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Laboratory note

Studies on the synthesis and biological evaluation of the metabolite of clausenamide CM_2 and its stereoisomers

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ABSTRACT

The synthesis and biological evaluation of 5-hydroxy clausenamide (CM_2), one of the major metabolites of clausenamide, and its stereoisomers have been carried out. The absolute configurations of (−)- and (+)-CM_2 were assigned as 3*S*,4*S*,5*S*,6*S* and 3*R*,4*R*,5*R*,6*R* respectively based on ^{1}H NMR spectroscopic investigation and their chemical correlation to (−)- and (+)-clausenamidone (**3**). Electrophysiological assay showed that compound (+)-CM_2 and its C_6 epimer (+)-**8a** had significant effects on synaptic transmission and thus induced the long-term potentiation of the dentate gyrus.

1. Introduction

Dementia is one of the age related mental problems and characteristic symptom of various neurodegenerative diseases including Alzheimer's disease. Nootropic agents are clinically used to address organic disorders in learning abilities and to improve memory, mood and behavior [1]. (±)-Clausenamide (**1**, as shown in Fig. 1) is a naturally occurring nootropic agent first isolated from the Chinese medicine *wampee* [2]. The synthesis and configuration determination of (±)-**1** and its two optical isomers (+)-**1** and (−)-**1** (Fig. 1) has been accomplished by our group [3,4]. Compound (−)-**1** has been found to possess potent nootropic activity in many physiological or behavioral experiments, but (+)-**1** does not have such properties [5]. Currently (−)-**1** is being investigated clinically for the treatment of senile dementia.

In vitro and *in vivo* metabolic studies have demonstrated that CM_2, (Fig. 1) (the C_5 hydroxylation product of clausenamide) is one of the major metabolites of clausenamide (**1**) [6]. The hydroxylation could take place on either side of the pyrrolidone ring of **1**, while the configurations of C_3, C_4 and C_6 remain unchanged in all cases. Thus CM_2 may be either (3*S*,4*S*,5*S*,6*S*)-5-hydroxy clausenamide (**2a**) or (3*S*,4*S*,5*R*,6*S*)-5-hydroxy clausenamide (**2b**) as shown in Fig. 1. To support the clinical studies of (−)-**1** and to investigate whether CM_2 is also a nootropic agent, the synthesis, characterization and biological evaluation of CM_2 and its isomers have been conducted and described herein.

2. Chemistry

CM_2 is the C_5 hydroxylation product of **1**. Direct introduction of a tertiary hydroxyl group at C_5 of **1** by means of traditional chemistry is difficult. Accordingly a *de novo* synthetic route via dihydroxylation of $\Delta^{5,6}$ clausenamide (**7**) or its acetylation product (**6**) was designed and used to prepare CM_2 and its stereoisomers (Scheme 1). (±)-Clausenamidone (**3**) or its optical active isomers (−)-**3** and (+)-**3** were chosen as the starting material, where the relative or absolute configurations had already been determined by our group [5] and thus were an aid to the determination of the configuration of CM_2.

(±)-Clausenamidone (**3**), prepared according to literature [3], was acetylated with acetic anhydride in pyridine to give (±)-**4** in quantitative yield. The ketone group on the side chain of compound (±)-**4** was reduced with sodium borohydride to afford (±)-**5** as the sole product in 95% yield. The relative configuration at C_6 of (±)-**5** is presumed to be *S* according to the literature precedent that reported similar transformation of clausenamidone to

