Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of plant cyclopeptide Astin C analogues and investigation of their immunosuppressive activity

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ARTICLE INFO

Keywords:
Astin C analogues
Solid-phase peptide synthesis
Immunosuppressive activity

ABSTRACT

To further investigate on the structure-activity relationships of immunosuppressive Astin C, seventeen analogues **1–17** were designed and synthetized *via* amino acid substitution strategy by the solid-phase peptide synthesis method for the first time. In comparison with Astin C ($IC_{50} = 12.6 \pm 3.3\,\mu M$), only compounds **2** ($IC_{50} = 38.4 \pm 16.2\,\mu M$), **4** ($IC_{50} = 51.8 \pm 12.7\,\mu M$), **5** ($IC_{50} = 65.2 \pm 15.6\,\mu M$), and **8** ($IC_{50} = 61.8 \pm 12.4\,\mu M$) exhibited immunosuppressive activity in the Lymph node cells of mice. These results showed that the Astin C analogues containing $_{D}$-amino acid residues, hydrophobic long-chain alkyl substituents, and aryl substituents performed better than those carrying hydrophilic amino acid residues and short-chain alkyl substituents. Moreover compounds **15**, **16**, and **17** had no immunosuppressive activity, which suggested that *cis*-3,4-dichlorinated proline played an important role in the immunosuppressive activity of Astin C.

In our project on unique structure and potential activity of cyclopeptides from traditional Chinese medicines (TCMs), we have found that Astins, a family of homomonocyclopeptides, named as Compositae-type cyclopeptides, were only isolated from the roots and rhizomes of *Aster tataricus* L. (Compositae)[1–4], which is one of TCMs used for relieving cough and eliminating phlegm.[5] It is noteworthy that Astins share an unusual structural similarity, which are mainly characterized by the presence of four unnatural amino acids ($_{L}$-Abu, $_{L}$-*allo*-Thr, β-Phe, and mono- or di-chlorinated $_{L}$-Pro residues) and one proteinogenic amino acid ($_{L}$-Ser).[1] So far, sixteen cyclopentapeptides (Astins A–I[1], K-P[2]) together with two unique skeleton cyclopeptides, Tataricins A and B[3] (Fig.1), have been isolated from *A. tataricus*. Previous studies have shown that Astin A, B, and C with the *cis*-3,4-dichlorinated proline residue played an important role in antitumor activity.[6,7] Cozzolino et al. have reported that synthetized antineoplastic cyclic astin analogues can kill tumor cells *via* caspase-mediated induction of apoptosis.[8] Recently we have demonstrated that Astin C could induce mitochondria-dependent apoptosis of activated T cells, and exhibited potential immunosuppressive activity *in vitro* and *in vivo*.[4] To the best of our knowledge, Astin C and its derivatives with a chlorinated proline have not been synthetized. There are only two reports about the synthesis of Astins. The first is Astin G, which was totally synthetized in 1999.[9] The second is Tataricins A and B, which were synthetized by our group in 2013.[3] However, study on the structure-immunosuppressive activity of Astin C has not been carried out to date. Herein, several amino acid substitution strategies have been conducted to modify Astin C and seventeen analogues were synthetized for the first time by the solid-phase peptide synthesis (SPPS) method. Their immunosuppressive activities were screened against Lymph node cells and the structure-activity relationships (SARs) have been discussed.

Astin C contains a cyclic pentapeptide core comprised of two $_{L}$-Abu, one $_{L}$-Ser, one β-Phe, and one strikingly *cis*-3,4-dichlorinated $_{L}$-Pro. Its structure was divided into two parts, *i.e.* substitution part and conserved sequence part (Fig. 2). Firstly, we synthetized two analogues by the SPPS method, which bear 3,4-dehydro $_{L}$-Pro in compound **15** and *cis*-3,4-dihydroxylated $_{L}$-Pro in compound **16**, to study the role of the *cis*-3,4-dichlorinated $_{L}$-Pro on the immunosuppressive activity against lymph node cells. Secondly, we synthetized other fifteen Astin C analogues **1–14** and **17** *via* amino acid substitution strategy by the SPPS method to study their SARs.

