Synthesis and biological evaluation of longanlactone analogues as neurotrophic agents

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Graphical Abstract

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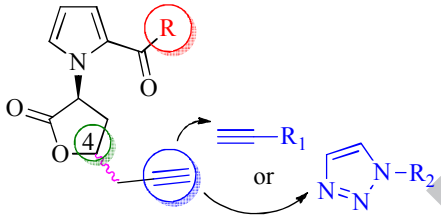

C4 = ▬, Longanlactone (**1**)
C4 = ┈┈, 4-*epi*-Longanlactone (**1a**)

Evaluation of neurotrophic activity of longanlactone along with its analogues has been achieved for the first time. A total of 12-analogues have been prepared including C4-epimers.

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ABSTRACT

Longanlactone analogues were synthesized using a route featuring Friedel-Crafts acylation, Sonogashira coupling and 1,3-dipolar cycloaddition reactions. Structure–activity relationships were investigated for neurotrophic activity. Compound **6** was found to have the most potent neurotrophic activity among all the synthesized analogues in Neuro2a cells as evidenced by a battery of in vitro/cell based assays for assessment of neurogenic and potential neurotrophic activity including neurite outgrowth assay and real time PCR for popular markers of augmented neurotrophic activity. Compound **6** might serve as a template for further development of highly effective neurotrophic molecules.

Natural products and their analogues play an important role as therapeutic agents in the treatment of many diseases. In this direction, the synthesis of various analogues of a natural product and evaluation of their effectiveness became the key topic of research among the medicinal chemistry research groups. Modern synthetic methodologies have emerged as key steps to strengthen the structure activity relationship (SAR) studies in natural product derivatives to improve their efficacy and therapeutic index.[1] Longanlactone (natural pyrrole lactone, **1**, Figure 1) was isolated by Zhen and co-workers from the longan (*Dimocarpus longan*) seeds,[2] which was used as a traditional medicine in China.[3] From the literature, it was found that the natural sources producing pyrrole alkaloids such as funebral (**2**), funebrine (**3**), were used as local folk medicines to control psychopathic fears.[4] In addition, longan fruit has several health benefits. Recently, the central nervous system (CNS) depressant, analgesic and antidiarrheal effects of the seed extracts of *Dimocarpus longan* have been studied.[5] Aqueous extract of longan has been reported to reduce the neural pain and swelling as well as to improve memory and learning,[6] principally by upregulating Brain-derived neurotrophic factor (BDNF) and its downstream markers leading to heightened cyclic AMP response element binding (pCREB) expression along with greater number of cells immunoreactive for 5'-bromo-2'-deoxyuridine (BrdU) and Doublecortin (DCX).[7] Amelioration of memory impairment in chemically induced cognitively impaired mice models is well reported using HX106N (a water soluble plant extract), one of whose many components is the extract from Longan.[8] However, the biological activity of natural product **1** has not been evaluated. In 2014, we reported the first asymmetric total synthesis of longanlactone (**1**), a pyrrole alkaloid, in six steps with 31% yield.[9]

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Figure 1. Structures of pyrrole-lactone based natural alkaloids

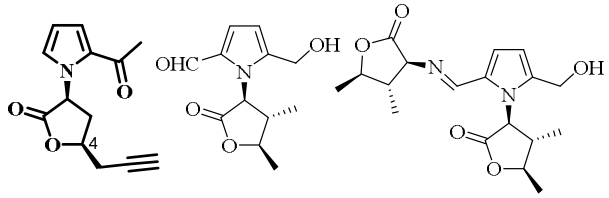
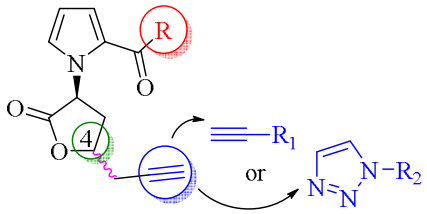


