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Original article

# Synthesis, bioactivity and functional evaluation of linker-modified allatostatin analogs as potential insect growth regulators

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ABSTRACT

Insect growth regulators play an important role in integrated pest management strategies. The FGLa–allatostatins (ASTs) are a family of neuropeptides that can inhibit juvenile hormone (JH) biosynthesis by the corpora allata (CA) of *Diploptera punctata in vitro*, are regarded as insect growth regulator candidates. In the search for new potential mimics and to explore the effect of linker length on inhibiting JH biosynthesis, a series of AST analogs were synthesized by modifying the linker of K24, which was found to have a significant effect on JH biosynthesis *in vitro* in our previous study. Functional evaluation demonstrated that all the target compounds can activate the *Dippu-AstR*, albeit with different potencies. Analog L6 with the longest linker ($n = 5$), exhibited not only a promising effect on inhibition of JH biosynthesis both *in vitro* and *in vivo*, but also good activity in inhibiting basal oocyte growth. Structure–activity relationships (SAR) studies showed that longer linkers provided greater contribution to activity.


## 1. Introduction

Juvenile hormone (JH) production in the corpora allata (CA) of *Diploptera punctata* is tightly controlled and highly predictable during the reproductive cycle. This characteristic provides an ideal model system to study the effect of compounds with anti-JH actions. FGLa–allatostatins (ASTs), a family of pleiotropic neuropeptides, were originally identified for their ability to inhibit JH biosynthesis by CA rapidly and reversibly [1]. Because of their high-efficiency, selectivity and safety to non-target organisms, FGLa–ASTs have been employed in the design of new potential IGRs [2–4]. Although ASTs inhibit JH biosynthesis effectively *in vitro*, they have some shortcomings as potential pesticides. First, the more amino acid residues, the higher the production costs. For ASTs, even the shortest AST peptide has six amino acids [5]. Second, ASTs are susceptible to metabolic inactivation by peptidases in hemolymph and midgut [3]. Third, difficulties in ASTs absorption through cuticle could prevent their effects *in vivo*. Therefore, much effort has been expended to overcome these problems and create better AST peptidomimetics.

Earlier structure–activity relationship (SAR) studies demonstrated that the C-terminal pentapeptide Y/FXFGLa (X = A, N, G, and S) represents the minimum sequence capable of eliciting inhibition of JH production *in vitro* [6–8]. Subsequently, based on the peptidomimetic approach and identification of catabolic cleavage sites of ASTs, several AST analogs were discovered to show activity *in vivo* as well as reduced susceptibility to metabolic inactivation [2–4]. As neuropeptides, ASTs exert their effects by binding to a G protein-coupled receptor (GPCR). In 2008, a single FGLa–AstR was isolated and functionally characterized from *D. punctata* [9]. Recently, the single receptor of *D. punctata* was activated with thirteen natural FGLa–ASTs [10]. However, whether AST analogs can activate the *D. punctata* receptor (*Dippu-AstR*) remains uncertain.

In our previous studies [11–15], four series of the pentapeptide analogs were designed and synthesized employing the peptidomimetic approach to probe the SAR of the core pentapeptide region of AST. The bioassay and hologram quantitative structure–activity relationships (HQSAR) calculation results suggested that the potent AST analogs should contain an aromatic group, an appropriate length of linker, and an FGLa moiety. Subsequently, a potent AST mimic H17 was found to have a significant inhibitory effect on JH biosynthesis by cockroach CA both *in vitro* and *in vivo* [11]. Additionally, owing to its high activity *in vitro* and its molecular flexibility, K24 was considered as another good lead. In the present work, a series of AST analogs (Fig. 1) were synthesized by modifying

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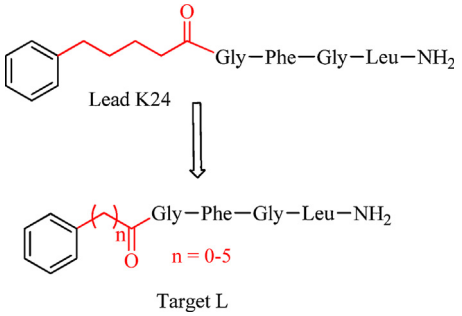



**Fig. 1.** Design strategy of target analog L.

the linker of K24 and their bioactivities were evaluated for the purpose of discovering new potential mimics and exploring the effect of linker length on inhibition of JH biosynthesis.

## 2. Experimental

### 2.1. Synthesis of AST analogs

All the solvents and reagents were purchased from commercial suppliers and used without further purification. The analogs structures were confirmed with high-resolution mass spectrometry (HRMS) and $^1$H NMR. HRMS data was obtained using Agilent Accurate-Mass-Q-TOF MS 6520 system equipped with an Electro Spray Ionization (ESI) source. All the MS experiments were detected in the positive ionization mode. For Q-TOF/MS conditions, fragment and capillary voltages were kept at 130 and 3500 V, respectively. Nitrogen was supplied as the nebulizing and drying gas. The temperature of the drying gas was set at 300 °C. The flow rate of the drying gas and the pressure of the nebulizer were 10 L/min and 25 psi, respectively. Full-scan spectra were acquired over a scan range of $m/z$ 80–1200 at 1.03 spectra $s^{-1}$. $^1$H NMR spectra was recorded on a Bruker AM-300 (300 MHz) spectrometer with DMSO-$d_6$ as the solvent and TMS as the internal standard. Chemical shifts ($\delta$) are reported in parts per million (ppm).

