.71-2.66 (m, 2H), 2.36-2.32 (m, 2H), 2.19-2.15 (m, 2H), 1.72-1.68 (m, 4H), Synthetic scheme is given in Scheme 4.



Scheme 4: Synthesis of 3- (3-Chloropropoxy) -7,8,9,10- tetrahydro- 6Hbenzo [c] chromen- 6-one

3.3.5 General Procedure for the Synthesis of the Title Compounds

The corresponding alkyl halide derivative (1.7 mmol, either **3** or **4** depending on THU and URO series), 0.38 g K_2CO_3 (2.75 mmol), and 0.38 g NaI (2.75 mmol) was mixed in ACN (15 mL) for 5 min. Then, 5.2 mmol appropriate amine derivative was added and the resulting content was mixed for additional 3 min in a 30mL microwave reaction vessel. The reaction was conducted in a microwave instrument (i.e., A CEM DISCOVER-SP W/ACTIVENT). It was heated 1hr at 105°C at the dynamic mode of the instrument automatically calibrate the radiation and temperature balance with respect to the change in pressure. At the end of the time specified, acetonitrile was distilled out under vacuum and the residue was mixed with 20 mL of K_2CO_3 solution (5.0%) and this mixture was heated at 60 °C for 1 h. Then the mixture was cooled down to room temperature and the aqueous phase was extracted 3 times with 25 mL of ethyl acetate. Combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give the product (i.e., free base). The free base was dissolved in 10 mL acetone and HCl gas was continuously passed until HCl salt precipitation took place. The synthetic scheme is given in Scheme 5.



Scheme 5**:** synthetic scheme of the title compounds and derivatives

3.4 Measurement of the Potential of the Title Compounds to Inhibit Cholinesterase Enzyme

Each original compound synthesized was tested for their potential to inhibit AChE and BChE enzymes. AChE and BChE inhibitory activities of the compounds were determined by modified spectrophotometric method of Ellman [125]. The human

recombinant AChE (rAChE) and human recombinant BuChE were obtained from Sigma. Acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates, respectively for AChE, and BuChE. 5,5´-Dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. In a general enzyme-catalyzed reaction, 50 mM Tris HCl buffer (pH 8.0), 6.8 mM DTNB, 2 µl of sample solutions (at varying concentrations), and 10 µl of AChE/BuChE solution were added by multichannel automatic pipette (Thermo Fisher Scientific, USA) in a 96-well microplate. The reaction was then initiated with the addition of 10 µl of acetylthiocholine iodide/butyrylthiocholine chloride. The reactions were incubated for 15 min at 27°C. The formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines was monitored at 412 nm utilizing a 96-well microplate reader (Varioskan Flash, Thermo Scientific, and Serial RS-232 C USA). The measurements and calculations were evaluated by using SkanIt Software 2.4.5 RE for Varioskan Flash software. Percentage of inhibition of hAChE and hBuChE was determined by comparison of rates of reaction of samples relative to blank sample (DMSO and methanol) using the formula (E-S)/E x 100, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate and the mean ± standard deviation was calculated. The IC_{50}s for each test compound including the reference compounds, rivastigmine (Sigma-Aldrich, USA), donepezil hydrochloride (Sigma-Aldrich,USA) and galantamine hydrobromide from Lycoris sp. (Sigma-Aldrich, St. Louis, MO, USA), were determined using a sigmoidal hill slope.

3.5 Measurement of the Potential of the Title compounds to Inhibit AChE-induced Aβ Aggregation

The aggregation of amyloid-beta (1-42 rat (Sigma-Aldrich, USA) (Aβ) induced by human recombinant acetylcholinesterase (rAChE) was studied by thioflavin T fluorescence spectroscopy. Incubation experiments were performed in the presence of title compounds, a known amyloid beta aggregation inhibitor, Phenol red (Sigma-Aldrich, USA), as well as donepezil hydrochloride (Sigma-Aldrich, USA) in order to find out their potential to prevent the rAChE-induced Aβ aggregation under the experimental conditions utilized.

1 mg Aβ was dissolved in 0.5 ml Hexafluoroisopropanol (HFIP) solution. Following the evaporation of the solvent DMSO was added to obtain 2.3 mM Aβ solution. Aliquots (2 μL) of Aβ in DMSO were transferred to 24-well multidish microplate (BioLite, Thermo Fisher Scientific, Korea) and incubated for 24 hours at room temperature in 0.215 M sodium phosphate buffer (pH 8.0) at a final Aβ concentration of 230 μM. For co-incubation experiments, aliquots (16 μL) of rAChE dissolved in the same buffer were added to obtain rAChE at a final concentration of 2.30 μM (Aβ/ AChE molar ratio ～100:1). The final volume of each assay was 20 μL. Each assay was run in duplicate. To quantify amyloid fibril formation, the thioflavin T fluorescence method was used [126].

For the thioflavin T-based fluorometric assay, analyses were performed with a (Varioskan Flash, Thermo Scientific, USA) using a 24-well multidish reader. Fluorescence was monitored at 446 nm (Aexc) and 490 nm (Aem) with excitation and emission slits of 5 nm bandwidth. After incubation, the solutions containing Aβ

plus AChE, or Aβ plus AChE in the presence of inhibitors were added to 50 mM glycine-NaOH buffer (pH = 8.5) containing 1.5 μM thioflavin T in a final volume of 2.0 mL. A time scan of fluorescence was performed and the intensity values reached at the plateau (around 300 s) were averaged after subtracting the background fluorescence from 1.5 μM thioflavin T and AChE.

The fluorescence intensities were compared and the percent inhibition with respect to the presence of test compounds was calculated. The percent inhibition of the rAChE-induced aggregation due to the presence of increasing test compound concentration was calculated by the following expression: 100-(IFi/IFo x 100) where IF; and IFo are the fluorescence intensities obtained for Ap plus HuAChE in the presence and in the absence of inhibitor, respectively.

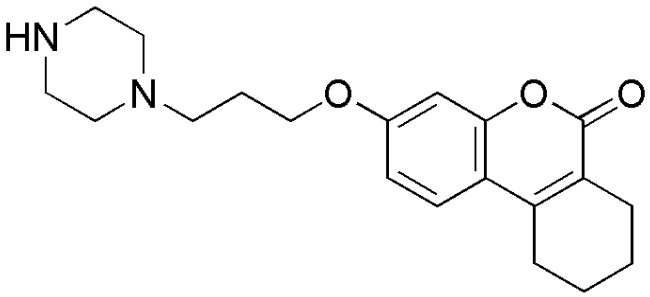
Chapter 4

RESULTS AND DISCUSSION

The synthesis of 3-hydroxy benzo[c]chromene (**1**, also referred to as **URO**) and 7,8,9,10-tetrahydro benzo[*c*]chromene (**2**, also referred to as **THU**) structures was considered as references for the present study and we synthesized again by the reported synthesis procedure [47]. A final microwave assisted step following the synthesis of alkylhalide derivatives were conducted according to the methodologies designed in chapter 3 materials and method section. The structural identification and purity analysis of the title compounds was performed employing, melting point, HPLC, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral analysis techniques.

4.1 Chemistry, Synthesis and Analyses of Title Compounds

4.1.1 Synthesis and Analysis of (THU 1)

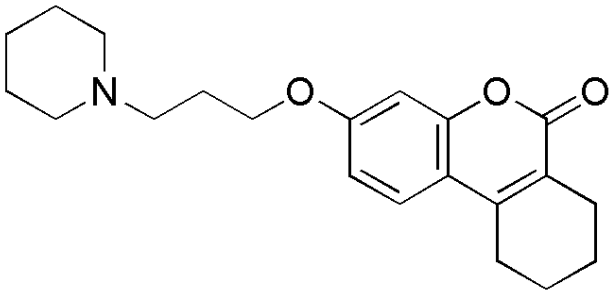


3-(3-(piperazin-1-yl)propoxy)-7,8,9,10-tetrahydrobenzo[c]chromen-6-one dihydrochloride

Off-white solid; yield: 56 %; mp: 182-184 °C; IR (cm^{-1}): 2509, 2443, 1704 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 9.84 (b, 1H, N-H), 7.56 (d, 1H, Ar-H), 6.90 (m, 2H, Ar-H), 4.14 (t, 2H, -O-$\underline{CH_2}$-CH_2-), 3.13-3.39 (m, 10H, -CH_2-CH_2-$\underline{CH_2}$-N- and piperazin protons), 2.70 (m, 2H, -CH_2-$\underline{CH_2}$-CCOO-), 2.36 (m, 2H, -CH_2-$\underline{CH_2}$-C=C-), 2.16 (m, 2H, -N-CH_2-$\underline{CH_2}$-CH_2-O-), 1.69-1.72 (m, 4H, -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.7 (C=O), 160.2, 152.9, 147.5, 119.5, 124.9, 119.5, 113.2, 101.0, 65.7 (CH_2-O), 53.2 (CH_2-N), 48.1 (CH_2-NH), 30.0, 24.6, 23.6, 23.5, 21.2, 20.8 (CH_2); HPLC purity: 100.00 %, R.T : 18.88 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{26}N_2O_3$, 343.2022; found, 343.2011.

R_f : S-1, S-2, S-3; 0.243, 0.051, 0.217 (UV lamp 254nm).

4.1.2 Synthesis and Analysis of (THU 2)

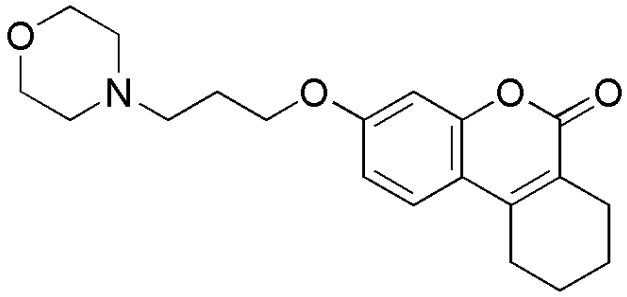


3-(3-(Piperidin-1-yl)propoxy)-7,8,9,10-tetrahydrobenzo[c]chromen-6-one hydrochloride

White solid; yield: 62 %; mp: 238-240 ˚C; IR (cm^{-1}): 2698, 2547, 1708 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 10.36 (b, 1H, N-H), 7.62 (d, 1H, Ar-H), 6.93 (m, 2H, Ar-H), 4.15 (t, 2H, -O-$\underline{CH_2}$-CH_2-), 3.15 (m, 6H, -CH_2-$\underline{CH_2}$-N-), 2.74 (m, 2H, -CH_2-$\underline{CH_2}$-CCOO-), 2.38 (m, 2H, -CH_2-$\underline{CH_2}$-C=C-), 2.23 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-), 1.76 (m, 10H, -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2- and -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.8 (C=O), 160.1, 152.9, 147.5, 125.0, 119.5, 113.2, 112.2, 101.0, 65.7 (CH_2-O), 53.2, 52.0 (CH_2-N), 24.6, 23.5, 23.1, 22.3, 21.4, 21.1, 20.8; HPLC purity: 99.4699%, R.T: 22.97 min.; HRMS (m/z): [M+H]+ calcd. for $C_{21}H_{27}NO_3$, 342.2069; found, 342.2052.

R_f : S-1, S-2, S-3; 0.117, 0.062, 0.189 (UV lamp 254nm).

4.1.3 Synthesis and Analysis of (THU 3)

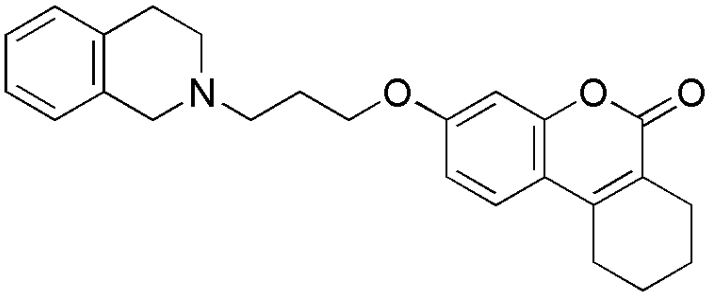


3-(3-Morpholinopropoxy)-7,8,9,10-tetrahydrobenzo[c]chromen-6-one hydrochloride

Off-white solid; yield: 90 %; mp: 138-140 °C; IR (cm^{-1}): 2510, 2459, 1706 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.59 (b, 1H, N-H), 7.56 (d, 1H, Ar-H), 6.91 (m, 2H, Ar-H), 4.15 (t, 2H, -O-$\underline{CH_2}$-), 3.92 (m, 4H, -$\underline{CH_2}$-O-$\underline{CH_2}$-), 3.1-3.24 (m, 6H, -CH_2-N-), 2.70 (m, 2H, -CH_2-$\underline{CH_2}$-CCOO-), 2.36 (m, 2H, -CH_2-$\underline{CH_2}$-C=C-), 2.25 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-), 1.70 (m, 4H, -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.8 (C=O), 160.1, 152.8, 147.4, 124.9, 119.5, 113.2, 112.1, 101.0, 65.6, 63.1 (CH_2-O), 53.3, 51.0 (CH_2-N), 24.6, 23.5, 22.8, 21.1, 20.8;HPLC purity: 100.00 %, R.T: 12.12 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{25}NO_4$, 344.1862; found, 344.1845.

R_f : S-1, S-2, S-3; 0.289, 0.112, 0.243 (UV lamp 254nm).

4.1.4 Synthesis and Analysis of (THU 4)

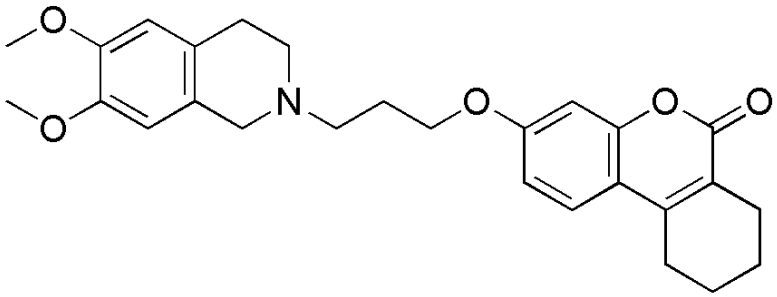


3-(3-(3,4-Dihydroisoquinolin-2(1H)-yl)propoxy)-7,8,9,10-tetrahydrobenzo[c]chromen-6-one hydrochloride

White solid; yield: 88 %; mp: 218-220 °C; IR (cm^{-1}): 2463, 2383, 1697 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 9.66 (b, 1H, N-H), 7.62 (d, 1H, Ar-H), 7.19-7.24 (m, 4H, Ar-H), 6.95 (m, 2H, Ar-H), 4.35 (t, 2H, -O-$\underline{CH_2}$-), 4.20 (m, 6H, -$\underline{CH_2}$-N-), 2.99 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-) 2.50 (m, 2H, -CH_2-$\underline{CH_2}$-CCOO-), 2.37 (m, 2H, -CH_2-$\underline{CH_2}$-C=C-), 1.72 (m, 6H, -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2- and -N-CH_2-$\underline{CH_2}$-C=C-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.8 (C=O), 160.1, 152.9, 147.5, 132.0, 131.5, 128.9, 128.6, 127.5, 126.6, 125.0, 119.5, 113.3, 112.2, 101.1, 65.7 (CH_2-O), 52.5, 51.7, 48.7 (CH_2-N), 24.8, 24.6, 23.5, 23.4, 21.2, 20.8; HPLC purity: 100.0%, R.T: 16.52 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{25}H_{27}NO_3$, 390.2069; found, 390.2083.

R_f : S-1, S-2, S-3; 0.114, 0. 612, 0.432 (UV lamp 254nm).

4.1.5 Synthesis and Analysis of (THU 5)

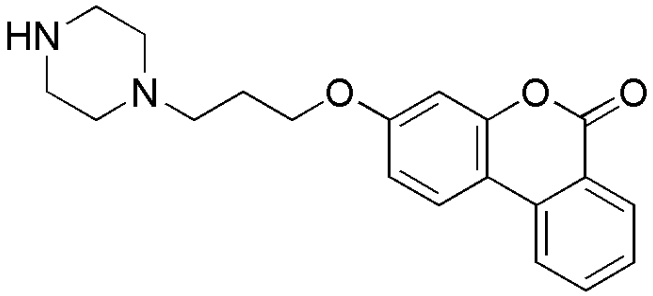


3-(3-(3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)propoxy)-7,8,9,10-tetrahydro-benzo [c]chromen-6-one hydrochloride

Off-white solid; yield: 93 %; mp: 101-103 ˚C; IR (cm^{-1}): 2545, 2411, 1701 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 9.65 (b, 1H, N-H), 7.60 (d, 1H, Ar-H), 6.95 (m, 2H, Ar-H), 6.77-6.79 (m, 2H, Ar-H), 4.20 (m, 2H, -O-$\underline{CH_2}$-), 4.10 (m, 6H, -$\underline{CH_2}$-N-), 3.72 (m, 6H, -O-$\underline{CH_3}$), 2.91 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-), 2.74 (m, 2H, -CH_2-$\underline{CH_2}$-COO-), 2.36 (m, 2H, -CH_2-$\underline{CH_2}$-C=C-), 1.72 (m, 6H, -CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2- and -N-CH_2-$\underline{CH_2}$-C=C-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.8, 160.1, 152.9, 148.3, 147.6, 125.0, 123.8, 120.4, 120.0, 119.5, 113.3, 112.2, 111.8, 109.9, 101.0, 66.0 (CH_2-O), 55.5 ($2CH_3$-O), 52.3, 51.3, 48.8 (CH_2-N), 24.4, 24.2, 23.5, 23.4, 21.2, 20.8; HPLC purity: 100.00 %, R.T: 9.23 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{27}H_{31}NO_5$, 450.2280; found, 450.2262.

R_f : S-1, S-2, S-3; 0.682, 0.125, 0.652 (UV lamp 254nm).