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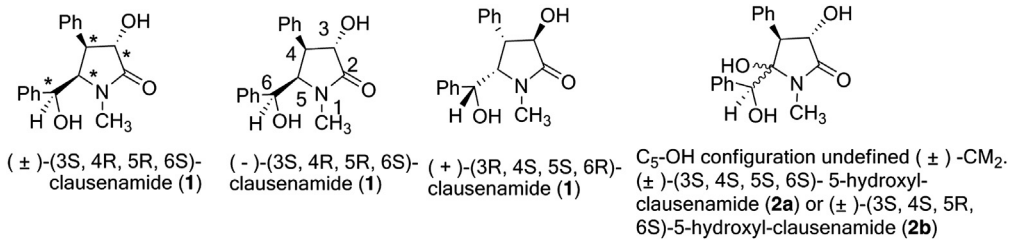
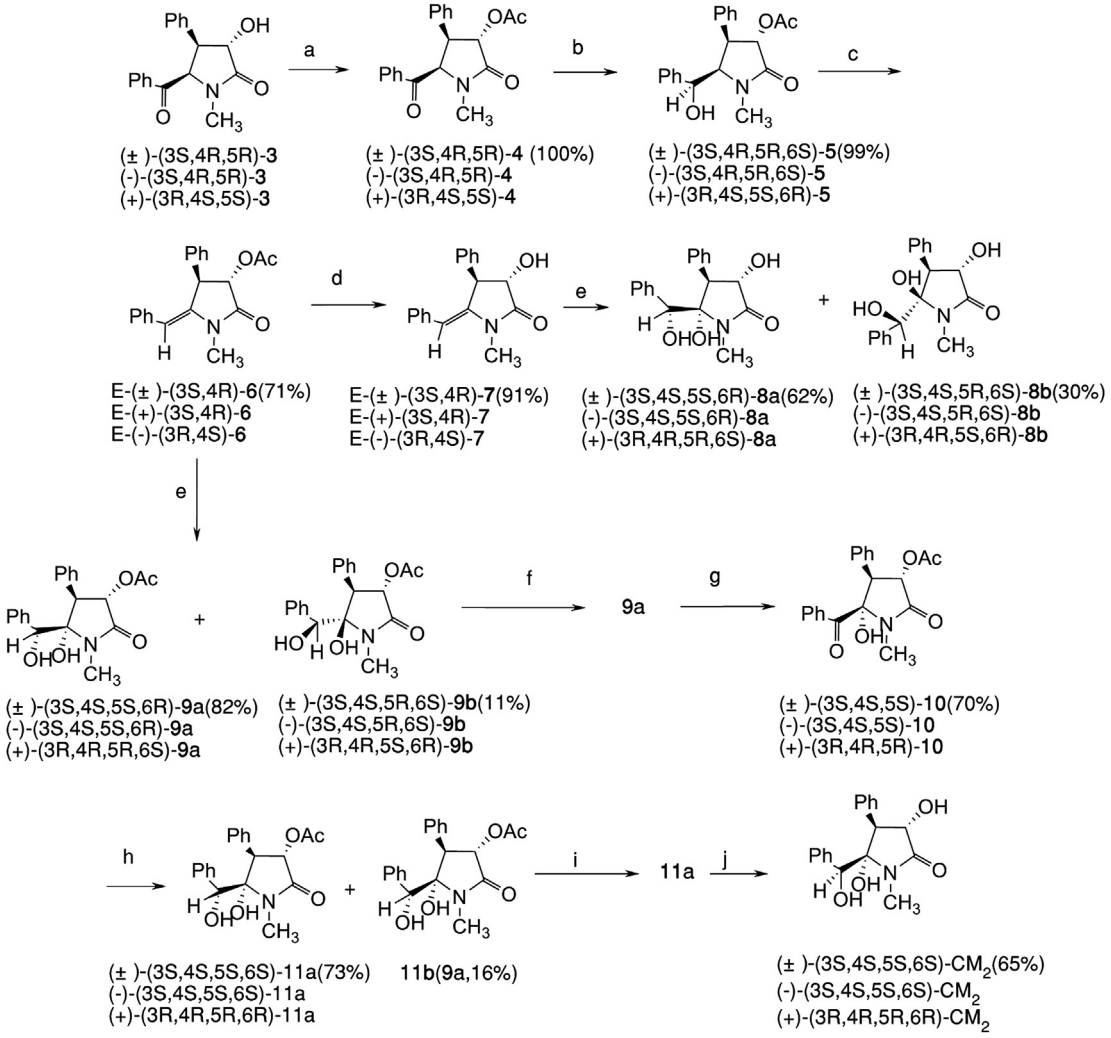


Fig. 1. The structure of clausenamide and CM_2.

clausenamide [3]. Dehydration of (±)-**5** with phosphorus oxychloride in pyridine provided the *E*-(±)-olefin-**6** exclusively in 70% yield. The *E* configuration was confirmed by NOE correlation between the olefin signal (δ 6.11) and *N*-methyl signal (δ 3.10). The *E*-(±)-olefin-**6** was deacetylated with NaOH in ethanol to give *E*-(±)-olefin-**7** in 91% yield. *Syn*-dihydroxylation of *E*-(±)-olefin-**7** with osmium tetroxide and *N*-methylmorpholine oxide (NMO) gave a crude mixture. Purification by chromatography on silica gel gave a major product (yield 62%), determined to be (±)-(3*S*,4*S*,5*S*,6*R*)-**8a** according to the positive NOE between C_4-**H** (δ 3.41) and C_5-O**H** (δ 6.42). The minor product, assigned as (±)-(3*S*,4*S*,5*R*,6*S*)-**8b** (no NOE between C_4-**H** (δ 3.19) and C_5-O**H** (δ 6.00) detected) was obtained in 30% yield. This result showed that the *cis*-dihydroxylation of *E*-(±)-olefin-**7** proceeded in a kinetically controlled manner and predominantly took place at the less hindered side of the double bond (opposite the C_4 phenyl). The gross structure of (±)-**8b** is the same as (±)-**2b**, one possible structure of (±)-CM_2 (Fig. 1). But, the physical constants and spectral data of (±)-**8b** were inconsistent with those reported for CM_2. Then, it is clear that (±)-CM_2 must (±)-(3*S*,4*S*,5*S*,6*S*)-5-hydroxy clausenamide (**2a**, Fig. 1).

To obtain (±)-CM_2, attempts were made to *trans*-dihydroxylate *E*-(±)-olefin-**6** using several methods, such as WO_3/H_2O_2 [7], silver acetate/I_2 [8], but none gave a successful result. Efforts were



Scheme 1. The preparation of CM_2 and its isomers. Reagents and conditions: a: Ac_2O/pyridine; b: $NaBH_4$/MeOH; c: $POCl_3$/pyridine; d: 10% NaOH in ethanol/CH_2Cl_2; e: OsO_4/NMO/THF/acetone; f: chromatography; g: DMSO/oxalyl chloride/THF/TEA; h: $NaBH_4$/MeOH; i: recrystallization or preparative thin-layer chromatography; j: Sm/I_2/methanol.