Firstly, we prepared the intermediate *cis*-3,4-diol-$_{L}$-Pro[10] **P-5a**, and the synthetic route is shown in Scheme 1. Compound **P-1** was prepared

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https://doi.org/10.1016/j.bmcl.2018.05.050
Received 27 March 2018; Received in revised form 24 May 2018; Accepted 26 May 2018
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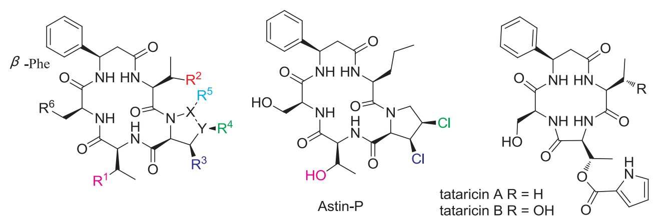


Astins	R^1	R^2	R^3	R^4	R^5	R^6	X-Y
Astin-A	H	OH	Cl	Cl	H	OH	CH-CH
Astin-B	OH	H	Cl	Cl	H	OH	CH-CH
Astin-C	H	H	Cl	Cl	H	OH	CH-CH
Astin-D	H	H	H	H	Cl	OH	C=C
Astin-E	OH	H	H	H	Cl	OH	C=C
Astin-F	H	H	Cl	H	H	OH	CH-CH
Astin-G	H	H	H	H	H	OH	CH-CH
Astin-H	H	OH	H	H	Cl	OH	C=H
Astin-I	H	H	OH	Cl	H	OH	CH-CH
Astin-K	OH	OH	Cl	Cl	H	OH	CH-CH
Astin-L	OH	OH	H	H	Cl	OH	C=C
Astin-M	H	H	H	Cl	H	OH	CH-CH
Astin-N	H	H	H	Cl	H	OH	C=C
Astin-O	H	H	Cl	Cl	H	OAc	CH-CH
Astin-Q	H	H	OH	H	H	OH	CH-CH

Fig. 1. Structures of Astins family.

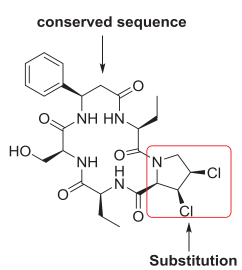
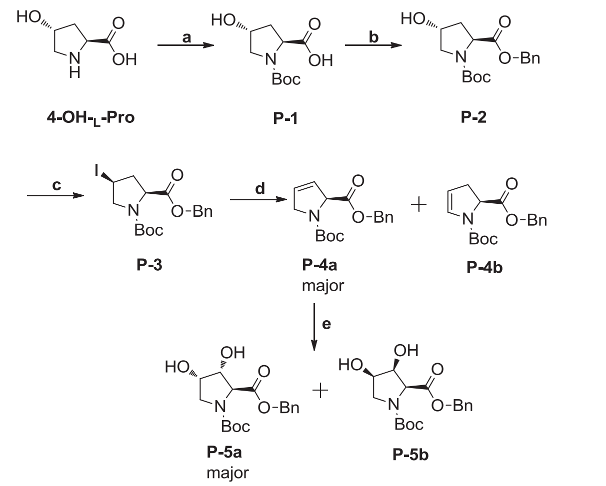


Fig. 2. Substitution of the structure of Astin C.

starting with commercially available *trans*-4-hydroxy-L-proline and Boc_2O in the presence of $NaHCO_3$ in dioxane/H_2O, which reacted with BnBr in DMF to give compound **P-2** in excellent yield. By using Appel-Lee reaction, compound **P-2** was successfully converted to compound **P-3** in 97% yield. Subsequently, elimination of compound **P-3** with DBU in ACN afforded compound **P-4a** as a major product, which is oxidized by $K_2OsO_2(OH)_4$ to the diol **P-5a** as the major product.

