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Peptidomimetics in the Discovery of New Insect Growth Regulators: Studies on the Structure−Activity Relationships of the Core Pentapeptide Region of Allatostatins

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ABSTRACT: Cockroach-type allatostatins (ASTs) were discovered in cockroaches through their capacity to inhibit the production of juvenile hormone by the corpora allata (CA). ASTs were considered as potential insect growth regulator (IGR) candidates, but several disadvantages, including the absence of the effect *in vivo* and rapid degradation *in vivo*, precluded their application in pest management. The CA were selected as the target, and the core pentapeptide region (YDFGL) was chosen as the lead sequence in the search for new IGRs based on the allatostatins. We designed and synthesized 24 analogues, which mimicked each amino acid of the core region, to determine structure−activity relationships and the possibility of shortening the ASTs in the core region while retaining activity. The results suggest that the sequence FGLa is more important than Y/FX because Y/FX mimics show strong effects *in vitro* and *in vivo*. In particular, compound I3 was synthesized by substitution of Y/FX with 6-phenylhexnoic acid and exhibits higher activity *in vitro* than the complete core region. Furthermore, compound I3 has a clear effect *in vivo* on juvenile hormone (JH) biosynthesis of *Diploptera punctata* females, providing a possible application for cockroach management. On the basis of the structure−activity relationship of pentapeptide analogues, a general structure of potential potent AST analogues is proposed here. A new approach using peptidomimetics in the discovery of IGRs is demonstrated in our study.

KEYWORDS: Insect growth regulators, peptidomimetics, allatostatins, pentapeptide, analogues, juvenile hormone, structure−activity relationships

1. INTRODUCTION

Insect growth regulators (IGRs) are compounds that can regulate the growth of insects.[1,2] Juvenile hormone analogues (JHAs), molting hormone analogues (MHAs), and chitin synthesis inhibitors are three principal groups of IGRs.[3,4] Owing to the virtue of high potency, good selectivity, and environmental safety for nontarget organisms, IGRs are regarded as "the ideal pesticides in the 21st century" and are playing an important role in integrated pest management systems.[3] The discovery of new IGRs and potential IGR targets has therefore become increasingly important to meet the requirements of pest management in agriculture and public health.

In many insect species, the reduction in juvenile hormone (JH) production profoundly influences insect growth, metamorphosis, and reproduction. JH plays a significant role in insect physiological processes,[5] and therefore, any compound that influences the JH production can be regarded as a potential lead for insect control agents. Allatostatins (ASTs), members of a family of insect neuropeptides originally isolated from the cockroach *Diploptera punctata*, can inhibit the *in vitro* biosynthesis of JH by the corpora allata (CA) and thereby control insect maturation and egg production.[6,7] These AST molecules, 6−18 amino acid peptides, also exhibit other biological properties, such as modulation of myotropic activity in both gut[8] and dorsal vessel,[9] inhibition of vitellogenin production in the fat body,[10] and stimulation of carbohydrate enzyme activity in the midgut.[11] These abilities to influence a number of physiological processes, especially inhibition of JH biosynthesis in some insect species, raise the possibility that ASTs could be potential leads for the discovery of new IGRs. In cockroaches, cockroach-type ASTs inhibit JH production at very low doses *in vitro* but have little promise as insect control agents. The major limitations of the ASTs, which have precluded their use for insect control, include their rapid degradation by peptidases and poor penetration through insect cuticle.[12] To find analogues that retain activity but overcome these limitations as promising leads in the development of IGRs, studies on structural modifications and structure−activity relationships (SARs) have been carried out in recent years.

Previous structure−activity studies demonstrated that the C-terminal pentapeptide, Tyr/Phe-Xaa-Phe-Gly-Leu-NH_2 (Y/FXFGLa), which was shared in all identified neuropeptides, was regarded as the minimum sequence to inhibit JH production and the "active core" region of the cockroach-type ASTs.[13] This region probably binds to the allatostatin receptors and elicits inhibition of JH production.[13,14] Using the Ala scanning technique, Hayes et al.[13] indicated that Leu[8], Phe[6], and Tyr[4] in Dippu-AST 4 (DRLYSFGLa) were the most important side chains for inhibition of JH biosynthesis. By altering the nature of the peptide bond with the aim of reducing the susceptibility of the bond to hydrolysis, Piulachs et al.[8] designed ketomethylene and methyleneamino pseudo-peptide analogues of allatostatin Dippu-AST 5 (DRLYSFGLa). Bioassay showed that

