行政院國家科學委員會專題研究計畫　成果報告

天然物乙醯膽鹼酯解胸抑制劑(-)quinolactacin A2 的全合成

計畫類別：個別型計畫
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執行單位：臺北醫學大學生化學科

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中　華　民　國 93 年 10 月 1 日

研究計畫中英文摘要：請就本計畫要點作一概述，並依本計畫性質自訂關鍵詞。

（一）計畫中文摘要。（五百字以內）

關鍵詞: 阿茲海默氏症，乙醯膽鹼酯解脢抑制劑，乙醯膽鹼酯解脢，quinolactacin A2，Pictet-Spengler condensation

解脢抑制劑的藥物不只抑制乙醯膽鹼酯解脢也同時抑制 butylcholinesterase(BuChE)而失智症是一種發生在中老年人的症候群，主要的特徵是認知及智力功能的衰退，包括記憶、思考、判斷力等。病患的日常生活及溝通能力會受到影響，需要他人長期照顧。而阿茲海默氏症（Alzheimer’s disease）是造成失智症的主要原因。隨著醫療技術的進步，人類壽命的延長，Alzheimer’s disease 成為許多已開發國家日趨嚴重的問題。許多科學研究明確指出，阿茲海默氏症患者之所以會喪失記憶力的原因之一，就是腦部的神經傳導物質--也就是被稱為乙醯膽鹼(acetylcholine)的腦部訊息物質大量減少。同時也發現乙醯膽鹼酯解脢（acetylcholinesterase）的酵素，參與部分的乙醯膽鹼分解。因此，下一步就是開發出可以抑制乙醯膽鹼酯解脢的藥物，藉以使得腦部有較多可使用的乙醯膽鹼。我們現在已有些被稱為乙醯膽鹼酯解脢抑制劑的藥物，它可延緩乙醯膽鹼的分解。但現有的乙醯膽鹼酯導發可能肝毒性的副作用，使醫生在給藥時無法用到高劑量，而使其療效無法完全發揮。開發針對抑制乙醯膽鹼酯解脢的藥物而不抑制 butylcholinesterase(BuChE)將有助於對阿茲海默氏症患者使用乙醯膽鹼酯解脢抑制劑的療效完全發揮。

從 fb90648 發酵槽中萃取出兩天然物 quinolactacin A1 (**1**)和 A2 (**2**)，這兩個天然物的化學結構是由不同的光譜分析鑑定而來。 quinolactacin A1 (**1**)和 A2 (**2**) 主要的化學結構是由一個 pyrrolo[3,4-b]quinolone 組合而成，天然物具有兩個 chiral centers 且之關係是非鏡像異構物(diastereomer)。這兩個天然物雖然具有有效抑制乙醯膽鹼酯解脢的功效，且 quinolactacin A2 (**2**)較對乙醯膽鹼酯解脢具有效力的抑制(IC_{50} = 19.8 μM)，而對 butylcholinesterase(BuChE)抑制較弱(IC_{50} = 653 μM)。應 quinolactacin A2 (**2**)選擇性抑制乙醯膽鹼酯解脢所以利用這天然物和此天然物的衍生物來探討這酵素的結構將可以進一步了解這酵素的結構因而幫助未來研發更有效和減少導發可能肝毒性的副作用的乙醯膽鹼酯解脢抑制劑，此抑制劑將有助於對阿茲海默氏症患者使用乙醯膽鹼酯解脢抑制劑的療效完全發揮。

此計畫為 enantioselective 天然物 quinolactacin A2 (**2**)的合成。我們利用氨基酸 L-isoleucine 的的兩個 chiral centers 運用於建立天然物的兩個 chiral centers，pyrrolo[3,4-b]quinolone 的化學結構將由 Pictet-Spengler condensation 組合而成。此天然物之主要化學結構已於 3 合成步驟完成，且 Pictet-Spengler condensation 為合成 pyrrolo[3,4-b]quinolone 的化學結構。經過許多測試，最後形成 lactame 環合成至今尚無研發出適當之合成方法，但尚有許多為嘗試合成途徑等待嘗試。