With 3,4-dehydro-L-Pro **P-4a** and *cis*-3,4-diol-L-Pro **P-5a** in hand, *cis*-3,4-dichlorinated L-Pro in Astin C was then modified. As depicted in Scheme 2, *cis*-3,4-dichlorinated L-Pro was substituted by Ala (Ala scan[11]), **P-4a**, and **P-5a**, respectively, and compounds **14**, **15**, and **16** were obtained by the SPPS method similar to Scheme 3. In addition, cyclotetrapeptide **17** was prepared by cutting the *cis*-3,4-dichlorinated proline residue.

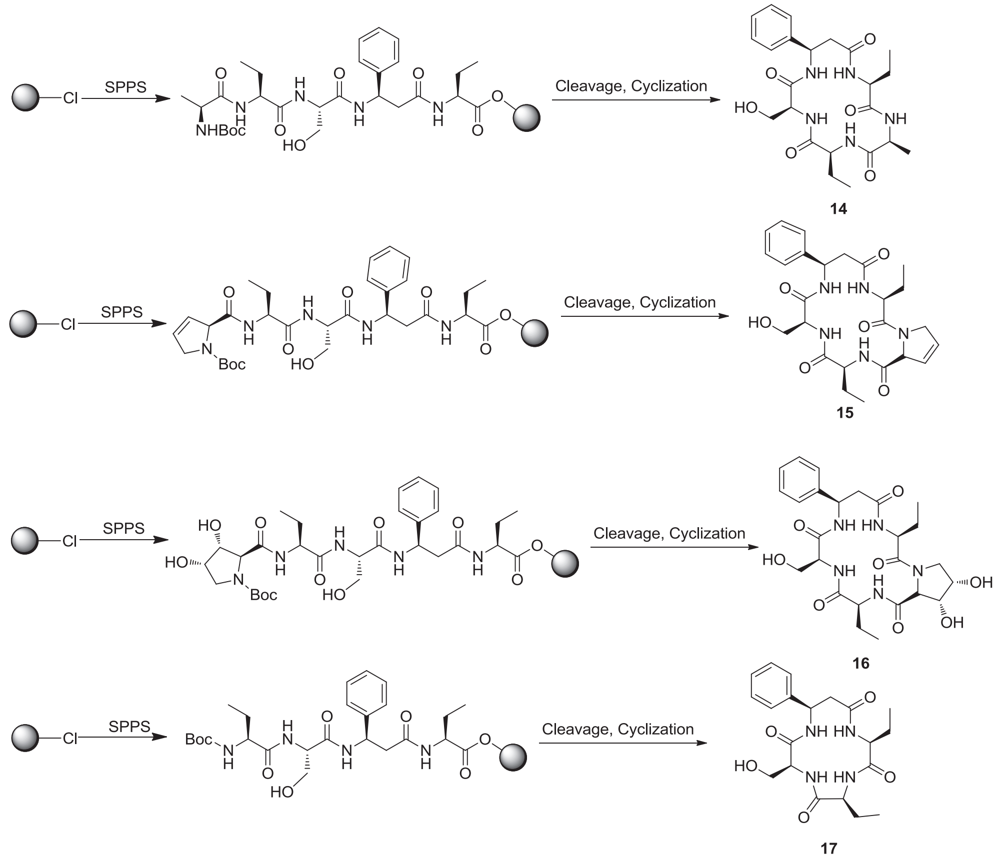


Scheme 1. Reagents and conditions: (a) Boc_2O, $NaHCO_3$, dioxane : H_2O = 1 : 1, rt, 24 h, 86%; (b) BnBr, K_2CO_3, DMF, rt, overnight, 92%; (c) Ph_3P, I_2, imidazole, DCM, N_2, reflux, 12 h, 97%; (d) DBU, ACN, reflux, overnight, 78%; (e) $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH : H_2O = 1 : 1, rt, 24 h, 83%.

