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Synthesis and biological evaluation of piperlongumine derivatives as potent anti-inflammatory agents

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ABSTRACT

Piperlongumine (PL) and its derivatives were synthesized by the direct reaction between acid chloride of 3,4,5-trimethoxycinnamic acid and various amides/lactams. Later their anti-inflammatory effects were evaluated in lipopolysaccharide (LPS)-induced RAW-264.7 macrophages. Of the piperlogs prepared in this study, the maximum (91%) inhibitory activity was observed with PL (IC_{50} = 3 μM) but showed cytotoxicity whereas compound **3** (IC_{50} = 6 μM) which possess α,β-unsaturated γ-butyrolactam moiety offered good level (65%) of activity with no cytotoxicity. This study revealed that amide/lactam moiety connected to cinnamoyl group with minimum 3 carbon chain length and α,β-unsaturation is fruitful to show potent anti-inflammatory activity.

Inflammation is the body's natural response to infection, disease and tissue damage and is an integral part of the immune response.[1] Symptoms of inflammation include swelling, redness of the area, pain, and sometimes loss of movement or function.[2] Inflammation is either acute or chronic and under specific circumstances the former could turn into the later one which will become a causative factor in the pathogenesis. Various molecules involved in the inflammatory response viz. prostaglandins (PG), leukotrienes (LT) and nitric oxide (NO) etc. PGs are produced by cyclooxeganases (COX, which mainly having two forms COX-1 and COX-2) and LTs are produced by lipoxygenases (LOX). Nitric Oxide (NO) is a short-lived, lipophilic, free-radical species generated from L-arginine by nitric oxide synthases (NOS). Among the NOS, inducible isoform of nitric oxide synthase (iNOS) is chiefly responsible for the large amounts of NO production in inflammatory lesions. NO is involved physiologically in vasorelaxation, neurotransmission, inhibition of platelet aggregation, immune defense, and intracellular signaling. However, NO reacts with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$), which is a powerful oxidant. NO bioactivity is related to the production of many reactive intermediates, but many of these reactive nitrogen species (RNS) are capable of damaging DNA or hindering DNA repair.[3] Therefore, control of the excess NO production by inhibition of iNOS may exert anti-inflammatory effects.

Non-steroidal anti-inflammatory drugs (NSAIDs) usage is common practice in the therapeutic approach to alleviate the symptoms associated with both acute and chronic inflammatory diseases. The activity of NSAIDs is most likely mediated through their ability to inhibit COX, LOX and iNOS. Although NSAIDs are effective, there are known risks. They can inhibit the housekeeping enzyme COX-1 along with COX-2. The problem is that COX-1 inhibition would 'switch off' functions like the repair and maintenance of stomach lining, which results in varying degrees of gastric health issues, the most significant one is gastrointestinal bleeding. Therefore, continuous efforts are on to search for more potent and selective anti-inflammatory drugs with minimal side-effects.

Piperlongumine (PL) **1** is a biologically active dihydropyridone alkaloid isolated from the various parts of long pepper, *Piper longum L.* along with other constituents viz. piperine, sylvatin, sesamin and diaeudesmin piperlongumine.[4] Long pepper is used to treat cough, respiratory infections, stomach pains and other diseases in Indian Ayurvedic medicine.[5] The chemical structure of PL is having 3,4,5-trimethoxycinnamoyl moiety, attached to dihydropyridone and is well characterized (Fig. 1). PL was previously reported to possess antibacterial and insecticidal properties.[6] In addition PL also showed several noteworthy activities which include anti-cancer,[7] anti-platelet aggregation,[8] anti-fungal[9] and anti-diabetic.[10] In recent studies, PL as a potential anti-cancer compound demonstrated that it would selectively kill 13 different human solid tumor cells (including melanoma, bladder, breast and lung cancer) without affecting their normal cells.[11]

