# New use of drug, Galanthamine as both Amyloid-Beta inhibitor and AChE inhibitor

**Group Member**

*Wenkai Fan*

**Instructor**

Professor Wang Shu Dr. Mingjun Teng

**School**

Beijing National Day School

**Location**

Beijing, China

北京十一学校

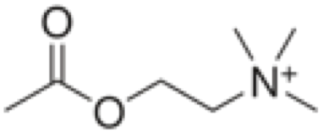
BEIJING NATIONAL DAY SCHOOL

## Table of content

Abstract:

The rapid research on the culprits of Alzheimer's Disease has increased in the theory of Amyloid-Beta protein. But, there is a lack of multi-functional drug to help stop or reserve the disease. Galanthamine, as the second generation of AChE inhibitor, shares the similar structure of Benzofuran rings with the existed Alzheimer's treating drug. Have the potential to act both function in the human brain. In this research, the author focus on the decoration of active site alongside Benzofuran structure, which could complement on the further decoration on this structure. Besides, the advance in the understanding across Alzheimer's Disease allows the design better cost-effective method to simulate alternative strategies to modulate neural function inside synapse of human brain. Adoption of such approaches should provide a wider understanding on the synapse terminals across in neural networks.

# List of Abbreviations

| | |
|---|---|
| Aβ | Amyloid Beta Protein aggregation |
| AChE | Acetylcholinesterase EC 3.1.1.7 |
| Glu | glutamate |
| CH3I | Iodomethane |
| RAZADYNE | (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 |
| GABA | gamma-aminobutyric acid |
| $CH_2Cl_2$ | dichloromethane |
| $Na_2CO_3$ | Sodium Carbonate |
| CH3CN | Acetonitrile |
| PBS | Phosphate buffered saline |
| NaOH | Sodium hydroxide |
| ThT | Thioflavin T |
| Galanthamine-Me | **(4aS,6R,8aS)- 5,6,9,10,11,12- hexahydro- 3-methoxy- 11-dimethyl- 4aH- [1]benzofuro[3a,3,2-ef] [2] benzazepin- 6-ol** |
| Galantamine | **Galanthamine** |
| Acetylcholine |  |

**Introduction:**

## 1.1 Introduction of Alzheimer's Disease:

Over a century a go, the German scientist, Alois Alzheimer, reported the brain anomaly in the 37th Meeting of South-West German Psychiatrists 37th Meeting of South-West German Psychiatrists. At that time, people don't known the cause of this disease. But, he describe this special disease as "A peculiar severe disease process of the cerebral cortex"(Hanns Hippius, ncbi). Five years after his death, this disease has first been reported as plaques and neurofibrillary in the cerebral cortex soon named as the Alzheimer's Disease. One major cost of dementia is the problem on diagnosis. Dementia patients are often diagnosis in the medial or late stages of AD, which the disease already become irreversible. With the aging problem around the globe, the number of AD patient is likely to be increased to 131 million by 2050. An estimate cost from the WHO will be US$818 billion on the worldwide cost of dementia. [1]

Currently, there is a need for developing medicines that would slow progression, halt, or prevent AD and other dementias from occurring. Although scientists do not yet know the underlying cause of this typical disease, several possible culprits has brought to the world to help understand the disease. [2]

### 1.1.1 The Cholinergic Hypothesis

Sims et al firstly brought up the cholinergic hypothesis in 198. In this theory, he postulated that the synthesis of acetylcholine, a neurotransmitter, was low in the neocortex of the brain in AD patients[3,4]. In supporting this notion, the level of choline acetyltransferase was clearly found down regulated in the hippocampus and frontal cortex, and cholinergic neuron counts in the nucleus basalis was generally lowered in AD condition

As extensively reviewed in this theory, acetylcholinesterase (AChE) is a key enzyme in the cholinergic nervous system. The function of AChE enzyme, is though returning back the Acetylcholine inside synapse. In a AD patient brain, the concentration of Acetylcholine reduced as the other types of neuron deteriorate. Beside the neuron, the source for synthesizing and The production of the Acetylcholine Both the acetylcholine-synthesizing enzyme and the functional acetylcholine-hydrolyzing enzyme enzyme are also effected. However, when the production of acetylcholine reduce, the AChE enzyme will keep functioning though returning back the neurons. Hence, the acetylcholine concentration in a AD patient brain will be significantly reduced .

According to the US national library of medicine, the AChE hypothesis, as the first brought theory that stand a solitary standpoint. in the researcher of Alzheimer's Disease. Acetylcholinesterase enzyme is the most studied proteins and the most designed therapy in the Alzheimer's field. Data shown in the library indicate about 1500 manuscripts indexed into the PubMed; the vast majority of reports in the field relate with treatment strategies associated with the use of AChE-I.

## 1.1.2 The Aβ hypothesis of Alzheimer's Disease:

Beside the AChE hypothesis, the amyloid cascade hypothesis was first brought in 1992 by professor Hardy and Higgins. They postulate that what Dc. Alzheimer saw in a dementia patient brain, include neurofibrillary tangles, cell loss, vascular damage was caused by amyloid-beta protein.

Inside human brain, the A-beta protein trend to misfiled from the separate of Amyloid Precursor protein, form soluble oligomers, aggregate in to insoluble plaques. In this hypothesis. Though which specific protein in the plaques are unknown to be toxic, but the oligomers re provide to be the most danger to the neurons. In this theory, the production, accumulation or disposal of beta-amyloid protein from the plaque is believe to the the main cause of Alzheimer's Disease. As the fundamental base of the research, the A-beta hypothesis will be further elaborate in the following part. [3]

The plaque of the A-beta protein contain many types, that is unknown of which is toxic to the brain cells. In this hypothesis, it is believed that the release of Cytokines when microglia triggering up the inflammatory that damage neurons, this release also damage the synapse from functioning.

In diagram, when the toxic oligomer of Amyloid plaques formed inside the brain, superficial, cerebral cortex, it will back the transformation of neurons by destroy the vesicle contain the neurotransmitters, AMPA and NMDA as two types of neurotransmitter carrier, are inhibited by the Amyloid protein, where the original terminals disfunction and deteriorate.