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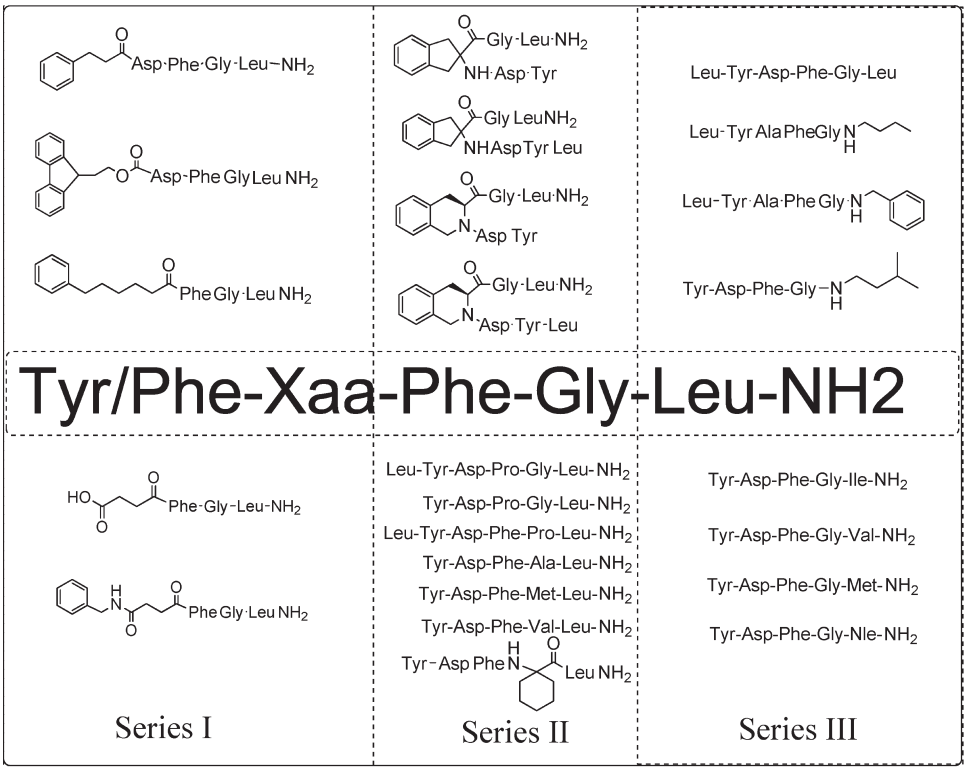


Figure 1. Principle of the design of AST analogues. The core pentapeptide region was divided into three parts, Tyr/Phe-Xaa (series I), Phe-Gly (series II), and Leu-NH_2 (series III), which were replaced by organic groups, restricting conformation components and different amino acids to determine the possibility of shortening the ASTs and the SARs of the core region, respectively.

both analogues were similarly active to the model peptides with respect to the inhibition of JH *in vitro* biosynthesis from CA of virgin *Blattella germanica*. To find analogues that have resistance to catabolism and retain inhibition of JH biosynthesis, Nachman et al.[12,15,16] designed a series of allatostatin analogues by replacing the first, third, or fourth amino acid residues of the C-terminal pentapeptide with sterically hindered amino acids or aromatic acids. These analogues showed significant *in vitro* inhibition of JH biosynthesis. The analogue AST(b)Φ2 (Hca-Asn-Phe-Cpa-Leu-NH_2, with Hca = hydrocinnamyl- and Cpa = cyclopropyl-Ala-) exhibited high resistance to degradation by enzymes in hemolymph and crude membrane preparation of brain and midgut and retained significant biological activity *in vitro* and *in vivo*.[12,15] The above efforts reveal the possibility for the discovery of IGRs that are neuropeptide-like but not readily degradable by peptidases.

However, the question of which amino acids of the core pentapeptide are most important to activity remains unresolved, and therefore, it is unclear if amino acids of the core region could be substituted further. The present work attempts to answer the above issues. In the present study, 24 analogues that mimicked amino acids of the core region pentapeptide were designed with the peptidomimetic approach (PA), one method widely used in the discovery of peptide-based pharmaceuticals,[17−20] by replacing portions of the peptides with unnatural or natural structures to overcome the shortage of natural peptides while retaining activity. The design strategy is shown in Figure 1. The three types of analogues were named as mimics of the Tyr/Phe-Xaa region (series I), the Phe-Gly region (series II), and the Leu-NH_2 region (series III). The *in vitro* effects on JH biosynthesis of all 24 compounds were tested, and the *in vivo* effects of some of the analogues were also evaluated. The SARs of the analogues were subsequently analyzed.