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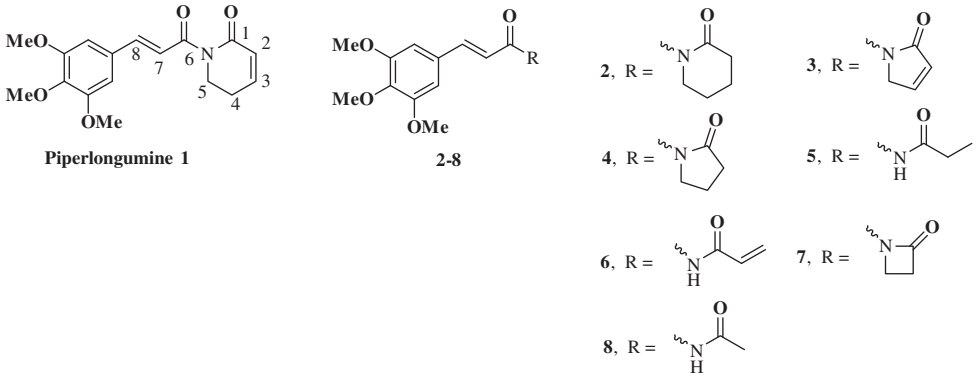
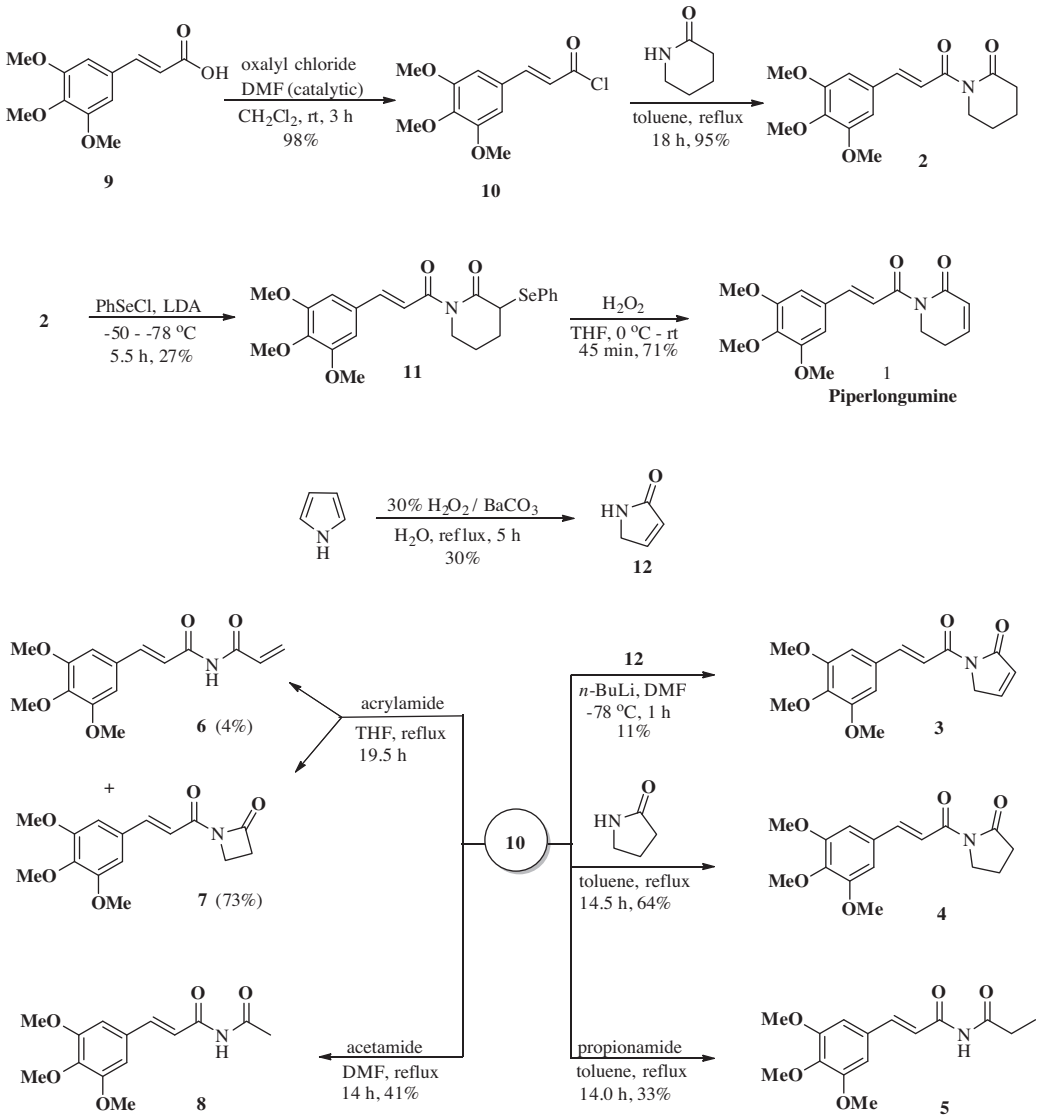