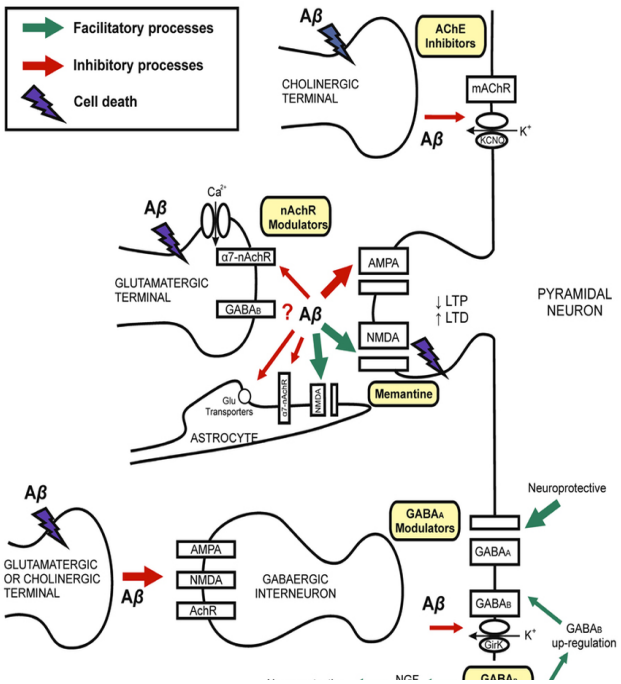



Figure1: The mechanism of synaptic disfunction cause by Amyloid-Beta protein

### 1.1.3 The Tau theory

From the timeline of the research, Tau protein aggregation hypothesis was raised as the third hypothesis on the cause of AD. This protein was originally a component of tangles. Where the health Tau protein are the component of the made on a series of track, and it stabilized the tube when Molecule are carried along axon, this series of track is named as microtubes,. But in Alzheimer's Disease, the Tau protein is no longer the health proteins but modified causing it to dissociate from the microtubules. The aggregation of the protein adopt abnormal shape and move form the axon to the cell body.

Like the A-beta theory, Tau proteins come from different forms, it's still unclear of which on contribute to the disease. And like beta, these forms either remain , or stick together and deposit. as the tangle that Dr. Alzheimer saw. Eventually, these process kill the neuron. In the race brain experiment, the researcher also find that the modified Tau protein can spread pathology across the brain, there make the healthy Tau protein start to misfiled as well. The patten of spread though the different brain regions share similar characteristics as the changing symptoms from early to late stages of Alzheimer's Disease, which fit the disease.

## 1.2.1 Introduction to Galathamine.

RAZATYNE (galantmine hydrobromide), as the second generation of cholinesterase inhibitor initial proved by FDA in 2001, is the focus of this research. Galanthamine Hydro-bromide is a natural product belonging to the Amaryllidaceae family of alkaloids which was discovered in the early 1950s. The molecule can be natural extracted from plant, with the function of maintaining the Acetylcholine concentration, RAZATYNE was first used to treat nerve pain and poliomyelitis.[5]

## 1.2.1 Function of the drug

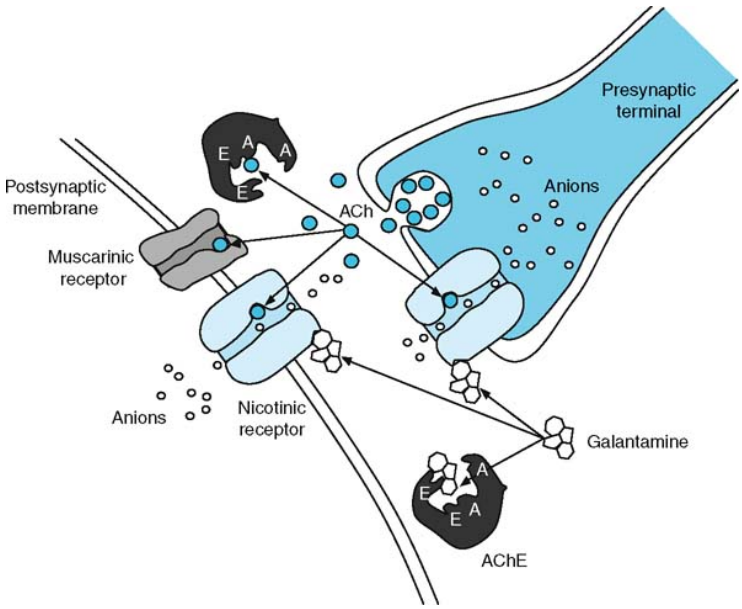



Figure2: The mechanism of Galanthamine binding to AChE enzyme[4]

1) Nootropic Agents, Parasympathomimetics,
2) Cholinesterase Inhibitors.

The main function of galanthamine is though enhancing the cholinergic function. It is achieved though two ways: first, it maintain acetylcholine concentration though competitive binding on the acetyl cholinesterase enzyme, and act as an allosteric potentiator to help the neurotransmitter to enter both nicotinic and muscarinic acetylcholine receptors. Secondly, it direct inhibits the hydrolysis of the neurotransmitter hence increase Acetylcholine concentration inside synaptic cleft

## 2.1.2 Common types of AChE inhibitor

Galantamine is not the only cholinesterase inhibitor. As figure three shown below, Donepezil, Rivastigmine, and Tacrine are included in the common types of AChE inhibitor and the use in the treatment of several brain disease. But, one feature that distinct Galanthamine from all other drugs, is the way Galanthamine bind with the enzyme inside synapse though competitive blocking. As previous example mentioned, the direct binding to the AChE enzyme distinguished galanthamine form the other AChE inhibitors[6]

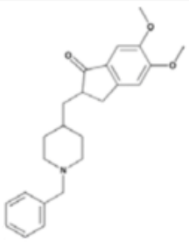
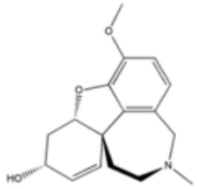
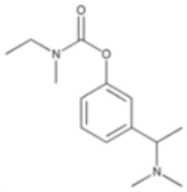
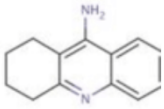
| | Donepezil | Galantamine | Rivastigmine | Tacrine |
|---|---|---|---|---|
| **Structure** |  |  |  |  |
| **Chemical class [29]** | Piperidine | Phenanthrene alkaloid | Carbamate | Acridinamide |
| **Target enzymes** | AChE | AChE | AChE and BuChE | AChE and BuChE |
| **Inhibition of target enzymes [29]** | Non-competitive<br>Rapidly-reversible (<1 ms) | Competitive<br>Rapidly-reversible (<1 ms) | Non-competitive<br>Very slowly reversible<br>(~6–8 h) | Non-competitive<br>Rapidly-reversible |
| **Metabolism [30]** | CYP2D6 and 3A4 | CYP2D6 and 3A4 | AChE and BuChE | CYP1A2 |
| **Recommended dose** | 10 mg/day (once daily) | 24 mg/day (twice daily) | 9.5 mg/24 h patch (once daily)<br>12 mg/day (twice daily) | 160 mg/day<br>(four times daily) |
| **Available formulations** | Tablets | Tablets<br>Oral solution<br>Once-daily controlled release | Transdermal patch<br>Capsules<br>Oral solution | Capsules |
| **Plasma half-life [29, 31]** | ~ 70 hours | ~ 7 hours | ~ 3 hours (patch)<br>~1 hour (capsule) | – |

Figure 3: Pharmacology of common types of AChE inhibitors and it's relation to Alzheimer's

## 1.3 The propose of this research,

Those the culprits on the cause of Alzheimer's Disease have been long brought to the world in last centuries, there is still no drug design for the culprits that can reverse or stop the deterioration of mental disorder. Though out the research on the the molecule galanthamine, the researcher find that this molecule share the similar Benzofuran with some existing Alzheimer's Drug, like Benzofuran-chalcone hybrids, has been proven to be effective on inhibiting the Amyloid-beta aggregation. On top of this, galanthamine is registered as AChE enzyme inhibitor. So, the researchr would like to see if this molecule can be applied to the both theories. Which, it's assumed that Galanthamine will success in inhibiting the se;f-assembly of Amyloid-Beta oligomer. Therefore, when the drug entering the synapse terminal in brain areas like cerebral cortex, this drug may share multi-functions to help further modify drug on this.