Table 1
The absolute configurations, melting point and optical rotation data of the synthesized compounds.

Compound	Melting point (°C)	Optical rotation data	Compound	Melting point (°C)	Optical rotation data
(−)-(3*S*,4*R*,5*R*)-**4**	157–160	$[\alpha]_D^{12} = -305$ (c, 0.343, $CHCl_3$)	(+)-(3*R*,4*S*,5*S*)-**4**	158–160	$[\alpha]_D^{12} = +306$ (c, 0.370, $CHCl_3$)
(−)-(3*S*,4*R*,5*R*,6*S*)-**5**	244–246	$[\alpha]_D^{14} = -167$ (c, 0.106, $CHCl_3$)	(+)-(3*R*,4*S*,5*S*,6*R*)-**5**	243–245	$[\alpha]_D^{15} = +173$ (c, 0.120, $CHCl_3$)
Z-(+)-(3*S*,4*R*)-**6**	119–120	$[\alpha]_D^{18} = +330$ (c, 0.870, $CHCl_3$)	*Z*-(−)-(3*R*,4*S*)-**6**	119–120	$[\alpha]_D^{18} = +326$ (c, 0.870, $CHCl_3$)
Z-(+)-(3*S*,4*R*)-**7**	125–127	$[\alpha]_D^{18} = +17.1$ (c, 0.700, $CHCl_3$)	*Z*-(−)-(3*R*,4*S*)-**7**	125–127	$[\alpha]_D^{18} = +16.7$ (c, 0.738, $CHCl_3$)
(−)-(3*S*,4*S*,5*S*,6*R*)-**8a**	Oil	$[\alpha]_D^{25} = -113$ (c, 0.505, CH_3OH)	(+)-(3*R*,4*R*,5*R*,6*S*)-**8a**	Oil	$[\alpha]_D^{25} = +117$ (c, 0.200, CH_3OH)
(−)-(3*S*,4*S*,5*R*,6*S*)-**8b**	147–149	$[\alpha]_D^{25} = -202$ (c, 0.510, CH_3OH)	(+)-(3*R*,4*R*,5*S*,6*R*)-**8b**	145–147	$[\alpha]_D^{25} = +208$ (c, 0.620, CH_3OH)
(−)-(3*S*,4*S*,5*S*,6*R*)-**9a**	125–128	$[\alpha]_D^{15} = -323$ (c, 0.870, CH_3OH)	(+)-(3*R*,4*R*,5*R*,6*S*)-**9a**	125–127	$[\alpha]_D^{15} = +327$ (c, 0.470, CH_3OH)
(−)-(3*S*,4*S*,5*S*)-**10**	125–128	$[\alpha]_D^{15} = -310$ (c, 0.360, $CHCl_3$)	(+)-(3*R*,4*R*,5*R*)-**10**	127–129	$[\alpha]_D^{18} = +312$ (c, 0.442, $CHCl_3$)
(−)-(3*S*,4*S*,5*S*,6*S*)-**11**	153–157	$[\alpha]_D^{15} = -31.9$ (c, 0.455, CH_3OH)	(+)-(3*R*,4*R*,5*R*,6*R*)-**11**	152–156	$[\alpha]_D^{18} = +31.7$ (c, 0.480, CH_3OH)
(−)-(3*S*,4*S*,5*S*,6*S*)-**CM_2**	Oil	$[\alpha]_D^{18} = -53.6$ (c, 0.470, CH_3OH)	(+)-(3*R*,4*R*,5*R*,6*R*)-**CM_2**	Oil	$[\alpha]_D^{18} = +54.1$ (c, 0.480, CH_3OH)

also made to prepare the (±)-*Z*-olefin-**6** through alternative dehydration methods, such as direct dehydration of (±)-**5** with AlO_3 [9], demesylation of the mesylate of compound **5** with DBU [10], with the aim of preparing (±)-CM_2 through *syn*-dihydroxylation of (±)-*Z*-olefin-**6** in the next step. However, these attempts were unsuccessful.

Therefore, a circuitous route was adopted to access the correct configuration of C_6, i.e. oxidation of the C_6-**OH** to a ketone and then reduction of the ketone to hydroxyl group in order to invert the configuration of C_6 from *R* to *S*. The (±)-*E*-olefin-**6** was *syn*-dihydroxylated with OsO_4 and NMO to produce a mixture of products. This mixture was recrystallized from ethyl acetate to give pure (±)-(3*S*,4*S*,5*S*,6*R*)-**9a** (NOE correlation observed between C_4-**H** (δ 3.74) and C_5-O**H** (δ 5.65)) as the major product in 82% yield. Preparative thin-layer chromatography of the mother liquors afforded the minor product (±)-(3*S*,4*S*,5*R*,6*S*)-**9b** in 11% yield. Compound (±)-**9a** was oxidized by Swern oxidation [11] to yield α-hydroxyketone (±)-(3*S*,4*S*,5*S*)-**10** in 70% yield. Reduction of the ketone group of (±)-**10** with $NaBH_4$ gave (±)-**11a** and (±)-**11b** in 73% and 16% yields respectively. Comparison of the ^{1}H NMR spectral data and Rf value of (±)-**11b** and (±)-**9a** suggests that they are the same compound. Accordingly, (±)-**11a** must be the C_6 epimer of (±)-**9a**, namely (±)-(3*S*,4*S*,5*S*,6*S*)-3-*O*-acetyl-5-hydroxy clausenamide. Attempted deacetylation of (±)-**11a** under acidic or basic conditions gave complex mixtures of products, whereas deacetylation proceeded smoothly under the mild and neutral conditions of Sm/I_2/MeOH [12], to afford a white solid in 65% yield, which showed identical spectral data to that of (±)-CM_2. The deacetylation did not affect the configuration, so the relative configuration of the (±)-CM_2 must be 3*S*,4*S*,5*S*,6*S* (Scheme 1). The structures of all new compounds were unambiguously characterized with ^{1}H NMR, ^{13}C NMR, and mass spectrometry or high-resolution mass spectrometry.

