The Chemistry and Biological Activities of (-)Clausenamide

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PREFACE

Clausenamide is a novel compound isolated from *Clausena lansium* (*Lour*) Skeels, which is a species of fruit tree commonly seen in southern China. As the content contained in the plant is very low, many scientists try to synthesize it. After long term of effort, this compound has now been chemically synthesized by our institute and the production art has reached semi-industry scale that satisfies the demand for clinical trail and hereafter therapeutic use.

Clausenamide research received extensive attention, the reasons for this are as follows: it is the first chiral compound having anti-dementia effect; clausenamide contains 4 chiral centers having 16 enantiomers. According to the biogenetic hypothesis proposed by Prof. Huang, synthesis of 16 enantiomers and optical active clausenamide as well as asymmetric synthesis of (−) and (+) clausenamide were achieved.

For pharmacology, (−)clausenamide shows multi-target effects which benefit complicate diseases including Alzheimer's disease and other neuro- degenerative disorders. According to the new theory "synaptic loss=AD", a good anti-dementia drug must be able to improve synaptic plasticity and promote synaptogenesis. Fortunately, (−)clausenamide happened to be such compound. As proved in our study that (−)clausenamide increased synaptic plasticity both in efficacy and structure. For latter, (−)clausenamide increased synaptic density (synaptogenesis) and expression of growth associate protein (GAP-43) in the brain significantly.

This book is aimed to review the recent advances and new discoveries in chemistry, pharmacology and anti-dementia mechanism of (−)clausenamide. Hope that readers will enjoy and get benefit from many stories and new findings written in this book.

All invited authors are engaged in clausenamide research for many years and have rich theoretical knowledge and practice experience. They contribute a lot to develop (−)clausenamide to Ⅱ phase of clinical trail. I also appreciate their articles which are full of new ideas and new thoughts. Some new findings are especially interesting. In addition, we have cooperated with Norkong Scientific

Company for about 8 years, they give us many support including economic support. Let us express our many thanks. We also extend our thanks to Chemical Industry Press for their patience in organizing and editing this book which is my third book in English version.

Zhang Jun-tian
2013.12.15

CONTENTS

Overview: Recent Advances in the Study of (−)Clausenamide on Chemistry, Biological Activities and Mechanism of Action

Zhang Jun-tian
Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College

1 Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive decline of cognitive function, appearance of senile plagues, neurofibrillary tangle, and loss of neurons and synapses. Yet few drugs have been found to be effective and safety for treatment of AD.

In China, increasing emphasis has been laid in recent years on research of natural products and traditional Chinese medicine. About 150 new drugs have been developed with different pharmacological actions, more than 20 affecting the nervous system. (−)Clausenamide is the first chiral anti-dementia compound isolated from *Clausena lansium* which is an evergreen plant distributed mainly in Asia and Pacific region. The percolate of the leaves of *Clausena lansium* was described in the traditional Chinese medical classic for the treatment of edema with yellow ting of skin and the fruit of *Clausena lansium* is eatable quite popular in southern China. Therefore, we believed that it is safe and may have some therapeutic effects. This paper highlights the recent advance in chemistry, pharmacology, pharmacokinetics and new findings of (−)clausenamide in anti-dementia research.

2 Chemistry of Clausenamide

From aqueous extract of the leaves of *Clausena lansium*, our chemists isolated

seven amides, one of them, clausenamide is a γ-lactam with four asymmetric carbons (3*R**4*S** 5*S**6*R**) having 16 enantiomers. The pharmacological screen, synthesis and resolution of 16 enantiomers were completed in Prof. Huang's laboratory. The structures and configurations of 16 enantiomers are shown in Figure 1. Pharmacological study showed that racemic clausenamide had nootropic effect proved by behavior and electrophysiological experiments. Then, a question was advanced that which one, (+) or (−)clausenamide is bioactive stereoisomer? Bayer Company and our institute attempt to synthesize racemic clausenamide and resolve into (−) and (+)clausenamide. Hartwig of Bayer Co. reported a 10-step synthesis of racemic clausenamide by building the skeleton first through C=ON and C4/C5 linking followed by stepwise stereoselective introduction of substituents. The total overall yield was 5.7%-6.4%. Huang Liang of our institute reported a biomimetic synthetic route based upon the biogenetic relationship perceived between the seven amides isolated. The total synthesis was reduced to 5-step and

