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Asymmetric synthesis and biological activities of natural product (+)-balasubramide and its derivatives

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Asymmetric synthesis and biological activities of natural product (+)-balasubramide and its derivatives

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ABSTRACT

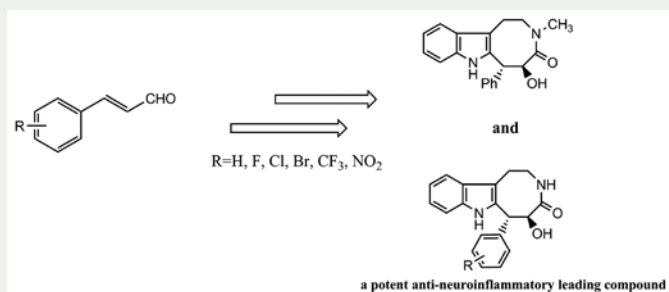
The natural product (+)-balasubramide (**3j**) and its derivatives (**3a–3i**) were synthesized using a two-step asymmetric synthesis, and the biological activities of **3a–3j** were determined *in vitro*. Methyl (2S,3R)-(+)-3-phenyloxirane-2-carboxylate (**1h**), the asymmetric synthesis of which was described in a previous paper, was selected as the starting material. Compounds **3a–3j** were evaluated for their neuroprotective, antioxidative, and anti-neuroinflammatory effects. (+)-Balasubramide and its derivatives with different electronegative groups in the 6-phenyl ring produced little neuroprotection and antioxidation, but induced potent anti-neuroinflammatory effects in BV-2 microglial cells (with the exception of **3g**). Compound **3c**, with a trifluoromethyl group in its 6-phenyl ring, was a particularly potent anti-neuroinflammatory agent. These results demonstrated that the electronegativity of the 6-phenyl ring of (+)-balasubramide is an important determinant of its inhibitory effect on neuroinflammation. More electronegative substituents result in more potent anti-neuroinflammatory effects. Moreover, cytotoxicity assays indicated no significant effects of the tested compounds.

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1. Introduction

(+)-Balasubramide is an eight-membered lactam compound with an absolute configuration of 5S,6R (Juárez-Calderón et al. 2013) that is extracted from the leaves of the Sri Lankan plant *Clausena indica* (Riemer et al. 1997) along with its biosynthetic precursor, (+)-prebalamide (Figure 1). Studies have shown that five-membered lactam compounds extracted from *C. indica* have extensive pharmacological uses. For example, clausenamide (Figure 1) produces hepatoprotective (Yang et al. 1987) and neuroprotective effects (Xue et al. 2008), inhibits apoptosis (Yao et al. 2001) and lipid peroxidation (Lin et al. 1992), and acts as an oxygen free radical scavenger (Jiang & Zhang 1998). However, eight-membered lactam compounds are rarely reported to have pharmacological activity. Total synthesis of (+)-balasubramide is difficult because of its eight-membered lactam ring and two chiral centers. Thus, the biological activities and preliminary structure-activity relationships (SARs) of related compounds have not yet been reported.

At present, although there are three synthetic routes regarding balasubramide (Johansen et al. 2007; Yang et al. 2007; Zheng et al. 2009), (+)-balasubramide, a chiral natural product, only could be obtained by one method (Johansen et al. 2007) which was synthesized by chiral resolution and the overall yield was only 17%.

We investigated an additional method of total synthesis of (+)-balasubramide to allow us to study its bioactivity and to perform a preliminary analysis of the SARs of (+)-balasubramide and derivative compounds. We previously reported a short asymmetric synthetic route of methyl (2S,3R)-(+)-3-phenyloxirane-2-carboxylate (**1h**) (Xuan et al. 2013). In this study, we used compound **1h** as the starting material to synthesize a series of (+)-balasubramide derivatives in order to explore their biological activities, including neuroprotection, anti-neuroinflammation, cytotoxic effects.

In recent years, as a catalytic method with the same status of enzyme catalysis and metal catalysis, asymmetric catalysis has been an important tool for building chiral molecular scaffold owing to the high efficiency and selectivity (Shi et al. 2015; Wang et al. 2015; Woźniak et al. 2015; Zhao et al. 2015). Our group initially reported a model reaction of cinnamaldehyde with organocatalyst, a diphenyl prolinol TES ether, to synthesize methyl (2S,3R)-(+)-3-phenyloxirane-2-carboxylate (**1h**) via a one-pot reaction with 73% yield and 95% enantioselectivity (Xuan et al. 2013). In this report, we used compound **1h** to prepare the linear amide **2j** by amine-ester interchange with N-methyltryptamine, followed by intramolecular cyclization using ytterbium(III) triflate ($\text{Yb}(\text{CF}_3\text{SO}_3)_3$) as a catalyst, resulting in (+)-balasubramide (**3j**) (Figure 2).