To testify suitable active site for the modification, the N-CH3 site is identified as a independent site else than the Benzofuran. Hence, the synthesis on decorating this site is taken.

## 2.1 Synthesis of Galanthamine-Me

## 2.2.1 Meterials and agents

Materials: All organic solvents were purchased from Beijing Chemical Works and used without further purification. Other chemicals were purchased from Acros, Sigma-Aldrich Chemical Company or Alfa-Aesar and used as received. Aβ42, The purity of the peptides was greater than 98%. The cell line PC12 was purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Deionized water (18.2 MΩ·cm) was obtained from a Milli-Q system (Millipore, Bedford, MA). Fetal bovine serum (FBS) was purchased from Sijiqing Biological Engineering Materials (Hangzhou, China). Modified RPMI 1640 (RP 1640) was purchased from HyClone/Thermofisher (Beijing, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

### 2.1.1The Preparation of Peptides

Aβ42, prepared as literature. Firstly, the lyophilized peptides were dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), bath-sonicated for 10 min and incubated with shaking at 4°C for 2 h. Subsequently, the samples were divided into aliquots, and the HFIP was evaporated and stored at -20°C. Aβ42 were dissolved in 10 mM sodium hydroxide solution and centrifuged at 4°C (10000 g, 10 min) before use.

### 2.1.2Detoxification of Aβ42

Rat pheochromocytoma PC12 cells were cultured in RP 1640 medium containing 10% fetal bovine serum under 5% CO2 at 37°C. The cells were seeded at a density of 7,000 cells per well. Aβ42 alone, with PPV-NP (ten-fold excess) or with PPV-OH (ten-fold excess) was incubated for 15 h at 37°C prior to the addition to cells. The samples were diluted with RP 1640 medium to Aβ42 concentration of 20 μM. After incubation for 48 h, the mixture in each well was replaced with MTT (1 mg/mL in medium, 100 μL/well) and incubation for 4 h, Then, the supernatant was replaced with 100 μL DMSO per well, and the plates were shaken for 10 min. Absorbance values of formazan were read with a microplate reader at 570 nm. Wells with neither PPVs nor Aβ42 were served as control group.

### 2.1.3Circular Dichroism(CD) Spectroscopy

200 μL of the sample was added to a 0.1 cm quartz cell for far-UV (190-260 nm) measurements. The bandwidth was 2 nm. The scanning speed is 100 nm/min with a response time of 4 s. Each

spectrum was an average of 3 scans.

2.1.4Transmission Electron Microscopy (TEM)

5 μL of the sample was dropped on the carbon-coated copper grid. After 10 min, the redundant sample was removed with filter paper and stained with 5 μL of 3% uranyl acetate for 1 min. The samples were then observed using TEM

**2.2.1Measurements:**

The 1H NMR and 13C NMR spectra were measured on Bruker Avance 400 or 600 MHz spectrometers. High resolution mass spectra (HR-MS) were taken on a Bruker 9.4T Solarix FT-ICR-MS spectrometer. Fluorescence spectra were recorded on Varioskan Flash (Thermo Scientific Company, USA). The absorbance for MTT analysis was measured on a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 570 nm. Cell counting was performed on an automated cell counter (Countess, Invitrogen). CD spectra were measured on a Hitachi Jasco-J815 circular dichroism spectrophotometer. TEM images were recorded on a Hitachi S-7700 transmission electron microscope.


Figure 4: Hitachi S-7700 TM

## 2.1.3. The process of synthesize

The common type of synthesis to stabilize a compound is towards methylation, which the active site of RAZATYNE are restricted without losing it's function. The CH group involved in the methylation can be replaced by a longer carbon chain to further decorate at the termination of the carbon chain. From the document, the benzofuro structure in Galathamine Hydro bromide is similar to the current AD drug, the nearby seven carbon ring with nitro-methyl group is the ideal active site for the decoration. Hence, decide to use this site as the reactive site.

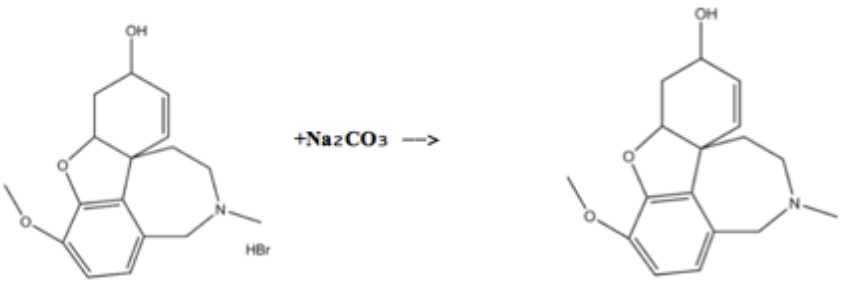
The synthesis of the compound was follow by three steps. Neutralization, extraction, and methylation.

3.2.1 Neutralization reaction

Add 30ml UltraPure water into a round-bottom flask(50ml). Put an appropriate size of magnate into the flask. Adding RAZATYNE into the flask(f1). Add excess amount of Sodium Carbonate(about 1.2 time more than the standard requirement of neutralization) into the flask.

Wait 30 minutes until the reaction finished completely.

Figure 5: the mechanism of the neutralization reaction




The polarity of the molecule shift form polar into none polar molecule, due to this property. The extraction measure can be used to help get the Galanthamine molecule only. Hence, $CH_2Cl_2$ is used in this step. The steps taken are as following: Prepare a separating funnel, Fold a filter paper with Triangle funnel on it. Then, take out the flask and gradually drainage it into the funnel. Pull 30ml

dichloromethane in to the separating funnel. Shake the flask, turn on the bottom to let the air flow out. Repeat for 3 times.

To testify if the separate of Bromide ion from the original compound, the use of irradiate with ultraviolet rays to testify the intensity of Br- in different layers of the solution. By using a Capillary to reach different layers, the researcher can tell if the Bromide ion exist in the organic solvent $CH_2Cl_2$.