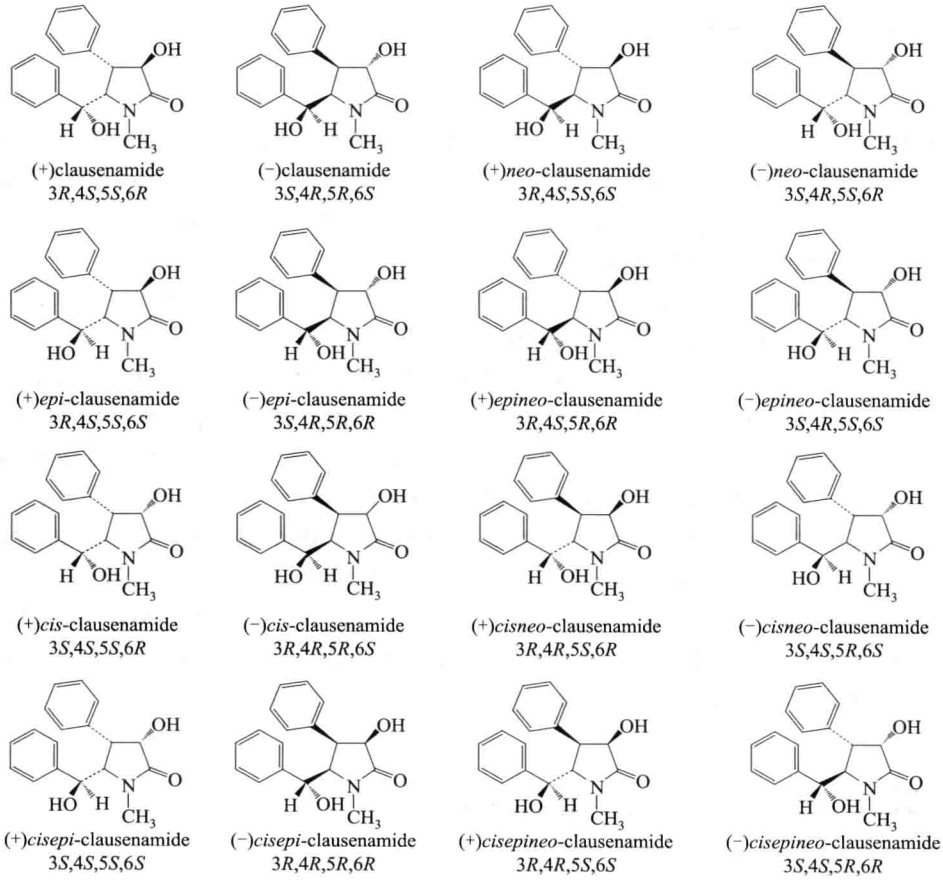

Figure 1 The structures and configurations of 16 enantiomers

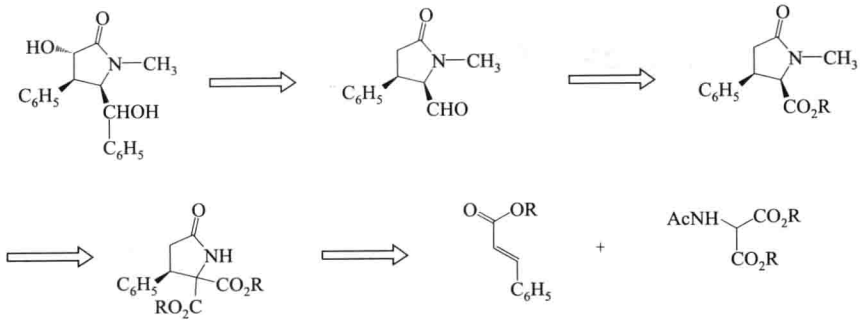

Figure 2 Hartwig's retrosynthesis scheme of recemic clausenamide

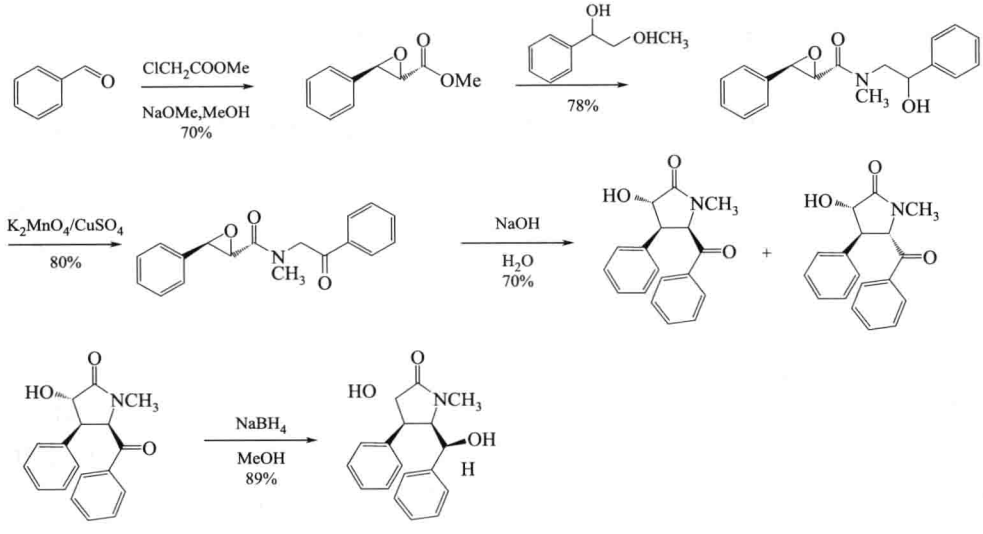

Figure 3 Huang Liang's synthesis scheme of recemic clausenamide

the total overall yield was increased to 15%-17%. Two synthetic schemes are shown in Figure 2 and Figure 3 respectively[1].

For ensuring (−)clausenamide to be effective, safe, stable and reliable in clinical use, methods for chiral separation, purity and quality control were developed, which include rapid and reliable method for enantiomers separation by capillary electrophoresis and chiral column. Optical purity was determined by high performance liquid chromatography (HPLC). Impurity substance was detected by HPLC method. According the methods as mentioned above, results showed that complete separation of (−)clausenamide and (+)clausenamide was achieved under a certain chromatography condition. The optical purity reached 99%. The impurity substance was identified as (−)*epi*-clausenamide with an approximate content of 0.3%.

3 Pharmacological Effects

3.1 Effect on memory impairments

10 animal models include APP transgenic mice, aged rats (24-27 month), diabetes mice, cerebral ischemia-reperfusion rats, A β and other 5 chemicals induced memory impairments models. Oral administration of (−)clausenamide at the dose of 5-10 mg/kg improved learning and memory significantly[2,3]. Its effective dosage was equal to that of donepezil and much more potent than piracetam.

3.2 Effect on long term potentiation of synaptic transmission