2. EXPERIMENTAL SECTION

2.1. Synthesis. *2.1.1. Materials.* Rink Amide-AM resin (0.52 mmol/g substitution), *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HOBt), 1-hydroxybenzotriazole anhydrate (HBTU), *N*,*N*′-diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), Fmoc-protected amino acids, Fmoc-1-amnocyclohexanecarboxylic acid, and Fmoc-L-Tic-OH were purchased from GL Biochem, Ltd. (Shanghai, China). High-performance liquid chromatography (HPLC)-grade *N*,*N*-dimethylformamide (DMF), dichloromethane (DCM), and acetonitrile were purchased from Dima Technology, Inc. (Richmond Hill, Ontario, Canada). Thioanisole, phenol, benzaldehyde, phenylmethanamine, hydrocinnamic acid, 6-phenylhexanoic acid, butanedioic anhydride, butyraldehyde, 3-methylbutanal, and $NaBH_4$ were purchased from Acros (Geel, Belgium).

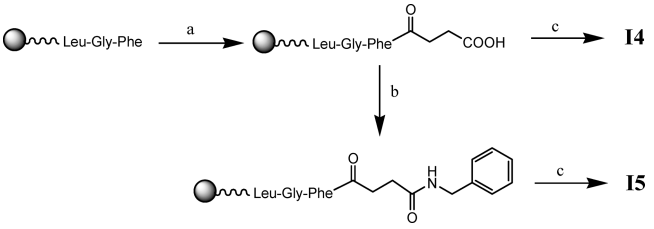
2.1.2. Synthesis of Peptides. The Dippu-AST 1 and pentapeptide were synthesized from Rink Amide-AM resin (198 mg, 0.1 mmol) using the standard Fmoc/tBu chemistry and HBTU/HOBt protocol.[25] Incoming amino acids were activated with HOBt (41 mg, 0.3 mmol), HBTU (114 mg, 0.3 mmol), and DIEA (105 μL, 0.6 mmol) in DMF (5 mL) for 5 min, and couplings were run for 2 h. Removal of the N-terminal Fmoc group from the residues was accomplished with 20% piperidine in DMF (5 mL) for 20 min. The peptides were cleaved from the resin with TFA (4 mL) containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h.

2.1.3. Synthesis of Series I Analogues

DFGL with Resin and FGL with Resin. The two key intermediates were synthesized from Rink Amide-AM resin using the standard Fmoc/tBu chemistry and HBTU/HOBt protocol as the above method in section 2.1.2.

I1. Hydrocinnamic acid (45 mg, 0.3 mmol) was coupled to the DFGL with resin (0.1 mmol) with HOBt, HBTU, and DIEA in DMF for 3 h at room temperature. **I1** was cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h.

Scheme 1. Synthesis of I4 and I5[a]



[a] Conditions: (a) butanedioic anhydride, DMAP, DMF, room temperature, and 3 h; (b) $PhCH_2NH_2$, HBTU, HOBt, DIEA, DMF, room temperature, and 24 h; and (c) TFA, 5% phenol, 2.5% thioanisole, 5% water, room temperature, and 2 h.

I2. Fmoc-Asp-OH (107 mg, 0.3 mmol) was coupled to the FGL with resin (0.1 mmol) with HOBt, HBTU, and DIEA in DMF for 3 h at room temperature. **I2** was cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h.

I3. 6-Phenylhexanoic acid (58 mg, 0.3 mmol) was coupled to the FGL with resin (0.1 mmol) with HOBt, HBTU, and DIEA in DMF for 3 h at room temperature. **I3** was cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h.

I4. Butanedioic anhydride (30 mg, 0.3 mmol) was coupled to the FGL with resin (0.1 mmol) with 4-dimethylaminopyridine (DMAP) (37 mg, 0.3 mmol) dissolved in DMF (5 mL) for 2 h at room temperature. **I4** was cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h (Scheme 1).

I5. Phenylmethanamine (54 mg, 0.5 mmol) was coupled to the intermediate **I4** with resin (0.1 mmol) with HOBt, HBTU, and DIEA in DMF for 24 h at room temperature. **I5** was cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h (Scheme 1).

2.1.4. Synthesis of Series II Analogues

II1−*II11.* They were obtained with the relevant Fmoc-protected amino acids using the same method as in section 2.1.2.