4.1.6 Synthesis and Analysis of (URO 1)

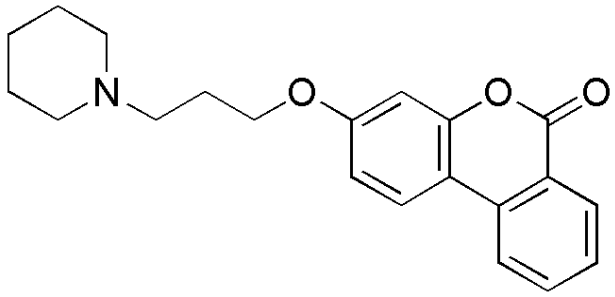


3-(3-(Piperazin-1-yl)propoxy)-6H-benzo[c]chromen-6-one dihydrochloride

Off-white solid; yield: 33 %; mp: 260-263 °C; IR (cm^{-1}): 2636, 2496, 1728 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 9.83 (b, 1H, N-H), 8.31-8.19 (m, 3H, Ar-H), 7.90 (t, 1H, Ar-H), 7.60 (t, 1H, Ar-H), 7.01 (m, 2H, Ar-H), 4.20 (t, 2H, -O-$\underline{CH_2}$-), 3.48 (m, 10H, -$\underline{CH_2}$-N-), 2.25 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.5 (C=O), 160.1, 152.0, 135.4, 134.7, 129.8, 128.2, 124.9, 122.0, 119.3, 112.7, 110.9, 110.1 (Ar), 65.5 (CH_2-O), 53.0 (CH_2-N), 47.8 (CH_2-NH), 25.5, 23.1; HPLC purity: 100.00%, R.T: 21.63 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{22}N_2O_3$, 339.1709; found, 339.1725.

R_f : S-1, S-2, S-3; 0.083, 0.062, 0.103 (UV lamp 254nm).

4.1.7 Synthesis and Analysis of (URO 2)

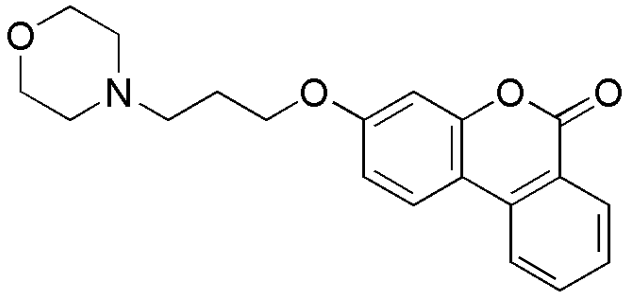


3-(3-(piperidin-1-yl)propoxy)-6H-benzo[c]chromen-6-one hydrochloride (URO 2)

Light brown solid; yield: 65 %; mp: 250-254 ˚C; IR (cm^{-1}): 2620, 2404, 1734 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 10.78 (b, 1H, N-H), 8.18-8.29 (m, 3H, Ar-H), 7.89 (t, 1H, Ar-H), 7.59 (t, 1H, Ar-H), 6.98 (m, 2H, Ar-H), 4.17 (t, 2H, -O-$\underline{CH_2}$-), 3.17 (m, 6H,-$\underline{CH_2}$-N-), 2.89 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-), 2.26 (m, 4H, -N-CH_2-$\underline{CH_2}$-), 1.81 (m, 2H, -CH_2-$\underline{CH_2}$-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.4 (C=O), 160.1, 152.0, 135.4, 134.6, 129.7, 128.2, 124.8, 122.0, 119.2, 112.6, 110.8, 102.1 (Ar), 65.7 (CH_2-O), 53.2, 52.0 (CH_2-N), 23.1, 22.3, 21.4; HPLC purity: 99.2%, R.T: 11.64 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{21}H_{23}NO_3$, 338.1756; found, 338.1776.

R_f : S-1, S-2, S-3; 0.219, 0.075, 0.400 (UV lamp 254nm).

4.1.8 Synthesis and Analysis of (URO 3)

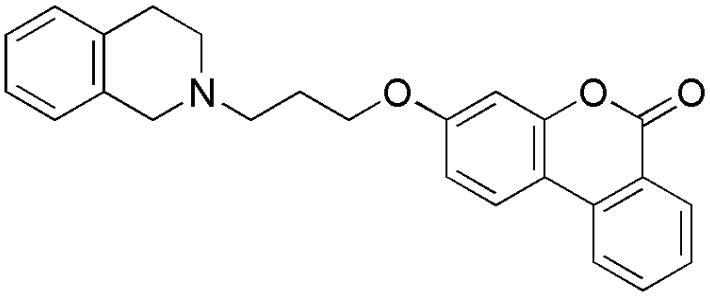


3-(3-Morpholinopropoxy)-6H-benzo[c]chromen-6-one hydrochloride

White solid; yield: 63 %; mp: 238-240 ˚C; IR (cm^{-1}): 2823, 2723, 1719 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 11.40 (b, 1H, N-H), 8.18-8.30 (m, 3H, Ar-H), 7.89 (t, 1H, Ar-H), 7.59 (t, 1H, Ar-H), 6.99 (m, 2H, Ar-H), 4.18 (t, 2H, -O-$\underline{CH_2}$-), 3.90 (m, 4H, -$\underline{CH_2}$-O-$\underline{CH_2}$-), 3.26 (m, 4H, -N-$\underline{CH_2}$-CH_2-O-), 3.09 (m, 2H, -O-CH_2-CH_2-$\underline{CH_2}$-N-), 2.25 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.4 (C=O), 160.1, 152.0, 135.3, 134.6, 129.8, 129.6, 128.1, 124.8, 122.0, 112.7, 110.9, 102.1 (Ar), 65.6, 63.2 (CH_2-O), 53.2, 51.0 (CH_2-N), 22.8; HPLC purity: 99.5 %, R.T: 11.09 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{20}H_{21}NO_4$, 340.1549; found, 340.1538.

R_f : S-1, S-2, S-3; 0.195, 0.125, 0.395 (UV lamp 254nm).

4.1.9 Synthesis and Analysis of (URO 4)

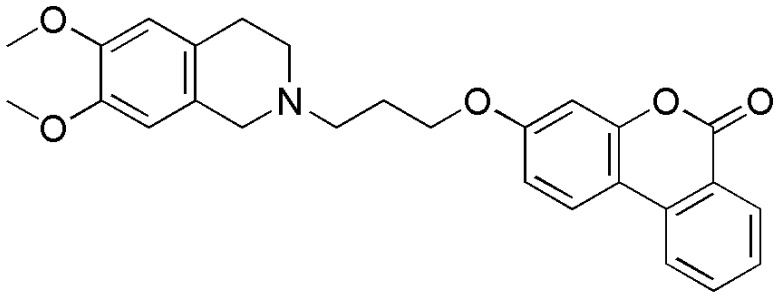


3-(3-(3,4-Dihydroisoquinolin-2(1H)-yl)propoxy)-6H-benzo[c]chromen-6-one

Light brown solid; yield: 63 %. mp: 115-117 °C; IR (cm^{-1}): 2532, 2421, 1729; 1H NMR (DMSO-d6, 400 MHz): δ = 9.75 (b, 1H, N-H), 8.25 (m, 1H, Ar-H), 8.17 (d, 2H, Ar-H), 7.86 (t, 1H, Ar-H), 7.56 (t, 1H, Ar-H), 7.07 (m, 4H, Ar-H), 6.96 (m, 2H, Ar-H), 4.15 (t, 2H, -O$\underline{CH_2}$-), 3.56 (m, 2H, -N-$\underline{CH_2}$-C=C-), 2.79 (t, 2H, -N-$\underline{CH_2}$-CH_2-C=C-), 2.64 (t, 2H, -O-CH_2-CH_2-$\underline{CH_2}$-N-), 2.59 (t, 2H, -N-CH_2-$\underline{CH_2}$-C=C-), 1.98 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) major rotamer: 160.6 (C=O), 160.4, 152.0, 135.2, 134.8, 134.2, 134.1, 129.7, 129.5, 128.3, 126.3, 125.9, 125.4, 124.5, 121.9, 119.2, 112.6, 110.5, 101.9, 66.5 (CH_2-O), 55.6, 54.1, 50.5 (CH_2-N), 28.7, 26.3; HPLC purity: 100.0 %, R.T: 17.35 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{25}H_{23}NO_3$, 386.1756; found, 386.1729.

R_f : S-1, S-2, S-3; 0.608, 0.662, 0.674 (UV lamp 254nm).

4.1.10 Synthesis and Analysis of (URO 5)



3-(3-(3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)propoxy)-6H-benzo[c]chromen-6-one

White solid; yield: 74 %; mp: 205-207 ˚C; IR (cm^{-1}): 2710, 2608, 1742; 1H NMR (400 MHz, DMSO-d6): δ = 9.48 (b, 1H, N-H), 8.33-8.20 (m, 3H, Ar-H), 7.91 (t, 1H, Ar-H), 7.61 (t, 1H, Ar-H), 7.02 (m, 2H, Ar-H), 6.79 (m, 2H, Ar-H), 4.23 (m, 2H, -O-$\underline{CH_2}$-), 4.11 (m, 6H, -$\underline{CH_2}$-N-), 3.72 (m, 6H, -O-$\underline{CH_3}$), 2.90 (m, 2H, -N-CH_2-$\underline{CH_2}$-C=C-), 2.34 (m, 2H, -O-CH_2-$\underline{CH_2}$-CH_2-N-); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) major rotamer: 160.5 (C=O), 160.2 , 152.0, 135.4, 134.7, 129.8, 128.2, 124.9, 123.8, 123.3, 122.0, 120.4, 119.9, 112.7, 111.8, 110.9, 109.9, 109.7, 102.1 (Ar), 65.7 (CH_2-O), 55.8 (CH_3-O), 55.6 (CH_3-O), 52.4, 51.2, 48.7 (CH_2-N), 24.4, 23.4; HPLC purity: 100.00%, R.T: 19.06 min.; HRMS (m/z): $[M+H]^+$ calcd. for $C_{27}H_{27}NO_5$, 446.1967; found, 446.1975.

R_f : S-1, S-2, S-3; 0.257, 0.125, 0.181 (UV lamp 254nm).

4.2 The potential of the Title Compounds to Inhibit Cholinesterase Enzyme

The potential of the compounds to inhibit human recombinant cholinesterase enzymes (i.e., human rAChE, and human rBuChE) were evaluated utilizing the Modified Ellman's method [127]. The obtained IC_{50}s for the title compounds concomitant to the references (i.e., rivastigmine, galantamine, and donepezil) are shown in Table 1.

Based on the results (Table 1), each title compound displayed AChE selectivity. The THU series were found to possess more potential to inhibit both enzymes in comparison to their corresponding URO analogues. These findings are in agreement with the previous findings that we have published on urolithins [47]. Both the AChE and the BuChE inhibitory potentials of the synthesized compounds were comparable to the activity of rivastigmine. The most active compound within the series (i.e., THU 2) displayed comparable activity in comparison to galantamine. However, none of the compounds were found as active as donepezil for the inhibition of AChE under the experimental conditions utilized.

Table 1: Cholinesterase inhibitory potential and selectivity of the title compounds and the references

Compounds	IC_{50}(μM)		Selectivity bias for AChE
	hAChE	hBuChE	
URO 1	12.4 ± 1.1	74.2 ± 2.2	∽ 6.0
URO 2	2.1 ± 0.2	11.5 ± 0.2	∽ 5.5
URO 3	16.9 ± 0.5	94.1 ± 1.7	∽ 5.6
URO 4	13.4 ± 0.9	22.4 ± 0.1	∽ 1.7
URO 5	8.3 ± 1.5	24.2 ± 0.8	∽ 1.9
THU 1	10.5 ± 0.4	61.6 ± 1.2	∽ 5.9
THU 2	0.5 ± 0.1	12.4 ± 1.0	∽ 24.8
THU 3	10.9 ± 1.0	82.3 ± 1.8	∽ 7.6
THU 4	11.3 ± 0.7	22.3 ± 1.2	∽ 2.0
THU 5	6.0 ± 0.7	24.1 ± 0.6	∽ 4.0
Rivastigmine	32.2 ± 0.3	12.6 ± 0.3	∽ 0.4
Galantamine	0.8 ± 0.1	23.1 ± 0.5	∽ 28.9
Donepezil	0.009 ± 0	6.5 ± 0.2	∽ 722

With respect to the aim of the study, modifications on the N-benzyl moiety within the title compounds pointed out important results. First of all, the isoquinoline analogues (i.e., the title compounds **URO 4-5** and **THU 4-5**) possessing the *N*-benzyl group did not display apparent superior activity in comparison to other title molecules lacking benzyl moiety. This implies that the C-N bond within the *N*-benzyl structure gains the optimum flexibility to fit to the active site of the enzyme in the presence of free rotation, as it is present in donepezil, and as it was obtained in the previous study on urolithin analogues [47]. Besides, the dimethoxy substituents on the isoquinoline ring appeared to yield out more active compounds in comparison to the non-substituted isoquinoline analogues. On the other hand, the title compounds without the N-benzyl group displayed variable results (i.e., **URO 1** to **3**, and **THU 1** to **3**). Among those compounds, morpholine, piperidine, and piperazine moieties

constitute the structural changes. Morpholine and piperazine both have only single heteroatom difference (i.e., the terminal Nitrogen, and Oxygen within the structures of piperazine, morpholine, respectively) in comparison to piperidine. This single heteroatom change revealed out drastic results. First of all, the nM level IC_{50} obtained for the piperidine derivative (i.e., the title compound **THU 2**) pointed out that the N-benzyl moiety is subject to criticism in terms of its necessity in aryl-spacer-*N*-Benzyl pharmacophore model. In other words, this result indicates that still potent AChE inhibitor molecules can be obtained without the presence of benzyl group. On the other hand, it was found that an additional heteroatom was not tolerated well (i.e., as it is present in piperazine and morpholine analogues) in the active site of the enzyme, since activities were decreased in the corresponding title compounds (i.e., **URO 1**, **URO 3**, **THU 1**, **THU 3**). This may be explained with the possible presence of a hydrophobic environment around the piperidine hydrocarbon backbone. This hydrophobic environment can lead to the weak accommodation of an additional heteroatom which is present in morpholine and piperazine.

4.3 The Potential of the Title Compounds to Inhibit AChE-induced Aβ Aggregation

In the final set of experiments, the title compounds were screened for their potential to prevent AChE-accelerated amyloid beta aggregation at 100 μM concentration, concomitant to the references, donepezil, and phenol red [128]. The results are shown in Table 2. According to amyloid beta aggregation results (Table 2), the compounds were shown to possess similar activity to donepezil, but they were not potent inhibitors. As it is suggested, amyloid beta aggregation inhibitors have different structural scaffolds, particularly non-polar groups [129]. Obviously, the title

compounds, designed for cholinesterase inhibition are lacking the structural requirements to prevent amyloid beta aggregation inhibition.

Table 2: The potential of the compounds to inhibit AChE induced amyloid beta aggregation

Compounds	Percent Inhibition *	Compounds	Percent inhibition
URO 1	12 ± 1 %	**THU 1**	14 ± 0.4 %
URO 2	8 ± 0.5 %	**THU 2**	9 ± 1 %
URO 3	5 ± 1 %	**THU 3**	8 ± 0.3 %
URO 4	8 ± 1 %	**THU 4**	8 ± 0.9 %
URO 5	6 ± 0.3 %	**THU 5**	9 ± 1.1 %
Phenol Red	84 ± 1.7 %	**Donepezil**	16 ± 0.3 %

* Each compound is tested at 100 μM.

4.4 Molecular Docking Study with the Most Active Compound

The basic function of the benzyl group in donepezil, and aryl-spacer-*N*-benzyl pharmacophore strategy was explained with the construction of the Pi-Pi interaction between the benzyl in donepezil and the tryptophan (i.e., W 86) at the active site of the AChE. A molecular docking study was performed using Molecular Operating Environment (MOE, 2015.10) in order to show possible interactions of the most active compound (i.e., **THU 2**) with hAChE [133]. For this purpose, the crystal structure of cholinesterase enzyme complexed with donepezil (code ID: 1EVE) were retrieved from protein data bank [130]. The co-crystallized ligand (i.e., donepezil) was removed. The 2D structures of ligands were sketched and converted to 3D format. The docking parameters were left as default. Finally, the interaction of the most favorable conformation with the minimum free energy of binding was illustrated using Discovery Studio [131]. The results are shown in [Figure 10, 11].

Accordingly, the docking study with the most active compound (i.e., **THU 2**) revealed out similar interactions with the hAChE in comparison to donepezil. The urolithin backbone indicated interaction with Tyr341 and Trp286 residues. Furthermore, the spacer moiety (i.e., propylene within the series) accommodated similar to donepezil spacer, led to the formation of an ion-dipole interaction between the tertiary Nitrogen and a water molecule as it is exactly seen in donepezil .