Figure 2. Structure of designed analogues

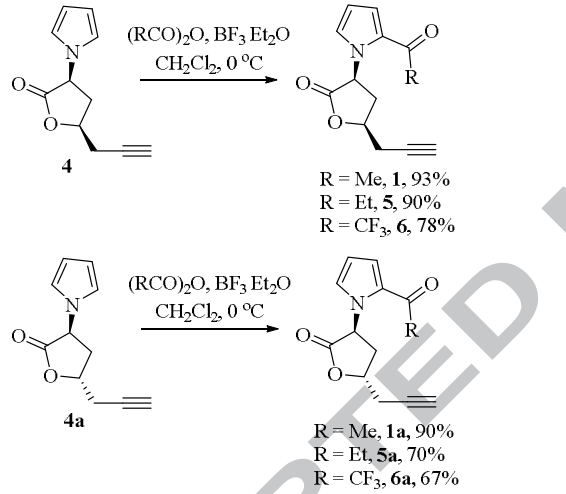


The natural product **1** was investigated for its neurotrophic potential on Neuro-2a cells at 0.1 µM concentration and a concurrent MTT cells cytotoxic assay was carried out to

substantiate the compound concentrations are non-cytotoxic. In neurite growth assay, **1** showed a considerable increase in neurite length at 0.1μM concentration as compared to control (i.e. 37.01% increase in the neurite length when compared to the control) [Table 1, Figure 3B)]. This preliminary outcome encouraged us to synthesize the derivatives of natural product **1** with an array of substitutions, which may lead us towards an enhancement in neurotrophic activity.

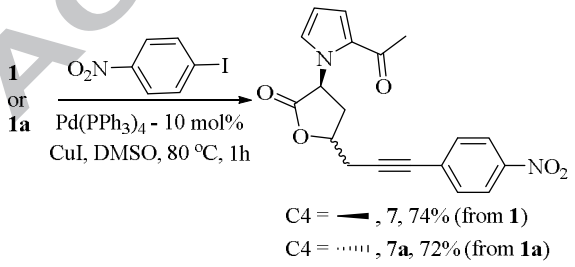
Based on the structure of longanlactone (**1**), analogues were designed with three-point diversity (Figure 2), such as i) C2-acylation of pyrrole, to generate the variations at 'R' group ii) aryl substitution on terminal alkyne (R_1) and iii) triazole formation with alkyne functionality using azides (R_2). Further, to verify the role of stereochemistry at the C4 centre on biological activity, synthesis of 4-*epi*-longanlactone (**1a**) analogues were also planned.

To synthesize the C-2 acylated derivatives, previously reported[9] pyrrole-lactone **4** was treated with different anhydrides such as acetic anhydride, propionic anhydride and trifluoroacetic anhydride in the presence of $BF_3{\cdot}Et_2O$ at 0 °C to afford the corresponding products longanlactone **1** and analogues **5**, **6** in good yield. Similarly, 4-*epi*-longanlactone **1a** and analogues **5a**, **6a** were synthesized from compound **4a**, without epimerization.



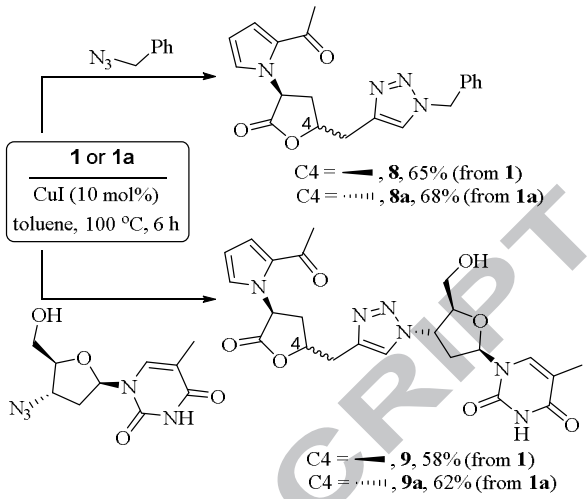
Scheme 1. Synthesis of acyl derivatives

Towards the arylation of the terminal alkyne, natural longanlactone **1** and its 4-epimer **1a** were independently subjected to a Sonogashira coupling reaction[10] with 4-iodo-nitrobenzene in the presence of palladium-catalyst to give the desired products **7** and **7a** in 74% and 72% yields, respectively (Scheme 2).