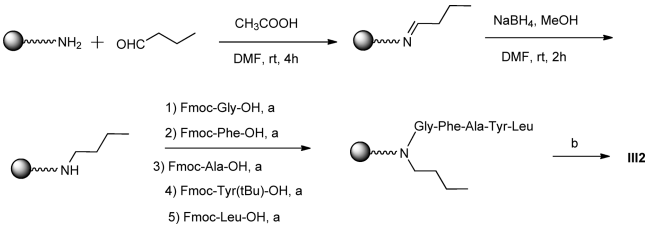
2.1.5. Synthesis of Series III Analogues

III1. **III1** was synthesized from Wang resin (67 mg, 0.1 mmol) using the standard Fmoc/tBu chemistry and HBTU/HOBt protocol. Incoming amino acids were activated with HOBt, HBTU, and DIEA in DMF for 5 min, and couplings were run for 3 h. Removal of the N-terminal Fmoc group from the residues was accomplished with 20% piperidine in DMF for 20 min. **III1** were cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h.

III2. Butyraldehyde (43 mg, 0.5 mmol) was coupled to Rink Amide-AM resin (198 mg, 0.1 mmol) with acetic acid (4 μL) in DMF (5 mL) for 4 h at room temperature. The resin was reduced by $NaBH_4$ (38 mg, 1 mmol) with methanol (0.4 mL) in DMF (5 mL) for 2 h at room temperature. Incoming amino acids were activated with HOBt, HBTU, and DIEA in DMF for 5 min, and couplings were run for 3 h. Removal of the N-terminal Fmoc group from the residues was accomplished with 20% piperidine in DMF for 20 min. **III2** were cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h (Scheme 2).

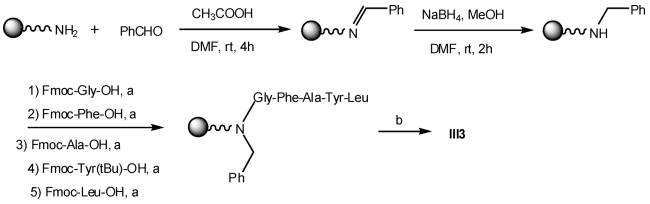
III3. Benzaldehyde (53 mg, 0.5 mmol) was coupled to Rink Amide-AM resin (198 mg, 0.1 mmol) with acetic acid (4 μL) in DMF (5 mL) for 4 h at room temperature. The resin was reduced by $NaBH_4$ (38 mg, 1 mmol) with methanol (0.4 mL) in DMF (5 mL) for 2 h at room temperature. Incoming amino acids were activated with HOBt, HBTU, and DIEA in DMF for 5 min, and couplings were run for 3 h. Removal of the N-terminal Fmoc group from the residues was accomplished with 20% piperidine in DMF for 20 min. **III3** was cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h (Scheme 3).

Scheme 2. Synthesis of III2[a]



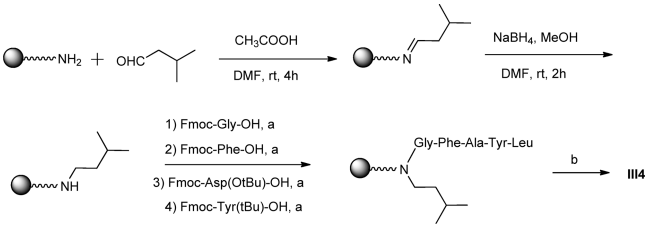
[a] Conditions: (a) HBTU, HOBt, DIEA, DMF, room temperature, and 3 h and (b) TFA, 5% phenol, 2.5% thioanisole, 5% water, room temperature, and 2 h.

Scheme 3. Synthesis of III3[a]



[a] Conditions: (a) HBTU, HOBt, DIEA, DMF, room temperature, and 3 h and (b) TFA, 5% phenol, 2.5% thioanisole, 5% water, room temperature, and 2 h.

Scheme 4. Synthesis of III4[a]



[a] Conditions: (a) HBTU, HOBt, DIEA, DMF, room temperature, and 3 h and (b) TFA, 5% phenol, 2.5% thioanisole, 5% water, room temperature, and 2 h.

III4. 3-Methylbutanal (43 mg, 0.5 mmol) was coupled to Rink Amide-AM resin (198 mg, 0.1 mmol) with acetic acid (4 μL) in DMF (5 mL) for 4 h at room temperature. The resin was reduced by $NaBH_4$ (38 mg, 1 mmol) with methanol (0.4 mL) in DMF (5 mL) for 2 h at room temperature. Incoming amino acids were activated with HOBt, HBTU, and DIEA in DMF for 5 min, and couplings were run for 3 h. Removal of the N-terminal Fmoc group from the residues was accomplished with 20% piperidine in DMF for 20 min. **III4** were cleaved from the resin with TFA containing 5% phenol, 2.5% thioanisole, and 5% water for 2 h (Scheme 4).

III5−*III8.* They were obtained with the relevant Fmoc-protected amino acids using the same method as in section 2.1.2.

