Bioorganic & Medicinal Chemistry Letters 19 (2009) 2112–2115

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and activity in enhancing long-term potentiation (LTP) of clausenamide stereoisomers

Zhiqiang Feng *, Xingzhou Li, Guojun Zheng, Liang Huang *

Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China

ARTICLE INFO

Article history:
Received 5 January 2009
Revised 2 March 2009
Accepted 5 March 2009
Available online 10 March 2009

Keywords:
Clausenamide
Stereoisomers
Long-term potentiation
Nootropic activity

ABSTRACT

Clausenamide, isolated from aqueous extract of dry leaves of *Clausena lansium*, a Chinese folk medicine, was found to have potent activity in enhancing LTP and show nootropic activity in animal tests. In order to discovery more potent stereoisomers and to analyze the relationship of structure–activity, the synthesis of 16 (8 pairs) optically pure stereoisomers of clausenamide with four chiral centers was achieved. The results of LTP assay showed that the nootropic activity of the stereoisomers of clausenamide is closely related to the configuration of stereoisomers.

Synaptic plasticity in mammalian brain is one of the most widely studied topics in neuroscience over the last decade. One of the clinical symptoms of Alzheimer's disease (AD) is disorder of memory that has been suggested to be related with synaptic plasticity.[1] LTP is an important form of synaptic plasticity that increases the strength of synapses, and it is a physiologic correlate of memory and a cellular model for study of learning and memory.[2]

The naturally occurring clausenamide is in racemic form and was found to have potent activity in enhancing LTP and show nootropic activity in animal tests.[3] The synthetic single enantiomer (−)-(3*S*,4*R*,5*R*,6*S*)-clausenamide (**1**) (Fig. 1) could improve Aβ induced impairment of spatial discrimination, potentiate basic synaptic transmission and HFS-induced LTP on either anesthetized or freely moving rats, and increase cortical ChAT activity, hippocampal synapses and Mossy fiber sprouting.[4] In addition, **1** was 50–100 times more active than the known nootropic drug, piracetam, and 5–10 times more active than the racemic clausenamide[5], which indicated that the nootropic activity may be related with the configuration of clausenamide.

There are four chiral centers (C3, C4, C5, C6) in clausenamide, which means that 16 (8 pairs) stereoisomers are possible (Fig. 2). In order to discover more potent stereoisomers and to analyze the relationship between structure and activity of the clausenamide stereoisomers, herein, all optically pure stereoisomers of clausenamide were prepared, their activity in enhancing LTP were evaluated, and the primary relationships of structure–activity were analyzed.

A number of synthetic routes are available to build into the lactam core structure in clausenamide.[6] Four optically pure intermediate compounds, (3*S*,4*R*,5*R*)-clausenamidone (**17**), (3*R*,4*S*,5*S*)-clausenamidone (**18**), (3*S*,4*R*,5*S*)-neo-clausenamidone (**19**) and (3*R*,4*S*,5*R*)-neo-clausenamidone (**20**), were obtained through chiral resolution of racemic clausenamidone and neo-clausenamidone from a biomimetic synthetic route,[7] using menthoxylacetic acid as chiral derivatizing reagent.[8] Reduction of **17** with $NaBH_4$ afforded single product **1**, but its C6 isomer, (3*S*,4*R*,5*R*,6*R*) *epi*-clausenamide was not found under various reduction conditions (Scheme 1) due to the steric hindrance of the C4 phenyl group.

Reduction of **20** with sodium boronhydride gave two C6 isomers, (3*R*,4*S*,5*R*,6*S*)-neo-clausenamide (**3**) and (3*R*,4*S*,5*R*,6*R*)-*epi*-neo-clausenamide (**5**) in almost equal yields after column chromatography. However, reduction with $Al(i\text{-}OPr)_3/i\text{-}PrOH$ afforded predominantly isomer **5** (mol ratio3:**5** = 1:20). Protection of C3–OH with dihydropyran and reduction with L-selectride mainly produced **3** (mol ratio **3**:**5** = 10:1) (Scheme 2).[9]

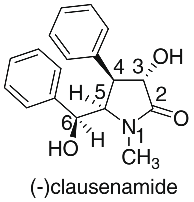


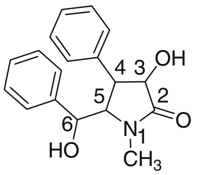
Figure 1. The structure of (−)clausenamide (**1**).