To help dehydrate the H2O molecules in the solution, Sodium Sulfate are used in this step. With the function of occupying the water molecule, the next step can help to filter both $Na_2CO_3$ and $H_2O$.

This step is name as Decompress distillation, this step also help gather the mass of the product, hence can calculate the product yield after neutralization. With a 250ml round-bottom flask with knowing mass, let the mixture of liquid to pass though the filter paper. Only the solvent($CH_2Cl_2$) and galanthamine will be able to penetrate the filter paper. Then, the liquid inside the false are ready for the Decompress distillation process. The mechanism of distillation is though the reduce of boiling point by Decompression of air pressure inside the flask. Hence, require less heat and speed up the reaction. Considering the common types of organic solvent like dichloromethane, have the boiling point lower than the water, with the addition o decompression of air pressure. It's easy to get the galanthamine powder. Hence, calculate for the yield of the product.

After taking out the product by using Acetonitrile into a new flask, the last reaction will undergo the overnight reaction though magnet stirring. Then, the methylation of galanthamine is complete(22h).

To get the final product from this original solution, filtering(using buchner funnel) and vacuum drying(using Phosphorus pentoxide) is designed to help clean out the Iodine ion and excess excess reagent of hydro bromide galanthamine. (The product yield is 92%)

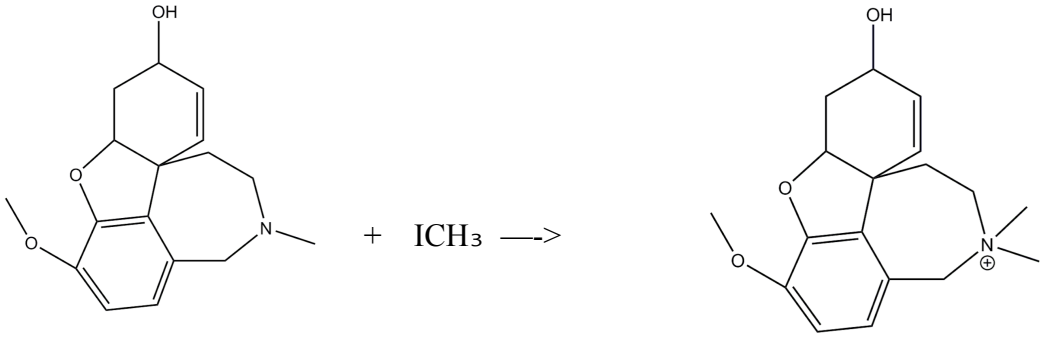



Figure 6: the methylation of the compound galanthamine-Me

The HMR data is collected though the dissolve of the synthesis sample in DMSO. The picture is shown as following:

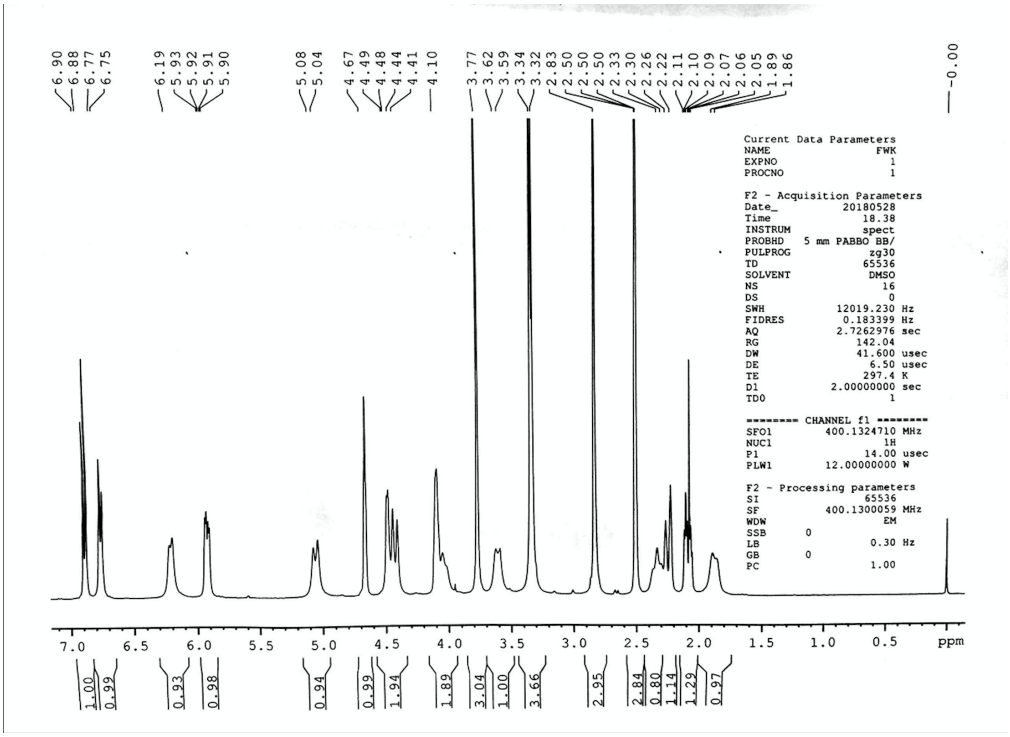
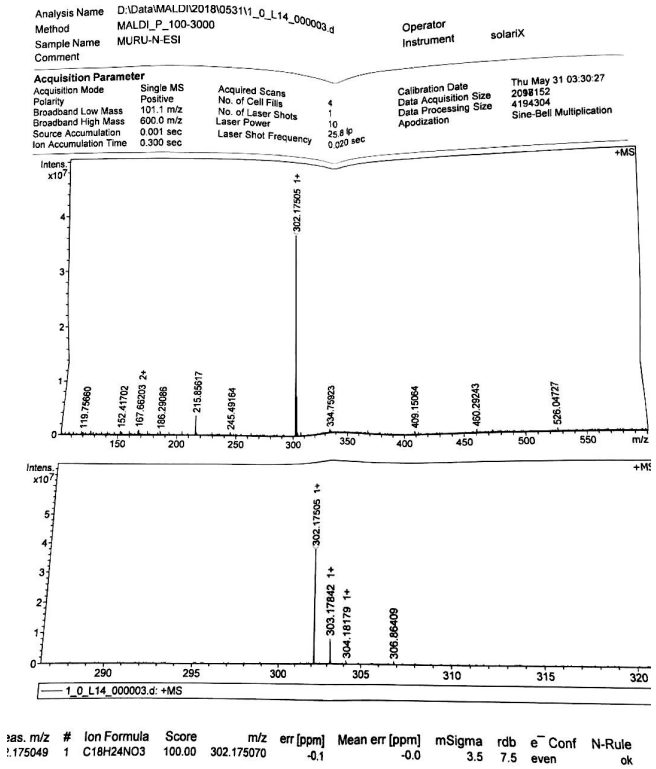