All of the crude compounds were purified on a C_{18} reversed-phase preparative column with a flow rate of 10 mL/min using acetonitrile/water (50:50) containing 0.06% TFA as an ion-pairing reagent. UV detection was at 215 nm.[21,25] The purity of each purified compound was greater than 95%. The structures of the analogues were confirmed by the presence of the following molecular ions using a 1100 series LC/MSD trap (VL) (Agilent Technologies, Santa Clara, CA). The structures, purity, and mass spectrometry (MS) data of all target compounds are shown in Table 1.

2.2. Bioassays. *2.2.1. Animals.* Newly emerged mated females of *D. punctata* (day 0) were isolated from stock cultures. Mating was confirmed by the presence of a spermatophore. Stocks and isolated

Table 1. Structures, Purity, MS Results, and IC_{50} Values of Analogues

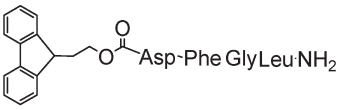
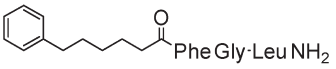
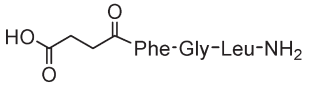
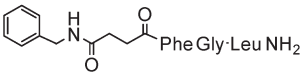
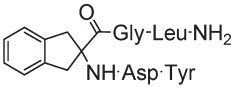
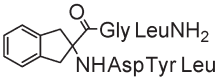
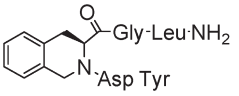
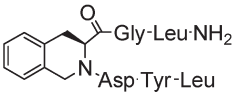
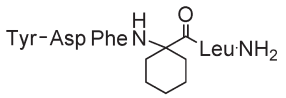
Compd.	Structure	Molecular weight	Mass result	Purity (%)	IC_{50}(μM) *in vitro*
Dip-AST 1	Leu-Tyr-Asp-Phe-Gly-Leu-NH_2	725.4	MH^+: 726.2	100	0.008
pentapeptide	Tyr-Asp-Phe-Gly-Leu-NH_2	612.7	MH^+: 613.3	98	0.13
I1		581.7	MNa^+:604.3	97	0.14
I2		671.7	MH^+: 672.3	99	0.99
I3		508.7	MNa^+:531.3	96	0.06
I4		434.5	MH^+: 435.3	95	0.87
I5		523.6	MH^+: 524.3	96	0.16
II1		625.3	MH^+: 625.7	100	1.21
II2		738.4	MH^+: 738.8	100	0.14
II3		625.2	MH^+: 625.7	97	No effect
II4		738.1	MH^+: 738.8	98	1.26
II5	Leu-Tyr-Asp-Pro-Gly-Leu-NH_2	676.4	MH^+: 676.8	98	No effect
II6	Tyr-Asp-Pro-Gly-Leu-NH_2	563.3	MH^+: 563.7	99	No effect
II7	Leu-Tyr-Asp-Phe-Pro-Leu-NH_2	766.5	MH^+: 766.9	97	No effect
II8	Tyr-Asp-Phe-Ala-Leu-NH_2	626.3	MH^+: 627.3	98	No effect
II9	Tyr-Asp-Phe-Met-Leu-NH_2	686.3	MH^+: 687.3	99	No effect
II10	Tyr-Asp-Phe-Val-Leu-NH_2	654.3	MH^+: 655.3	98	No effect
II11		680.3	MH^+: 655.3	98	No effect
III1	Leu-Tyr-Asp-Phe-Gly-Leu	726.8	MH^+: 727.6	100	No effect
III2		624.4	MH^+: 625.5	95	3.43

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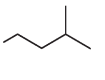
Table 1. Continued

Compd.	Structure	Molecular weight	Mass result	Purity (%)	IC_{50}(μM) *in vitro*
III3	Leu-Tyr-Ala-Phe Gly-N(H)- 	658.8	MNa^+:681.4	95	0.13
III4	Tyr-Asp-Phe-Gly-N(H)- 	569.3	MH^+: 570.3	95	No effect
III5	Tyr-Asp-Phe-Gly-Ile-NH_2	612.3	MH^+: 613.3	97	27.2
III6	Tyr-Asp-Phe-Gly-Val-NH_2	598.3	MH^+: 599.3	98	12.4
III7	Tyr-Asp-Phe-Gly-Met-NH_2	630.3	MH^+: 631.3	98	No effect
III8	Tyr-Asp-Phe-Gly-Nle-NH_2	612.3	MH^+:613.3	99	No effect

females, fed Lab Chow and water *ad libitum*, were kept at 27 ± 1 °C and relative humidity of 50 ± 5% with a 12 h light/12 h dark cycle.

