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The efficient synthesis and biological evaluation of justicidin B

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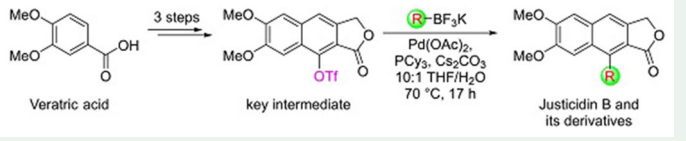
ABSTRACT

A facile new synthetic method for the preparation of a Type-A 1-arylnaphthalene lactone skeleton was developed and used to synthesise justicidin B and several derivatives. Key synthesis steps included Hauser–Kraus annulation of a phthalide intermediate and Suzuki–Miyaura cross coupling between a triflated naphthalene lactone intermediate and various potassium organotrifluoroborates. With two exceptions, the derivatives showed significant inhibitory effect on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse macrophages. Moreover, several compounds, including justicidin B, had marked cytotoxicity towards six human tumour cell lines.



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Justicidin B; total synthesis; natural products; annulation; cross coupling

1. Introduction

Naturally occurring 1-arylnaphthalene lactones are a plant-derived subclass of lignans (Ward 1995; Man et al. 2012; Schmidt et al. 2012; Tadross and Stoltz 2012; Jullian-Pawlicki et al. 2015; Hemmati and Seradj 2016; Teponno et al. 2016; Li et al. 2020). They possess anti-tumour (Yu et al. 2010; Gui et al. 2011; Shi et al. 2012), anti-platelet (Chen et al. 1996; Weng et al. 2004), and anti-bacterial (Kawazoe et al. 2001) properties as well as phosphodiesterase (Ukita et al. 1999), 5-lipooxygenase (Delorme et al. 1996), and HIV reverse transcriptase inhibitory functions (Chang et al. 1995; Lee et al. 1996). The 1-arylnaphthalene lactone structure may be used as a template to develop lead compounds for novel therapeutics derived from natural products (Nandy et al. 2009; Kumar and Waldmann 2009a, 2009b; Zhao et al. 2021). 1-Arylnaphthalene lactone lignans are classified as Type-A, Type-B, Type-C and Type-D (Scheme 1). The Type-A 1-

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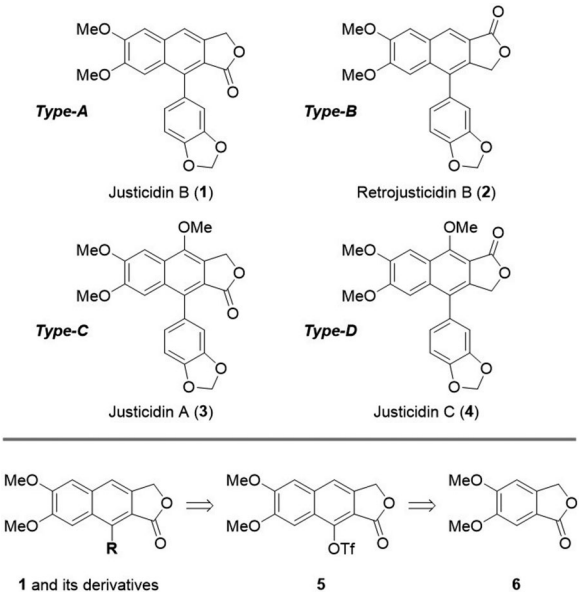
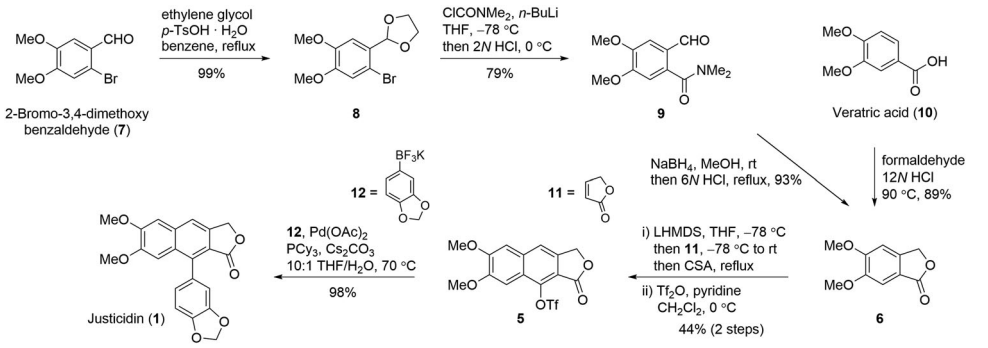