(−)-CM_2 and (+)-CM_2 were prepared from the corresponding (−)-(3*S*,4*R*,5*R*)-clausenamidone (**3**) or (+)-(3*R*,4*S*,5*S*)-clausenamidone (**3**) by the same process used to synthesize (±)-CM_2. The absolute configurations, optical rotation data and melting points of intermediates and final products are listed in Table 1. The absolute configurations of (−)-CM_2 and (+)-CM_2 were assigned as 3*S*,4*S*,5*S*,6*S* and 3*R*,4*R*,5*R*,6*R* respectively (Fig. 2) based on their chemical correlation to (−)- and (+)-**3**.

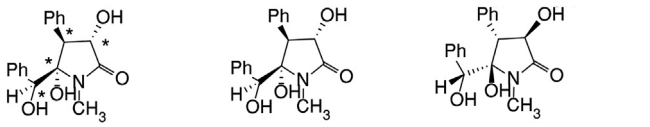


Fig. 2. The structure of (−)- and (+)-CM_2.

3. Pharmacology

Repeated stimulation on hippocampal neurons can induce an immediate and prolonged increase in synaptic strength that is called long-term potentiation (LTP). It is believed that LTP in the hippocampus is the key form of long-lasting synaptic plasticity and it connects the behavior of learning and memory with the plasticity of neurons. Therefore, LTP has been considered as an important index of cognitive activities in cellular and synaptic levels [13].

The nootropic activity of (−)-CM_2 and (+)-CM_2 and their stereoisomers were evaluated herein with LTP assays, with the nootropic parent (−)-clausenamide as the control. As shown in Table 2, at an estimated final brain concentration of 1 mM, (−)-clausenamide caused an increase of 32–58% of population spike amplitude (PSA) above the basal level ($P < 0.01$), whereas, its enantiomer (+)-clausenamide is inactive in LTP-inducing activity. Unexpectedly, (−)-CM_2, which is the metabolite of active (−)-clausenamide, exhibited little effect on the LTP-inducing activity, but (+)-CM_2, the metabolite of inactive (+)-clausenamide, caused 61% ($P < 0.01$) and 88% ($P < 0.001$) increase relative to basal PSA at 45 min and 60 min After Administration (A.A.) respectively. The effect (PS amplitude) of (+)-CM_2 is greater than that of (−)-clausenamide. These findings suggest that the introduction of a hydroxyl group at C_5 position of (−)- or (+)-clausenamide exerts a dramatic influence on the nootropic activities. Compound (+)-**8a**, the C_6 epimer of (+)-CM_2, caused 94% ($P < 0.05$) and 133% ($P < 0.05$) increase relative to basal PSA at 45 min and 60 min A.A. respectively. Its effect (PS amplitude) was greater than that of (+)-CM_2 and (−)-clausenamide, which indicates that (+)-**8a** is a more potent nootropic agent than (+)-CM_2 and (−)-clausenamide. The other three CM_2 stereoisomers (−)-**8a**, (−)-**8b** and (+)-**8b** exhibited little effect on LTP-inducing activity. The common "3*R*,4*R*,5*R*" configuration in compounds (+)-**8a** and (+)-CM_2 demonstrated that the "3*R*,4*R*,6*R*" configuration might be responsible for the nootropic activities. While, the absolute configuration of C_6 may be less important to the nootropic activities, 6S may be preferred over 6R consistent with the different activities between (+)-CM_2 and (+)-**8a**. Our findings suggest that the configuration of 5-hydroxy clausenamide has important implications for the LTP-inducing activities. This may provide a better understanding of the SAR of clausenamide derivatives and may benefit the study of the biological mechanisms of LTP.

4. Conclusion

In summary, an efficient and economic synthesis of CM_2 and its stereoisomers has been described. The absolute configurations of (−)- and (+)-CM_2 were assigned as 3*S*,4*S*,5*S*,6*S* and 3*R*,4*R*,5*R*,6*R* respectively based on ^{1}H NMR spectroscopic studies and chemical correlations to (−)-(3*S*,4*R*,5*R*)-**3** and (+)-(3*R*,4*S*,5*S*)-**3**. The activities

Table 2
LTP screening results of CM_2 and its stereoisomers at a concentration of 1 μM.

