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⁄ M"«⁄AæQ`x˘P ›, Q§ ¤ fl(–)quinolactacin A2 "˝¥ ƒX ƒ¤

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Pictet-Spengler condensation

butylcholinesterase (BuChE)

Alzheimer's disease

Alzheimer's disease

acetylcholinesterase

butylcholinesterase (BuChE)

quinolactacin A1 (1)

quinolactacin A2 (2)

pyrrolo[3,4-b]quinolone

chiral centers

quinolactacin A2 (2),

(IC_{50} = 19.8 μM)

butylcholinesterase (BuChE)

(IC_{50} = 653 μM)

quinolactacin A2 (2)

enantioselective

quinolactacin A2 (2)

L-isoleucine

chiral centers

pyrrolo[3,4-b]quinolone

Pictet-Spengler condensation

Pictet-Spengler condensation

pyrrolo[3,4-b]quinolone

lactame

¡] ⁄ G ¡ ^ › p e¡^⁄] ″K»nƒ¡Ĉ ƒ¥H⁄″¡^

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.

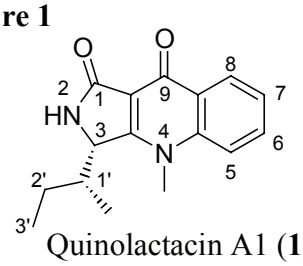
The fungal metabolites, quinolactacin A1 (**1**) and A2 (**2**) were isolated from fermentation strain fb90648 are novel ChEIs. Both natural products **1** and **2** exhibited inhibitory activity against AChE with IC_{50} value of 280 and 19.8 μM respectively. Quinolactacin A2 (**2**) is a weak BuChE 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.

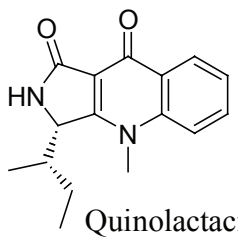
Introduction

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**)

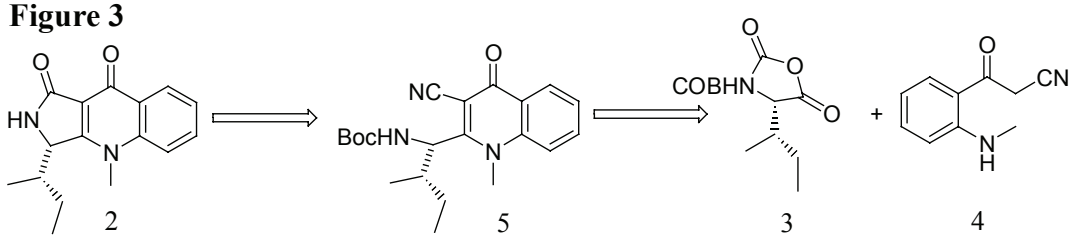
Synthesis of quinolactacin A2 (**2**) is compelling due to its AChE antagonist activity, the synthetic challenges posed by the structure, and the status as potential new leads in drug discovery efforts. The synthesis herein is an asymmetric synthesis of quinolactacin A2 (**2**). The total synthesis of 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

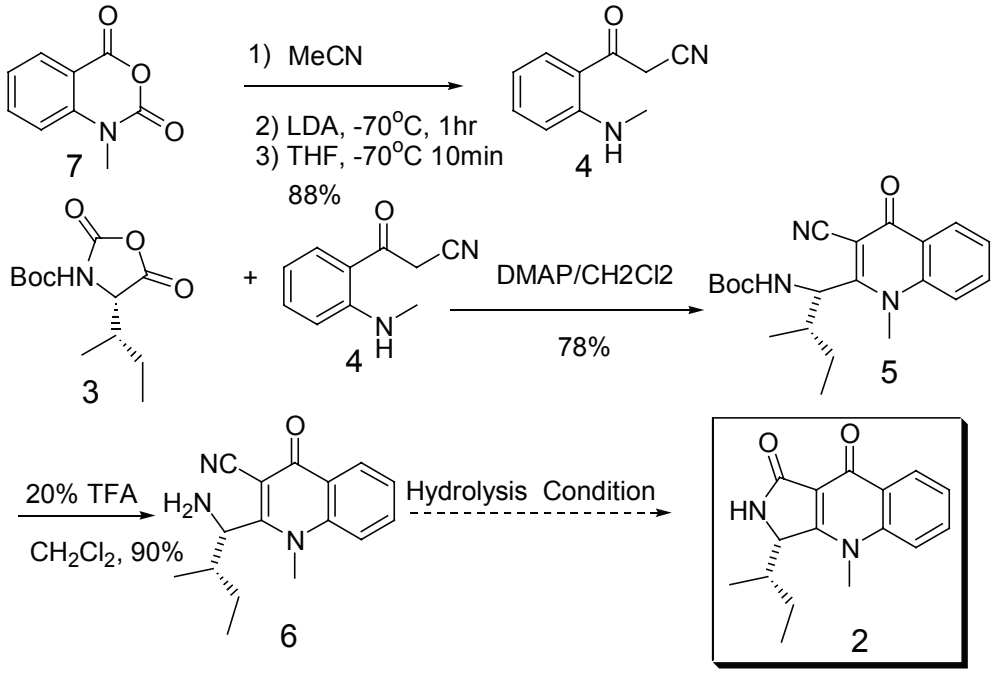


2 5 3 4

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°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°C was added 0.02 mole of n0butyllithium (1.6M in hexane). The LDA solution was cooled further at -70°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°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°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°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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