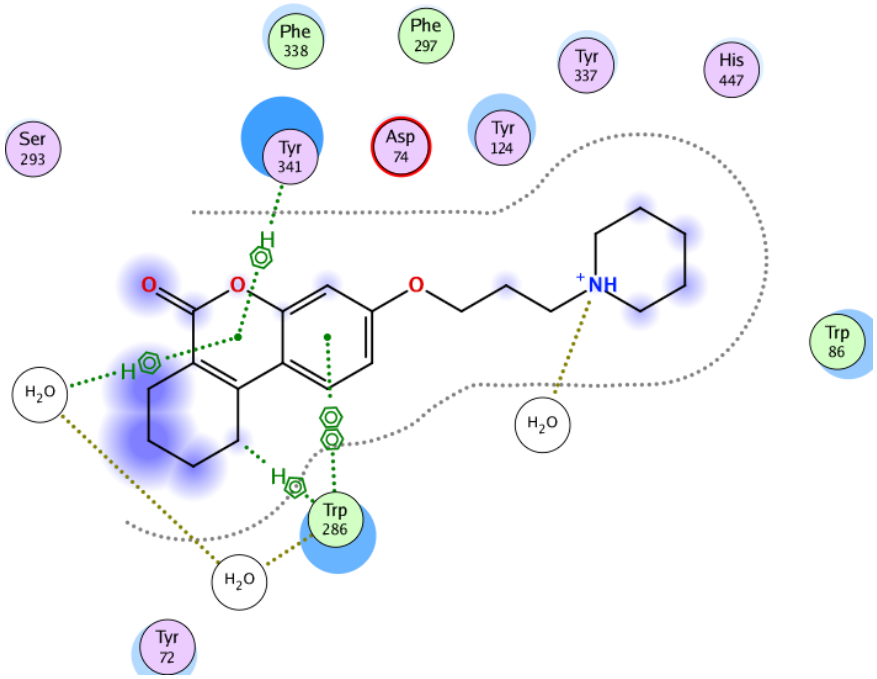


Figure 10: THU 2 interaction with AChE active site

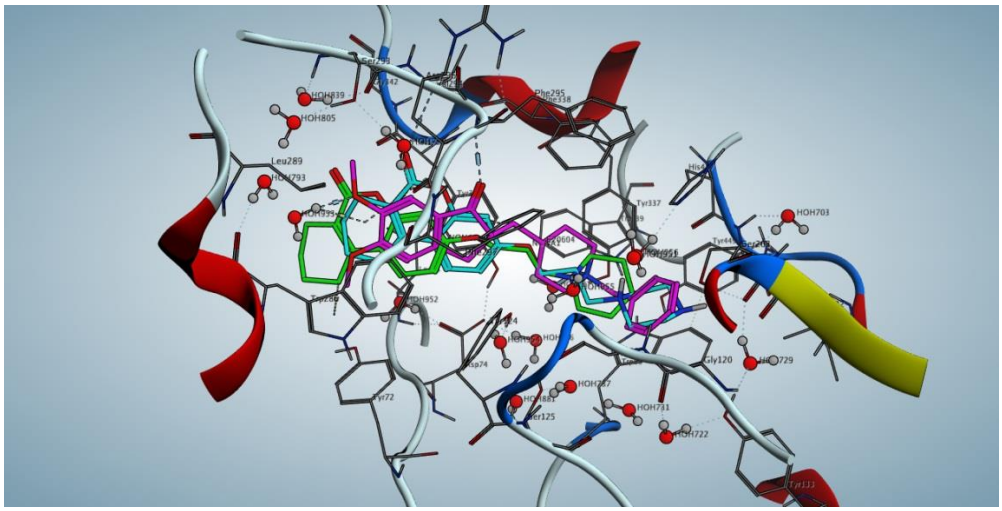


Figure11: Superimpose view of THU 2 (green molecule) and donepezil (purple molecule) in the AChE active site

Chapter 5

CONCLUSION

Overall our study, on the continuation of a previous study that we have published on the design of novel cholinesterase inhibitor agents employing the urolithines, has shown that the benzyl group is not always a necessity in the design of cholinesterase inhibitor agents employing the aryl-spacer-N-benzyl pharmacophore mto the activity of galantamine and rivastigmine in terms of inhibition of AChE. The THU-2 compound, the most active compounds within the series, has come out as a lead molecule for the continuation of the current work.

On the other hand, the significance of the flexibility within the carbon-nitrogen bond is strictly shown by employing the isoquinoline analogues. Obviously, the title compounds, **THU 4-5**, and **URO 4-5**, designed to prove this hypothesis definitely reinforced this idea. In other words, the results absolutely indicated that the employment of N-benzyl group within the Aryl-Spacer-N-Benzyl group must utilize a free N-C bond rotation for the optimal activity.

These results all together indicate that urolithins are still important building blocks for the design of potent cholinesterase inhibitor agents. Considering the fact that urolithins are bioavailable molecules, the title compounds are also promising to have drug-likely properties. However, further biological experiments are required to display this features.

REFERENCES

[1] Brookmeyer, R., Gray, S., & Kawas, C. (1998). Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *American journal of public health*, *88*(9), 1337-1342.

[2] Kumar, A., & Singh, A. (2015). A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacological Reports*, *67*(2), 195-203.

[3] Cheung, K. H., Mei, L., Mak, D. O. D., Hayashi, I., Iwatsubo, T., Kang, D. E., & Foskett, J. K. (2010). Gain-of-function enhancement of InsP3 receptor modal gating by familial Alzheimer's disease-linked presenilin mutants in human cells and mouse neurons. *Science signaling*, *3*(114), ra22.

[4] Fleisher, A. S., Sowell, B. B., Taylor, C., Gamst, A. C., Petersen, R. C., & Thal, L. J. (2007). Clinical predictors of progression to Alzheimer disease in amnestic mild cognitive impairment. *Neurology*, *68*(19), 1588-1595.

[5] Goedert, M., & Spillantini, M. G. (2006). A century of Alzheimer's disease. *science*, *314*(5800), 777-781.

[6] Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *science*, *297*(5580), 353-356.

[7] Tarawneh, R., & Holtzman, D. M. (2012). The clinical problem of symptomatic Alzheimer disease and mild cognitive impairment. *Cold Spring Harbor perspectives in medicine*, *2*(5), a006148.

[8] Herrera-Rivero, M. (2013). Late-Onset Alzheimer's Disease: Risk Factors, Clinical Diagnosis and the Search for Biomarkers. In *Neurodegenerative Diseases*. InTech.

[9] Mattsson, N., Andreasson, U., Persson, S., Carrillo, M. C., Collins, S., Chalbot, S., ... & Hsiung, G. Y. R. (2013). CSF biomarker variability in the Alzheimer's Association quality control program. *Alzheimer's & Dementia*, *9*(3), 251-261.

[10] Herrera-Rivero, M. (2013). Late-Onset Alzheimer's Disease: Risk Factors, Clinical Diagnosis and the Search for Biomarkers. In *Neurodegenerative Diseases*. InTech.

[11] Blennow, K. (2004). Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx*, *1*(2), 213-225.

[12] Sharma, S., & Lipincott, W. (2017). Biomarkers in Alzheimer's Disease-Recent Update. *Current Alzheimer research*.

[13] Stokking, R., Zuiderveld, K. J., Pol, H. E. H., van Rijk, P. P., & Viergever, M. A. (1997). Normal fusion for three-dimensional integrated visualization of SPECT and magnetic resonance brain images. *The Journal of Nuclear Medicine*, *38*(4), 624.

[14] Herrera-Rivero, M. (2013). Late-Onset Alzheimer's Disease: Risk Factors, Clinical Diagnosis and the Search for Biomarkers. In *Neurodegenerative Diseases*. InTech.

[15] https://pubchem.ncbi.nlm.nih.gov/compound/2826731#section=Top

[16] Kumar, A., & Dogra, S. (2008). Neuropathology and therapeutic management of Alzheimer's disease--An update. *Drugs of the Future*, *33*(5), 433-446.

[17] Fisher, A. (2012). Cholinergic modulation of amyloid precursor protein processing with emphasis on M1 muscarinic receptor: perspectives and challenges in treatment of Alzheimer's disease. *Journal of neurochemistry*, *120*(s1), 22-33.

[18] Kurz, A., & Perneczky, R. (2011). Novel insights for the treatment of Alzheimer's disease. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(2), 373-379.

[19] Kumar, A., & Dogra, S. (2008). Neuropathology and therapeutic management of Alzheimer's disease--An update. *Drugs of the Future*, *33*(5), 433-446.

[20] Engel, T., Goñi-Oliver, P., Lucas, J. J., Avila, J., & Hernández, F. (2006). Chronic lithium administration to FTDP-17 tau and GSK-3β overexpressing mice prevents tau hyperphosphorylation and neurofibrillary tangle formation, but pre-formed neurofibrillary tangles do not revert. *Journal of Neurochemistry*, *99*(6), 1445-1455.

[21] Javed, H., Khan, M. M., Ahmad, A., Vaibhav, K., Ahmad, M. E., Khan, A., ... & Safhi, M. M. (2012). Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. *Neuroscience*, *210*, 340-352.

[22] Antanitus, D. S. (1998). A theory of cortical neuron-astrocyte interaction. *The Neuroscientist*, *4*(3), 154-159.

[23] Medina, M., & Avila, J. (2014). New perspectives on the role of tau in Alzheimer's disease. Implications for therapy. *Biochemical pharmacology*, *88*(4), 540-547.

[24] Schelterns, P., & Feldman, H. (2003). Treatment of Alzheimer's disease; current status and new perspectives. *The Lancet Neurology*, *2*(9), 539-547.

[25] Qaseem, A., Snow, V., Cross, J. T., Forciea, M. A., Hopkins, R., Shekelle, P., ... & Santaguida, P. (2008). Current Pharmacologic Treatment of Dementia: A Clinical Practice Guideline from the American College of Physicians and the American Academy of Family PhysiciansCurrent Pharmacologic Treatment of Dementia. *Annals of internal medicine*, *148*(5), 370-378.

[26] Ishii, M., & Kurachi, Y. (2006). Muscarinic acetylcholine receptors. *Current pharmaceutical design*, *12*(28), 3573-3581.

[27] Abrams, P., Andersson, K. E., Buccafusco, J. J., Chapple, C., Groat, W. C., Fryer, A. D., ... & Wein, A. J. (2006). Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. *British journal of pharmacology*, *148*(5), 565-578.

[28] Dvir, H., Silman, I., Harel, M., Rosenberry, T. L., & Sussman, J. L. (2010). Acetylcholinesterase: from 3D structure to function. *Chemico-biological interactions*, *187*(1), 10-22.

[29] Parsons, C. G., Danysz, W., Dekundy, A., & Pulte, I. (2013). Memantine and cholinesterase inhibitors: complementary mechanisms in the treatment of Alzheimer's disease. *Neurotoxicity research*, *24*(3), 358-369.

[30] Bresink, I., Benke, T. A., Collett, V. J., Seal, A. J., Parsons, C. G., Henley, J. M., & Collingridge, G. L. (1996). Effects of memantine on recombinant rat NMDA receptors expressed in HEK 293 cells. *British journal of pharmacology*, *119*(2), 195-204.

[31] Boyett, K. W., DiCarlo, G., Jantzen, P. T., Jackson, J., O'leary, C., Wilcock, D., ... & Gordon, M. N. (2003). Increased fibrillar β-amyloid in response to human C1q injections into hippocampus and cortex of APP+ PS1 transgenic mice. *Neurochemical research*, *28*(1), 83-93.

[32] Takasugi, N., Tomita, T., Hayashi, I., & Tsuruoka, M. (2003). The role of presenilin cofactors in the gamma-secretase complex. *Nature*, *422*(6930), 438.

[33] Guglielmotto, M., Aragno, M., Autelli, R., Giliberto, L., Novo, E., Colombatto, S., ... & Tamagno, E. (2009). The up-regulation of BACE1 mediated by hypoxia and ischemic injury: role of oxidative stress and HIF1α. *Journal of neurochemistry*, *108*(4), 1045-1056.

[34] Bhatia, N., & Hall, G. (2013). Untangling the role of tau in Alzheimer's disease: A unifying hypothesis. *Translational Neuroscience*, *4*(2), 115-133.

[35] Smith, M. A., Harris, P. L., Sayre, L. M., & Perry, G. (1997). Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proceedings of the National Academy of Sciences*, *94*(18), 9866-9868.

[36] Racchi, M., Mazzucchelli, M., Porrello, E., Lanni, C., & Govoni, S. (2004). Acetylcholinesterase inhibitors: novel activities of old molecules. *Pharmacological Research*, *50*(4), 441-451.

[37] Giacobini, E. (2002). Long-term stabilizing effect of cholinesterase inhibitors in the therapy of Alzheimer'disease. *Ageing and Dementia: Current and Future Concepts*, *62*, 181.

[38] Giacobini, E. (2001). Do cholinesterase inhibitors have disease-modifying effects in Alzheimer's disease?. *CNS drugs*, *15*(2), 85-91.

[39] Nordberg, A., & Svensson, A. L. (1998). Cholinesterase inhibitors in the treatment of Alzheimer's disease. *Drug safety*, *19*(6), 465-480.

[40] Qizilbash, N., Birks, J., López Arrieta, J., Lewington, S., & Szeto, S. (1999). Tacrine for Alzheimer's disease. *The Cochrane Library*..

[41] Olivares, D., K Deshpande, V., Shi, Y., K Lahiri, D., H Greig, N., T Rogers, J., & Huang, X. (2012). N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. *Current Alzheimer Research*, *9*(6), 746-758.

[42] Wilkinson, D., Windfeld, K., & Colding-Jørgensen, E. (2014). Safety and efficacy of idalopirdine, a 5-HT 6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. *The Lancet Neurology*, *13*(11), 1092-1099..

[43] https://pubchem.ncbi.nlm.nih.gov/compound/3152.

[44] Giacobini, E., & Pepeu, G. (Eds.). (2006). *The Brain Cholinergic System*. Informa Health Care.

[45] Buerger, K., Otto, M., Teipel, S. J., Zinkowski, R., Blennow, K., DeBernardis, J., ... & McCulloch, C. (2006). Dissociation between CSF total tau and tau protein phosphorylated at threonine 231 in Creutzfeldt–Jakob disease. *Neurobiology of aging*, *27*(1), 10-15.

[46] Kaduszkiewicz, H., Zimmermann, T., Beck-Bornholdt, H. P., & van den Bussche, H. (2005). Cholinesterase inhibitors for patients with Alzheimer's disease: systematic review of randomised clinical trials. *Bmj*, *331*(7512), 321-327.

[47] Gulcan, H. O., Unlu, S., Esiringu, İ., Ercetin, T., Sahin, Y., Oz, D., & Sahin, M. F. (2014). Design, synthesis and biological evaluation of novel 6H-benzo [c] chromen-6-one, and 7, 8, 9, 10-tetrahydro-benzo [c] chromen-6-one derivatives as potential cholinesterase inhibitors. *Bioorganic & medicinal chemistry*, *22*(19), 5141-5154.

[48] Gibney, G., Camp, S., Dionne, M., MacPhee-Quigley, K., & Taylor, P. (1990). Mutagenesis of essential functional residues in acetylcholinesterase. *Proceedings of the National Academy of Sciences*, *87*(19), 7546-7550.

[49] Rakonczay, Z. (1988). Cholinesterase and its molecular forms in pathological states. Progress in neurobiology, 31(4), 311-330.

[50] Singh, M., Kaur, M., Kukreja, H., Chugh, R., Silakari, O., & Singh, D. (2013). Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection. *European journal of medicinal chemistry*, *70*, 165-188.

[51] Rivera, E. J., Goldin, A., Fulmer, N., Tavares, R., Wands, J. R., & de la Monte, S. M. (2005). Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. *Journal of Alzheimer's Disease*, *8*(3), 247-268.

[52] Klein, W. L., Krafft, G. A., & Finch, C. E. (2001). Targeting small Aβ oligomers: the solution to an Alzheimer's disease conundrum?. *Trends in neurosciences*, *24*(4), 219-224.

[53] Nagele, R. G., Wegiel, J., Venkataraman, V., Imaki, H., Wang, K. C., & Wegiel, J. (2004). Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. *Neurobiology of aging*, *25*(5), 663-674.

[54] Ribé, E. M., Pérez, M., Puig, B., Gich, I., Lim, F., Cuadrado, M., ... & Gómez-Ramos, P. (2005). Accelerated amyloid deposition, neurofibrillary degeneration and neuronal loss in double mutant APP/tau transgenic mice. *Neurobiology of disease*, *20*(3), 814-822.

[55] Bono, G. F., Simão-Silva, D. P., Batistela, M. S., Josviak, N. D., Dias, P. F. R., Nascimento, G. A., ... & Furtado-Alle, L. (2015). Butyrylcholinesterase: K variant, plasma activity, molecular forms and rivastigmine treatment in Alzheimer's disease in a Southern Brazilian population. *Neurochemistry international*, *81*, 57-62.

[56] Savelev, S., Okello, E., Perry, N. S. L., Wilkins, R. M., & Perry, E. K. (2003). Synergistic and antagonistic interactions of anticholinesterase terpenoids in Salvia lavandulaefolia essential oil. *Pharmacology Biochemistry and Behavior*, *75*(3), 661-668.

[57] R Sridhar, G. (2011). Proteins of the esterase family: patents for some proteins in search of metabolic functions. *Recent Patents on Biomarkers*, *1*(3), 205-212.

[58] Estrada Serrato, C. Polimorfismos del gen Butirilcolinesterasa responsables de reacciones adversas en pacientes consumidores de "cocaína".

[59] Bono, G. F., Simão-Silva, D. P., Batistela, M. S., Josviak, N. D., Dias, P. F. R., Nascimento, G. A., ... & Furtado-Alle, L. (2015). Butyrylcholinesterase: K variant, plasma activity, molecular forms and rivastigmine treatment in Alzheimer's disease in a Southern Brazilian population. *Neurochemistry International*, *81*, 57-62.

[60] Chautard-Freire-Maia, E. A., Primo-Parmo, S. L., Picheth, G., Lourenço, M. A. C., & Vieira, M. M. (1991). The C5 isozyme of serum cholinesterase and adult weight. *Human heredity*, *41*(5), 330-339.

[61] Bono, G. F., Simão-Silva, D. P., Batistela, M. S., Josviak, N. D., Dias, P. F. R., Nascimento, G. A., ... & Furtado-Alle, L. (2015). Butyrylcholinesterase: K variant, plasma activity, molecular forms and rivastigmine treatment in Alzheimer's disease in a Southern Brazilian population. *Neurochemistry International*, *81*, 57-62.

[62] Brimijoin, S., & Hammond, P. (1988). Butyrylcholinesterase in human brain and acetylcholinesterase in human plasma: Trace enzymes measured by two-site immunoassay. *Journal of Neurochemistry*, *51*(4), 1227-1231.

[63]Greig, N. H., Utsuki, T., Ingram, D. K., Wang, Y., Pepeu, G., Scali, C., ... & Chen, D. (2005). Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer β-amyloid peptide in rodent. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(47), 17213-17218.

[64] VandenBos, G. R. (2007). APA dictionary of psychology. American Psychological Association.

[65] Valenstein, E. S. (2002). The discovery of chemical neurotransmitters. Brain and cognition, 49(1), 73-95.

[66] Drachman, D. B. (1974). The role of acetylcholine as a neurotrophic transmitter. Annals of the New York Academy of Sciences, 228(1), 160-175.

[67] Tuccaron, S. (1990). The synthesis of acetylcholine: twenty years of progress. Progress in brain research, 84, 467-477.