---

* Corresponding authors. Tel.: +86 10 63189351; fax: +86 10 83155752 (Z.F.); tel.: +86 10 63165259 (L.H.).

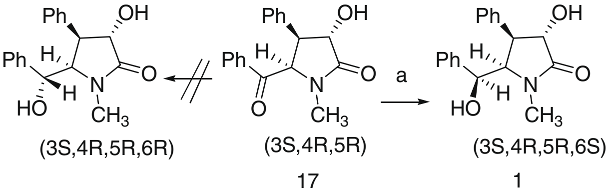
E-mail address: fengzhq@imm.ac.cn (Z. Feng).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.018

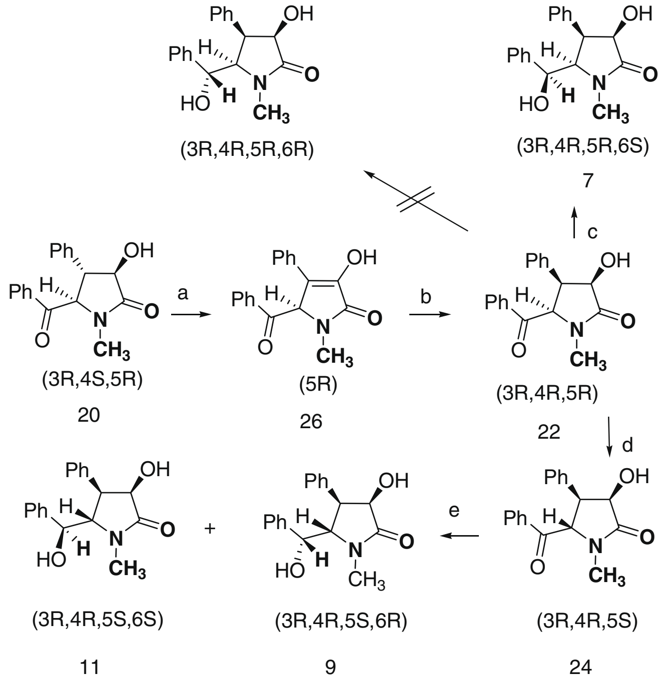


1 (3S,4R,5R,6S)	**2** (3R,4S,5S,6R)	clausenamide
3 (3R,4S,5R,6S)	**4** (3S,4R,5S,6R)	neoclausenamide
5 (3R,4S,5R,6R)	**6** (3S,4R,5S,6S)	epi-neoclausenamide
7 (3R,4R,5R,6S)	**8** (3S,4S,5S,6R)	cis-clausenamide
9 (3R,4R,5S,6R)	**10** (3S,4S,5R,6S)	cis-neoclausenamide
11 (3R,4R,5S,6S)	**12** (3S,4S,5R,6R)	epi-cis-neoclausenamide
13 (3R,4R,5R,6R)	**14** (3S,4S,5S,6S)	epi-cis-clausenamide
15 (3S,4R,5R,6R)	**16** (3R,4S,5S,6S)	epi-clausenamide

Figure 2. The configuration of 16 optically active stereoisomers of clausenamide.



Scheme 1. Reagents and condition: $NaBH_4/CH_3OH$ (85%).



Scheme 3. Reagents and conditions: (a) $Na_2Cr_2O_7/H_2SO_4/H_2O/CH_2Cl_2$, 30–40 °C (76%); (b) $NaBH_4/HOAc/CH_2Cl_2$, 0 °C–rt (79%); (c) $NaBH_4/CH_3OH$ (85%); (d) KOH/CH_3OH, rt (90%); (e) $NaBH_4/CH_3OH$, 0 °C–rt (80%).

cis-Clausenamide has three substituent groups, C3–OH, C4–Ph, and C5-side chain on the same sides of the lactam ring. The congested structure might encounter difficulty in preparation. Indeed inversion of C3–OH in clausenamidone by Mitsunobu reaction or CsOAc/18-Crown ether failed. An attempt of de novo synthesis using cis epoxy cinnamate ester instead of trans epoxy in the clausenamide synthesis was also unsuccessful. Finally a revised synthetic approach by oxidation of C3–OH of neoclausenamidone for rebuilding the chirality of C4 into the desired configuration was designed and carried out (Scheme 3).

