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Aza-cycloisodityrosine analogue of RA-VII, an antitumor bicyclic hexapeptide

Yukio Hitotsuyanagi, Akihiro Miyazawa, Taka-aki Hinosawa, Yoshie Nakagawa, Tomoyo Hasuda, Koichi Takeya *

School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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ABSTRACT

An aza-cycloisodityrosine analogue of RA-VII, **3**, was designed and synthesized. The key aza-cycloisodityrosine unit was prepared by copper(II)-acetate-mediated intramolecular phenylamine/arylboronic acid coupling of dipeptide followed by connection with the tetrapeptide segment to afford a hexapeptide. Subsequent macrocyclization of the hexapeptide with EDC·HCl and HOOBt under dilute conditions gave **3**. Analogue **3** showed significant cytotoxic activity against human promyelocytic leukemia HL-60 cells and human colon carcinoma HCT-116 cells, but its activity was weaker than that of parent peptide RA-VII (**1**).

RA-VII (**1**) is a bicyclic hexapeptide isolated from *Rubia cordifolia* L. and *Rubia akane* Nakai (Rubiaceae).[1,2] Its related peptide, bouvardin (NSC 259968, **2**), was isolated from *Bouvardia ternifolia* (Cav.) Schltdl. (Rubiaceae).[3] Both structures are characterized by the incorporation of a unique and strained 14-membered cycloisodityrosine unit in the molecule (Fig. 1). These peptides have promising antitumor activity and peptide **1** had previously undergone phase I clinical trials as an anticancer drug in Japan.[4] The antitumor activity of these peptides is believed to be due to the inhibition of protein synthesis through the interaction with eukaryotic ribosomes.[5,6] Peptide **1** has been shown also to induce conformational changes in F-actin, stabilizing actin filaments and inducing G2 arrest.[7] In this Letter, we describe the synthesis of a novel analogue of RA-VII (**1**) where the diphenyl ether oxygen of the cycloisodityrosine moiety in **1** is replaced by an amine nitrogen, and the evaluation of its cytotoxicity.

In the RA series of peptides, the cycloisodityrosine moiety is considered essential for the expression of the cytotoxic activity. This moiety not only affects the conformation of the 18-membered macrocycle of the peptides,[8,9] but also appears to participate in the expression of the activity.[10,11] Thus, we sought to investigate the effect of the electron density of the phenyl rings of the isodityrosine moiety on the cytotoxic activity. The electron density of an aromatic compound is often modulated by introducing an electron-donating or an electron-withdrawing group on the aromatic ring. A survey of a limited number of RA analogues having a substituent on the aromatic rings of the isodityrosine moiety revealed that the introduction of a hydroxy group at the εa or the εb position of Tyr-5[12] or at the δa or the εa position of Tyr-6[13] reduces the cytotoxic activity of RA-VII (**1**). Although such modification increases the electron density of the phenyl ring to which the hydroxy group is attached, it is not clear whether the reduction of the activity is due to changes in the electron density of the phenyl rings, the steric bulkiness of the introduced hydroxy group, and/or the polarity of the hydroxy group. It is also known that in RA-VII (**1**), the introduction of a hydroxy group at either the εa or the εb position of the Tyr-5 residue affects the orientation of the cycloisodityrosine phenyl ring by forming a hydrogen bond between the hydroxy proton and the methyl ether oxygen of Tyr-6.[12] Those conformational changes in the cycloisodityrosine moiety may be responsible for the reduced activity of those analogues. To obtain information about the effects of the electron density of the cycloisodityrosine phenyl rings in peptide **1** on the cytotoxic activity, we designed an RA-VII analogue in which the diphenyl ether oxygen of the cycloisodityrosine moiety was replaced by an amine nitrogen. We considered that this modification would increase the electron density of the phenyl rings of both Tyr-5 and Tyr-6 residues with minimal structural changes, as the steric demand between **1** and **3** is expected to be almost the same. Comparison of the energy-minimized structure of analogue **3** obtained by the Monte Carlo conformational search[14] with the X-ray crystal structure of RA-VII (**1**)[11] indicated that their 3D structures were very similar and almost superimposable (Fig. 2).