[68] Coleman, C. G., Baghdoyan, H. A., & Lydic, R. (2006). Dialysis delivery of an adenosine A2A agonist into the pontine reticular formation of C57BL/6J mouse increases pontine acetylcholine release and sleep. Journal of neurochemistry, 96(6), 1750-1759.

[69] Buckwalter, M. S., Coleman, B. S., Buttini, M., Barbour, R., Schenk, D., Games, D., ... & Wyss-Coray, T. (2006). Increased T Cell Recruitment to the CNS after Amyloid β1–42 Immunization in Alzheimer's Mice Overproducing Transforming Growth Factor-β1. Journal of Neuroscience, 26(44), 11437-11441.

[70] Nordberg, A. (2001). Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications. Biological psychiatry, 49(3), 200-210.

[71] Coyle, J. T., Price, D. L., & Delong, M. R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. Science, 219(4589), 1184-1190.

[72] Scheuermann, S., Hambsch, B., Hesse, L., Stumm, J., Schmidt, C., Beher, D., ... & Multhaup, G. (2001). Homodimerization of amyloid precursor protein and its implication in the amyloidogenic pathway of Alzheimer's disease. Journal of Biological Chemistry, 276(36), 33923-33929.

[73] Gouras, G. K., Almeida, C. G., & Takahashi, R. H. (2005). Intraneuronal Aβ accumulation and origin of plaques in Alzheimer's disease. Neurobiology of aging, 26(9), 1235-1244.

[74] Yan, R. (2016). Stepping closer to treating Alzheimer's disease patients with BACE1 inhibitor drugs. Translational neurodegeneration, 5(1), 13.

[75] Vassar, R. (2014). BACE1 inhibitor drugs in clinical trials for Alzheimer's disease. Alzheimer's research & therapy, 6(9), 89.

[76] John, V., Beck, J. P., Bienkowski, M. J., Sinha, S., & Heinrikson, R. L. (2003). Human β-secretase (BACE) and BACE inhibitors. Journal of medicinal chemistry, 46(22), 4625-4630.

[77] Bentahir, M., Nyabi, O., Verhamme, J., Tolia, A., Horré, K., Wiltfang, J., ... & Strooper, B. (2006). Presenilin clinical mutations can affect γ-secretase activity by different mechanisms. Journal of neurochemistry, 96(3), 732-742.

[78] Esler, W. P., Kimberly, W. T., Ostaszewski, B. L., Ye, W., Diehl, T. S., Selkoe, D. J., & Wolfe, M. S. (2002). Activity-dependent isolation of the presenilin–γ-secretase complex reveals nicastrin and a γ substrate. *Proceedings of the National Academy of Sciences*, *99*(5), 2720-2725.

[79] De Strooper, B., Vassar, R., & Golde, T. (2010). The secretases: enzymes with therapeutic potential in Alzheimer disease. Nature Reviews Neurology, 6(2), 99-107.

[80] Kopan, R., & Ilagan, M. X. G. (2004). [gamma]-Secretase: proteasome of the membrane, Nature reviews. Molecular cell biology, 5(6), 499.

[81] Wolfe, M. S. (2008). Inhibition and modulation of γ-secretase for Alzheimer's disease. Neurotherapeutics, 5(3), 391-398.

[82] Kukar, T. L., Ladd, T. B., Bann, M. A., Fraering, P. C., Narlawar, R., Maharvi, G. M., ... & Moore, B. (2008). Substrate-targeting γ-secretase modulators. Nature, 453(7197), 925.

[83] Shoghi-Jadid, K., Small, G. W., Agdeppa, E. D., Kepe, V., Ercoli, L. M., Siddarth, P., ... & Barrio, J. R. (2002). Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. The American Journal of Geriatric Psychiatry, 10(1), 24-35.

[84] Jagannatha Rao, K. S. (2007). Studies on alpha-Synuclein Aggregation and the effect of Dietary Curcumin Derivative: Relevance to Parkinson's Disease (Doctoral dissertation, University of Mysore).

[85] Robinson, S. (2014). Vital networks: the biological turn in computation, communication, and control. Queen's University (Canada).

[86] Rostami, E., Davidsson, J., Ng, K. C., Lu, J., Gyorgy, A., Walker, J., ... & Risling, M. (2012). A model for mild traumatic brain injury that induces limited transient memory impairment and increased levels of axon related serum biomarkers. Frontiers in neurology, 3.

[87] Lewis, S. A., Wang, D., & Cowan, N. J. (1988). Microtubule-associated protein MAP2 shares a microtubule binding motif with tau protein. Science, 242(4880), 936.

[88] Necula, M., Chirita, C. N., & Kuret, J. (2005). Cyanine dye N744 inhibits tau fibrillization by blocking filament extension: implications for the treatment of tauopathic neurodegenerative diseases. Biochemistry, 44(30), 10227-10237.

[89] Cisek, K., L Cooper, G., J Huseby, C., & Kuret, J. (2014). Structure and mechanism of action of tau aggregation inhibitors. Current Alzheimer Research, 11(10), 918-927.

[90] Horoupian, D. S., & Chu, P. L. (1994). Unusual case of corticobasal degeneration with tau/Gallyas-positive neuronal and glial tangles. Acta neuropathologica, 88(6), 592-598.

[91] Bialonska, D., Kasimsetty, S. G., Khan, S. I., & Ferreira, D. (2009). Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. Journal of Agricultural and Food Chemistry, 57(21), 10181-10186.

[92] Koponen, J. M., Happonen, A. M., Mattila, P. H., & Törrönen, A. R. (2007). Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. Journal of Agricultural and Food Chemistry, 55(4), 1612-1619.

[93] Bialonska, D., Kasimsetty, S. G., Khan, S. I., & Ferreira, D. (2009). Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. Journal of Agricultural and Food Chemistry, 57(21), 10181-10186.

[94] Bialonska, D., Kasimsetty, S. G., Khan, S. I., & Ferreira, D. (2009). Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. Journal of Agricultural and Food Chemistry, 57(21), 10181-10186.

[95] Bialonska, D., Kasimsetty, S. G., Khan, S. I., & Ferreira, D. (2009). Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. Journal of Agricultural and Food Chemistry, 57(21), 10181-10186.

[96] Landete, J. M. (2011). Ellagitannins, ellagic acid and their derived metabolites: a review about source, metabolism, functions and health. Food Research International, 44(5), 1150-1160.

[97] Espín, J. C., González-Barrio, R., Cerdá, B., López-Bote, C., Rey, A. I., & Tomás-Barberán, F. A. (2007). Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans. Journal of Agricultural and Food Chemistry, 55(25), 10476-10485.

[98] Beserra, A. M. S. E. S., Calegari, P. I., Souza, M. D. C., dos Santos, R. A. N., Lima, J. C. D. S., Silva, R. M., ... & Martins, D. T. D. O. (2011). Gastroprotective and ulcer-healing mechanisms of ellagic acid in experimental rats. *Journal of Agricultural and Food Chemistry*, *59*(13), 6957-6965.

[99] Piwowarski, J. P., Kiss, A. K., Granica, S., & Moeslinger, T. (2015). Urolithins, gut microbiota-derived metabolites of ellagitannins, inhibit LPS-induced inflammation in RAW 264.7 murine macrophages. *Molecular nutrition & food research*, *59*(11), 2168-2177.

[100] Doyle, B., & Griffiths, L. A. (1980). The metabolism of ellagic acid in the rat. *Xenobiotica*, *10*(4), 247-256.

[101]Tomás-Barberan, F. A., Espín, J. C., & García-Conesa, M. T. (2009). Bioavailability and metabolism of ellagic acid and ellagitannins. In *Chemistry And Biology Of Ellagitannins: An Underestimated Class of Bioactive Plant Polyphenols* (pp. 273-297).

[102]Espín, J. C., González-Barrio, R., Cerdá, B., López-Bote, C., Rey, A. I., & Tomás-Barberán, F. A. (2007). Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans. *Journal of Agricultural and Food Chemistry*, *55*(25), 10476-10485.

[103] Wang, L. (2013). *Pomegranate Juice and Pomegranate Derived Natural Products as Alternative Treatment for Cancer Progression and Metastasis*. University of California, Riverside.

[104] Larrosa, M., Yañéz-Gascón, M. J., Selma, M. V., González-Sarrías, A., Toti, S., Cerón, J. J., ... & Espín, J. C. (2009). Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. *Journal of agricultural and food chemistry*, *57*(6), 2211-2220.

[105] Bialonska, D., Kasimsetty, S. G., Khan, S. I., & Ferreira, D. (2009). Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. *Journal of Agricultural and Food Chemistry*, *57*(21), 10181-10186.

[106] Bialonska, D., Kasimsetty, S. G., Khan, S. I., & Ferreira, D. (2009). Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. *Journal of Agricultural and Food Chemistry*, *57*(21), 10181-10186.

[107] Kasimsetty, S. G., Bialonska, D., Reddy, M. K., Ma, G., Khan, S. I., & Ferreira, D. (2010). Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. Journal of agricultural and food chemistry, 58(4), 2180-2187.

[108] MUÑOZ, M. C. G. (2013). *Ecole doctorale Sciences des Procédés–Sciences des Aliments Unité Mixte de recherche (UMR)* (Doctoral dissertation, Institut de recherche pour le développement).

[109] Furlanetto, V., Zagotto, G., Pasquale, R., Moro, S., & Gatto, B. (2012). Ellagic acid and polyhydroxylated urolithins are potent catalytic inhibitors of human topoisomerase II: an in vitro study. *Journal of agricultural and food chemistry*, *60*(36), 9162-9170.

[110] Sharma, M., Li, L., Celver, J., Killian, C., Kovoor, A., & Seeram, N. P. (2009). Effects of fruit ellagitannin extracts, ellagic acid, and their colonic metabolite, urolithin A, on Wnt signaling. *Journal of agricultural and food chemistry*, *58*(7), 3965-3969.

[111] Walsh, H. (2014). *Functional properties of whey protein and its application in nanocomposite materials and functional foods*. The University of Vermont and State Agricultural College.

[112] Yuan, T., Ma, H., Liu, W., Niesen, D. B., Shah, N., Crews, R., ... & Seeram, N. P. (2015). Pomegranate's neuroprotective effects against Alzheimer's disease are mediated by urolithins, its ellagitannin-gut microbial derived metabolites. *ACS chemical neuroscience*, *7*(1), 26-33.

[113] Yuan, T., Ma, H., Liu, W., Niesen, D. B., Shah, N., Crews, R., ... & Seeram, N. P. (2015). Pomegranate's neuroprotective effects against Alzheimer's disease are mediated by urolithins, its ellagitannin-gut microbial derived metabolites. *ACS chemical neuroscience*, *7*(1), 26-33.

[114] Seeram, N. P., & Heber, D. (2014). *U.S. Patent No. 8,648,112*. Washington, DC: U.S. Patent and Trademark Office.

[115] Cozza, G., Gianoncelli, A., Bonvini, P., Zorzi, E., Pasquale, R., Rosolen, A., ... & Moro, S. (2011). Urolithin as a converging scaffold linking ellagic acid and coumarin analogues: design of potent protein kinase CK2 inhibitors. *ChemMedChem*, *6*(12), 2273-2286.

[116] De Sarkar, S., Grimme, S., & Studer, A. (2010). NHC catalyzed oxidations of aldehydes to esters: chemoselective acylation of alcohols in presence of amines. *Journal of the American Chemical Society*, *132*(4), 1190-1191.

[117] Wu, J., Lan, J., Guo, S., & You, J. (2014). Pd-Catalyzed C–H Carbonylation of (Hetero) arenes with Formates and Intramolecular Dehydrogenative Coupling: A Shortcut to Indolo [3, 2-c] coumarins. *Organic letters*, *16*(22), 5862-5865.

[118] Tomás-Barberán, F. A., González-Sarrías, A., García-Villalba, R., Núñez-Sánchez, M. A., Selma, M. V., García-Conesa, M. T., & Espín, J. C. (2017). Urolithins, the rescue of "old" metabolites to understand a "new" concept: Metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. *Molecular nutrition & food research*, *61*(1).

[119] Kudou, K., Yamamoto, N., Ban, M., & Ohno, A. (2014). *U.S. Patent No. 8,802,869*. Washington, DC: U.S. Patent and Trademark Office.

[120] Nealmongkol, P., Tangdenpaisal, K., Sitthimonchai, S., Ruchirawat, S., & Thasana, N. (2013). Cu (I)-mediated lactone formation in subcritical water: a benign synthesis of benzopyranones and urolithins A–C. *Tetrahedron*, *69*(44), 9277-9283.

[121] Rinsch, C. L., Blanco-Bose, W., Schneider, B., Mouchiroud, L., Ryu, D., Andreux, P., & Auwerx, J. (2013). *U.S. Patent Application No. 13/929,455*.

[122] Rinsch, C., Blanco-Bose, W., Schneider, B., Thomas, C., Sandi, C., Auwerx, J., ... & Genoux, D. (2016). *U.S. Patent Application No. 15/218,663*.

[123] Kasimsetty, S. G., Bialonska, D., Reddy, M. K., Ma, G., Khan, S. I., & Ferreira, D. (2010). Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. *Journal of agricultural and food chemistry*, *58*(4), 2180-2187.

[124] Rinsch, C. L., Blanco-Bose, W., Schneider, B., Mouchiroud, L., Ryu, D., Andreux, P., & Auwerx, J. (2013). *U.S. Patent Application No. 13/929,455*.

[125] Hariri, A. T., Moallem, S. A., Mahmoudi, M., & Hosseinzadeh, H. (2011). The effect of crocin and safranal, constituents of saffron, against subacute effect of diazinon on hematological and genotoxicity indices in rats. Phytomedicine, 18(6), 499-504.

[126] Hung, T. M., Thuong, P. T., Nhan, N. T., Mai, N. T. T., Quan, T. L., Choi, J. S., ... & Bae, K. (2011). Cholinesterase inhibitory activities of alkaloids from Corydalis tuber. Nat Prod Sci, 17(2), 108-112.

[127] Bartolini, M., Bertucci, C., Cavrini, V., & Andrisano, V. (2003). β-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. Biochemical pharmacology, 65(3), 407-416.

[128] Reinke, A. A., & Gestwicki, J. E. (2007). Structure–activity Relationships of Amyloid Beta-aggregation Inhibitors Based on Curcumin: Influence of Linker Length and Flexibility. Chemical biology & drug design, 70(3), 206-215.

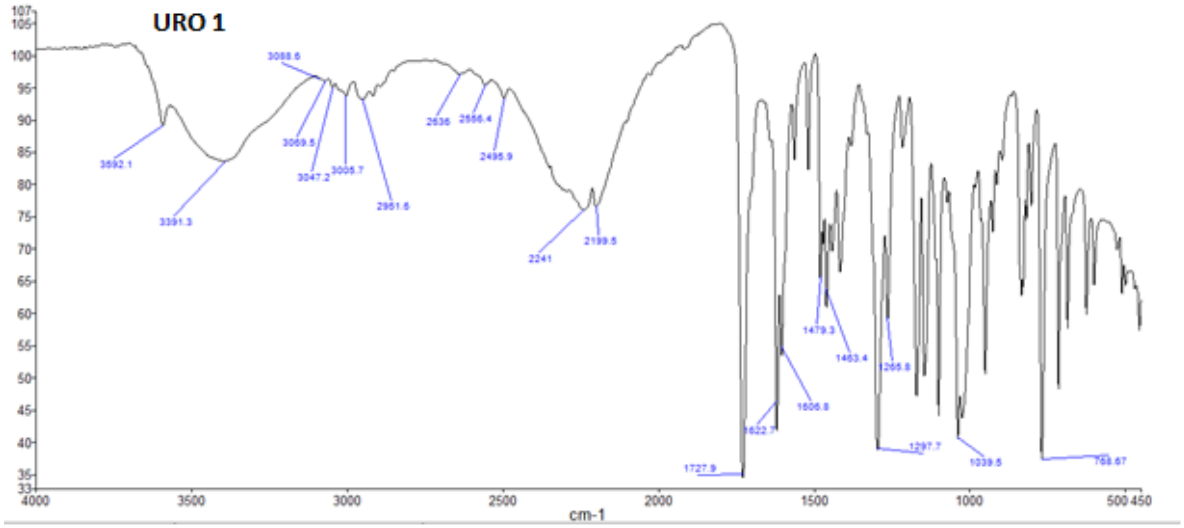
[129] Molecular Operating Environment (MOE), 2015.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015.

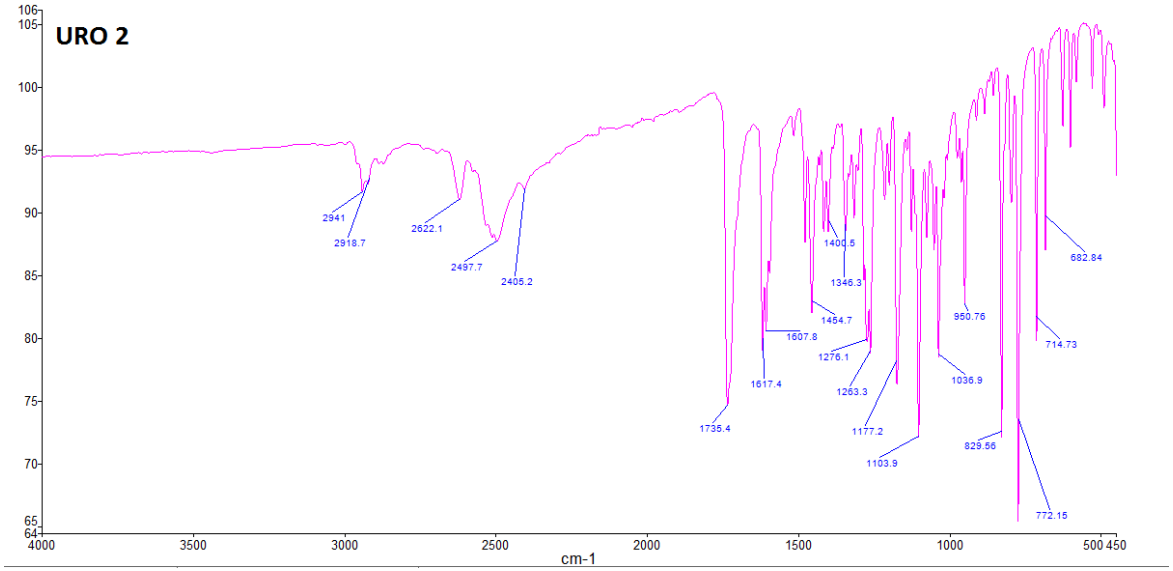
[130] Kryger, G., Silman, I., & Sussman, J. L. (1999). Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs. Structure, 7(3), 297-307.