Scheme 1. Typical structure of 1-arylnaphthalene lactone lignans and the retrosynthesis of Type-A justicidin B (**1**).

arylnaphthalene lactone, justicidin B (**1**), is the main active component of *Phyllanthus piscatorum*, exhibiting anti-fungal and anti-proliferative properties (Gertsch et al. 2003; Rao et al. 2006; Luo et al. 2014; Ai et al. 2020).

Since the chemical structure was first reported in the 1960s, justicidin B (**1**) has been efficiently prepared using several synthetic strategies: naphthalene construction from a seven membered ring system with ether linkage (Munakata et al. 1967), cyclisation of a cinnamyl phenylpropiolate ester (Stevenson and Block 1971), Michael initiated ring closure (Kamal et al. 1994), conjugate addition–aldol reaction of cyanohydrin (Ogiku et al. 1995), regio-controlled benzannulation of *gem*-dichlorocyclopropane (Nishii et al. 2005) and continuous photoflow intramolecular Diels–Alder reaction of 3-phenylprop-2-yn-1-yl 3-phenylpropiolate (Ge et al. 2021). Despite many synthetic studies on justicidin B (**1**), the synthetic strategies of the common intermediate **5** from Hayat et al. (2015) and Mal and Jana (2016) are most suitable for Type-A derivative synthesis. However, to the best of our knowledge, this is the first study to report the chemical derivatisation of **5** for biological studies. Herein, we report a short and facile new synthetic method for the preparation of justicidin B (**1**) derivatives and the biological evaluation of anti-inflammatory and cytotoxic activities.

Our synthesis and derivatisation strategy were based on the efficient construction of the 1-arylnaphthalene lactone framework. The retrosynthetic analysis of 1-arylnaphthalene lactone structure predicted the formation of a triflated naphthalene lactone intermediate and corresponding potassium organotrifluoroborates. Owing to the efficient synthesis of the natural 1-arylnaphthalene lactone, this compound could contribute to structure–activity relationship (SAR) compound libraries to investigate the anti-inflammatory and anti-tumour activities of these compounds.



Scheme 2. Synthesis of justicidin (**1**) from 2-bromo-3,4-dimethoxybenzaldehyde (**7**) or veratric acid (**10**).

2. Research and discussion

2.1. Chemistry

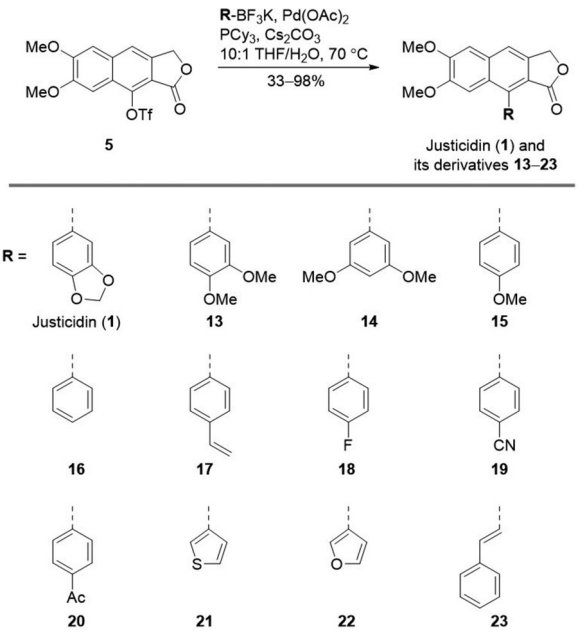
Justicidin (**1**) synthesis is described in Scheme 2. First, the synthesis of phthalide **6**. *N*,*N*-Dimethylamide **9** was prepared from commercially available 2-bromo-3,4-dimethoxybenzaldehyde (**7**) using our previous report on the synthesis of Type-C and Type-D 1-arylnaphthalene lactones (Kim et al. 2017). Then, sodium borohydride reduction of the formyl group was followed by acidic treatment with hydroxylmethyl, generating phthalide **6**. An alternative approach to phthalide **6** via lactonisation (Singh and Khade 2011) of veratric acid (**10**) using formaldehyde under acidic conditions was also generated.