2.2.2. Bioassays in Vitro. All radiochemical assays for JH biosynthesis were performed using individual pairs of CA from day 7 mated females. Compounds were dissolved in medium 199 for assay as described previously.[22,23] All test compounds were assayed on the same day that the samples were prepared. The solutions were discarded at the end of each day. Rates of JH release were determined using the modified *in vitro* radiochemical assay.[22,23] This assay measures the incorporation of the radiolabeled S-methyl moiety of radiolabeled methionine into JH III in the final step of biosynthesis by CA maintained *in vitro*. CA were incubated for 3 h in 100 μL of medium 199 (GIBCO, 1.3 mM Ca^{2+}, 2% Ficoll, methionine-free) containing L-[^{14}C-*S*-methyl]methionine (40 μM, specific radioactivity of 1.48−2.03 GBq/mmol, Amersham). Samples were extracted, and the JH release was determined. Each data point on the dose−response figures represent replicate incubations of 10−27 experimental CA compared to control CA (i.e., no analogue added).

2.2.3. Bioassays in Vivo. The pentapeptides, **I1**, **I3**, and **I4** were dissolved in H_2O (stock final concentration of 10 μM). A total of 5 μL was injected into *D. punctata* females on day 1, and animals were assayed for JH biosynthesis at day 3.[15] The assay for JH biosynthesis is identical to the *in vitro* assay described above. All injections were administered to non-anaesthetized mated female *D. punctata*. Assuming a hemolymph volume of 50 μL for day 1 adult female *D. punctata*,[24] the concentrations of the injected analogue in the hemolymph were approximately 1 μM. Injections were administered using a 26-gauge 10 μL Hamilton syringe. The needle was inserted into the membranous joint between the coxa and femur on the metathoracic leg. For control samples, insects were injected with 5 μL of double-distilled water. Each group of compound-injected animals was compared to a group of water-injected animals treated concurrently.

3. RESULTS AND DISCUSSION

3.1. Choice of Lead Peptide. The length of the lead peptide directly affects the difficulty in designing peptidomimetics; i.e., the longer the lead peptide, the more difficult the design. The cockroach-type ASTs in *D. punctata* contain 6−18 amino acid residues. To choose an appropriate lead peptide is the first step in the AST peptidomimetic approach. Previous studies[12,14−16] showed that AST analogues, which modify or protect the "active core" pentapeptide, retained significant *in vitro* activity. This demonstrates that the C-terminal pentapeptide could be the lead.

In this study, a pentapeptide, YDFGLa, was synthesized and investigated as the lead compound. The native Dippu-AST 1, LYDFGLa, was chosen as the control. Treatment of adult female *D. punctata* with the pentapeptide showed a significant effect on JH production *in vitro*, with an IC_{50} value of 0.13 μM. The IC_{50} value for native Dippu-AST 1 was 0.008 μM. Inhibition of JH biosynthesis by the pentapeptide is 16-fold less than that of Dippu-AST 1, but the structure of the pentapeptide is simpler than that of the natural AST. A good lead compound in pesticide discovery should be bioactive and easily modified. Therefore, taking the pentapeptide as the lead compound and Dippu-AST 1 as the control, three series of analogues were designed and synthesized successfully in the present work.

3.2. Design and Effects of Series I. In series I, aromatic acids and dicarboxylic fatty acids were used as mimics of the Tyr/Phe-Xaa region of pentapeptide. In previous work, AST(b)Φ2, in which Tyr was replaced by 3-phenylpropanoic acid, showed resistance to catabolism and high activity.[12,15] In our work, we chose aromatic acids to mimic the Tyr/Phe-Xaa region to design **I1**, **I2**, **I3**, and **I5**. In comparison to the lead (pentapeptide in Table 1), these analogues showed significant inhibition of JH biosynthesis. Compound **I1**, in which 3-phenylpropanoic acid mimicked the Tyr of the core region, had the same IC_{50} value as that of the pentapeptide. The bioactivity of **I2** was almost 10-fold less than that of the pentapeptide, probably because the hydrophobic group of (9*H*-fluoren-9-yl) methyl hydrogen carbonate is larger than either Tyr or Phe. In the structures of **I3** and **I5**, 6-phenylhexanoic acid and 3-(benzylcarbamoyl)propanoic acid were used to mimic the Y/FX sequence, respectively. The bioactivity of **I3** was higher than that of the pentapeptide, and the IC_{50} value of **I5** was similar to that of the pentapeptide. Compound **I4**, in which succinic acid was used to replace the Xaa sequence of XFGLa, was also able to inhibit JH production. A comparison of the activities of **I4** and **I5** indicates that the aromatic group (benzene ring) contributes to elevate biological activity to the analogues. The significant activity of series I analogues, which only contained the "FGLa" region, suggests that the Y/FX region could be substituted in analogue design,