Figure 7, NMR result of the synthesized product

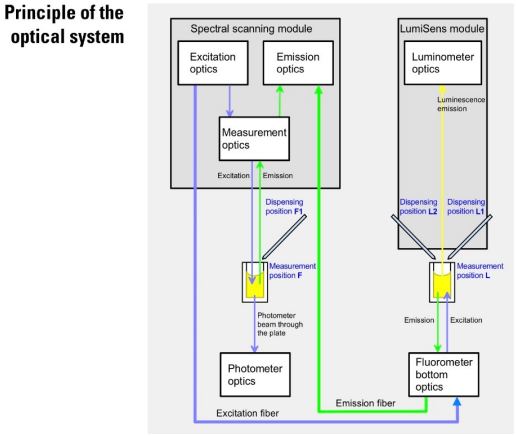
Figure 8: Mass Spectrometry results on the target product



## 2.2.1 The ThT measurement of the research

Both RAZATYNE(Galanthamine Hydro-Bromide), and Galanthamine-Me have similar structure of benzoforun rings, to testify if the benzoforun play the only role in inhibiting the self-assembly of Abeta from oligomer to fabric structure. A series of experiment were designed to determining the aggregation.

## 2.1.1 Introduction to the principle of the ThT



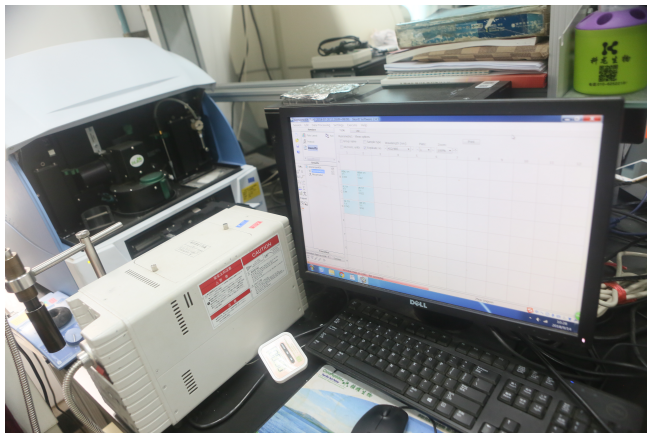



Figure 9: the principle of the optical system
Figure 10: the varioskan flash instrument

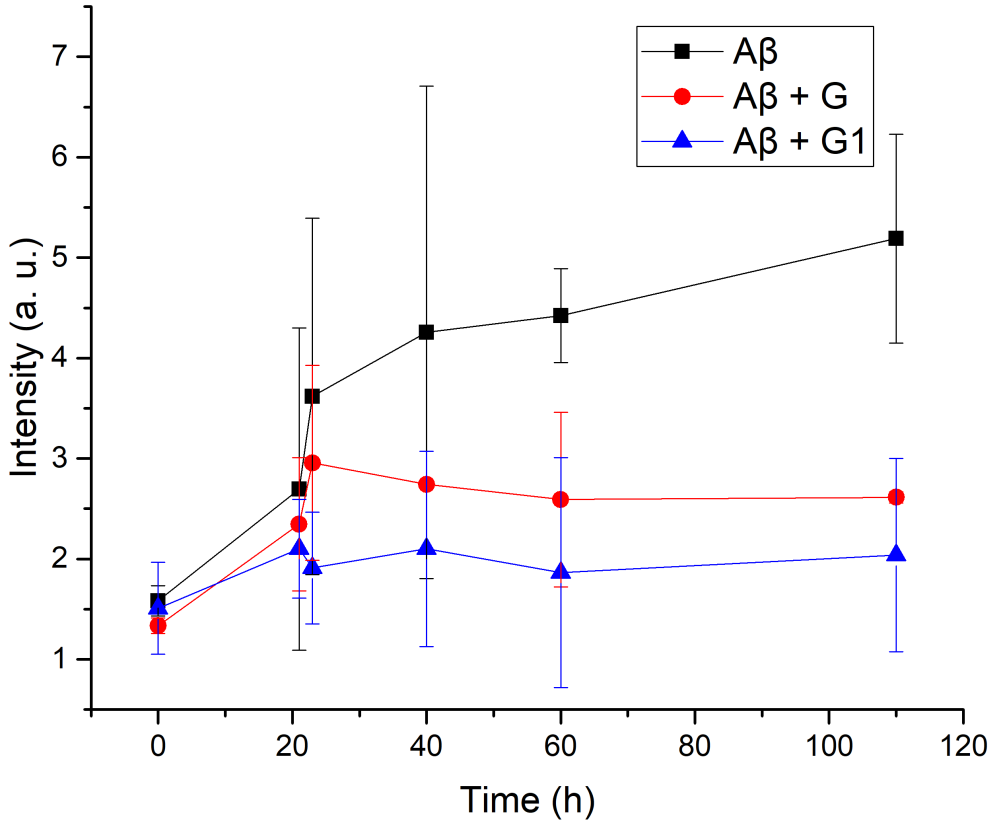
## 2.1.2The preparation and condition of ThT Fluorescence test

In a 96 slot plate, 3 parallel experiment for pure A-beta, galanthamine hydro-bromide, galanthamine-Me were inject to the slot. Eachneaction(200μL) were mixed with 20μL of 20 μM ThT in 10mM phosphate buffer (PBS), pH 7.4, each trial were recorded every 24 hours in total time 110h. The incubation timer the individual fluorescence are 5 minutes. The wavelength in the measurement were at λex = 452 nm and λem = 485 nm, the equipment used were shown above.

2.3.3 Condition for the Aβ protein Used in the Aggregation Assays

| | | Incubation (5min) | | |
|---|---|---|---|---|
| | | Temperature | agitation | assay |
| Aβ42(control group) | Preparation A:were dissolved in 60 mM sodium hydroxide (10% of the final volume), followed by addition of 45% of Ultra pure water, followed by 45% of PBS. | 37 | + | ThT |
| Aβ42 protein with 1 time, 5 times, 10 times excessive RAZATYNE | Preparation B:were dissolved in 60 mM sodium hydroxide (10% of the final volume), followed by addition of 45% of RAZATYNE , followed by 45% of PBS. | 37 | + | ThT |
| Aβ42 protein with 1 time, 5 times, 10 times excessive RAZATYNE | Preparation C:were dissolved in 60 mM sodium hydroxide (10% of the final volume), followed by addition of 45% of Galanthamine , followed by 45% of PBS. | 37 | + | ThT |