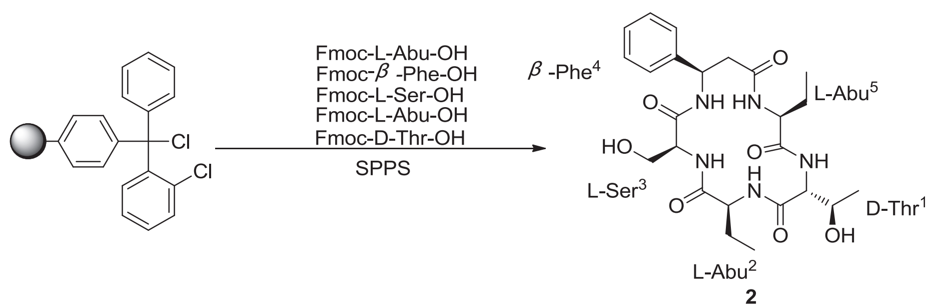
Next, solid-phase synthesis of other Astin C analogues were carried out by substituting the *cis*-3,4-dichlorinated proline residue with various amino acids. Considering that Astins is all consisted of L-amino acid, we firstly introduced D-Thr and D-*allo*-Thr to synthetize Astin C analogues **2** and **4**. Moreover, hydrophobic amino acid residues (L-Ada, L-Val, L-Leu, *β*-Phe, L-Tyr) and hydrophilic amino acid residues (L-Thr, L-*allo*-Thr, L-Lys, L-Arg, L-His, (2*S*, 3*S*)-3-O-(pyrrole-2-carbonyloxy)-*allo*-Thr) were also introduced to prepare Astin C analogues **1**, **3**, and **5–13**, respectively. Because of the same sequence of linear tetrapeptide with L-Abu[5]-*β*-Phe[4]-L-Ser[3]-L-Abu[2] in **1–16**, we designed to cyclize at the Abu[5]/substituted amino acid[1] site. As an example, the synthetic route of compound **2** was shown in Scheme 3.

Compound **2** was synthetized using the Fmoc-Abu-CTC Resin. Fmoc group protecting amino acids were successively coupled by HBTU and DIPEA in DMF. 20% piperidine/DMF was used for deblocking. The reaction was monitored by ninhydrin color reaction. After the last step, the peptide resin was washed with DMF (three times), and MeOH (three times), then dried by N_2 bubbling over night. TFA in DCM was added to the flask containing the peptide resin to release the crude linear peptide from the resin. Cyclization was accomplished by using HATU and DIPEA. The progress of the cyclization was monitored by LC-MS. The solvent was removed under reduced pressure and the crude peptide was purified by Prep-HPLC to get the final product **2**.[12] Similarly, compounds **1**, **3–13** were prepared by the above synthetic method.

With Astin C and seventeen analogues **1–17** in hand (Fig. 3), we have screened immunosuppressive activity against lymph node cells, in which CsA was used as the positive control. As shown in Table 1, Astin C shows potential immunosuppressive activity. Compared with Astin C (IC_{50} = 12.6 ± 3.3 μM), compound **2** (IC_{50} = 38.4 ± 16.2 μM) with D-Thr residue and compound **4** (IC_{50} = 51.8 ± 12.7 μM) with D-*allo*-Thr residue showed comparable immunomodulatory activity, which opened doors to us that the introduction of D-amino acids is a moderate strategy. On the other hand, compound **5** (IC_{50} = 65.2 ± 15.6 μM) with hydrophobic long-chain alkyl substituent and compound **8** (IC_{50} = 61.8 ± 12.4 μM) with *β*-Phe residue (aryl substituent) also exhibit comparable immunosuppressive activity. Unfortunately, we



Scheme 2. Solid-phase synthesis of compounds **14**, **15**, **16**, and **17**.