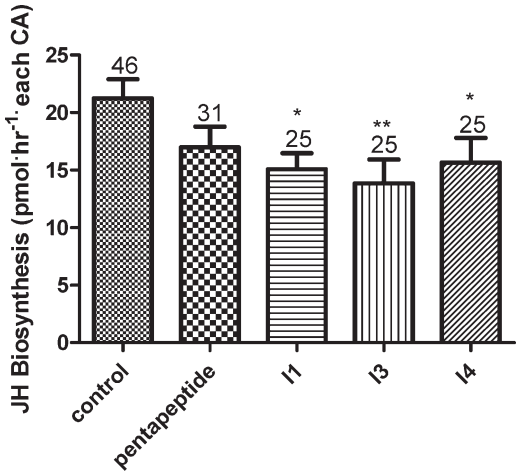


Figure 2. Inhibitory effects *in vivo* of the pentapeptide and AST analogues on JH biosynthesis by CA. The concentrations were 1 μM in the hemolymph of female cockroach *D. punctata* at day 1 and 1.1 μM at day 3. Each column represents the mean ± scanning electron microscopy (SEM) for the number of individual measurements indicated at the top of error bars. Asterisks indicate significant differences between peptide- and water-injected groups of animals as determined by Dunnett's multiple comparison test following one-way analysis of variation (ANOVA): (∗) $0.01 < p < 0.05$ and (∗∗) $0.001 < p < 0.01$. Significance determined by a Student's *t* test was as follows: pentapeptide, not significant (NS); **I1** and **I4**, <0.05; and **I3**, <0.01.

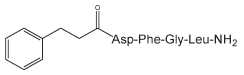
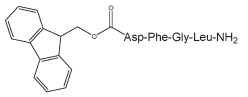
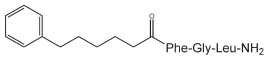
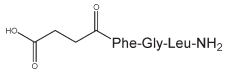
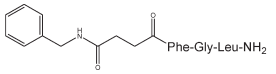
and the presence of an aromatic group could help to retain the significant activity.

Although the natural ASTs exert a strong *in vitro* inhibitory effect on JH biosynthesis by cockroach CA, they have little or no effect *in vivo* because of the major limitations, including their rapid degradation by peptidases and poor penetration through the insect cuticle.[12] Accordingly, the effect *in vivo* on JH biosynthesis of the pentapeptide and series I analogues **I1**, **I3**, and **I4** was determined. The concentrations of the compounds were 1 μM in the hemolymph of *D. punctata* females at day 1 and 1.1 μM at day 3. Figure 2 shows that the pentapeptide has no effect *in vivo* but the three analogues do exert some effect *in vivo*. Previous work showed that the analogue AST(b)Φ2 containing only four true amino acids (XFGLa) retain *in vitro* and *in vivo* activity.[12] It is striking that **I4**, which contains only three true amino acids (FGLa), also had some effect *in vivo*. Compound **I3**, whose *in vitro* bioactivity was significantly better than that of pentapeptide, showed an effect *in vivo*. This compound offers considerable potential for the use of allatostatin analogues in cockroach management.

Hemolymph enzymes primarily cleave the peptide in the N-terminal region outside of the peptide core region, and cleavage by membrane preparation of brain, gut, and CA occurs between the residues Tyr-Xaa.[17,25] In series I, both cleavages are prevented because Tyr is replaced by a nonpeptide structure. The log *P* values of series I were calculated by the prediction system of log *P* (PSLogP) and are shown in Table 2. The log *P* value of all of the series I analogues is higher than that of lead pentapeptide and Dippu-AST 1. This indicates that it is easier for these compounds to penetrate the insect cuticle than the pentapeptide, and consequently, they exhibit an effect *in vivo*.

3.3. Design and Effects of Series II. D-Amino acid replacements suggested that a secondary structural element(s) at the C terminus of Dippu-AST 5 could be important to the biological activity. A turn in the active core in residues XFGL could

Table 2. Log *P* Prediction Results of Series I

Compd.	Structure	logP[a]
Dippu-AST 1	Leu-Tyr-Asp-Phe-Gly-Leu-NH_2	-0.53
pentapeptide	Tyr-Asp-Phe-Gly-Leu-NH_2	-1.2
I1		1.03
I2		3.56
I3		3.8
I4		-0.01
I5		1.32

[a] The PSLogP was developed by Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. All of the predicted values were performed by Dr. Jian-hua Yao.

represent a plausible active conformation, in agreement with the study using protein secondary structural prediction methods and energy minimizations.[13] In addition, Nachman et al.[16] synthesized allatostatin analogues incorporating β-turn-promoting moieties, some of which exhibited activity similar to the model peptide. Thus, series II analogues incorporating turn-promoting moieties were designed with the replacement of the Phe-Gly region by aminoindane carboxylic acid (Aic), L-1,2,3,4-tetrahydroisoquino line-3-carboxylic acid (Tic), L-proline (Pro), Ala, Met, Val, and 1-aminocyclohexanecarboxylic acid to validate the hypothesis of the turn conformation in the core region.