Figure 1. Structure of piperlongumine (**1**) and its derivatives (**2**–**8**).



Scheme 1. Synthesis of PL and its derivatives.

In continuation of our work[12] on the synthesis of bioactive natural products and their analogues, herein we wish to describe the synthesis and anti-inflammatory activity evaluation of PL and its derivatives.

Our approach for the synthesis of PL and its derivatives is outlined in Scheme 1. The synthesis was initiated by the conversion of 3,4,5-trimethoxycinnamic acid (**9**) to it acid chloride (**10**) (Scheme 1). Treatment of compound **9** with oxalyl chloride and catalytic amount of DMF afforded the acid chloride **10** in 98% yield. Keeping **10** in hand, we first attempted the PL derivative **2** synthesis by the direct reaction between **10** and piperidin-2-one and the product was formed in 95% yield. Treatment of compound **2** with

Table 1
Anti-inflammatory activities of piperlogs **1–8**

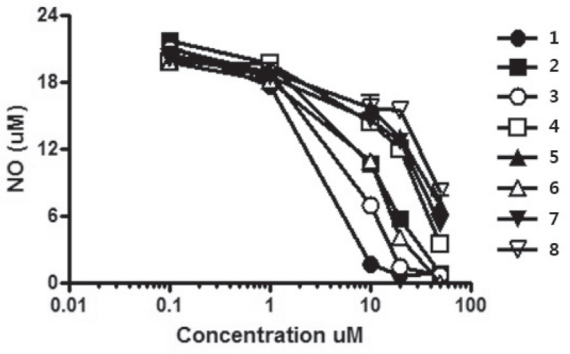
Compound	NO production (% inhibition)	
	1 μM	10 μM
Medium (MED)	2 ± 1 (98)***	2 ± 1 (98)***
1	89 ± 2 (11)***	9 ± 1 (91)***
2	99 ± 2 (1)	54 ± 11 (46)***
3	95 (5)**	35 ± 1 (65)***
4	99 ± 1 (1)	72 ± 1 (28)***
5	101 ± 3 (−1)	84 ± 6 (16)
6	97 ± 2 (3)	59 ± 1 (41)***
7	99 ± 1 (1)	78 ± 1 (22)***
8	101 ± 2 (−1.0)	84 ± 2 (16)**
LPS	100 ± 1 (0)	100 ± 1 (0)

The results are reported as mean value ± SEM for $n = 3$. Statistical significance is based on the difference when compared with LPS-treated groups (**P <0.01, ***P <0.001).
% Inhibition is based on LPS as shown in parenthesis.

Table 2
Proliferation effect of piperlogs **1–8**

Compound	Proliferation	
	1 μM	10 μM
Medium (MED)	100 ± 4	100 ± 4
1	115 ± 3	77 ± 1**
2	107 ± 4	101 ± 2
3	101 ± 3	113 ± 2
4	105 ± 2	102 ± 8
5	97 ± 7	103 ± 6
6	103 ± 1	83 ± 2*
7	97 ± 5	92 ± 8
8	104 ± 6	104 ± 3

The results are reported as mean value ± SEM for $n = 3$. Statistical significance is based on the difference when compared with Medium groups (*P <0.05, **P <0.01).



IC_{50} Values 1: 3 μM, 2: 14 μM, 3: 6 μM, 4: 80 μM, 5: 64 μM, 6: 17 μM, 7: 64 μM, 8: 341 μM

Figure 2. IC_{50} values for NO production..

phenylselenyl chloride gave the α-phenylseleno imide **11** which subsequently subjected to oxidation with hydrogen peroxide to furnish piperlongumine **1** in 71%.