Scheme 3. Solid-phase synthesis of compound **2**.

found that all of hydrophilic L-amino acid residues were inactive (IC_{50} > 100), including compounds **1**, **3**, and **10–13**. These results show that the Astin C analogues containing D-amino acid residues, hydrophobic long-chain alkyl substituents, and aryl substituents performed better than those carrying hydrophilic amino acid residues and short-chain alkyl substituents. To further explore the role of the *cis*-3,4-dichlorinated L-Pro, we synthetized compounds **14–17**, which resulted in loss of activity against lymph node cells (IC_{50} > 100), suggesting that the significance of *cis*-3,4-dichlorinated proline to the immunosuppressive activity.

In summary, seventeen Astin C analogues **1–17** were designed and synthesized by the SPPS method and their immunosuppressive activity was evaluated against lymph node cells for the first time. The results indicated that compounds **2**, **4**, **5**, and **8** exhibited immunosuppressive activity. Analysis of the structure-activity relationships indicated that D-amino acid residues, hydrophobic long-chain alkyl substituents, and aryl substituents might act as a guideline for the further modification of Astin C. Moreover, we found that *cis*-3,4-dichlorinated proline plays an

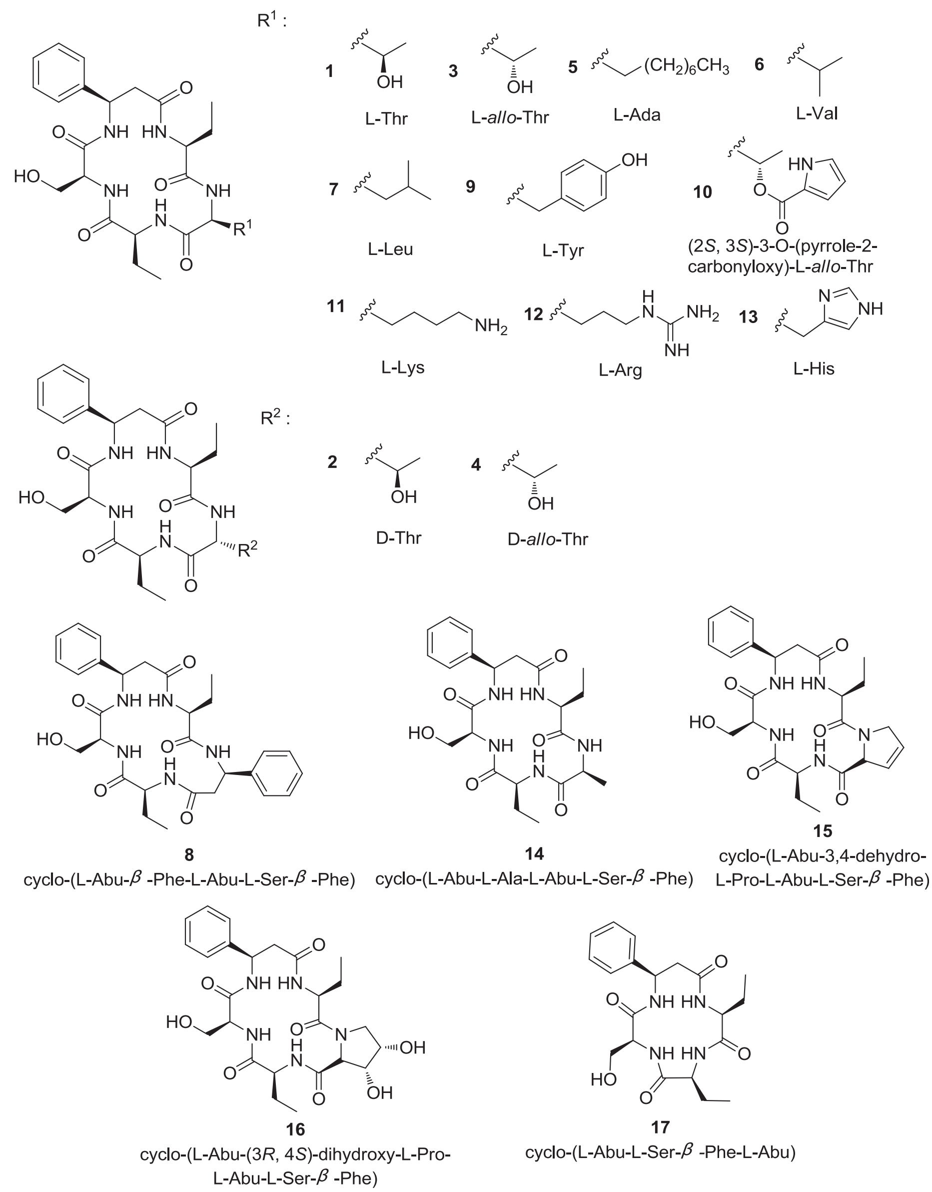


Fig. 3. Structures of Astin C analogues **1**–**17**.

Table 1
Screening of immunosuppressive activity.

Compunds	IC_{50} (mean ± SD, μM)
Astin C	12.6 ± 3.3
1	> 100
2	38.4 ± 16.2
3	> 100
4	51.8 ± 12.7
5	65.2 ± 15.6
6	> 100
7	> 100
8	61.8 ± 12.4
9–17	> 100
CsA	0.5 ± 0.04

CsA was used as the positive control.