After incorporation of turn-promoting moieties, **II1**, **II2**, **II3**, and **II4** were designed as peptidomimetics, in which the FG moiety was mimicked by Aic and L-Tic (Table 1). The phenyl is coupled by methylene in Aic and Tic. The IC_{50} value of **II1** is 10-fold less than that of the pentapeptide, whereas the bioactivity of **II2** is almost 18-fold less than that of Dippu-AST 1. Inhibition of JH biosynthesis by **II4** is 160-fold less than that of Dippu-AST 1. A dose−response curve for **II3** with respect to the inhibition of JH biosynthesis *in vitro* could not be generated because of the low levels of inhibition. Similarly, in analogue **II5**, the Phe was replaced with Pro to hold the turn conformation. The IC_{50} values of II5 could not be calculated because they showed no effect.

In earlier studies, Ala[13] and Cpa[16] were used to replace Gly. These analogues showed lower activity than their leads. In analogues **II6**−**II11**, the Gly was replaced with Pro, Ala, Met, Val, and 1-aminocyclohexanecarboxylic acid, respectively, in which residues are larger organic groups than Gly. These analogues have no effect on JH biosynthesis. The IC_{50} values of series II analogues suggest that the phenyl group in the FG region is critical to bioactivity and its position must be considered carefully in the design of analogues. The free space of the Gly residue should also remain small. If some bigger organic groups, such as Ala, Cpa, Met, Val, Pro, or 1-aminocyclohexanecarboxylic acid are used to replace Gly, the analogue should be inactive or less active.

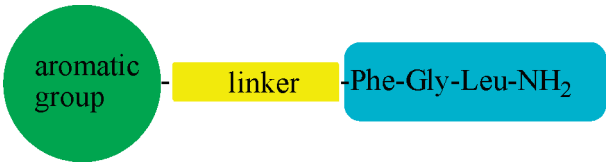


Figure 3. General approach for the design of potential AST analogues. Aromatic groups mimic the phenol and phenyl groups of the Y/F residue in the core region. The linker is the bridge between the aromatic group and the FGLa moiety.

3.4. Design and Effects of Series III. In series III, we synthesized **III1**, which shares the same sequence with Dippu-AST 1 but without the C-terminal amide to validate the function of the C-terminal amide. Bioassay showed that **III1** did not show any bioactivity on JH biosynthesis. The C terminus ($CONH_2$) was ignored in **III2**, **III3**, and **III4**. The IC_{50} values of **III2** and **III3** are less than that of Dippu-AST 1, and **III4** show no effect on the inhibition of JH biosynthesis, suggesting that the hydrogen bonds of the C terminus with the receptor are important to AST activity. Substitution of Leu with Ile, Val, Met, and Nle demonstrated little inhibition of JH biosynthesis by **III5** (<200-fold) and **III6** (<100-fold) relative to the penptapeptide, and **III7** and **III8** showed no effect on JH biosynthesis. Thus, in the potent AST analogues, the Leu-NH_2 region cannot readily be reduced.

3.5. Primary SARs of AST Analogues. When the structures and bioactivities of all of the analogues are compared to the pentapeptide and Dippu-AST 1, the primary SARs of the core region can be summarized as follows: (1) The Y/FX region can be substituted in the new potent AST analogues, and the aromatic group is able to mimic the phenyl group in the Tyr/Phe region. (2) The Phe-Gly region could also be modified, although this did not result in significant activity. (3) The C-terminal Leu-NH_2 region must appear in all new bioactive AST analogues. On the basis of our studies, we suggest a general formula for potential potent AST analogues in Figure 3. Substituent and non-substituent aryl groups mimic the phenol and phenyl groups of the Y/F residue in the core region. The linker in Figure 3 should be the bridge between the aromatic group and the FGLa moiety. Asp, Gly, and some organic segments can be the linker, but the length and most suitable structures of the linker require further study. These AST mimics should be helpful for determining which residues of the pentapeptide can be replaced by nonpeptide moieties while retaining JH inhibitory activity and for designing new potential AST analogues.

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