Next, a Hauser–Kraus annulation between phthalide **6** using γ-crotonolactone (**11**) was performed in the presence of lithium bis(trimethylsilyl)amide (LHMDS) in tetrahydrofuran at −78 °C, followed by aromatisation using camphorsulfonic acid in one pot to generate an unstable naphthol intermediate. The subsequent formation of the triflate group using trifluoromethanesulfonic anhydride (Tf_2O) and pyridine in CH_2Cl_2, yielded the key intermediate **5** for the synthesis of justicidin B (**1**) and derivatives.

Finally, the Suzuki–Miyaura cross-coupling reaction of **5** was successfully performed using potassium 1,3-benzodioxol-5-yltrifluoroborate (**12**) in four steps to yield 36.4% from the veratric acid (**10**). The other derivatives, i.e., **13**–**23** were similarly synthesised (Scheme 3) from the common intermediate triflate **5**. The structures of the final compounds **1** and **13**–**23** were identified from spectral data (^1H & ^{13}C nuclear magnetic resonance and mass spectrometry). Analysis data of all the synthesized compounds are available in Supplementary Material.

2.2. Bioassays

Before investigating the inhibitory effects of the synthesized compounds on lipopolysaccharide (LPS)-induced nitric oxide (NO) production, their cytotoxicity was evaluated in RAW264.7 cells. The compounds, except for **1** (IC_{50} = 10.1 μM), **15** (IC_{50} = 33.3 μM) and **23** (IC_{50} = 25.4 μM), did not affect cell viability at concentrations up to 40 μM (Table S1 in Supplemental Material). The following experiment was performed in the



Scheme 3. Synthesis of justicidin (**1**) and derivatives.

concentration range of those without cytotoxicity. The derivatives **13**, **14**, **16**–**22** significantly inhibited NO production in a concentration-dependent manner (Figure S33 in Supplemental Material).

Next, cytotoxicity of justicidin B (**1**) and derivatives (**13**–**23**) was evaluated in six human cancer cell lines: AGS (gastric), A549 (lung), HCT116 (colon), PC-3 (prostate), HepG2 (liver) and MDA-MB-231 (breast). Table S2 in Supplemental Material reveals that the compounds **1**, **15**, **17** and **23** exerted cytotoxic effect on the cancer cells. In particular, the derivatives **15**, **17** and **23** were more cytotoxic against PC-3 and HepG2 cells than VP-16, which is used as a drug for cancer, although justicidin B (**1**) was the most effective.

3. Conclusions

We demonstrated a facile new synthetic preparative approach for a naphthalene lactone skeleton using Hauser–Kraus annulation of phthalide **6**. Moreover, the Suzuki–Miyaura cross-coupling reaction of triflated naphthalene lactone intermediate **5** with the corresponding potassium organotrifluoroborates generated justicidin B (**1**) and derivative (**13**–**23**) formation. Additionally, compounds were screened for their inhibitory effects on the NO production in LPS-induced RAW 264.7 and on cytotoxicity in six human tumour cell lines. The derivatives **13**, **14**, **16 − 22** had the most potent inhibitory activity towards NO production in LPS-stimulated RAW267.4 cells. In addition, the compounds **1**, **15**, **17** and **23** exerted cytotoxic effect on various cancer cell lines. Further studies on structure optimisation and biological activity mechanisms are underway in our laboratory.

Disclosure statement

No potential conflict of interest was reported by the authors.

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