Many evidence supported the idea that long lasting activity-dependent plasticity of synaptic transmission is of fundamental importance in the development of neural circuity and storage of information. As a typical form of this plasticity, long-term potentiation of synaptic transmission (LTP) in the hippocampus is the neuromolecular basis of learning and memory. It is also a good experimental model for investigating cognitive function at cellular and synaptic level. Surgery and electrophysiological recordings were conducted as described in our many published papers and books[4-7].

By comparing (−) and (+)clausenamide, results are obtained as follows: (−)clausenamide at concentration of 10^{-5}-10^{-7}/mol increased basic synaptic transmission and amplitude of LTP induced by high frequency stimulation in anesthetized or freely moving rats at a concentration-dependent and time-dependent manner (Figure 4,5). (+)Clausenamide showed no effect on LTP. There are two forms of LTP, one is dependent on the activation of NMDA receptor, and the other is mediated by entry of calcium via VDCC. It was demonstrated that (−)clausenamide induced LTP was inhibited by nimodipine, a specific antagonist of L-type VDCC. But AVP, a specific antagonist of the NMDA glutamate receptor had no inhibitory effect on (−)clausenamide induced LTP, indicating that VDCC rather than NMDA receptors are required for an effect of (−)clausenamide to take place. We have also screened the 16 enantiomers of clausenamide by testing their effects on the synaptic transmission in the hippocampus and found 4 out of them had potentiation effects. Nimodipine, piracetam and donepezil showed no effect on LTP[8].