[131] Diego, S. (2007). Discovery Studio Modeling Environment Release 2.1. Accelerys Software Inc.

APPENDICES

Appendix A: Infrared Spectra of Title Compound




URO 2
106
105
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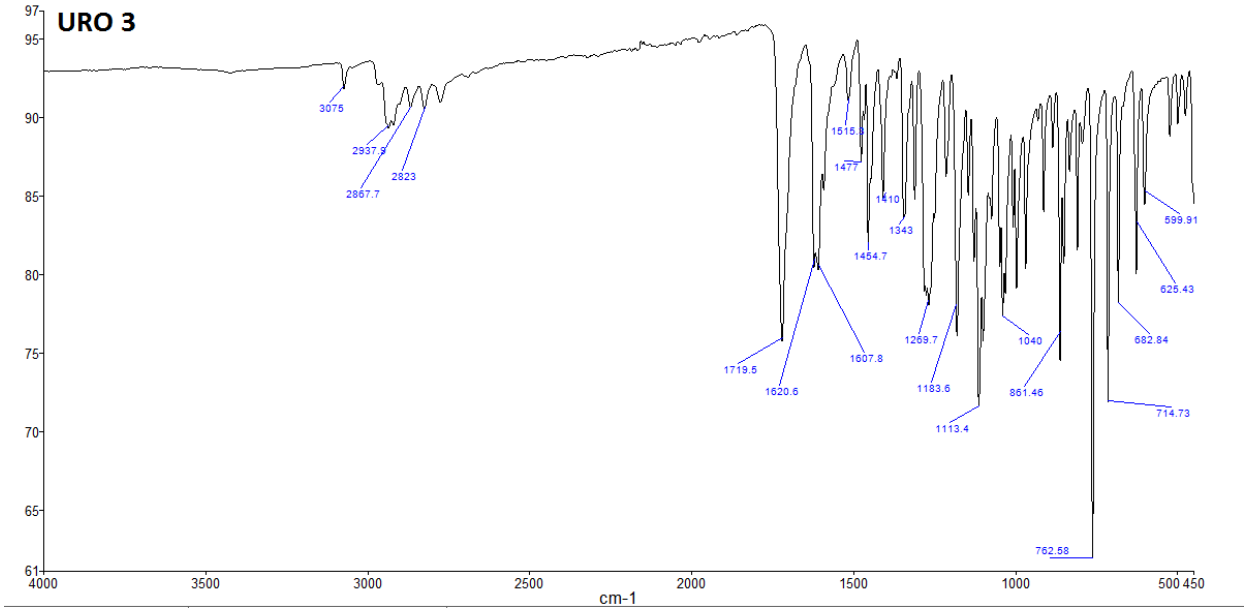
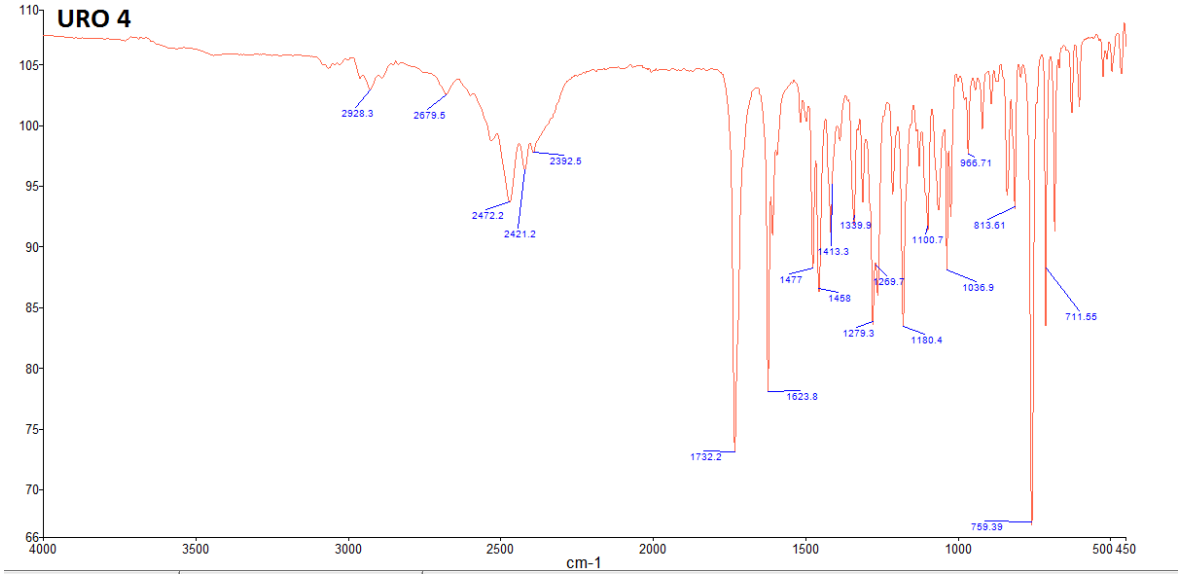
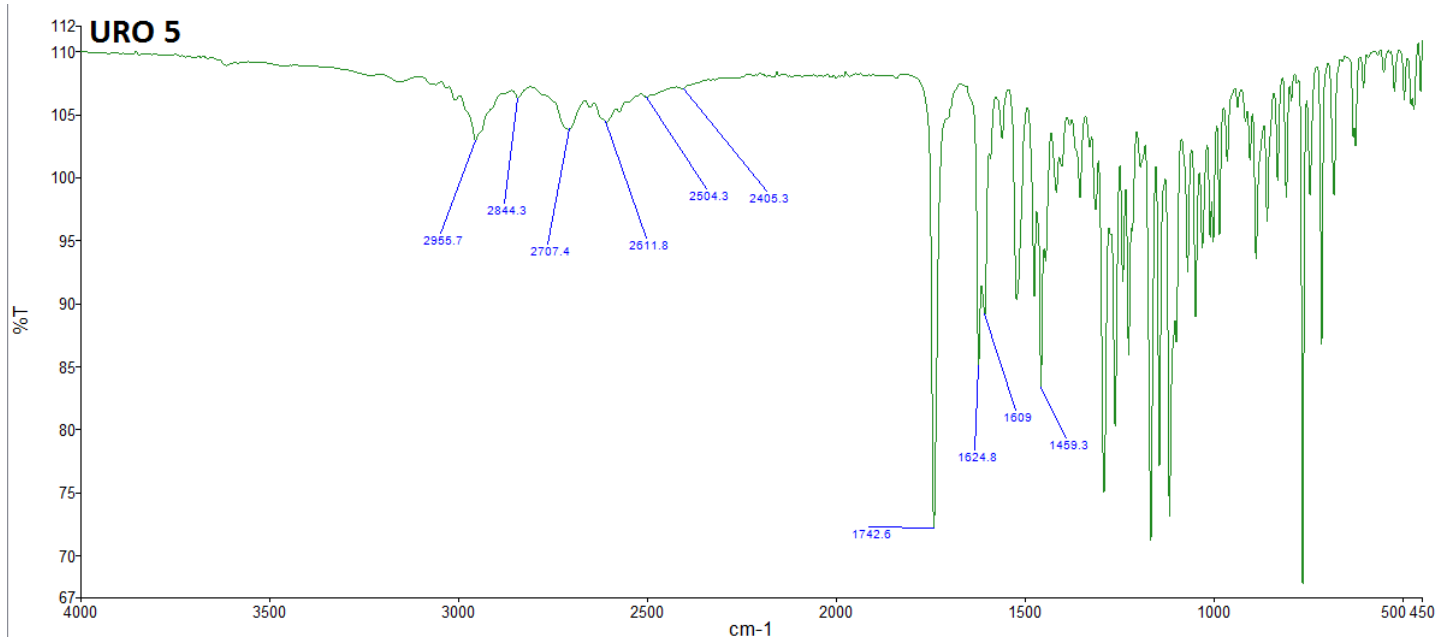
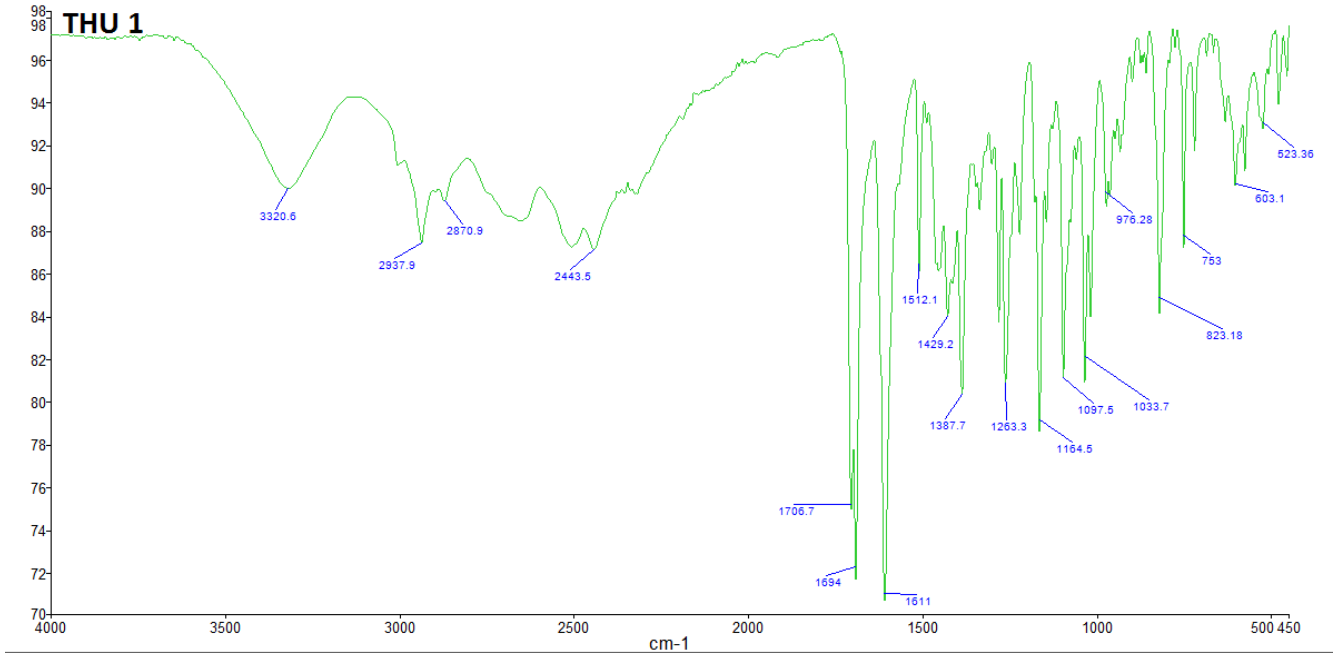
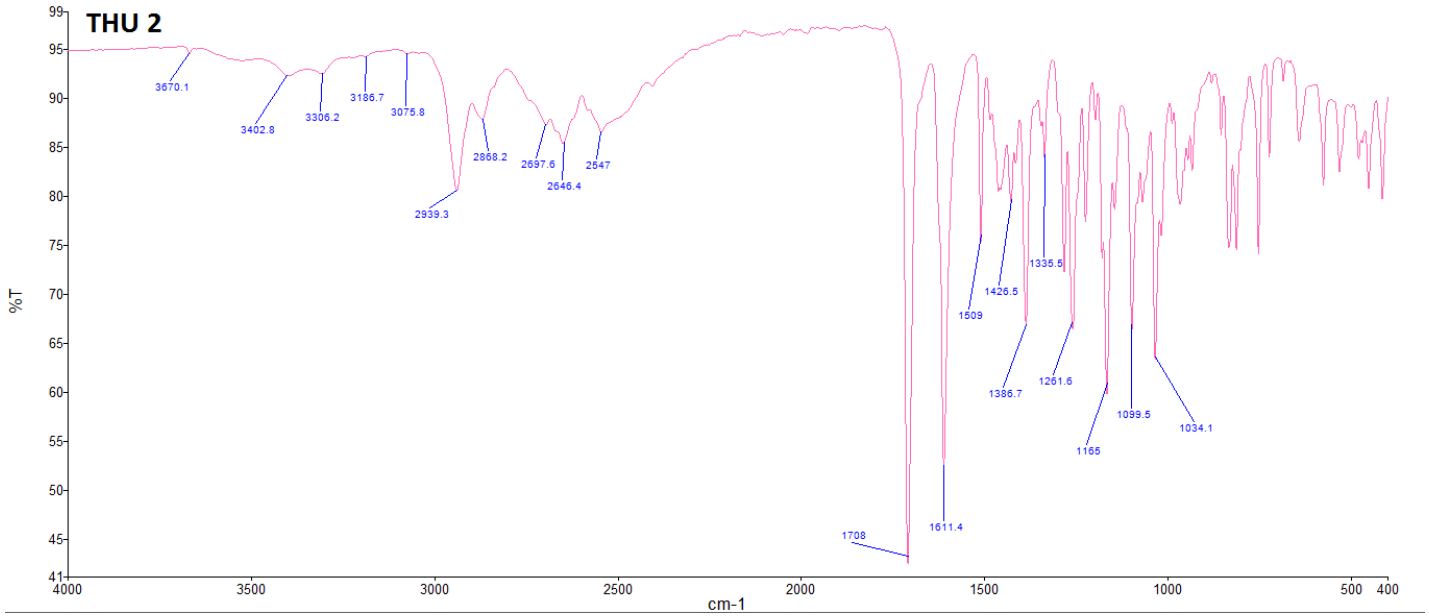
2497.7
2405.2
1735.4
1617.4
1607.8
1454.7
1400.5
1346.3
1276.1
1263.3
1177.2
1103.9
1036.9
950.76
829.56
772.15
714.73
682.84

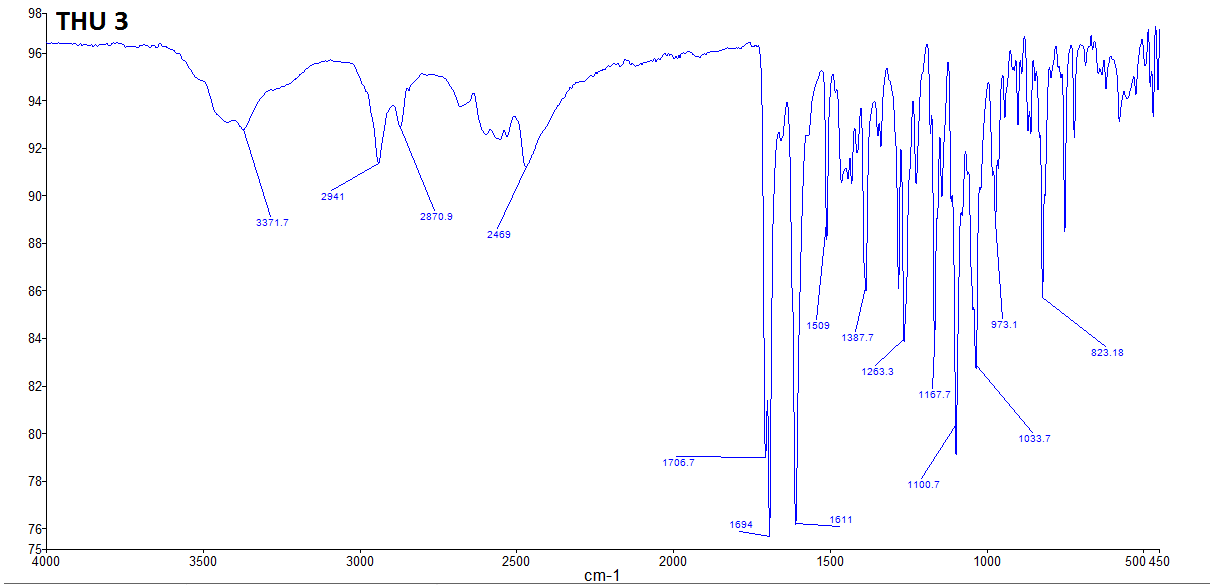

URO 3
97
95
90
85
80
75
70
65
61
4000
3500
3000
2500
2000
1500
1000
500 450
cm-1
3075
2937.9
2867.7
2823
1719.5
1620.6
1607.8
1515.9
1477
1454.7
1410
1343
1269.7
1183.6
1113.4
1040
861.46
762.58
682.84
714.73
625.43
599.91