In order to introduce substituents with varying electronegativity into the 6-phenyl ring, cinnamaldehydes with different substitutions were employed to prepare (+)-balasubramide

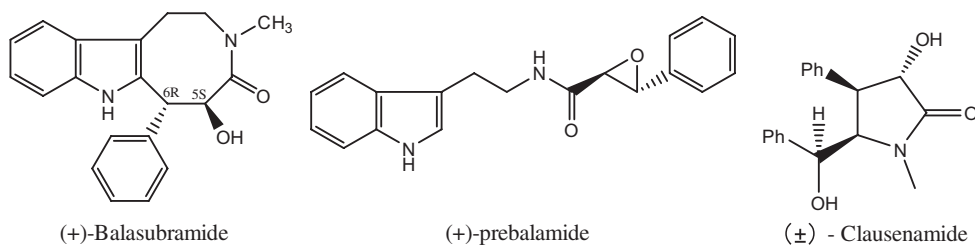


Figure 1. Structures of (+)-balasubramide, (+)-prebalamide and (±)-clausenamide.

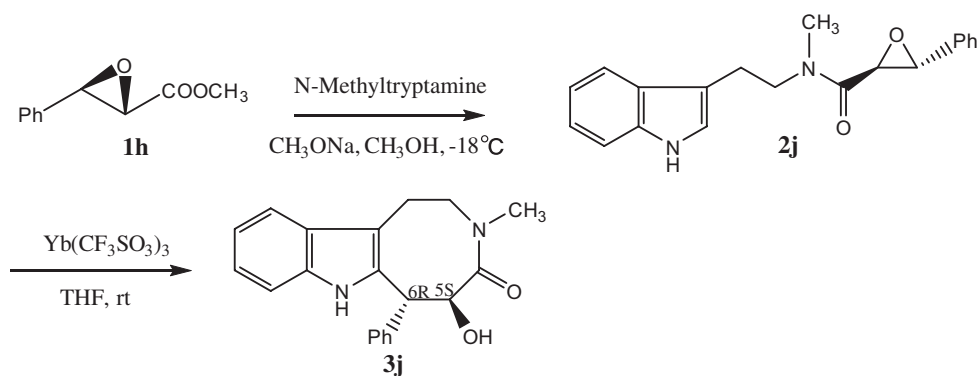


Figure 2. Total asymmetric synthesis of natural product (+)-balasubramide.

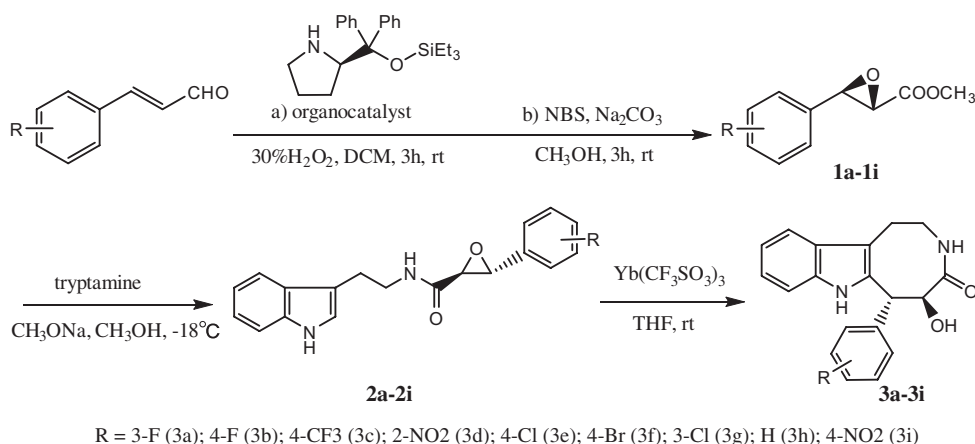


Figure 3. Asymmetric synthesis of natural product (+)-balasubramide derivatives.

analogues using organocatalyst followed by asymmetric epoxidation, oxidative esterification, an amine–ester interchange reaction, and intramolecular cyclization (Figure 3).

2. Results and discussion

2.1. Chemistry

In the conversion of methyl (2S,3R)-(+)-3-phenyloxirane-2-carboxylate into the linear amide **2j**, an amine–ester interchange reaction with N-methyltryptamine was carried out at room temperature in CH_3OH for 10 h, but resulted in only a 25% yield. Therefore, we investigated reaction conditions using temperatures less than -18°C and added catalytic basic additives such as NaHCO_3 , K_2CO_3 , Na_2CO_3 , *t*-BuOK, and CH_3ONa . When CH_3ONa was used as the additive, the process resulted in a good yield of compound **2j**. Despite our best efforts, the resultant amide **2j** could not be separated from at least one other unidentified compound, we speculated that the compound in column chromatography may be decomposed due to its instability, but the mixture could once again be subjected to next step without using purification.

The intramolecular cyclization of compound **2j** was found to be greatly influenced by the use of Lewis acid activities. When AlCl_3 , FeCl_3 , or CuCl were used, compound **3j** was not

Table 1. Yield and enantiomeric excess of (+)-balasubramide derivatives.

Entry ^a	R	Product	Yield (%)	ee ^b (%)
1	3-F	3a	76	97
2	4-F	3b	70	>99
3	4-CF ₃	3c	65	96
4	2-NO ₂	3d	60	>99
5	4-Cl	3e	75	>99
6	4-Br	3f	72	98
7	3-Cl	3g	72	98
8	H	3h	75	>99
9	4-NO ₂	3i	62	97

^aUnless otherwise stated, all reactions were performed with **2** (2 mmol) and Yb(CF₃SO₃)₃ (0.4 mmol) in THF.