Compound	Animal number	Relative PS (PSA %)				
		P.A.[a]	15 min A.A.[b]	30 min A.A.	45 min A.A.	60 min A.A.
DMSO	3	100	107.1 ± 10.1	103.5 ± 6.1	97.8 ± 3.2	96.0 ± 11.1
(−)-(3*S*,4*S*,5*S*,6*R*)-**8a**	3	100	78.9 ± 13.9	83.7 ± 13.6	94.6 ± 8.9	102.7 ± 2.9
(+)-(3*R*,4*R*,5*R*,6*S*)-**8a**	4	100	144.9 ± 49.5	168.0 ± 49.2	194.3 ± 56.7*[c]	233.2 ± 55.6*
(−)-(3*S*,4*S*,5*R*,6*S*)-**8b**	3	100	110.6 ± 7.3	103.1 ± 10.1	94.5 ± 10.5	88.4 ± 14.4
(+)-(3*R*,4*R*,5*S*,6*R*)-**8b**	3	100	81.5 ± 6.1	77.3 ± 8.9	82.6 ± 1.8	99.9 ± 6.3
(−)-(3*S*,4*S*,5*S*,6*S*)-**CM_2**	3	100	92.8 ± 17.5	87.1 ± 30.5	97.3 ± 25.7	98.3 ± 28.6
(+)-(3*R*,4*R*,5*R*,6*R*)-**CM_2**	4	100	91.7 ± 17.2	117.8 ± 14.9	161.1 ± 13.9**	188.0 ± 5.3***
(+)-(3*R*,4*S*,5*S*,6*R*)-**1**	6	100	90.6 ± 0.3	110.1 ± 13.1		106.4 ± 4.1
(−)-(3*S*,4*R*,5*R*,6*S*)-**1**	6	100	131.8 ± 0.4*	138.5 ± 8.9*		158.1 ± 4.2*

[a] P.A.: Prior administration.
[b] A.A.: After administration.
[c] Test $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ *vs.* control.

of (−)-CM_2, (+)-CM_2 and their optically active stereoisomers for inducing long-term potentiation (LTP) of the dentate gyrus were tested by electrophysiological assay. Preliminary results showed that compounds (+)-(3*R*,4*R*,5*R*,6*S*)-**8a** and (+)-(3*R*,4*R*,5*R*,6*R*)-CM_2 had significant effects on synaptic transmission and thus induced the LTP of the dentate gyrus, and that the configuration of CM_2 and its stereoisomers has important implications for their LTP-inducing activities. These findings may provide a better understanding of the SAR of clausenamide derivatives and may assist the study on the biological mechanisms of LTP.

5. Experiment

5.1. Chemistry

All the reagents were commercially available and used without further purification. Melting points were determined using a Yanaco apparatus and the thermometer is uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured using a Bruker-400 or YS-300. Mass spectra were obtained from VG300, ZAD-2F or API3000 instruments.

5.1.1. (±)-(3S,4S,5R)-3-O-acetyl-clausenamidone (*4*)

Clausenamidone (**3**) (2.00 g, 6.78 mmol) was dissolved in a mixture of acetic anhydride and pyridine. The mixture was stirred overnight at 0 °C, and then was poured into cold water. The precipitated solid was collected by suction, washed with water and air dried to give title compound as white solid 2.15 g (yield: 94%), which was pure enough to be used in next step without further purification. mp: 163–165 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.07 (3H, s), 2.89 (3H, s), 4.02 (1H, dd, $J = 6.0$, 8.8 Hz), 5.42 (1H, d, $J = 8.8$ Hz), 6.13 (1H, d, $J = 6.0$ Hz), 7.00–7.52 (10H, m). HRMS (QFT-ESI): calculated for $C_{20}H_{20}N_1O_4$ (MH^+) 338.1387, found 338.1389.

5.1.2. (±)-(3S,4S,5R,6S)-3-O-acetyl-clausenamide (*5*)

To a solution of (±)-(3*S*,4*S*,5*R*)-3-O-acetyl-clausenamidone (**4**) (1.20 g, 3.56 mmol) in 10 mL methanol, was added sodium borohydride (480 mg, 12.70 mmol). The reaction mixture was stirred at ambient temperature for 30 min. The precipitated solid was collected by suction, washed with water and dried to give pure title compound as white solid (1.15 g, yield: 95%), which was pure enough to be used in the next step without further purification, mp: 230–232 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.05 (3H, s), 2.89 (3H, s), 3.99 (1H, dd, $J = 11.2$, 8.0 Hz), 4.16 (1H, dd, $J = 8.0$, 4.0 Hz), 4.82 (1H, d, $J = 4.0$ Hz), 5.87 (1H, d, $J = 11.2$ Hz), 6.86–7.30 (10H, m). HRMS (QFT-ESI): calculated for $C_{20}H_{22}N_1O_4$ (MH^+) 340.1543, found 340.1560.