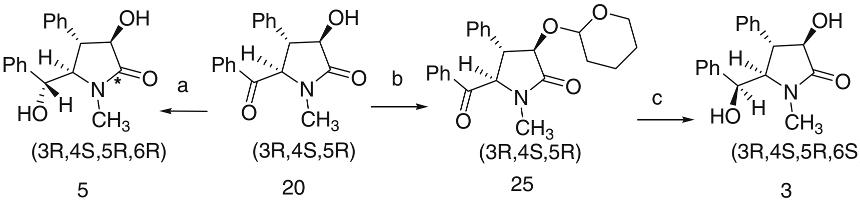
The oxidation of C3–OH of **20** gave a product (5*R*)-3,4-dehydroclausenamidone (**26**). An attempt to reduce the double bond in **26** under catalytic hydrogenation in ethanol gave a complex mixture. While treatment with $NaBH_4$ in the presence of acetic acid in dichloromethane, a single compound (3*R*,4*R*,5*R*)-*cis*-clausenamidone (**22**) was given. Its structure was confirmed by ^1H NMR and NOESY1D, in which strong NOEs between C3–H and C4–H, C4–H and C5–H were observed. The cis relation among C3, C4, C5 was established.

According to the structure of **22**, the reduction of C6=O should favor the Si face to give C6(*S*) configuration, (3*R*,4*R*,5*R*,6*S*)-*cis*-clausenamide, as the Re face is highly hindered by C4 phenyl group. Indeed, the reduction of **22** with $NaBH_4$ in methanol at 0 °C gave a product **7**.

The treatment of (3*R*,4*R*,5*R*)-*cis*-clausenamidone (**22**) with K_2CO_3 in methanol afforded quantitatively a single C5 isomer **24**, which was recrystallized three times in acetone until specific rotation was consistent, giving white crystals with mp: 143–145 °C, $[\alpha]_D^{20}$ +140.5 (*c* 0.56, $CHCl_3$).

Reduction of **24** with $NaBH_4$ in methanol gave a mixture of two C6 isomer **9** and **11** in 80% combined yield, which was separated by column chromatography, with a ratio of **11**:**9** being 2:1. Then they were purified by repeated crystallization, **11** with mp: 248–250 °C, $[\alpha]_D^{22}$ +19.2 (*c* 0.525, CH_3OH), **9** with mp: 164.5–166 °C, $[\alpha]_D^{22}$ +66.7 (*c* 0.46, CH_3OH). Their structure was confirmed by spectra, MS_{FAB} showed: FW = 297, and ^1H NMR data of **11** and **9** showed that the configuration of C6 in **11** is different from that of C6 in **9**, which was inferred from the clear difference between **11** and **9**, $J_{5,6}$ = 2.7 Hz in **11**, $J_{5,6}$ = 5.4 Hz in **9**.

The absolute configuration of C6 in **11** could be deduced by comprehensive analysis for the dominant conformation, the reduction mechanism of **24** and the spectra of **11** and **24**. The reduction of C6=O should favor the Si face to give C6(*S*) configuration, (3*R*,4*R*,5*S*,6*S*)-*epi-cis*-neoclausenamide (**11**) as a main isomer, which is identical with the result of **11**:**9** = 2:1 (mol ratio). The product **9** should be (3*R*,4*R*,5*S*,6*R*)-*cis*-neo-clausenamide.



Scheme 2. Reagents and conditions: (a) Al(*i*-OPr)$_3$/*i*-PrOH (90%); (b) 3,4-dihydro-2H-pyran/CH_2Cl_2 (85%); (c) L-selectride/THF; TsOH/EtOH, rt (two steps, 75%).