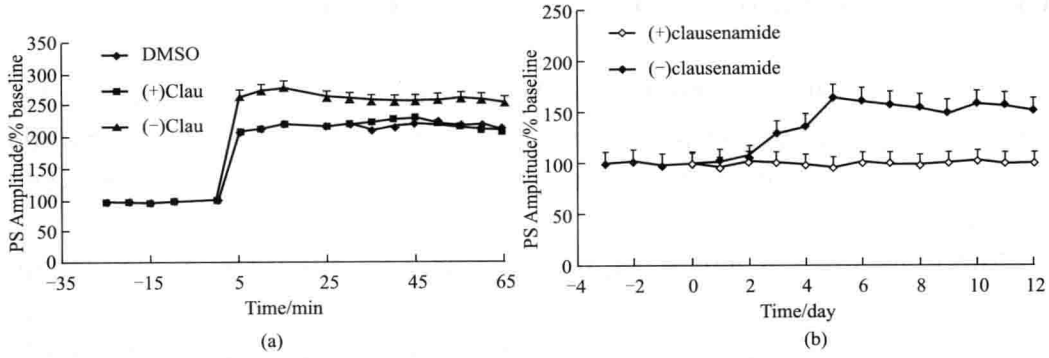

Figure 4 Effect of (−) or (+)clausenamide on LTP in the dentate gyrus (DG) of anesthetized (a) and freely moving (b) rats induced by high Frequency stimuli

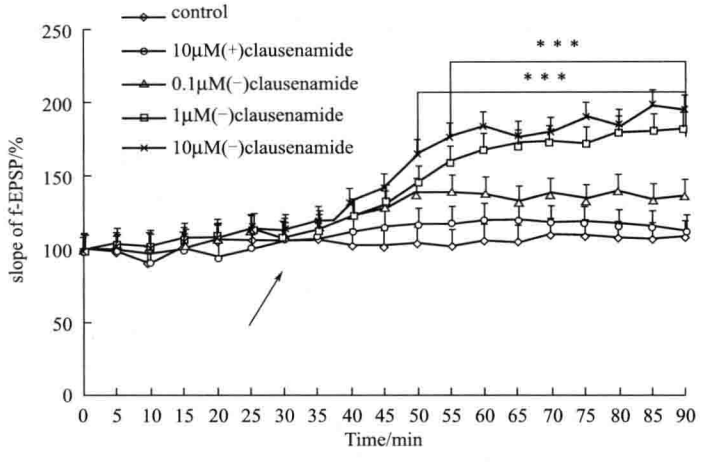

Figure 5 Effect of (−)clausenamide on LTP with different doses. ***P<0.01 vs control animals

4 Nootropic Mechanism of (−)Clausenamide

As mentioned above, (−)clausenamide improved learning and memory in 10 models of memory impairment and increased synaptic plasticity in anesthetized or freely moving rats. For elucidation of nootropic mechanism of (−)clausenamide, many aspects of researches were conducted and found that moderate increase of $[Ca^{2+}]_i$, stimulation of central cholinergic neurons and enhancement of synaptogenesis are important factors in inducing nootropic effects of (−)clausenamide.

4.1 (−)Clausenamide increased $[Ca^{2+}]_i$ at moderate level

Calcium ions are ubiquitous intracellular second messengers that acts as key regulators of innumerable processes, such as the control of cell growth and differentiation, maintenance of cytoskeletal integrate, learning, memory and synaptic activity.

There are many machineries, for example, Ca^{2+} influx and accumulation, Ca^{2+} clearance, cytoplasmic Ca^{2+} buffering, controlling the temporal and special distribution of Ca^{2+} in the neurons. These mechanisms maintain $[Ca^{2+}]_i$ at extremely low level, so that a relatively small or localized change in $[Ca^{2+}]_i$ can be used by the cell as a signal to trigger a distinct physiological process. For a drug, control elevation of $[Ca^{2+}]_i$ to a moderate level is important that might activate neuroprotective pathway. The well known ability of KCl to promote survival of neurons in cell culture may be explained by the moderate elevation of $[Ca^{2+}]_i$ induced by KCl. Several neurotrophic factors such as NGF and BDNF are also known to induce small elevations of $[Ca^{2+}]_i$ in neurons in which they promote long term survival and protect against excitability toxicity insults[9, 10].

In our investigation on (−)clausenamide induced Ca^{2+} signaling in primary culture of cortical neurons by using laser confocal microscope, it was demonstrated that (−)clausenamide induced only small $[Ca^{2+}]_i$ change after application of 20 mM Mg^{2+} extracellularly[11]. In this condition, (−)clausenamide could improve learning and memory, increase synaptic plasticity in both efficacy and structure, increase acetylcholine release and activate nootropic signal transduction pathway and so on.