5.1.3. (±)-(3S,4S)-3-O-acetyl-$\Delta^{5,6}$ clausenamide (*6*)

To a solution of (±)-(3*S*,4*S*,5*R*,6*S*)-3-O-acetyl-clausenamide (**5**) (1.00 g, 2.94 mmol) in anhydrous pyridine (6 mL), a solution of distilled phosphorous oxychloride (1.0 mL) in anhydrous pyridine (4 mL) was added dropwise over half an hour at 0 °C. The mixture was stirred at 0 °C for 24 h and then poured into ice water. The precipitate was separated by filtration. The filter cake was dissolved with methylene dichloride, washed with water and brine successively, dried over anhydrous sodium sulfate, and the solvent evaporated off to give a crude solid. The solid was purified by column chromatography eluted with methylene dichloride and methanol to give title compound as white solid (746 mg, yield: 71%), mp: 148–150 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.13 (3H, s), 3.30 (3H, s), 4.33 (1H, s), 5.14 (1H, s), 6.11 (1H, s), 7.02–7.36 (10H, m); HRMS (QFT-ESI): calculated for $C_{20}H_{20}N_1O_3$ (MH^+) 322.1438, found 322.1440.

5.1.4. (±)-(3S,4S)-$\Delta^{5,6}$ clausenamide (*7*)

To a solution of (±)-(3*S*,4*S*)-3-O-acetyl-$\Delta^{5,6}$ clausenamide **6** (400 mg, 1.24 mmol) in methylene dichloride (15 mL), was added 10% sodium hydroxide alcohol solution (3 mL) and the mixture was stirred for 3 min. The mixture was neutralized with 2 M HCl, diluted with methylene dichloride (20 mL), washed with 1 M sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate, and then concentrated to give title compound as white solid (330 mg, yield: 95%), mp: 162–163 °C. ^{1}H NMR (300 MHz, CD_3COCD_3): δ 3.18 (3H, s), 4.08 (1H, dd, $J = 5.1$, 1.8 Hz), 4.36 (1H, d $J = 1.8$ Hz), 5.32 (1H, d, $J = 5.1$ Hz (D_2O exchangeable)), 6.12 (1H, s), 6.95–7.22 (10H, m). HRMS (QFT-ESI): calculated for $C_{18}H_{18}N_1O_2$ (MH^+) 280.1332, found 280.1338.

5.1.5. (±)-(3S,4S,5R,6S)-5-hydroxy clausenamide (*8a*) and (±)-(3S,4S,5S,6R)-5-hydroxy clausenamide (*8b*)

To a solution of (±)-(3*S*,4*S*)-$\Delta^{5,6}$ clausenamide (**7**) (260 mg, 0.931 mmol) in acetone (1 mL) and THF (5 mL), *N*-methylmorpholine *N*-oxide (545 mg, 4.66 mmol) and 4% OsO_4 aqueous solution (0.6 mL, 0.093 mmol) were added with a syringe. After stirring at room temperature for 24 h, a mixture of anhydrous sodium sulfite (650 mg) and water (3 mL) was added. The resulting mixture was stirred for 1 h, diluted with methylene dichloride, washed with water then brine and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded an oily product (280 mg), which was purified by chromatography to give (±)-(3*S*,4*S*,5*R*,6*S*)-5-hydroxy clausenamide (**8a**) (150 mg, yield: 50%) and (±)-(3*S*,4*S*,5*S*,6*R*)-5-hydroxy clausenamide (**8b**) (70 mg, yield: 24%.).

8a, White solid, mp: 134–136 °C. ^{1}H NMR (400 MHz, DMSO-d6): δ 2.11 (3H, s), 3.39 (1H, d, $J = 10.08$ Hz), 4.06 (1H, d, $J = 4.2$ Hz), 4.92 (1H, d, $J = 10.08$ Hz), 5.40 (1H, d, $J = 4.2$ Hz, (D_2O exchangeable)),

5.60 (1H, brs, (D_2O exchangeable)), 6.42 (1H, s, (D_2O exchangeable)), 6.90–7.53 (10H, m); ^{13}C NMR (DMSO-d6): δ 26.18, 61.53, 70.03, 71.98, 90.10, 126.67, 126.83, 127.81, 127.86, 128.88, 136.35, 141.44, 173.67. FAB-MS (*m*/*e*, %) 314 (MH^+, 60), 296 (7), 277 (11), 185 (90), 93 (100). HRMS (QFT-ESI): calculated for $C_{18}H_{20}N_1O_4$ (MH^+) 314.1387, found 314.1389.

8b, White solid, mp: 127–129 °C, ^1H NMR (400 MHz, DMSO-d6): δ 2.87 (3H, s), 3.18 (1H, d, J = 9.9 Hz), 3.51 (1H, d, J = 8.9 Hz), 4.19 (1H, d, J = 4.8 Hz), 4.69 (1H, d, J = 4.2 Hz), 5.52 (1H, bs, (D_2O exchangeable)), 5.78 (1H, d, J = 4.2 Hz, (D_2O exchangeable)), 6.00 (1H, s, (D_2O exchangeable)), 6.90–7.36 (10H, m); ^{13}C NMR (DMSO-d6): δ 24.72, 50.90, 72.37, 75.12, 90.18, 125.84, 126.66, 126.74, 126.87, 127.77, 130.19, 138.16, 140.03, 173.67; FAB-MS (*m*/*e*, %): 314 (MH^+, 15), 312 (20), 176 (100), 120 (29), 93 (71). HRMS (QFT-ESI): calculated for $C_{18}H_{20}N_1O_4$ (MH^+) 314.1387, found 314.1390.