（二）計畫英文摘要。（五百字以內）

Keywords: Alzheimer disease, acetylcholinesterase inhibitor, acetylcholinesterase (AChE), quinolactacin A2, Pictet-Spengler condensation

Alzheimer disease (AD) is a neurodegenerative disorder that is the most common cause of dementia among elderly. This disease is mainly affect the central nervous system (CNS) characterized especially by premature senile mental deterioration. AD patients exhibit marked decline in cognitive ability and severe behavioral abnormalities such as irritability, anxiety, depression, disorientation, and restlessness. The precise mechanism causing the disease is still unknown; however, at the cellular level, there is a marked reduction in the levels of neurotransmitters such as acetylcholine (ACh), serotonin, noradrenaline, dopamine, glutamate and substance P; and the depletion of acetylcholine is the most important event. The major focus on the drug development in this area is symptomatic treatments aimed at repletion deficient neurotransmitters. One of an approach to enhance cholinergic deficit in AD patients is through cholinesterase inhibitors (ChEI), which block the ChE enzyme activity thereby invigorating cholinergic activity to enhance cognitive function. Inhibition acetylcholinesterase (AChE) activities in AD patients will boost endogenous level of acetylcholine (ACh) thereby invigorating cholinergic activity to enhance cognitive function. The AChE has been the target for drug development treatment for AD.

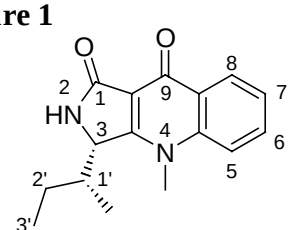
The fungal metabolites, quinolactacin A1 (**1**) and A2 (**2**) were isolated from fermentation strain fb90648 are novel ChEIs. Both natural products **1** and **2** exhibited inhibitory activity against AChE with IC_{50} value of 280 and 19.8 μM respectively. Quinolactacin A2 (**2**) is a weak BuChE inhibitors with IC_{50} values of 650 μM. The natural products **1** and **2** are diastereomers. The architectural framework that is common in **1** and **2** is an unique pyrrolo[3,4-b]quinolone skeleton. The only difference between **1** and **2** are found in the stereo configuration of chiral center on C1`. The quinolactacin A1 (**1**) bears a R configuration on C1'. On the other hand, compound **2** bears a S configuration on C1`. Since quinolactacin A2 exhibited 33 time more potent inhibitory effect toward AChE than BuChE, the selective inhibition property on AChE imposed on this chemical structure has attracted attention as lead structure for AChE inhibitors development.

The progress of an efficient asymmetric synthesis of (-) quinolactacin A2 (**2**) was reported herein. We will utilize the stereo configuration in L-isoleucine as the chiral starting material for this asymmetric total synthesis. The core structural of this natural product was completed in 3 steps. The key step of this synthesis was a Pictet-Spengler condensation of the anhydride **3** and the β nitrile aniline **4** for construction of the quinolone ring skeleton. The final ring lactame ring closure was futile after various trials. Several other approaches toward the completion of the synthesis were in progress.