Scheme 2. Synthesis of 4-nitrophenyl alkynyl analogues of **1** and **1a**

Later, triazole analogues **8, 9, 8a** and **9a** were obtained in 1,4-regioselective manner from the reaction of **1** and **1a** with benzyl azide and AZT-derived azide in the presence of CuI catalyst in toluene (Scheme 3).[11]



Scheme 3: Synthesis of triazole derivatives of **1** and **1a**

The synthesized natural product derivatives (**1a**, **4-9** and **4a-9a**) were evaluated for their in vitro cell cytotoxicity against mouse neuroblastoma cells (Neuro 2a) by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[12,13] The results of in vitro cytotoxicity revealed that the tested compounds were relatively less cytotoxic as compared to the untreated control (see the supplementary information).

Table 1: Morphometric analysis of neurite outgrowth of differentiated Neuro2a cells[a]

Treatment	Average neurite length (μm) ± SEM		Percent change from control (%)	
Untreated control	77.29±2.38		-	
DMSO	82.43±3.79		6.65	
NGF (100ng/mL)	151.73±5.47*		96.31	
Compound	Treatment dose- 0.01 μM	Treatment dose- 0.1 μM	Treatment dose- 0.01 μM	Treatment dose- 0.1 μM
1	83.5±3.59	105.90±3.66*	**8.03**	**37.01**
1a	84.28±3.75	93.48±4.03	9.04	20.95
4	82.36±4.52	85.72±4.91	6.56	10.90
4a	86.68±3.13	88.55±4.78	12.15	14.56
5	86.28±4.45	84.62±4.82	11.63	9.47
5a	80.87±2.76	84.63±4.62	4.63	9.50
6	**103.37±5.56**	**109±4.41**	**33.74**	**41.02**
6a	83.29±3.58	94.47±3.57	7.76	22.22
7	71.44±3.51	84.33±4.88	-7.58	9.11
7a	78.39±3.32	87.55±5.01	1.43	13.27
8	81.48±4.05	89.37±3.57	5.41	15.62
8a	75.57±3.15	90.45±3.53	-2.23	17.02
9	80.13±3.07	82.40±4.23	3.67	6.61
9a	84.65±2.61	88.73±4.48	9.52	14.80

[a]Neuro2a cells cultured for 24 h in DMSO (0.1%), Compounds (0.01μM & 0.1 μM). The tabulated data and bar graphs were expressed as mean±SEM where n= 60 and *p<0.01 vs control.

This preliminary observation was promising to explore the neurotrophic potential in Neuro2a cells using standard in vitro protocol.[12] Initially, neurite outgrowth assay was performed to investigate the neurotrophic potential of natural product derivatives (**1a**, **4-9**, **4a-9a**). In this study, the Neuro 2a Cells were serum starved for 6 hours and were exposed to the synthesized compounds (**1a**, **4-9**, **4a**-**9a**) along with Nerve growth factor (NGF) as a positive control at concentrations of 0.1 µM and 0.01 µM for 48 hours.[13]