important role in the immunosuppressive activity against lymph node cells. This work will be help for exploring the structure-activity relationships of Astin C to design better immunosuppressive compounds in the future.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31470428), the National New Drug Innovation Major Project of China (2017ZX09309027), the Fund of Chinese Academy of Sciences (XDA09030301-4, Hundred Talents Program), the Program for Jiangsu Province Innovative Research Team, and the Fund for Introduction of High-level Talents from China Pharmaceutical University.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2018.05.050.

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12. Data for **2**: ^{1}H NMR (600 MHz, C_5D_5N) δ: 10.38 (1 H, brs), 10.12 (1 H, brs), 8.99 (1 H, d, *J* = 6.1 Hz), 8.63-8.59 (2 H, overlap), 7.58 (2 H, overlap), 7.23-7.21 (2 H, overlap), 7.15 (1 H, t, *J* = 7.3 Hz), 5.41 (1 H, brs), 5.26-5.23 (1 H, m), 5.00-4.97 (1 H, overlap), 4.80 (1 H, dd, *J* = 8.2, 5.2 Hz), 4.76-4.72 (1 H, m), 4.65-4.60 (1 H, m), 4.53 (1 H, dd, *J* = 11.0, 5.9 Hz), 4.48 (1 H, dd, *J* = 10.8, 4.7 Hz), 3.84 (1 H, t, *J* = 12.2 Hz), 2.97 (1 H, dd, *J* = 12.5, 2.7 Hz), 2.34-2.30 (1 H, m), 1.98-1.95 (1 H, m), 1.87-1.83 (1 H, m), 1.79-1.74 (1 H, m), 1.58 (3 H, d, *J* = 6.2 Hz), 1.06 (3 H, t, *J* = 7.4 Hz), 0.91 (3 H, t, *J* = 7.4 Hz); ^{13}C NMR (150 MHz, C_5D_5N) δ: 175.5, 174.5, 173.2, 173.0, 171.6, 142.1, 128.9, 127.8, 127.4, 66.8, 63.8, 62.9, 57.1, 56.3, 55.7, 54.5, 40.2, 25.1, 24.9, 21.6, 11.7, 11.2; IR (KBr) νmax 3423, 3299, 2969, 2932, 1646, 1538, 1383, 1064, 699, 586 cm^{-1}; $[\alpha]^{D}$21 -41.7 (c 0.10, MeOH); HRMS(ESI) calcd for $C_{24}H_{35}N_5NaO_7$ $[M+Na]^+$: 528.2434; found: 528.2437.