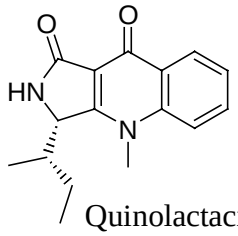
Introduction

The fungal metabolites quinolactacin A1 (**1**) and A2 (**2**) which are isolated from fermentation strain fb90648 are recent discover potent AChEI. The structure of both **1** and **2** were assigned based on their physical and spectral characteristic (figure 1).[1] The natural products **1** and **2** are diastereomers. The architectural framework that is common in **1** and **2** is an unique pyrrolo[3,4-b]quinolone skeleton. The only difference between **1** and **2** is found in the stereo configuration on C1`. The quinolactacin A1 (**1**) bears a R configuration on C1'. On the other hand, compound **2** bears a S configuration on C1`. Both natural products **1** and **2** exhibited inhibitory activity against AChE with IC_{50} value of 280 and 19.8 μM respectively.[1] Quinolactacin A2 (**2**) is competitive inhibitor for AChE with K*i* and K*m* values of $2.39X10^{-5}$M and $1.09X10^{-4}$M respectively.[1] Quinolactacin A2 (**2**) is a weak BuChE inhibitors with IC_{50} values of 650 μM.[18] Since quinolactacin A2 exhibited 33 time more potent inhibitory effect toward AChE than BuChE, the selective inhibition property on AChE imposed on this chemical structure has attracted attention as lead structure for AChE inhibitors development.

Figure 1



Quinolactacin A1 (**1**)



Quinolactacin A2 (**2**)

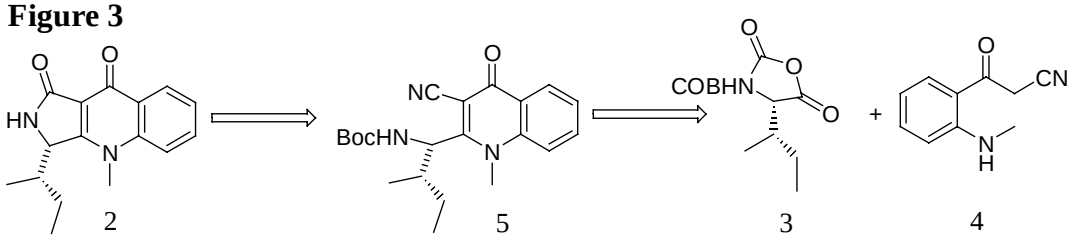
Synthesis of quinolactacin A2 (**2**) is compelling due to its AChE antagonist activity, the synthetic challenges posed by the structure, and the status as potential new leads in drug discovery efforts. The synthesis herein is an asymmetric synthesis of quinolactacin A2 (**2**). The total synthesis of this natural product will be useful for possible future derivatives construction.

Result and Discussion

The retro-synthetic scheme of (-) quinolactacin A2 (**2**) is outlined in figure 2.

Carefully analyzed the (-) quinolactacin A2 (**2**) structure, there are two chiral centers C3 and C1` in the alkyl side chain of the structure (figure 2). We envision that the structure of the alkyl side chain in **2** is closely resembled to the alkyl side chain of L-isoleucine. Closely exam the stereo configuration on the C3 and C1' of **2**, they match with the stereo configuration of the α carbon and the only chiral center in alkyl side chain of the L-isoleucine respectively. We will utilize the stereo configuration in L-isoleucine as the chiral starting material for this asymmetric total synthesis. The starting enantiomeric pure anhydride **3**, which is the derivative of the L-isoleucine, contains the necessary absolute stereo configuration in **2**. The quinolone skeleton formation via the Pictet-Spengler condensation of the anhydride **3** and the β nitrile aniline **4**[2] will be the key step of this total synthesis.[3.4] Finally, the γ-lactam ring will be closed by intramolecular amide formation in **5** to gives the target natural products **2**.