^bThe enantiomeric excess was determined by high-performance liquid chromatography (Daicel Chiralcel AD-H column).

obtained, but when LaCl₃, *p*-TSA, or Yb(CF₃SO₃)₃ was used, compound **3j** was obtained with varying yields. Yb(CF₃SO₃)₃ produced the best yield with >99% enantioselectivity. Although the solvent had no effect on enantioselectivity in the last reaction, a polar solvent was found to increase the yield, as evidenced by the improvement produced by the addition of tetrahydrofuran.

Toward the synthesis of (+)-balasubramide analogues, compounds **1a–1i** were asymmetrically synthesized via a one-pot reaction with a variety of α,β-unsaturated aldehydes in organocatalyst. When compounds **1a–1i** were treated with tryptamine instead of N-methyltryptamine, intermediates **2a–2i** were obtained in good yields after purification. In the intramolecular cyclization of **2a–2i**, (+)-balasubramide analogues **3a–3i** were successfully produced using Yb(CF₃SO₃)₃ as the catalyst (Table 1).

2.2. In vitro biological evaluation

The (+)-balasubramide (**3j**) and its derivatives **3a–3i** were evaluated for their *in vitro* biological activities, including neuroprotective, antioxidative and anti-neuroinflammatory effects. Our results in Tables S1–3 showed that compounds **3a–3j** didn't exhibit the significant neuroprotective effects in primary neurons against glutamate or nutrient deprivation and antioxidative effects in PC12 neuronal cells against H₂O₂ incubation.

All compounds were further subjected to bioassay to test their *in vitro* anti-neuroinflammatory effects against LPS-induced pro-inflammatory cytokine TNFα expression in microglial cells. The results are shown in Figure 4 and Table S4. With the exception of

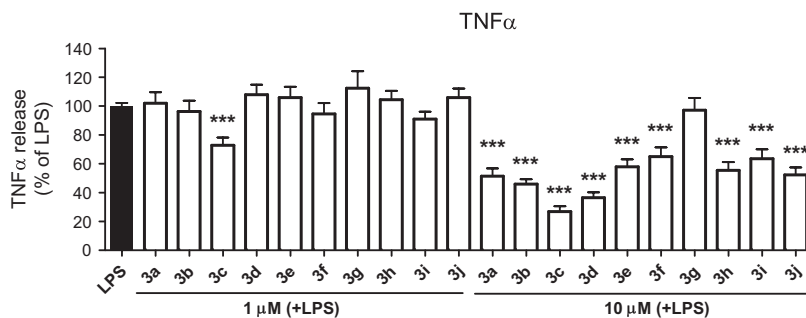


Figure 4. Inhibitory effects of target compounds on LPS-induced TNFα production in BV-2 microglial cells. ****p* < 0.001 compared to LPS group.

compound **3g** (3-Cl-substituted), the target compounds at 10 μ M markedly inhibited LPS-induced pro-inflammatory cytokine TNF α release in BV-2 microglial cells. Compound **3c**, with a strongly electronegative substituent (4-CF₃), at 1 and 10 μ M, exhibited significant inhibition of LPS-induced TNF α release in a dose-dependent manner. In addition, compound **3c** dose-dependently inhibited LPS-induced TNF α gene expression (Figure S1). This indicated that a strongly electronegative substituent in the 6-phenyl ring might be required for the anti-neuroinflammatory effects of (+)-balasubramide derivatives. Meanwhile, in the MTT assay for cytotoxicity of these compounds in BV-2 microglial cells (Figure S2), (+)-balasubramide and its derivatives **3a–3j** did not show cytotoxic effects.

3. Conclusions

In this report, we describe a convenient and efficient method for the synthesis of natural (+)-balasubramide with a 44% overall yield and excellent enantioselectivity (>99%). Substituents with varying electronegativity were introduced into the 6-phenyl ring of (+)-balasubramide and the resulting target compounds **3a–3j** were evaluated for neuroprotective, antioxidative, and anti-neuroinflammatory effects. Our results indicated that natural (+)-balasubramide and its derivatives had little neuroprotective and antioxidative effects, but significantly inhibited neuroinflammation (with the exception of **3g**). These results show that electronegativity in the 6-phenyl ring of (+)-balasubramide and its derivatives is an important determinant of their inhibitory effects on neuroinflammation; substituents with stronger electronegativity produced more potent anti-neuroinflammatory effects. Our preliminary SAR study provided information that could facilitate the design of novel anti-neuroinflammatory drug candidates or leads. These molecular structures may be worthy of future study with the aim of developing new anti-neuroinflammatory agents. Further studies to improve anti-neuroinflammatory activity and clarify the molecular mechanism of these compounds are in progress.

Supplementary material

All experimental sections relating to this article are available online, alongside Tables S1–4 and Figures S1–43.

Disclosure statement

No potential conflict of interest were reported by the authors.

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