## 3.2 discussion of results



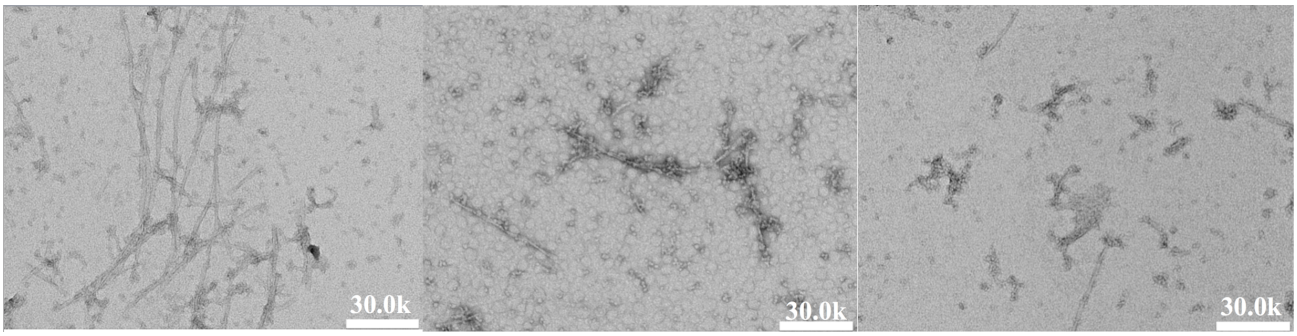




Figure 11: ThT result of of A-beta self assembly, Galanthamine-Me, Galanthamine-HBr
Figure 12: TEM data of f A-beta self assembly, Galanthamine-Me, Galanthamine-HBr

In view of the results, the researcher asked the question: to what extent is the inhibition of aggregation by both galanthamine compound was a general phenomenon. To answer this question, the result of ThT diagram is assisted by the TEM and CD graph. In the original design, and the experimental section. The self-assembling of the amyloid-beta protein was considered as a independent control group. In the research, the researcher collected the data on the growth of A-beta on the basis of ThT intensity. Which the ThT flash device is designed for the detecting the intensity of fluorescence by eliminating the background intensity of the fluorescence.

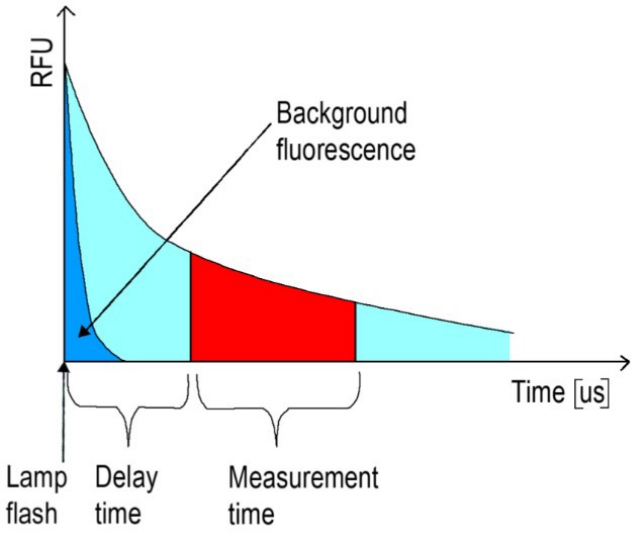



Figure 13: Mechanism of fluorescence detection

On the basis of number, location, and relative abundance of the Amyloid-beta protein(TEM), the result of both ThT and TEM supported the phenomenon of success inhibition of protein aggregation.

Since both molecules succeed in the inhibition process, though the observation on the relative abundance. And the difference in figure11. The Galanthamine hydro-bromide have a better inhibition on the aggregation.

In review of this result, the researcher asked for another question: is the Benzofuran the only functional group inside. By comparing the structure of Galanythamine-HBr and Galanthamine-Me. The dimethyl nitro group on the seven carbon ring avianize the original structure. Which the weak-base environment is more suitable for the molecule for the inhibition.

The author also recognized that the active site he decorated is not the only active site available for modifications. For future decoration. The synthesis can focus on OH group or O-CH3 group that are attached to the benzofuran ring.