Figure 3

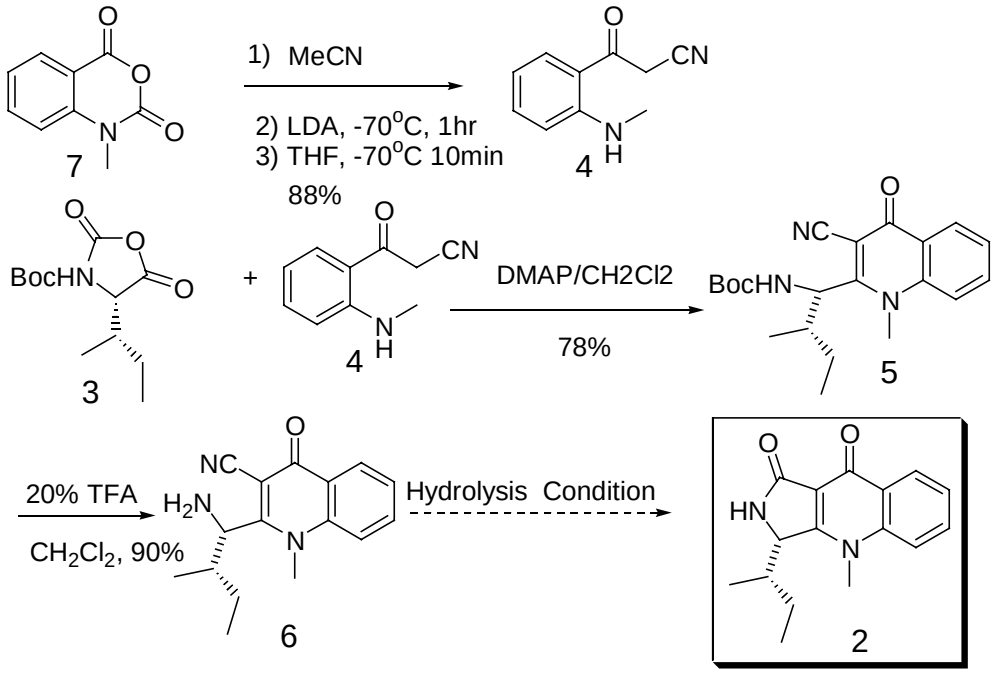


2 5 3 4

The syntheses of (-) quinolactacin A2 (**2**) can be completed in 4 steps from two starting materials, and the detail synthetic scheme is outlined in figure **4**. The synthesis begins with the β nitrile aniline **4**. Treatment of methyl nitrile with 2 equivalents of LDA at -70^{o}C generated the corresponding enolated of methylnitrile (not shown), and it was allowed to react with *N*-methylisatoic anhydride yield product **4**. The Pictet-Spengler condensation of the anhydride **3** and the β carbonyl aniline **4** will proceed under basic condition, DMAP in methylene chloride, to give quinolin-4-one **5** in 78% yield, and other non-nucleophilic base, such as Et3N, and DBU gave unsatisfactory yield. Removal of Boc in **5** with 20% TFA in methylene chloride gave the unprotected amine **6** in 90% yield. Several attempts on the

hydrolysis of nitrile in **6** followed by the spontaneously intramolecular γ-lactam ring closures to give tricyclo **2** had failed, and the conditions of the nitrile hydrolysis were listed bellowed. Several other attempts on the on the hydrolysis of nitrile in **6** followed by the spontaneously intramolecular γ-lactam ring closures to give tricyclo **2** were underway.

Figure 4



Hydrolysis Condition for **6**.

Entry	**Condition**	**Resluts**
1	EtOH/ 20% H_2SO_4, rt	No reaction
2	NaOH/EtOH/ H_2O_2	Unknown materials
3	50% TFA/CH_2Cl_2	No reaction
4	1 N HCl/ THF	No reaction
5	$RuH(PPh_3)_4/(MeOCH_2)_2$, 160°C, 24h[5]	Unknown materials
6	AcOH/H_2O/MeOH, 80°C	No reaction
7	BF_3OEt2/ CH_2Cl_2, Molecular Sieves[6]	Unknown materials
8	EtOH/ 50% H_2SO_4, rt	Unknown materials

Methods

General. Proton and carbon NMR were obtained on a Bruker AMX-500 spectrometer. NMR spectra were recorded in $CDCl_3$ solution, expect as otherwise stated. Chemical shifts were reported in ppm downfield to tetramethylsilane (δ units). Fast atom bombardment (FAB) mass spectra and elemental analyses were recorded on a Micromass ZAB spectrometer and Perkin-Elmer 2400 elemental analyzer repetitively at the Analytical Facility of The National Taiwan University. IR spectra were obtained on Perkin Elmer Spectrum RXI FT-IR system. Silica gel TLC was performed on 60F-254 pre-coated sheets (E. Merck) and column chromatography was done on silica gel (60-120 mesh). All of chemicals were used directly as purchased from Acros, Aldrich, or TCI unless otherwise noted.