5.1.6. (±)-(3S,4S,5S,6R)-3-O-acetyl-5-hydroxy clausenamide (**9a**) and (±)-(3S,4S,5S,6R)-3-O-acetyl-5-hydroxy clausenamide (**9b**)

To a solution of (±)-(3*S*,4*S*)-3-O-acetyl-$\Delta^{5,6}$ clausenamide (**6**) (240 mg, 0.748 mmol) in THF (4 mL) and acetone (1 mL), *N*-methylmorpholine *N*-oxide (581 mg, 3.738 mmol) and 10 mg/mL OsO_4 aqueous solution (1.9 mL, 0.093 mol) were added. After stirring at room temperature for 48 h, anhydrous sodium sulfite (500 mg) and water (10 mL) was added. The resultant mixture was stirred for 1 h more, diluted with methylene dichloride, washed with water then brine and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 300 mg of an oil, which was recrystallized from ethyl acetate to give 150 mg of (±)-(3*S*,4*S*,5*S*,6*R*)-3-O-acetyl-5-hydroxy clausenamide (**9a**). Separation of the mother liquors with preparative thin-layer chromatography (eluted with ethyl acetate) gave 70 mg of (±)-(3*S*,4*S*,5*S*,6*R*)-3-O-acetyl-5-hydroxy clausenamide (**9b**) and 67 mg of **9a**.

9a, White solid, yield: 82%, mp: 134–136 °C. ^1H NMR (300 MHz, CD_3COCD_3): δ 1.99 (3H, s), 2.23 (3H, s), 3.74 (1H, d, J = 10.5 Hz), 4.31 (1H, d, J = 4.5 Hz), 4.76 (1H, d, J = 4.5 Hz (D_2O exchangeable)), 5.66 (1H, s (D_2O exchangeable)), 6.59 (1H, d, J = 10.5 Hz), 7.20–7.63 (10H, m). ^{13}C NMR (DMSO-d6): δ 20.60, 26.54, 58.53, 70.77, 71.88, 90.83, 127.08, 127.35, 127.79, 128.30, 128.50, 134.46, 140.80, 138.87, 169.86. HRMS (QFT-ESI): calculated for $C_{20}H_{22}N_1O_5$ (MH^+) 356.1493, found 356.1501.

9b, White solid, yield: 11%, mp: 146–148 °C. ^1H NMR (300 MHz, CD_3COCD_3): δ 1.93 (3H, s), 3.00 (3H, s), 3.60 (1H, d, J = 8.4 Hz), 4.98 (1H, d, J = 4.5 Hz), 4.98 (1H, s (D_2O exchangeable)), 5.07 (1H, d, J = 4.5 Hz (D_2O exchangeable)), 5.75 (1H, d, J = 8.4 Hz), 7.04–7.52 (10H, m). HRMS (QFT-ESI): calculated for $C_{20}H_{22}N_1O_5$ (MH^+) 356.1493, found 356.1503.

5.1.7. (±)-(3S,4S,5S)-5-hydroxy clausenamidone (**10**)

To a solution of re-distilled oxalyl chloride (453 μL, 5 mmol) in THF (5 mL) at −50–60 °C, DMSO (767 μL, 10 mmol) was carefully added under nitrogen atmosphere. After stirring for 5 min, a solution of (±)-(3*S*,4*S*,5*S*,6*R*)-3-O-acetyl-5-hydroxy clausenamide (**9a**) (150 mg, 0.423 mmol) in THF (10 mL) was added and stirred for a further 2 h at the temperature, then Et_3N (1 mL) was added. The cooling bath was removed, and the reaction mixture was allowed to warm to room temperature. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate. The extract was washed with 1 M hydrochloric acid, aqueous sodium bicarbonate, saturated sodium chloride solution and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the crude oil (250 mg) was purified by column chromatography (eluted with CH_2Cl_2 and MeOH) to give the title compound as white solid (95 mg, yield: 71%). mp: 103–106 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 2.10 (3H, s), 2.81 (3H, s), 4.16 (1H, d, J = 10.2 Hz), 4.64 (1H, s (exchangeable)), 6.09 (1H, d, J = 10.2 Hz), 7.04–8.22 (10H, m). ^{13}C NMR (DMSO-d6): δ 20.73, 25.88, 55.99, 70.62, 93.56, 127.98, 128.15, 128.40, 128.51, 128.55, 131.60, 133.30, 135.43, 169.66, 169.93, 198.57. FAB-MS (*m*/*e*, %) 354 (MH^+, 100), 276 (80), 376 (MNa^+, 20), 131 (82), 105 (PhCO, 53). HRMS (QFT-ESI): calculated for $C_{20}H_{22}N_1O_5$ (MH^+) 354.1336, found 354.1339.