* Corresponding author. Tel.: +81 42 676 3007; fax: +81 42 677 1436.
E-mail address: takeyak@ps.toyaku.ac.jp (K. Takeya).

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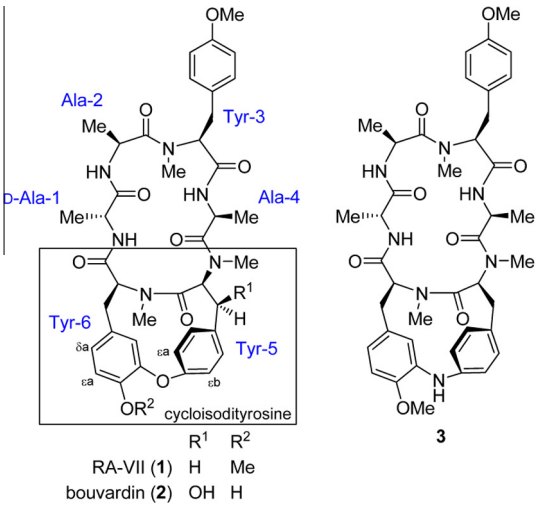


Figure 1. Structures of RA-VII (**1**), bouvardin (**2**), and analogue **3**.

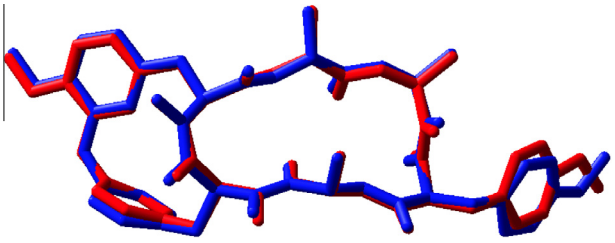
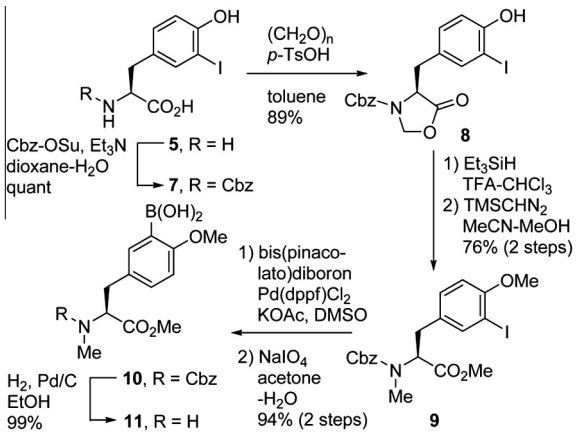
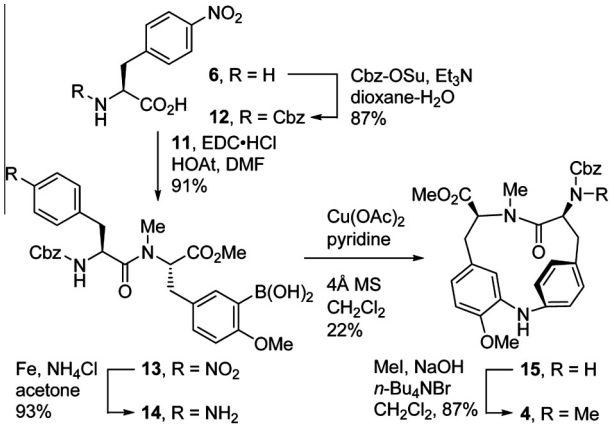


Figure 2. Superposition of the crystal structure of RA-VII (**1**, red) and the energy-minimized structure of analogue **3** (blue).



Scheme 1. Synthesis of boronic acid **11**.