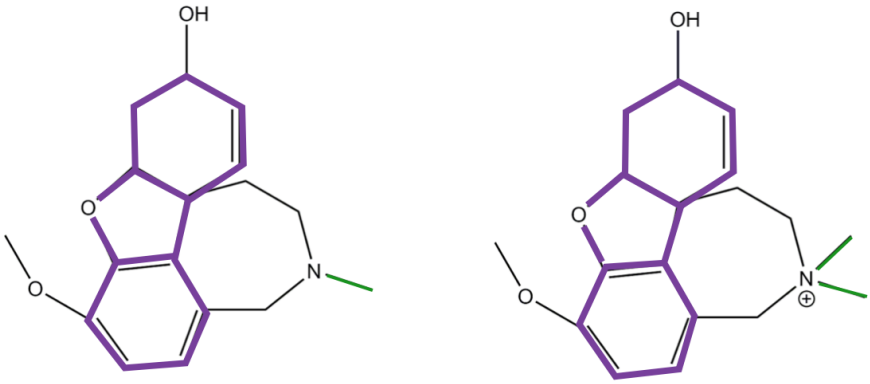


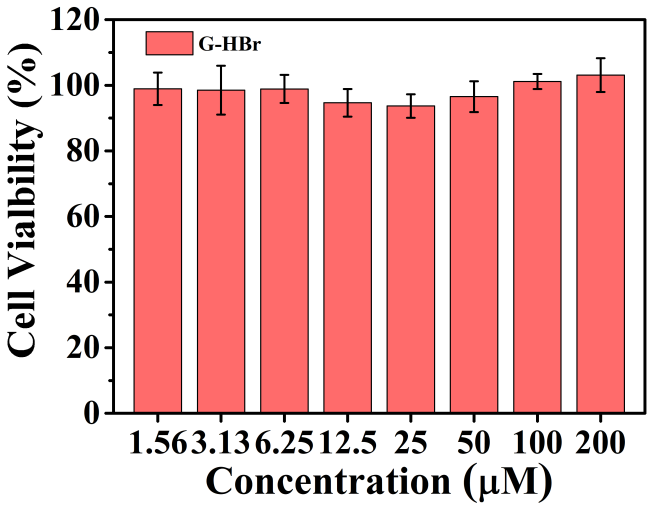

Figure 14: Comparing the structural formula of Galanthamine compounds

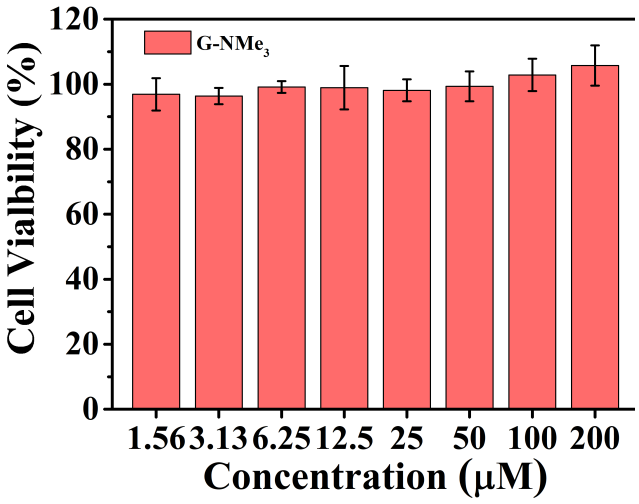
## 3.3 Supplementary test

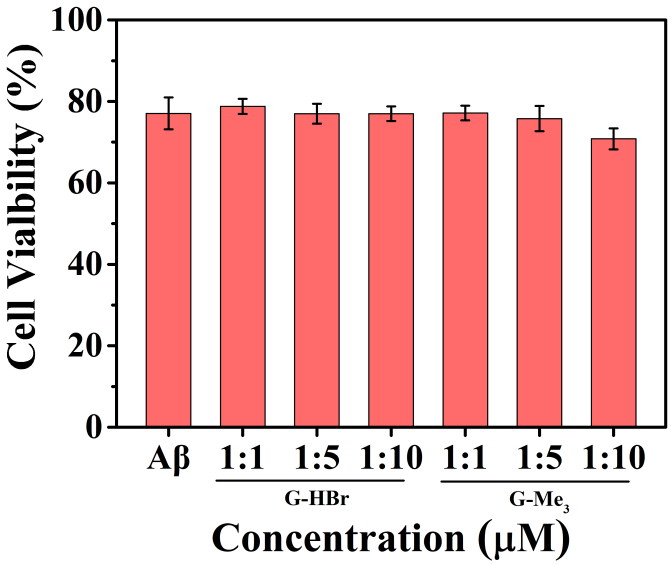
### 3.3.1 Supplementary information of cell assay

Considering human health as one major concern in the drug design, the cell culture assay is prepared to hep formulate proper concentration of the drug.

The goal of this experiment is to give a understanding on the concentration of drug appropriate for human health. This experiment is commonly used in a laboratory condition to give a considerable range on the dosage. But, this can not replace the clinical trial in determining LD50 or TD50.



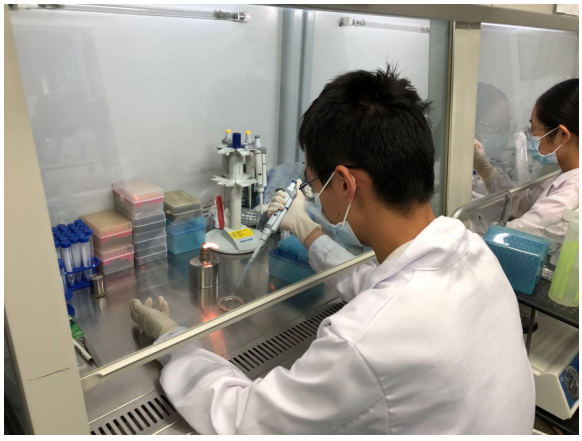



Figure 15: the cell assay of both galanthamine-HBr, and Galanthamine-Me

### 3.3.2 Supplementary information of CD experiment:

After gathering the data from the THT, the author recognized that the galanthamine molecules have the ability to inhibit the aggregation of the protein, And the cell assay for finding the proper range. The author chose 1 time, 5 times, and 10times excess of both drug into the CD spectrometer. Father the result as following:

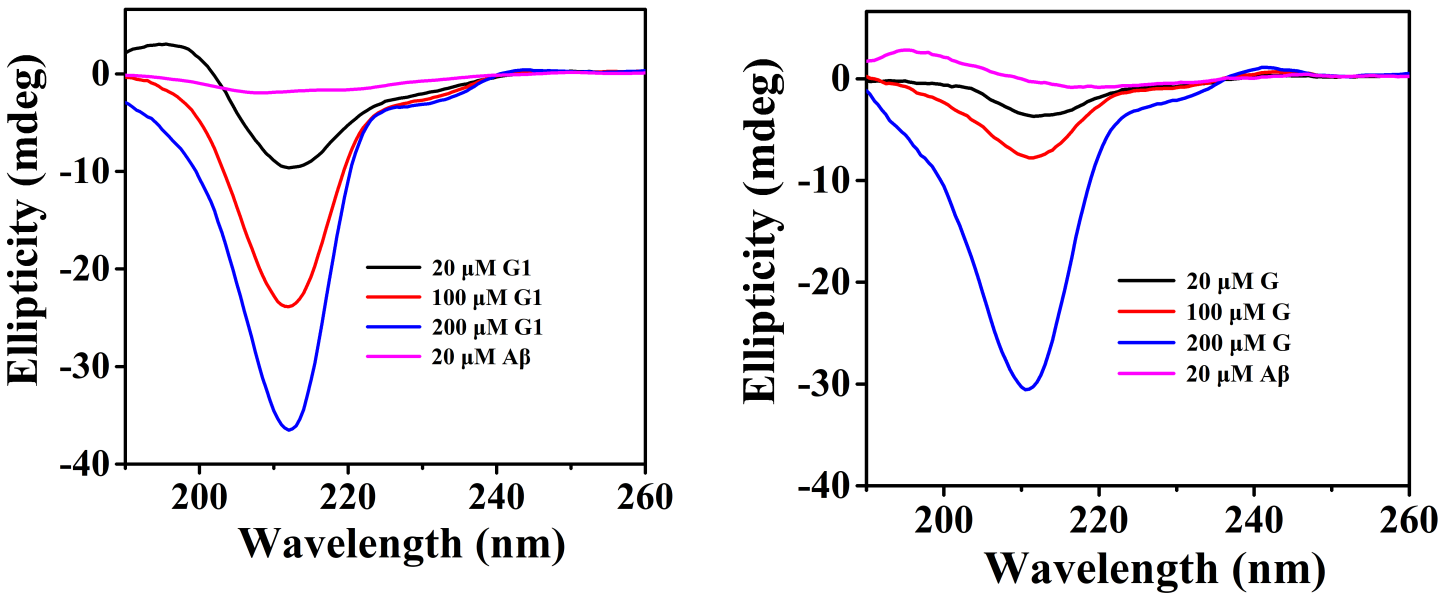
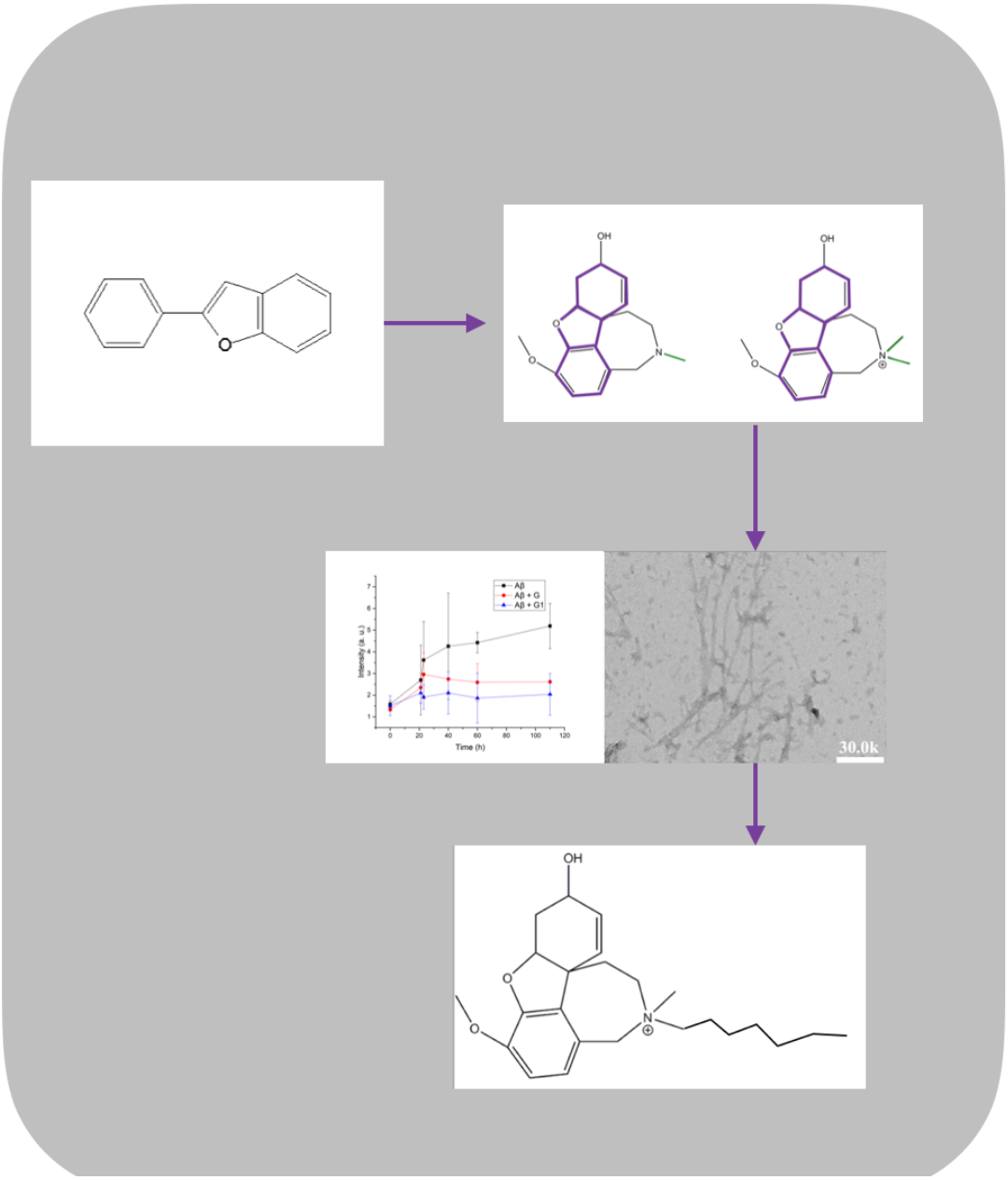