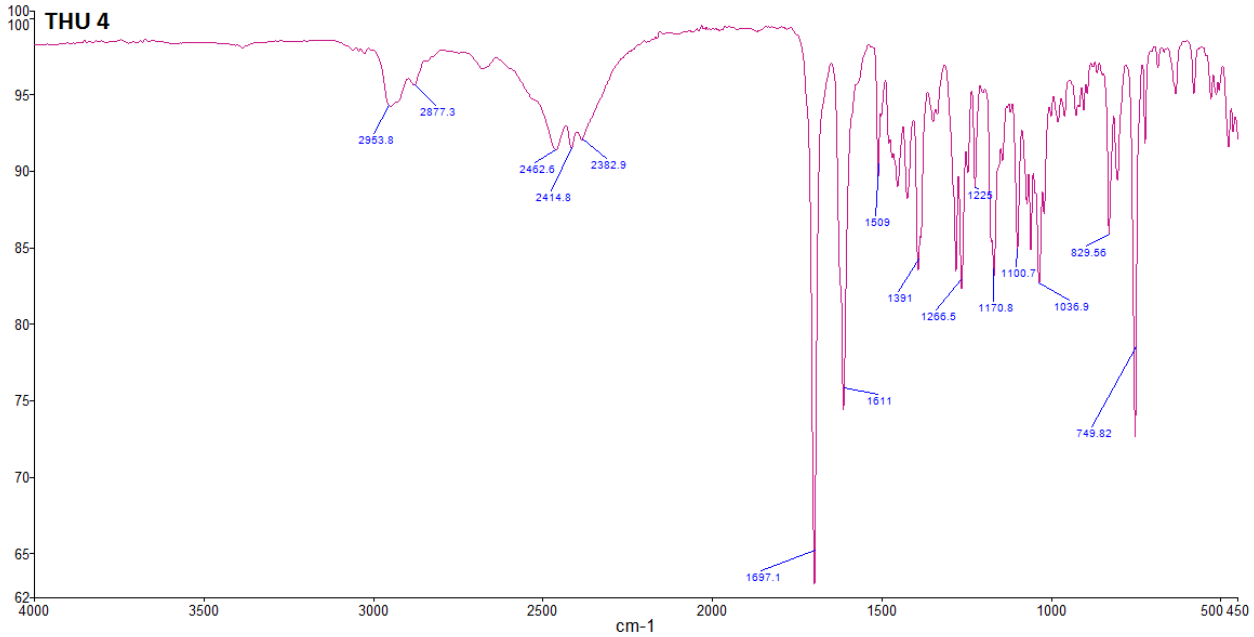

URO 4
110
105
100
95
90
85
80
75
70
66
4000
3500
3000
2500
2000
1500
1000
500 450
cm-1
2928.3
2679.5
2472.2
2421.2
2392.5
1732.2
1623.8
1477
1458
1413.3
1339.9
1279.3
1269.7
1180.4
1100.7
1036.9
966.71
813.61
759.39
711.55

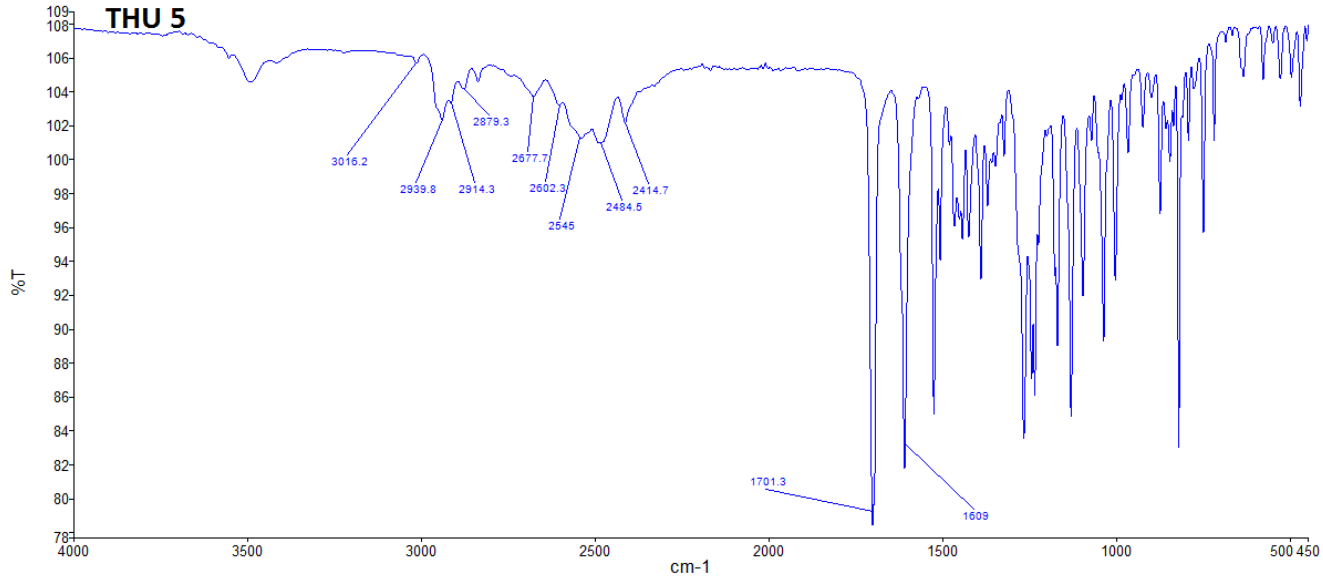

URO 5
%T
cm-1
2955.7
2844.3
2707.4
2611.8
2504.3
2405.3
1742.6
1624.8
1609
1459.3


THU 1
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
4000
3500
3000
2500
2000
1500
1000
500 450
cm-1
3320.6
2937.9
2870.9
2443.5
1706.7
1694
1611
1512.1
1429.2
1387.7
1263.3
1164.5
1097.5
1033.7
976.28
823.18
753
603.1
523.36

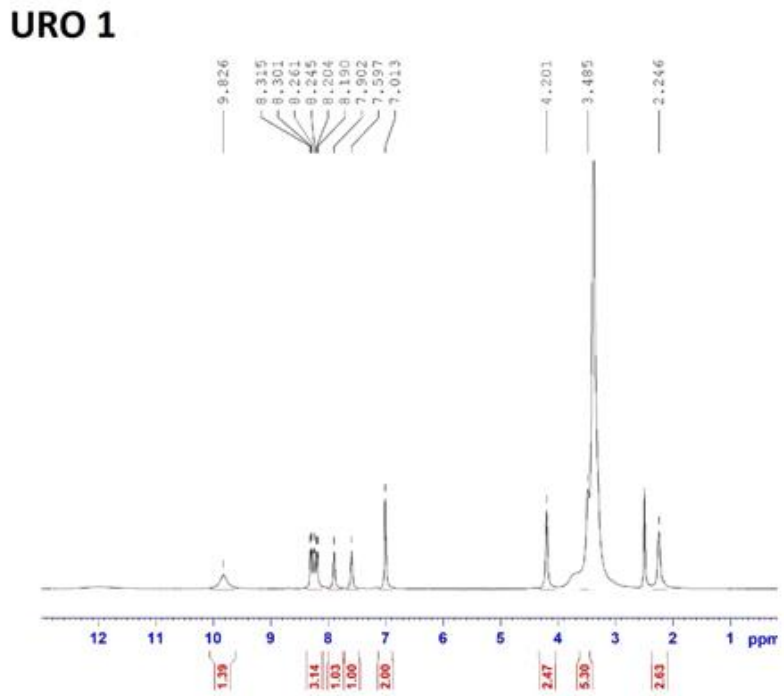

THU 2
%T
cm-1
3670.1
3402.8
3306.2
3186.7
3075.8
2939.3
2868.2
2697.6
2646.4
2547
1708
1611.4
1509
1426.5
1386.7
1335.5
1261.6
1165
1099.5
1034.1


THU 3
98
96
94
92
90
88
86
84
82
80
78
76
75
4000
3500
3000
2500
2000
1500
1000
500 450
cm-1
3371.7
2941
2870.9
2469
1706.7
1694
1611
1509
1387.7
1263.3
1167.7
1100.7
1033.7
973.1
823.18

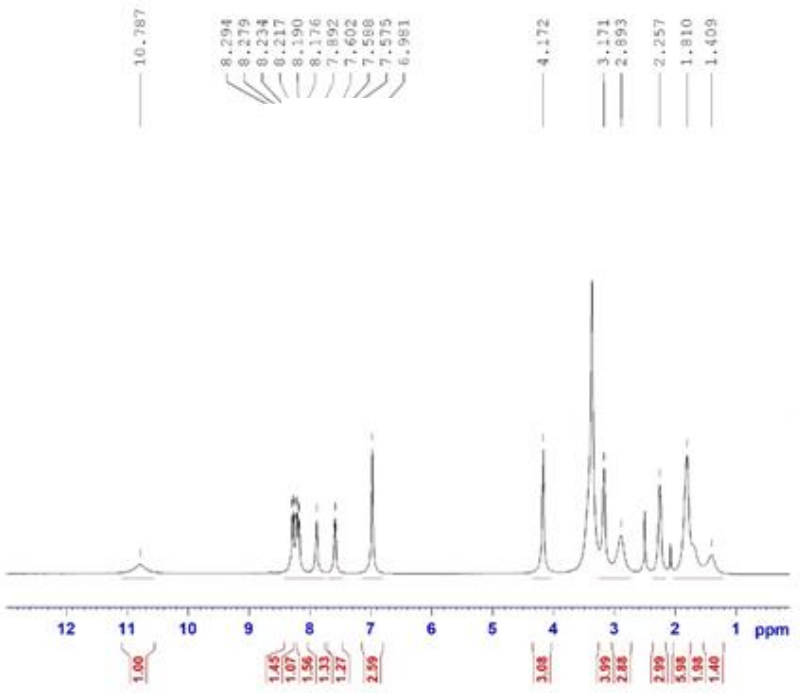

THU 4
100
100
95
90
85
80
75
70
65
62
2963.8
2877.3
2462.6
2414.8
2382.9
1697.1
1611
1509
1391
1266.5
1225
1170.8
1100.7
1036.9
829.56
749.82
4000
3500
3000
2500
2000
1500
1000
500 450
cm-1


THU 5
%T
cm-1
3016.2
2939.8
2914.3
2879.3
2677.7
2602.3
2545
2484.5
2414.7
1701.3
1609

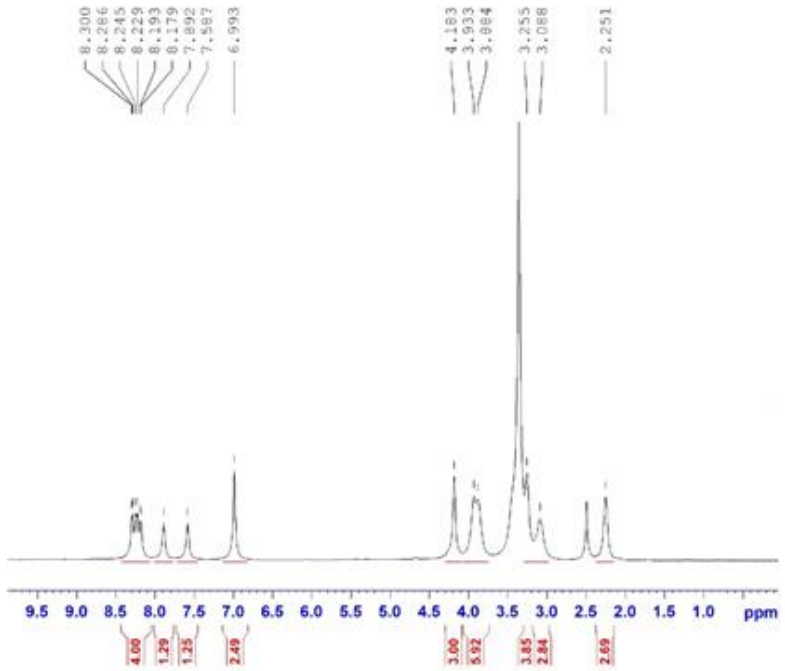
Appendix B: ^{1}H NMR of Title Compound



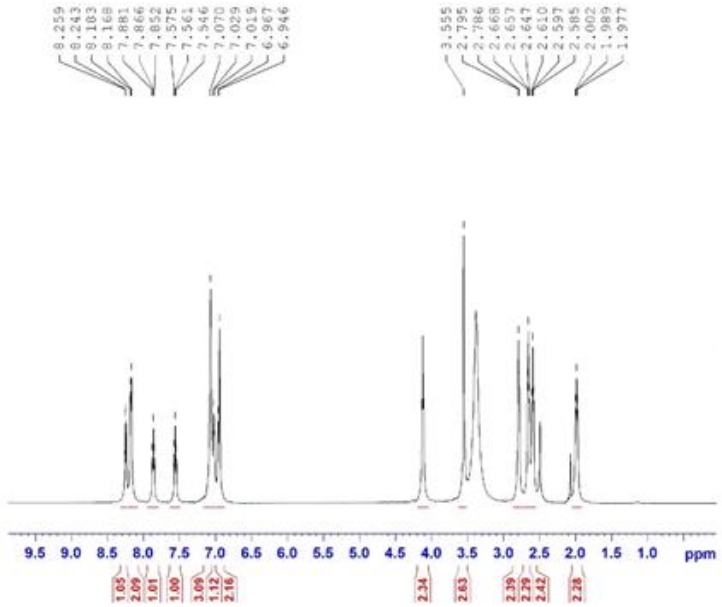
URO 2


10.787
8.294
8.279
8.234
8.217
8.190
8.176
7.892
7.602
7.588
7.575
6.981
4.172
3.171
2.893
2.257
1.810
1.409
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.45
1.07
1.56
1.33
1.27
2.59
3.08
3.99
2.88
2.99
5.98
1.98
1.40

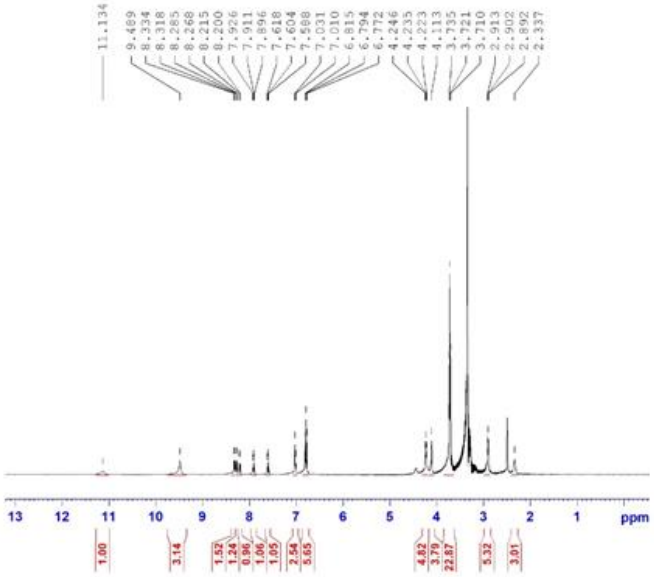
URO 3


8.300
8.266
8.245
8.229
8.193
8.179
7.892
7.587
6.993
4.183
3.933
3.864
3.255
3.088
2.251
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
4.00
1.29
1.35
2.49
3.00
5.92
3.85
2.84
2.69

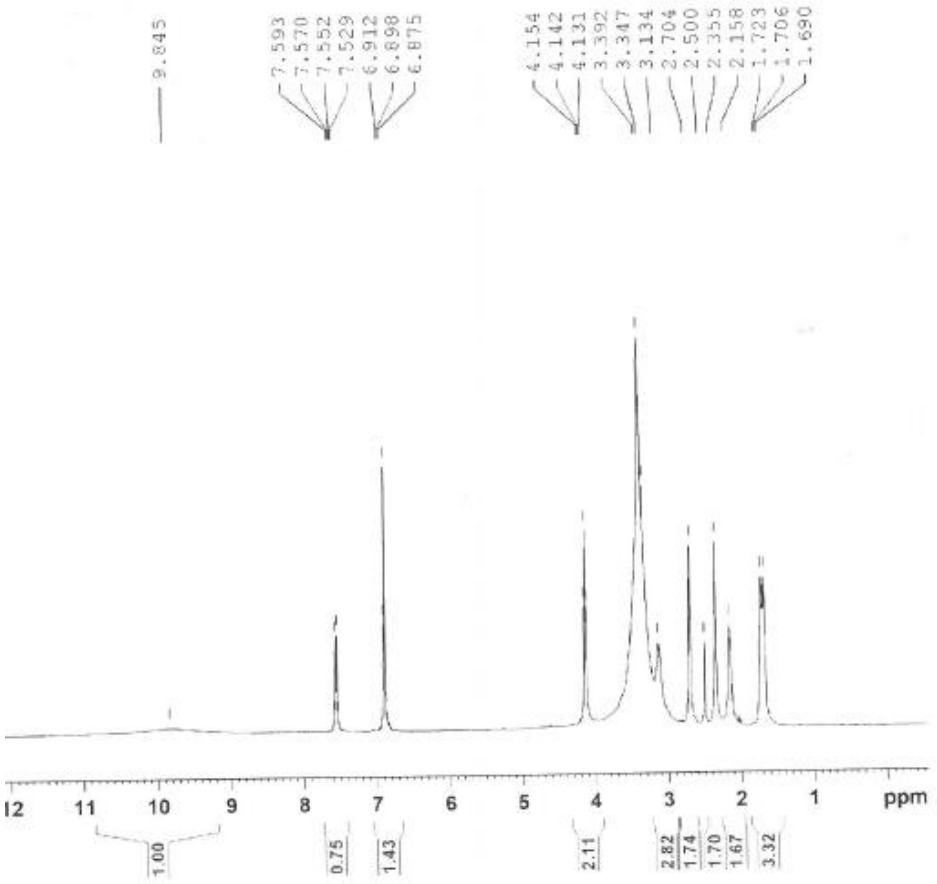
URO 4


8.259
8.243
8.103
8.168
7.881
7.866
7.852
7.575
7.561
7.546
7.070
7.029
7.019
6.967
6.946
3.555
2.795
2.786
2.668
2.657
2.647
2.610
2.597
2.585
2.002
1.989
1.977
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.05
2.09
1.01
1.00
3.09
1.12
2.16
2.34
2.63
2.39
2.29
2.42
2.28