*General synthetic procedure:* The synthesis of analogs was illustrated in Scheme 1. GFGL with resin was synthesized from Rink Amide-AM resin (440 mg, 0.3 mmol) using the standard Fmoc/*t*Bu chemistry and HBTU/HOBt protocol [15]. Incoming amino acids were activated with HBTU (456 mg, 1.2 mmol), HOBt (163 mg, 0.3 mmol) and DIEA (210 μL, 0.6 mmol) in DMF (5 mL) for 5 min, couplings were run for 2 h at room temperature. Removal of the N-terminal Fmoc group from the residues was accomplished with 20% piperidine in DMF (5 mL) for 20 min. Relative phenyl acids were coupled to the GFGL with resin with HBTU, HOBt and DIEA in DMF for 3 h at room temperature. Then, analogs were cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole and 5% water for 2 h at room temperature. The crude peptides were then washed with anhydrous ethyl ether, and lyophilized.

The crude compounds were purified on a C18 reversed-phase preparative column (250 mm × 4.6 mm, 10 μm) with a flow rate of 10 mL/min using the ratio of acetonitrile/water (v/v) 50:50 containing 0.1% TFA. UV detection was at 215 nm. Purification by reversed phase high performance liquid chromatography (HPLC) yielded peptides with over 95% purity. The physical and identification data of target compounds L1–L6 are given in the Supporting information.

**Table 1**
Inhibitory effect on JH release ($IC_{50}$) *in vitro*, potency in activation of *Dippu-AstR* ($EC_{50}$), retention time and log *P* calculation of AST analogs.

| Compd. | *n* | $IC_{50}$ (nmol/L) [a] | $EC_{50}$ (nmol/L) [b] | $t_R$ [c] (min) | log *P* [e] |
|---|---|---|---|---|---|
| AST-1 | / | 9.57[d] | 1.3 | / | / |
| L1 | 0 | 957 | 2952 | 7.465 | 1.69 |
| L2 | 1 | 93.5 | 472.7 | 7.748 | 1.49 |
| L3 | 2 | 77.0 | 153.3 | 9.598 | 1.87 |
| L4 | 3 | 69.5 | 443.3 | 11.832 | 2.34 |
| L5 (K24) | 4 | 37.8 | 62.6 | 15.765 | 2.77 |
| L6 | 5 | 27.2 | 65.5 | 22.598 | 3.24 |

[a] $IC_{50}$ values were determined by radiochemical assay (RCA).
[b] $EC_{50}$ was determined by the *Dippu-AstR* activation assay in CHO-WTA11 cells.
[c] Retention time of AST analogs was determined by reversed phase high performance liquid chromatography (HPLC).
[d] $IC_{50}$ value of AST 1 is as reported by Tobe *et al.* [5].
[e] log *P* calculation of AST analogs was made in the Prediction System of log *P* Version 1.0 and done by Dr. Jianhua Yao.

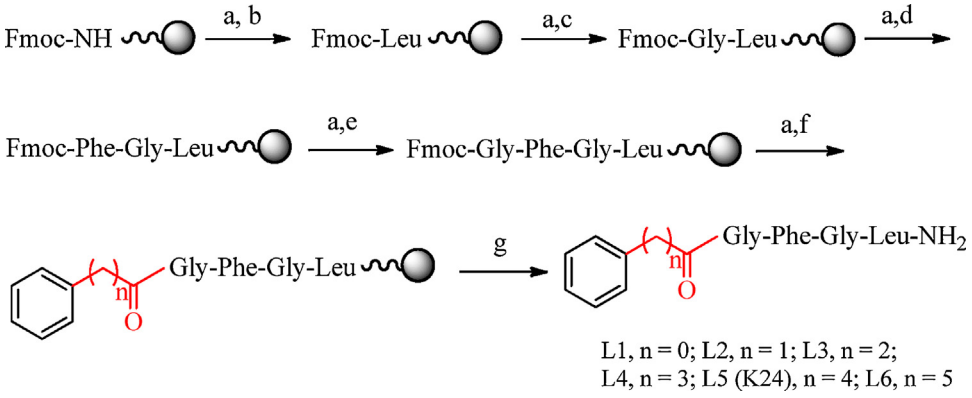
### 2.2. Bioassays

The detailed *in vitro* and *in vivo* biological evaluations and functional assays of *Dippu-AstR* in response to AST analogs are also shown in the Supporting information.

## 3. Results and discussion

### 3.1. In vitro effect of AST analogs on the inhibition of JH biosynthesis

The ability of target analogs to inhibit JH biosynthesis was evaluated *in vitro* using the CA of the cockroach *D. punctata*. As shown in Table 1, all target analogs exhibit differing potencies as a




**Scheme 1.** Synthetic route of lead compound L5 (K24) and target compounds L1–L4, L6. Reagents: (a) 20% piperidine/DMF, 20 min. (b) Fmoc-L-Leu-OH, HBTU, HOBT, DIEA, r.t., 2 h. (c) Fmoc-Gly-OH, HBTU, HOBT, DIEA, r.t., 2 h. (d) Fmoc-L-Phe-OH, HBTU, HOBT, DIEA, r.t., 2 h; (e) Fmoc-Gly-OH, HBTU, HOBT, DIEA, r.t., 2 h; (f) Relative phenyl acids, HBTU, HOBT, DIEA, r.t., 3 h; (g) 20% piperidine/DMF, 20 min; then 90% TFA, 5% phenol, 2.5% $H_2O$, 2.5% TIS, r.t., 2 h.