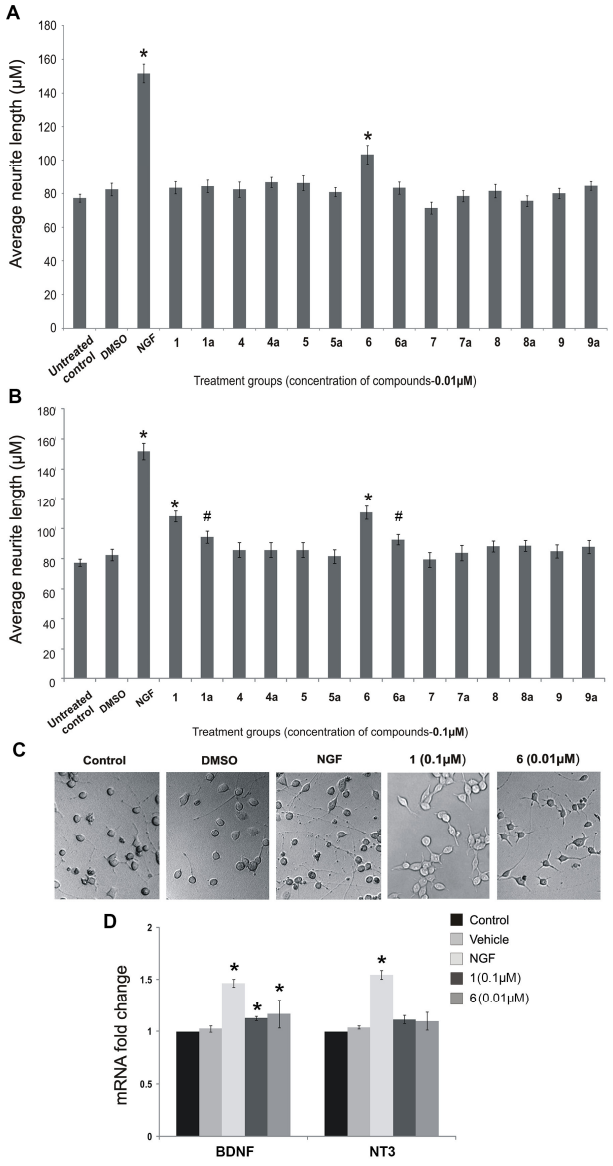


Figure 3. (A & B): Average neurite outgrowth at 48 hours for control, NGF and test compounds at a concentration of 0.01 µM and 0.1 µM respectively. There was significant increase in neurite length in NGF and compound **6** treated cells after 48 hours compared to control at 0.01µM concentration. Data were analyzed by student's t-test [(Where * $p<0.05$ and #$p<0.1$; (n=60)]. (C): Represents the pictorial micrographs (bright field) of different treatment groups taken at 10X magnification using Motic AE31 microscope. (D): Showing mRNA fold change in BDNF and NT3 after 2 h of treatment for control, NGF and test compounds **6** at 0.01 µM and **1** at 0.1 µM concentrations. There was significant increase in BDNF fold change in compound **1** and **6** treated cells as compared to control whereas no significant fold change for NT3 was seen. Data were analyzed by student's t-test. [(Where * $p<0.05$ (n=60)].

The results illustrated in Figure 3B revealed that compound **6** having -CF_3 substitution showed significant increase in neurite length at 0.1µM concentration as compared to control and other tested compounds (Figure 3B). Interestingly, compound **6** displayed better neuritogenic potential in neurite outgrowth assay even at 0.01 µM concentration (i.e. 33.74% increase in the neurite length when compared to the control) whereas decreased activity was observed for natural product **1** at lower concentration (0.01 µM, Figure 3A). In addition, compound **1a, 4a, 5** and **9a** also showed noticeably increased neurite length as compared to DMSO. Furthermore, the compounds exhibited promising results in neurite outgrowth assay were screened for BDNF gene expression,[14,15] which suggested the mRNA fold change in BDNF after the exposure of compound **1** and **6** at 0.1 µM and 0.01 µM respectively (Figure 3C). These results clearly demonstrated that the involvement of BDNF mediation in this activity. However, no significant mRNA fold change was observed in NT3 (Figure 3D).

In summary, various analogues of a pyrrole-lactone natural product, longanlactone, have been synthesized. In addition, we have studied the neurotrophic effects of the natural product **1** as well as its analogues for the first time. Interestingly, compound **1** and **6** showed considerable neurotrophic activity on neuro2a cells at 0.1 µM and 0.01 µM concentrations, respectively. Further analysis of these compounds on BDNF gene expression demonstrated the mRNA fold change in BDNF. Although more studies are required to discover the target at the molecular level of this molecule, its mode of action is supposedly *via* the upregulation of BDNF as shown by real time PCR. Thus, we might safely surmise that this compound holds potential as a framework for further development of highly neuroactive compounds that might contribute significantly to CNS therapy.

Acknowledgments

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Highlights

- Longanlactone, a natural pyrrole-lactone alkaloid, analogs were synthesized.
- Neurotrophic effects of Langanlactone analogs were studied for the first time
- mRNA Fold change was observed in BDNF gene expression study of Longanlactone