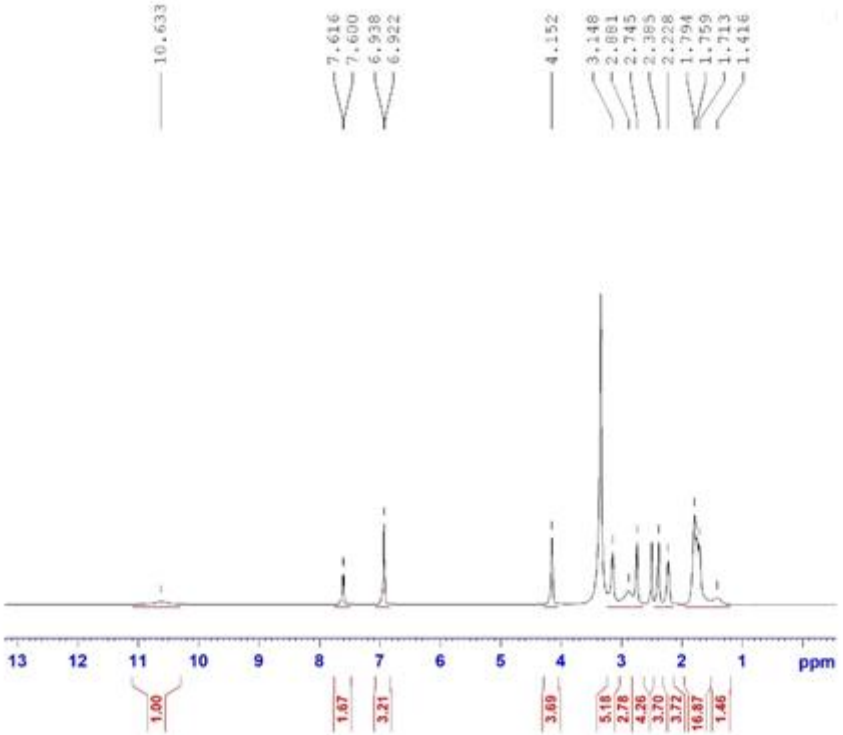
URO 5


11.134
9.469
8.334
8.318
8.285
8.268
8.215
8.200
7.926
7.941
7.896
7.618
7.604
7.588
7.031
7.010
6.815
6.794
6.772
4.246
4.235
4.223
4.113
3.735
3.721
3.710
2.913
2.902
2.892
2.337
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
3.14
1.52
1.24
0.96
1.06
1.05
2.54
5.65
4.82
3.79
22.87
5.32
3.01

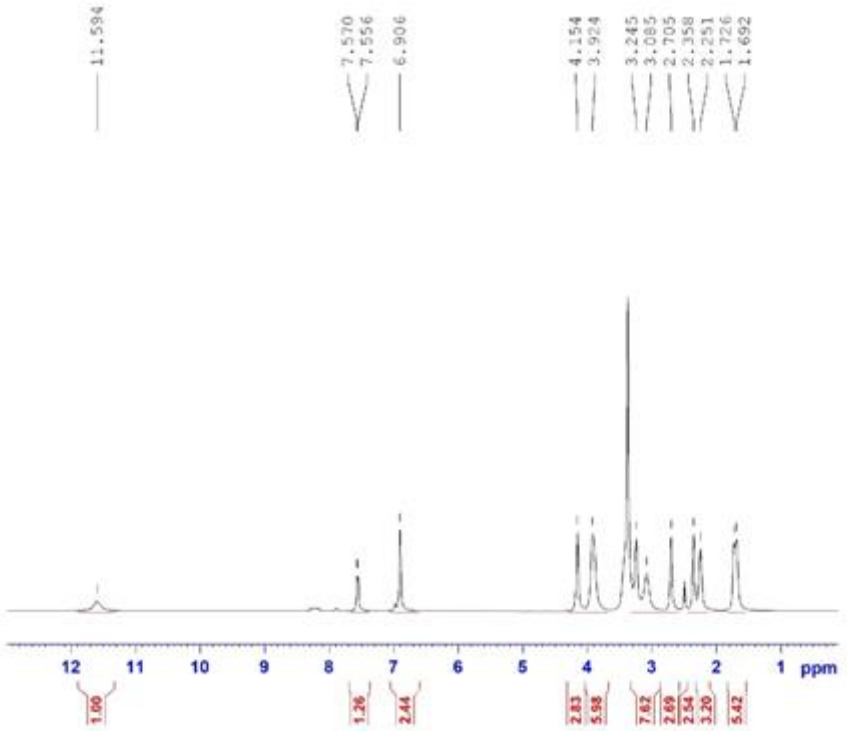
THU1


9.845
7.593
7.570
7.552
7.529
6.912
6.898
6.875
4.154
4.142
4.131
3.392
3.347
3.134
2.704
2.500
2.355
2.158
1.723
1.706
1.690
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
0.75
1.43
2.11
2.82
1.74
1.70
1.67
3.32

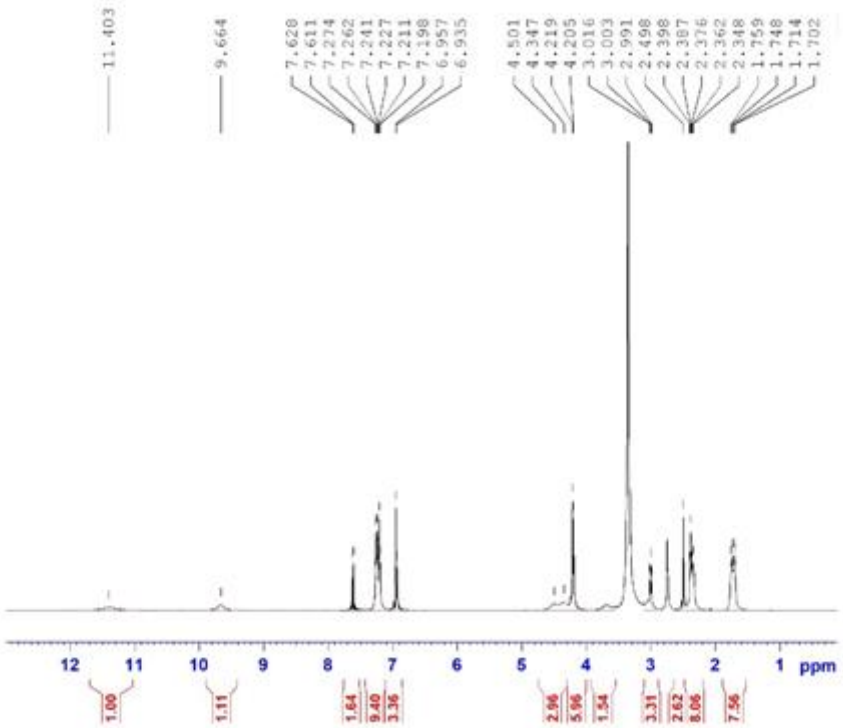
THU 2


10.633
7.616
7.600
6.938
6.922
4.152
3.148
2.881
2.745
2.385
2.228
1.794
1.759
1.713
1.416
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.67
3.21
3.69
5.18
2.78
4.26
3.70
3.72
16.87
1.46

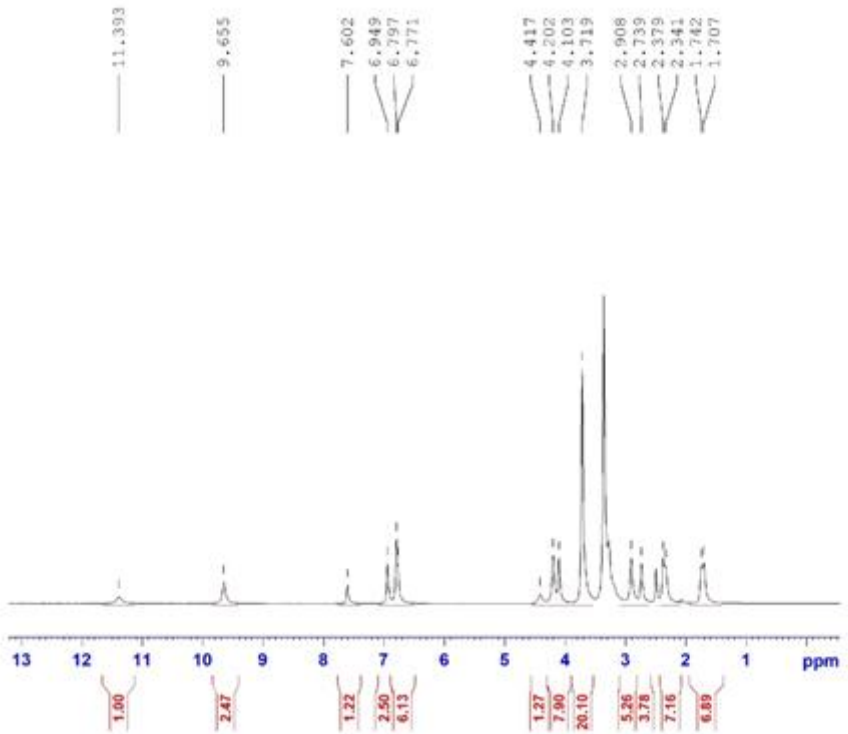
THU 3


11.594
7.570
7.556
6.906
4.154
3.924
3.245
3.085
2.705
2.358
2.251
1.726
1.692
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.26
2.44
2.83
5.58
7.62
2.69
2.54
3.20
5.42

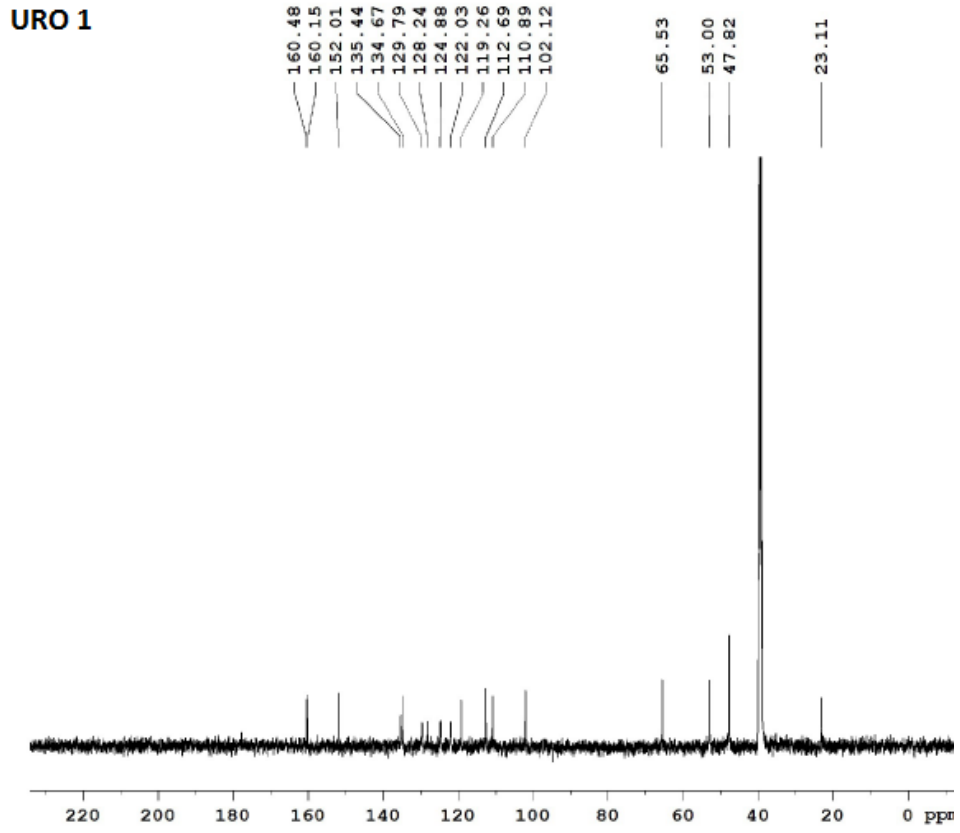
THU 4


11.403
9.664
7.628
7.611
7.274
7.262
7.241
7.227
7.211
7.198
6.957
6.935
4.501
4.347
4.219
4.205
3.016
3.003
2.991
2.498
2.398
2.387
2.376
2.362
2.348
1.759
1.748
1.714
1.702
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.11
1.64
9.40
3.36
2.96
5.96
1.54
3.31
2.62
8.06
7.56

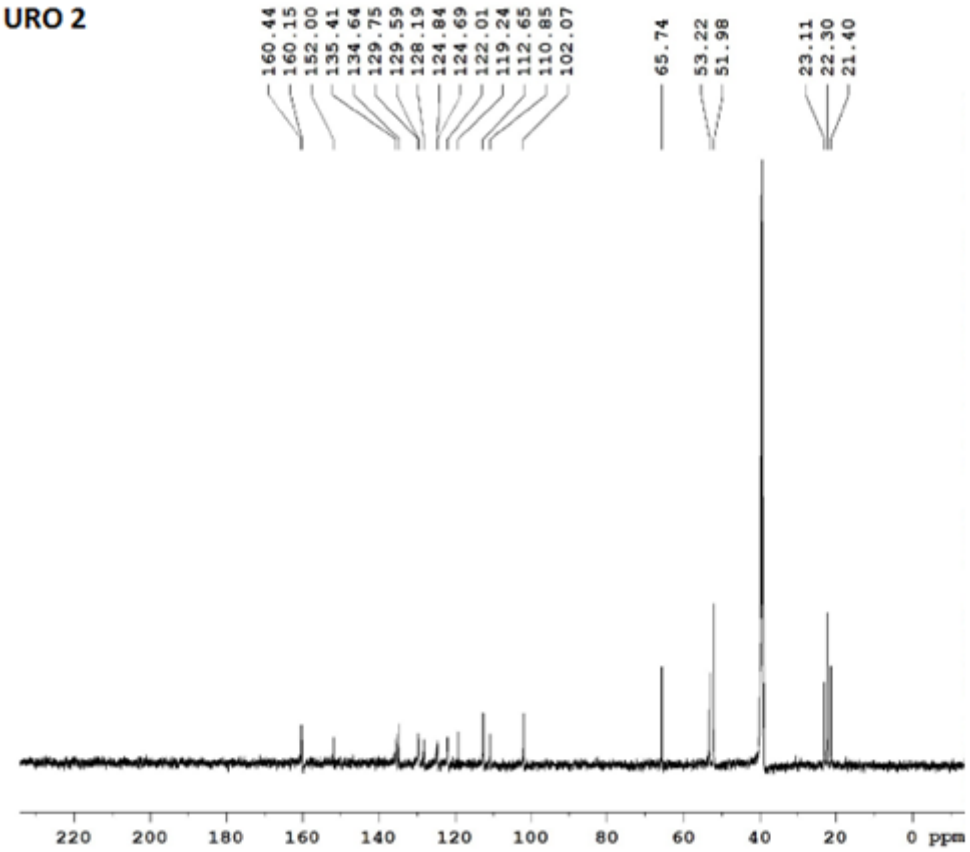
THU 5


11.393
9.655
7.602
6.949
6.797
6.771
4.417
4.202
4.103
3.719
2.908
2.739
2.379
2.341
1.742
1.707
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
2.47
1.22
2.50
6.13
1.27
7.90
20.10
5.26
3.78
7.16
6.89

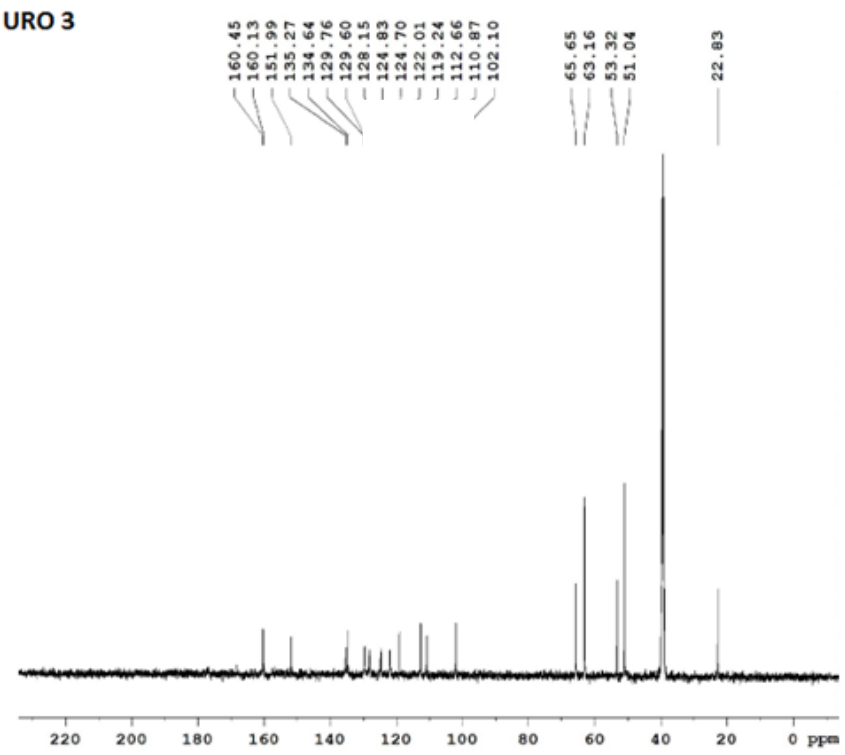
Appendix C: ^{13}C NMR of Title Compounds



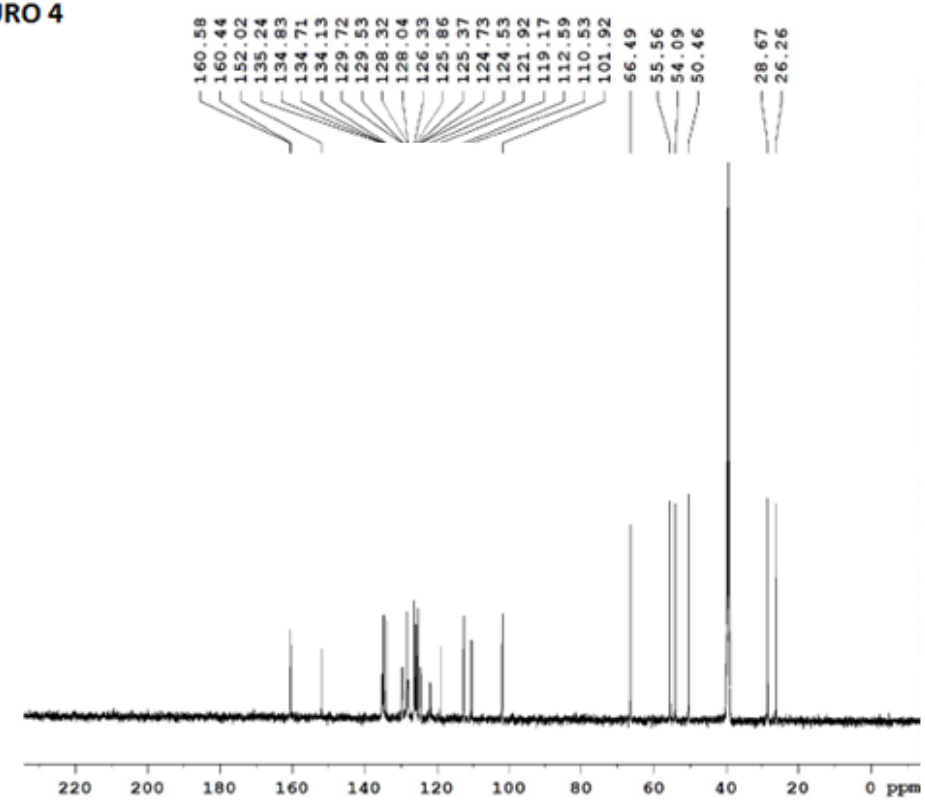
URO 2


160.44
160.15
152.00
135.41
134.64
129.75
129.59
128.19
124.84
124.69
122.01
119.24
112.65
110.85
102.07
65.74
53.22
51.98
23.11
22.30
21.40
220
200
180
160
140
120
100
80
60
40
20
0 ppm