*Preparation of β carbonyl aniline **4*** Lithium diisopropylamide (LDA) was prepared under a nitrogen atmosphere in the following manner: to a solution of 0.02 mole of diisopropylamine in 30 ml of dry THF at -30^{o}C was added 0.02 mole of n0butyllithium (1.6M in hexane). The LDA solution was cooled further at -70^{o}C then a solution of 0.01 mole of the methyl nitrile in 10 ml of THF was added dropwised. The mixture was stirred at -70^{o}C for 1 hr then a solution of 0.01 mole of **7** in 35 ml of THF was added slowly. The mixture was stirred at -70^{o}C for 10 min, the reaction was quenched with saturated aqueous ammonium chloride. The organic phase was separated and the aqueous layer was extracted twice with CH_2Cl_2. The organic layer were combined and dried over $MgSO_4$. Removal of the solvent under reduced pressure furnished essentially pure **4** as off white solid (88% yield). Mp:135-136^{o}C ^{1}H NMR ($CDCl_3$): δ 2.94 (s, 3H), 4.05 (s, 2H), 6.61 (t, 1H, *J* = 7.4 Hz), 6.74 (d, 1H, *J* = 8.6 Hz), 7.44 (t, 1H, *J* = 7.4 Hz), 7.47(d, 1H, *J* = 8.6 Hz). MS (EI) m/z: 174.

Preparation of quinolin-4-one **5** To a solution of 0.001 mole of **4** was added 0.001 mole of **3,** and 0.001 mole of DMAP in 1.2 ml of CH_2Cl_2, and the mixture was stirred in room temperature for 6 hr; then the reaction was quenched with saturated aqueous ammonium chloride. The organic phase was separated and the aqueous layer was extracted twice with

CH_2Cl_2. The organic layer were combined and dried over $MgSO_4$. Removal of the solvent under reduced pressure furnished crude **5.** The crude 5 was chromatographed on a silica gel using hexans and ethyl acetate (1:2) to afford pure 5 as yellow oil (78% yield). ^{1}H NMR ($CDCl_3$): δ 0.81 (d, 3H, *J* = 6.6 Hz), 0.98 (t, 3H, *J* = 7.4 Hz), 1.40 (s, 9H), 2.42 (m, 1H), 4.93(t, 1H, *J* = 9.5 Hz), 5.81 (d, 1H, *J* = 7.4), 7.49 (t, 1H, *J* = 7.4 Hz), 7.64 (d, 1H, *J* = 8.6 Hz), 7.76 (t, 1H, *J* = 7.4 Hz), 8.45(d, 1H, *J* = 8.6 Hz). MS (EI) m/z: 369.

*Preparation of amine **6*** To a solution of 0.001 mole of **5** was 0.24ml of TFA in 1.2 ml of CH_2Cl_2, and the mixture was stirred in room temperature for 6 hr; then the reaction was quenched with saturated bicarbonated. The organic phase was separated and the aqueous layer was extracted twice with CH_2Cl_2. The organic layer were combined and dried over $MgSO_4$. Removal of the solvent under reduced pressure furnished crude **6.** The crude **6** was chromatographed on a silica gel using ethyl acetate to afford pure **6** as yellow oil (90% yield). ^{1}H NMR ($CDCl_3$): δ 0.84 (d, 3H, *J* = 6.6 Hz), 1.05 (t, 3H, *J* = 7.4 Hz), 2.45 (m, 1H), 4.95(t, 1H, *J* = 9.5 Hz), 5.88 (d, 1H, *J* = 7.4), 7.65 (t, 1H, *J* = 7.4 Hz), 7.77 (d, 1H, *J* = 8.6 Hz), 7.86 (t, 1H, *J* = 7.4 Hz), 8.61(d, 1H, *J* = 8.6 Hz). MS (EI) m/z: 312.

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