Figure 17: CD detection of Galanthamine-HBr, and Galanthamine-Me

# 4 Conclusion:

By observing microscopically. The molecule galanthamine effective in inhibiting the assembly fibril structure of Amyloid beta protein. As a original Acetylcholine inhibitor, the galanthamine molecule share function beyond it's original medical use as an AChE inhibitor. The research shows that this molecule can direct interact with the fibril structure aggregation side human brain.

By synthesizing compound with difference functional group on the basis of Benzofuran rings , the researcher also give thoughtful explanation to the function of active sites inside the brain. Which have brought a way for the further application of this drug to allow the decoration on these active site. Beside from modifying into a dimethyl group, the same active site and be attached to another molecule. Or forming a bilayer with hydrophobic layer to increase it's solubility in liquid for better entering the blood-brain barrier, and a hydrophilic inner layer for better dissolve of the galanthamine molecule.



Despite from the bright future on the drug design base on this benzofuran structure. In face of treating of Alzheimer Disease, problem and limitations are needed to identify. With the decoration of the drug, the researcher question the original function of competitive binding to the original enzyme—AChE. If this process will keep successful when the lab environment do no allows the author to simulate synaptic terminals inside the brain. Besides, the less access to modern medical condition also leave millions of lives in LEDC countries to have no access on diagnosing and reporting this disease, when the synapse were already broken, the chemical stimulation may not be able to reconstruct certain component in the brain like an nicotinic receptor.

## 5 Reference:

[1]What Is Alzheimer's? Alzheimer's Association. https://www.alz.org/alzheimers-dementia/what-is-alzheimers. Accessed September 13, 2018.

[2]Decarli C. Concepts, Conjectures, and Hypotheses in Dementia. Alzheimer Disease & Associated Disorders. 2009;23(3):185. doi:10.1097/wad.0b013e3181b85eae.
O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Whitehouse Station, NJ: Merck and Co., Inc., 2001., p. 771

[4]Figure 2f from: Irimia R, Gottschling M (2016) Taxonomic revision of Rochefortia Sw. (Ehretiaceae, Boraginales). Biodiversity Data Journal 4: e7720. https://doi.org/10.3897/BDJ.4.e7720. doi:10.3897/bdj.4.e7720.figure2f.

[5]Decarli C. Concepts, Conjectures, and Hypothesis in dementia. Alzheimer's Disease& Associated Disorders. 2009;23(3:185)
[6]NatureVideoChannel. Inside Alzheimer's disease. YouTube. https://www.youtube.com/watch?v=zTd0-A5yDZI. Published January 3, 2017. Accessed September 15, 2018.
[7]Figure 2f from: Irimia R, Gottschling M (2016) Taxonomic revision of Rochefortia Sw. (Ehretiaceae, Boraginales). Biodiversity Data Journal 4: e7720. https://doi.org/10.3897/BDJ.4.e7720. doi:10.3897/bdj.4.e7720.figure2f.
[8]Macpherson S. Galanthamine: the new treatment of choice in Alzheimer’s Disease Inpharma Weekly. 1995;&NA;(1002):3-5. doi:10.2165/00128413-199510020-00002.

6 Acknowledgements:

I would like to appreciate the guidance and instructions of my instructor professor Wangshu, who provided the research lab and resources of Insitute of Chemistry, Chinese
Academy of Sciences. during the activity of Yingcai program.
Dr. Mingjun Teng who offered valuable feedbacks for my academic writing; the China YouthAssociation for Science and Technology Center (CYSCC) which is the sponsor of Yingcai program; Dr. Sun Han who gave me instruction in the experiment

本参赛团队声明所提交的论文是在指导老师指导下进行的研究工作和取得的研究成果。尽本团队所知，除了文中特别加以标注和致谢中所罗列的内容以外，论文中不包含其他人已经发表或撰写过的研究成果。若有不实之处，本人愿意承担一切相关责任。

参赛队员： 范文楷 指导老师： 王树、滕明俊