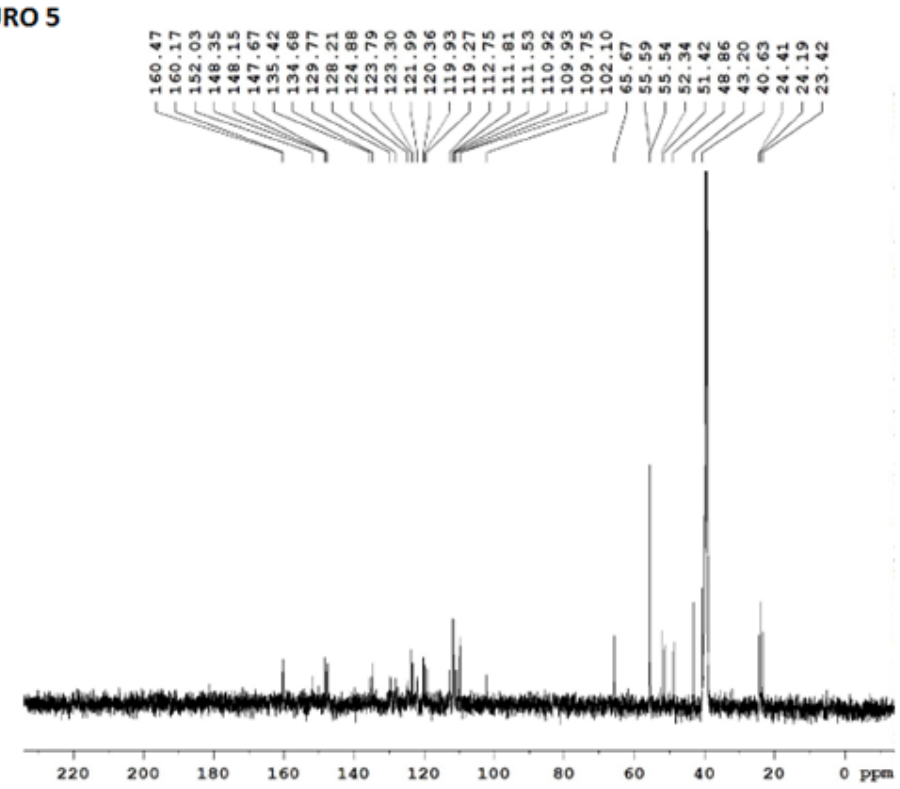
URO 3


160.45
160.13
151.99
135.27
134.64
129.76
129.60
128.15
124.83
124.70
122.01
119.24
112.66
110.87
102.10
65.65
63.16
53.32
51.04
22.83
220
200
180
160
140
120
100
80
60
40
20
0 ppm

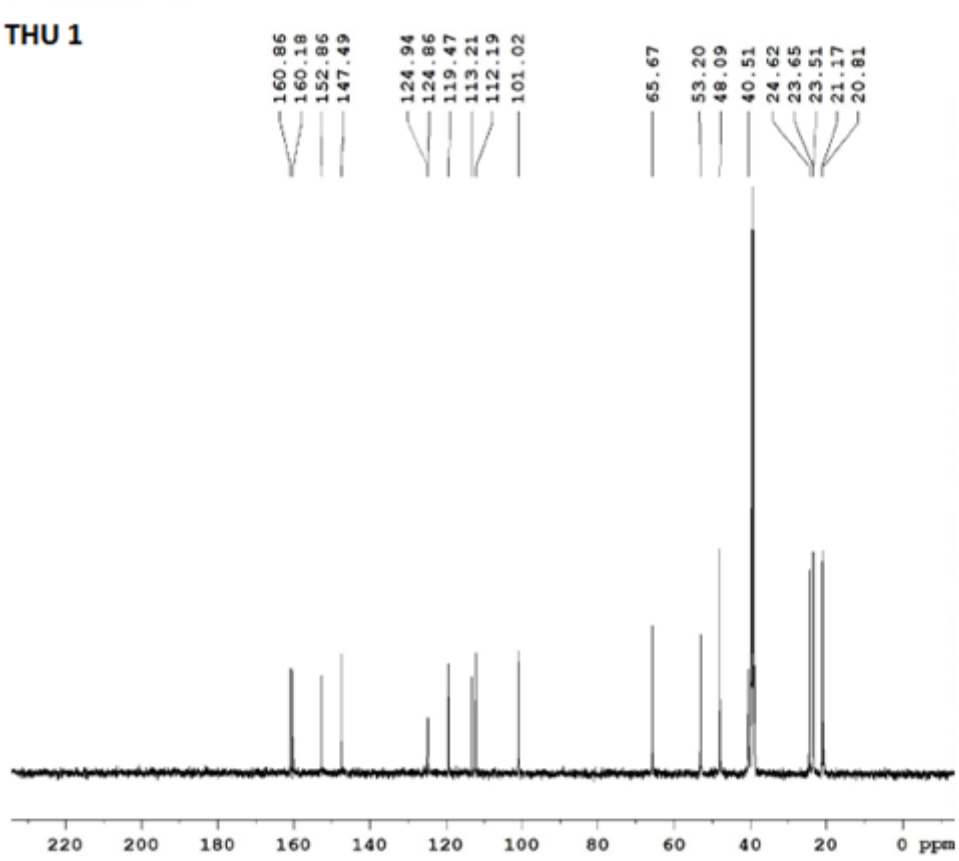
URO 4


160.58
160.44
152.02
135.24
134.83
134.71
134.13
129.72
129.53
128.32
128.04
126.33
125.86
125.37
124.73
124.53
121.92
119.17
112.59
110.53
101.92
66.49
55.56
54.09
50.46
28.67
26.26
220
200
180
160
140
120
100
80
60
40
20
0 ppm

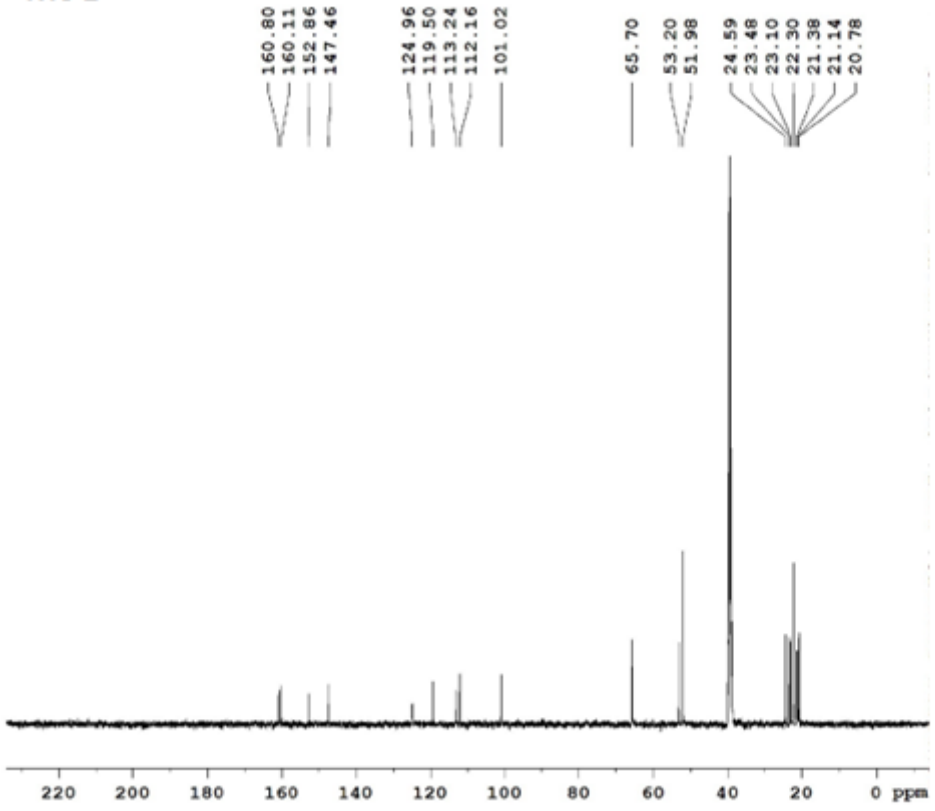
URO 5


160.47
160.17
152.03
148.35
148.15
147.67
135.42
134.68
129.77
128.21
124.88
123.79
123.30
121.99
120.36
119.93
119.27
112.75
111.81
111.53
110.92
109.93
109.75
102.10
65.67
55.59
55.54
52.34
51.42
48.86
43.20
40.63
24.41
24.19
23.42
220
200
180
160
140
120
100
80
60
40
20
0 ppm

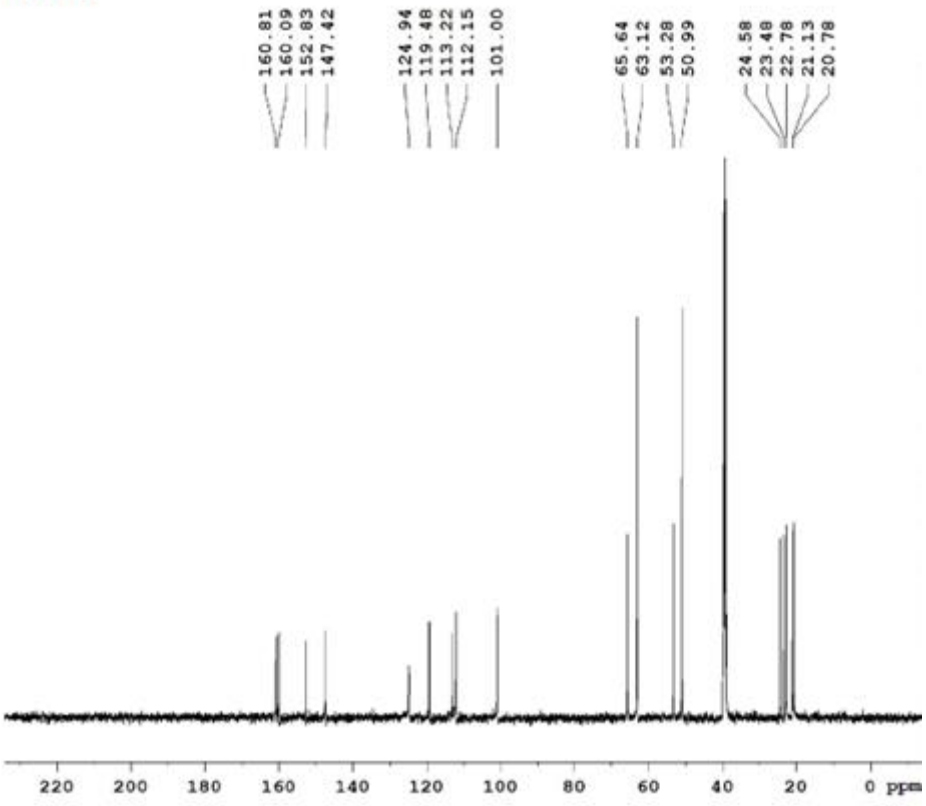
THU 1


160.86
160.18
152.86
147.49
124.94
124.86
119.47
113.21
112.19
101.02
65.67
53.20
48.09
40.51
24.62
23.65
23.51
21.17
20.81
220
200
180
160
140
120
100
80
60
40
20
0 ppm

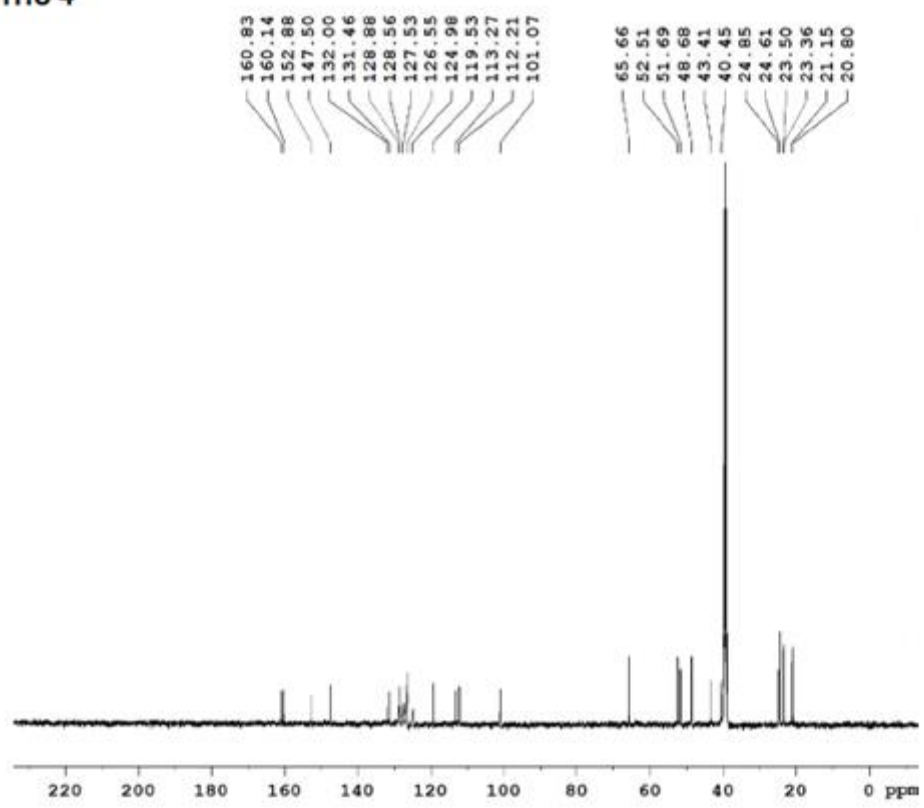
THU 2


160.80
160.11
152.86
147.46
124.96
119.50
113.24
112.16
101.02
65.70
53.20
51.98
24.59
23.48
23.10
22.30
21.38
21.14
20.78
220
200
180
160
140
120
100
80
60
40
20
0 ppm

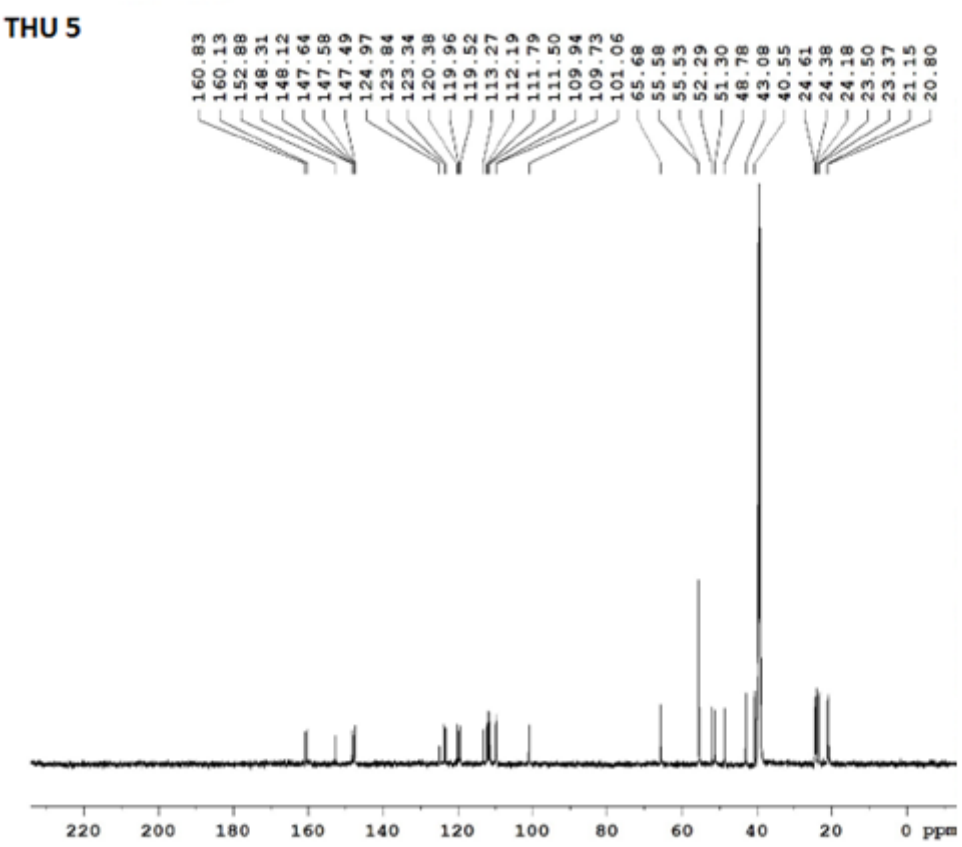
THU 3


160.81
160.09
152.83
147.42
124.94
119.48
113.22
112.15
101.00
65.64
63.12
53.28
50.99
24.58
23.48
22.78
21.13
20.78
220
200
180
160
140
120
100
80
60
40
20
0 ppm

THU 4


160.83
160.14
152.88
147.50
132.00
131.46
128.88
128.56
127.53
126.55
124.98
119.53
113.27
112.21
101.07
65.66
52.51
51.69
48.68
43.41
40.45
24.85
24.61
23.50
23.36
21.15
20.80
220
200
180
160
140
120
100
80
60
40
20
0 ppm

THU 5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160.13
152.88
148.31
148.12
147.64
147.58
147.49
124.97
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119.96
119.52
113.27
112.19
111.79
111.50
109.94
109.73
101.06
65.68
55.58
55.53
52.29
51.30
48.78
43.08
40.55
24.61
24.38
24.18
23.50
23.37
21.15
20.80
220
200
180
160
140
120
100
80
60
40
20
0 ppm