5.1.8. (3S,4S,5S,6S)-3-O-acetyl-5-hydroxy clausenamide (**11a**)

(±)-(3*S*,4*S*,5*S*)-3-O-acetyl-5-hydroxy clausenamidone (**10**) (82 mg, 0.260 mmol) was dissolved in methanol (3 mL) and cooled to 0 °C. Sodium borohydride (30 mg, 0.78 mmol) was added and the mixture stirred for 10 min. The mixture was neutralized carefully with 1 M hydrochloric acid and the organic solvents were distilled off under reduced pressure .The residue was extracted with ethyl ether, washed with brine, dried over anhydrous sodium sulfate, and the solvent distilled off to give a crude oil, which was purified by column chromatography (eluted with CH_2Cl_2 and MeOH) to give the title compound (60 mg, yield: 73%.) as white solid, mp: 144–147 °C, ^1H NMR (300 MHz, CD_3COCD_3): δ 1.93 (3H, s), 3.04 (3H, s), 3.77 (1H, d, J = 11.1 Hz), 4.73 (1H, d, J = 4.2 Hz), 5.05 (1H, d, J = 4.2 Hz, (D_2O exchangeable)), 5.20 (1H, d, J = 11.1 Hz), 5.96 (1H, s, (D_2O exchangeable)), 6.72–7.29 (10H, m). ^{13}C NMR (DMSO-d6): δ 20.51, 25.14, 55.25, 69.80, 73.81, 90.78, 126.45, 126.66, 127.05, 127.64, 127.81, 128.31, 133.38, 139.14, 167.91, 169.59. HRMS (QFT-ESI): calculated for $C_{20}H_{22}N_1O_5$ (MH^+) 356.1493, found 356.1503.

5.1.9. (±)-(3S,4S,5S,6S)-5-hydroxy clausenamide (**CM$_2$**)

(±)-(3*S*,4*S*,5*S*,6*S*)-3-O-acetyl-5-hydroxy clausenamide (**11a**) (50 mg, 0.142 mmol) was dissolved in anhydrous methanol (5 mL), to the mixture was added iodine (38 mg, 0.149 mmol) and samarium (23 mg, 0.149 mmol). The resultant mixture was stirred in air at room temperature for 12 h and then partitioned between ethyl acetate and water. The organic layer was washed with 25% sodium hyposulfite solution, brine, dried over sodium sulfate and concentrated *in vacuo*. The resulting light yellow oil was purified by column chromatography (eluted with CH_2Cl_2 and MeOH) to give the title compound, which was recrystallized from ethyl acetate/petroleum ether to give a white solid (25 mg, yield: 56%), mp: 114–116 °C. ^1H NMR (300 MHz, CD_3COCD_3): δ 3.11 (3H, s), 3.58 (1H, d, J = 8.4 Hz), 4.79 (1H, d, J = 4.2 Hz), 3.45 (1H, dd, J = 8.4, 4.5 Hz), 4.39 (1H, d, J = 4.5 Hz, (D_2O exchangeable)), 5.00 (1H, d, J = 4.2 Hz, (D_2O exchangeable)), 5.88 (1H, s, (D_2O, exchangeable)), 6.67–7.53 (10H, m), ^{13}C NMR (DMSO-d6): δ 25.89, 57.10, 68.73, 75.48, 89.84, 126.72, 127.01, 127.30, 127.51, 127.68, 128.04, 128.68, 135.19, 139.29, 172.44. FAB-MS (*m*/*e*, %) 314 (MH^+, 100), 296 (15), 289 (83). HRMS (QFT-ESI): calculated for $C_{18}H_{20}N_1O_4$ (MH^+) 314.1387, found 314.1390. Elemental Analysis: $C_{18}H_{19}N_1O_4$ found (calc.) C: 68.59, 68.85 (68.88), H: 5.98, 5.95 (6.11), N: 4.47, 4.48 (4.47).

5.1.10. Preparation of optically active (−)-(3S,4S,5R,6S)-**8b**, (+)-(3R,4R,5S,6R)-**8b**, (−)-(3S,4S,5S,6R)-**8a**, (+)-(3R,4R,5R,6S)-**8a** and (−)-(3S,4S,5S,6S)-**CM$_2$** and (+)-(3R,4R,5R,6R)-**CM$_2$**

These compounds were prepared from the corresponding (−)-(3*S*,4*R*,5*R*) clausenamidone (**1**) or (+)-(3*R*,4*S*,5*S*) clausenamidone (**1**) by the same process as that described above for their racemic counterpart. The absolute configurations, melting point and optical rotation data of the synthesized compounds are listed Table 1.

5.2. Pharmacology

5.2.1. Effects of our compounds on LTP induced in rat hippocampus

Male adult SD rats (5 per group) were anesthetized with 20% (w/v) urethane carbamate (1.0 g kg^{-1}, ip) and fixed in a stereotaxic head holder. Recording electrodes (stainless steel needle of 0.2 mm diameter, insulated except for 0.2 mm tip) were placed in the dentate gyrus granule cell layer. Our compounds were injected into

lateral ventricle at a concentration of 10^{-6} mol L^{-1}. The stimulating electrodes (two stainless steel needles of 0.15 mm diameter coated with Teflon except for the 0.2 mm tip, and the distance between two needles was 0.5 mm) were planted between the fiber of perforant path (PP) in rat. Continuous pulse was provided by electronic stimulator, and delivered to PP through stimulus isolator and stimulating electrodes. Electrode depth was then adjusted until a typical excitatory postsynaptic potential (EPSP) was observed. Electric current was magnified by amplifier and collected by computer, then processed by DataWave software. The stimulus intensity was adjusted to produce population spike (PS) with a slope that was ≈50% of the maximum. Single-pulse test stimuli were at an interval of 30 s during the experiment. Population spike amplitude (PSA) was recorded and the relatively percent of PSA was calculated according to the formula (PSA/basic value). The result was shown in Table 2.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.04.048.

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