Next, we prepared 1*H*-pyrrol-2(5*H*)-one **12** from pyrrole following the literature procedure[13] which was further used in the PL derivative **3** preparation. Treatment of compound **12** with *n*-BuLi at −78 °C followed by slow addition of **10** resulted **3**. PL derivatives **4**, **5** and **8** were obtained by direct reaction of **10** with the commercially available pyrrolidin-2-one, propionamide and acetamide in 64%, 33% and 41%, respectively. Finally, reaction of **10** with acrylamide offered PL derivative **7** as a major product along with compound **6**. The structures of all the products were settled from their spectral (^{1}H and ^{13}C NMR and MS) data.

In order to evaluate the anti-inflammatory effects of the prepared piperlongumine (**1**) and its derivatives (**2–8**), we measured the amount of nitric oxide (NO) which is one of the essential mediators on inflammation, in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages.[14]

Anti-inflammatory activity: Effect of PL (**1**) and its derivatives (**2–8**) on NO generation by induced macrophages was monitored (Table 1). Lipopolysaccharide (LPS) treated RAW 264.7 has been used to stimulate the production of NO through the activation of iNOS. Of the piperlogs prepared in the present study, only four compounds (i.e., PL, compound **2**, **3** and **6**) showed significant activities at 10 μM. Among the 8 compounds, the maximum inhibitory activity was observed with PL (91%) followed by compound **3** (65%), **2** (46%) and **6** (41%). The cell viability assay at 10 μM concentration was not affected by compounds **2**, **3**, **4**, **5** and **8** indicating no cytotoxicity as shown in Table 2. IC_{50} values of these compounds (**1–8**) were evaluated by using GraphPad Prism 4.0 software and showed 3, 14, 6, 80, 64, 17, 64 and 341 μM, respectively, (Fig. 2). From the aforementioned pharmacological results, we can conclude that amide/lactam moiety connected to cinnamoyl group with minimum 3 carbon chain length and α,β-unsaturation is fruitful as the compound **3** was found to show potent anti-inflammatory activity with no cytotoxicity (Fig. 3).

In summary, we have described the synthesis of piperlongumine (**1**) and its derivatives (**2–8**) by the direct reaction between the acid chloride of 3,4,5-trimethoxycinnamic acid and various amides/lactams. Later, the anti-inflammatory effects of these compounds were evaluated in lipopolysaccharide (LPS) stimulated RAW-264.7 macrophages. Of the piperlogs prepared in this study, the maximum inhibitory activity was observed with PL (91%) but showed cytotoxicity whereas compound **3** which possess α,β-unsaturated γ-butyrolactam moiety offered good level (65%) of activity with no cytotoxicity. This study revealed that amide/lactam moiety connected to cinnamoyl group with minimum 3 carbon chain length and α,β-unsaturation is fruitful to show potent anti-inflammatory activity. Gaining boost from this study, further investigation about the mechanism of action is under progress and will be disclosed in due course.

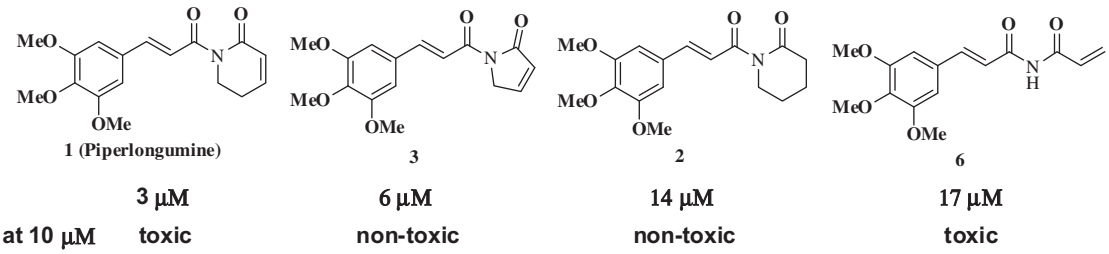


Figure 3. Comparison of IC_{50} values with cytotoxicity of active compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.054.

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