4.2 Stimulation of central cholinergic neurons

The deficits in cognition and memory storage observed in age-associated memory impairment (AAMI) and AD are associated with a deficiency of the central cholinergic system. Thus, cholinergic stimulants may be therapeutically used in the treatment of AD patients. It was demonstrated that single administration of (−)clausenamide (10, 20, 50 ng/kg) significantly ameliorated the reduction of acetylcholine induced by anisodine (a M-cholinergic receptor antagonist, 10 mg/kg i.p.) in a dose-dependent manner. But (+)clausenamide

had no effect on the decrease in similar doses. Further study showed that (−)clausenamide increased choline acetyltransferase (chAT) activity in cortex, hippocampus and striatum significantly. As we know, chAT is key enzyme for synthesis of Acetylcholine (Ach). Obviously, the protective effect of (−)clausenamide against Ach reduction by anisodine is the result of increasing Ach synthesis and release[12,13]. Furthermore, (−)clausenamide exhibited neurotrophic action, i.e. stimulated proliferation of cholinergic neurons, supported survival and neurite outgrowth of cholinergic neurons, (−)clausenamide and neural growth factor (NGF) had the similar neurotrophic action but they were mediated by different mechanisms.

4.3 (−)Clausenamide increased synaptogenesis

Basic and clinical study in recent years showed that senile plaque and neurofibrillary tangle are not related to the degree of cognitive decline. Synaptic loss occurs in all kinds of dementias and other neurodegenerative diseases. Synaptic loss suspended signal deliver and transduction, damaged the receptors, ion channels and proteins contained in front and posterior synaptic membrane, leading to the neuropsychological dysfunction, memory deficit and dementia. Therefore synaptic loss was considered as main cause of dementia, even some scientists proposed that "synaptic loss=AD". For this reason, the new strategy for treatment of AD is limiting synaptic loss and increasing synaptogenesis[14, 15].

In order to ascertain the basis of morphology of nootropic action of (−)clausenamide, the effect of (−)clausenamide on brain development was studied. (−)Clausenamide at dosage of 5 and 10 mg/kg by gavage to mice once a day for 4 weeks, facilitated learning and memory acquisition in step-down and step-through tests and increased thickness of cerebral cortex and synapses density significantly in the dentate cells over pyramidal cells in hippocampal region using quantitative technique of synapses analysis[16]. In another study, (−)clausenamide increased mossy fiber sprouting and expression of growth-associate protein (GAP-43). Synapses are the essential structure in the CNS through which signals are transmitted, processed and integrated among neurons. (−)Clausenamide increased synaptic plasticity in both efficacy and structure which provide morphological and physiological evidences to support nootropic effects of (−)clausenamide.

5 Activation of Learning and Memory Signal Transduction Induced by (−)Clausenamide [17, 18]

Study on the learning and memory signal transduction pathway is essential for elucidation of nootropic mechanism. This study can provide us much information, for example, finding of target receptor, gene encoding key proteins, crosstalk between different signaling cascades, explanation for long term memory formation at molecular level, and so on. There are two kinds of nootropic signaling pathway induced by (−)clausenamide.

5.1 PLC-PKC mediated signaling pathway

Studies showed that (−)clausenamide had no specific binding to NMDA receptors, but it could increase NMDA receptors density and glutamate release. Furthermore, (−)clausenamide was proved as potassium channel antagonist which blocked potassium outflux, induced membrane depolarization. The latter effect could remove Mg^{2+} around NMDA receptors, thus glutamate binding to NMDA receptor tightly. After ligand induced receptor activation, the signal transduction was initiated beginning with the activation of PLC via G protein. PLC hydrolyzes PIP2 into IP3 and DAG, the latter activated PKC which in turn phosphorylated a series of protein kinase including CREB in nuclei. Phosphorylated CREB transcripted and expressed memory related genes. In this study, the gene products were zif/268 and BDNF.

5.2 CaMK Ⅱ-ERK mediated signaling pathway

Britain in 1997 reported that MRK played a role in modulating synaptic plasticity. ERK was regulated by several protein kinases in upstream, such as PKA, PKC, CaMK Ⅱ, CaMKⅣ, etc. Among them, CaMK Ⅱ is the main protein contained in the postsynaptic density (PSD), its amount reached 20%-40%. (−)Clausenamide (10^{-5} mol/L) increased population spike of hippocampal dentate gyrus, the peaks were at 5 min and 30 min, separately. (−)Clausenamide also promoted the transient enhancement of upstream Ca^{2+} and activated CaMK Ⅱ at 2nd min *in vitro*. The downstream protein, CREB was phosphorylated at 9th min. Pretreated with KN93, a CaMK Ⅱ antagonist and PD98059, a ERK inhibitor, could partially