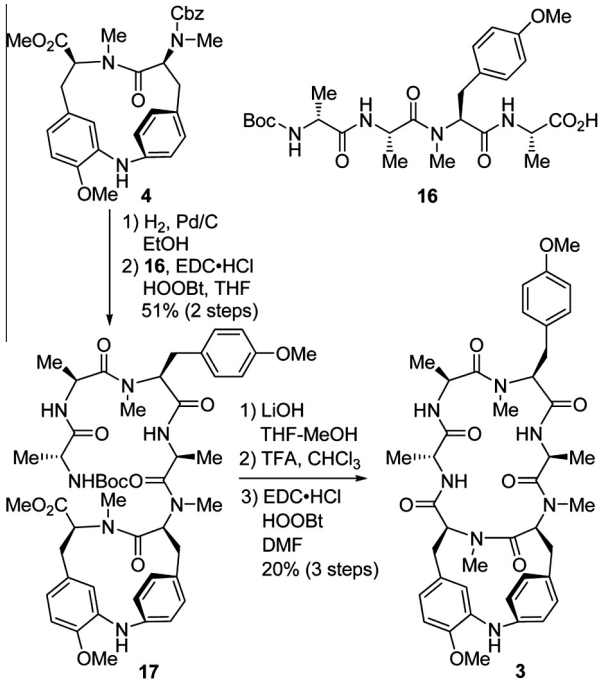
Scheme 2. Synthesis of aza-cycloisodityrosine **4**.

We synthesized analogue **3** by drawing on our previous successful synthesis of related compounds, which is characterized by the construction of the core cycloisodityrosine unit by the copper(II)-acetate-mediated intramolecular phenol/arylboronic acid coupling and the macrocyclization of the linear hexapeptide between the Tyr-6 and D-Ala-1 residues to construct the 18-membered cyclopeptide ring.[11] Key aza-cycloisodityrosine unit **4**, which corresponds to the Tyr-5 and Tyr-6 residues of **3**, was synthesized from commercially available chiral amino acids, 3-iodo-L-tyrosine (**5**) and 4-nitro-L-phenylalanine (**6**) (Schemes 1 and 2). Amino acid **5** was N-protected by a Cbz group (**7**) and then treated with paraformaldehyde in the presence of a catalytic amount of *p*-toluenesulfonic acid in toluene to give oxazolidinone **8** (Scheme 1). Reduction of **8** with triethylsilane in a trifluoroacetic acid (TFA)–chloroform mixture and subsequent treatment of the resulting carboxylic acid with (trimethylsilyl)diazomethane in MeCN–MeOH gave *N*-methyltyrosine derivative **9**. The iodine atom of **9** was substituted by a pinacolatoboron group by bis(pinacolato)diboron in the presence of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$) catalyst and KOAc in dimethyl sulfoxide (DMSO), and subsequent treatment of the crude boronic acid pinacol ester with sodium periodate in acetone–H_2O gave boronic acid **10** in excellent yield. Removal of the Cbz group of **10** gave amine **11**.

N-protected 4-nitro-L-phenylalanine **12**, derived from **6**, was coupled with amine **11** using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxy-7-azabenzotriazole (HOAt) in *N*,*N*-dimethylformamide (DMF) to afford dipeptide **13** (Scheme 2). Reduction of the nitro group of **13** with iron powder and ammonium chloride in acetone gave amine **14**, which was then treated with copper(II) acetate and pyridine in the presence of 4 Å molecular sieves (MS) in dichloromethane[15] to give desired aza-cycloisodityrosine **15** in 22% yield. Use of 4-(dimethylamino)pyridine or triethylamine in lieu of pyridine as the additive amine did not improve the yield of **15**, although the former amine significantly enhanced the yield of the C–O coupling product in the cycloisodityrosine synthesis.[16] Treatment of compound **15** with iodomethane and powdered NaOH in CH_2Cl_2 under phase-transfer catalysis (*n*-Bu_4NBr) conditions selectively methylated the N-terminus carbamate nitrogen to give **4**.

After removal of the Cbz group, compound **4** was coupled with tetrapeptide carboxylic acid **16**, corresponding to residues 1–4 of **3**, using EDC·HCl and 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOOBt) to provide hexapeptide **17** in 51% yield (Scheme 3). The methyl ester group at the C-terminus and the Boc group at the N-terminus of peptide **17** were sequentially removed by alkaline hydrolysis followed by treatment with TFA. The resulting deprotected hexapeptide was subjected to macrocyclization with EDC·HCl and HOOBt under dilute conditions (0.0012 M) in DMF at room temperature for 3 days to afford analogue **3**[17] in 20% yield from **17**.

The solution structure of analogue **3** was analyzed by NMR spectroscopy. The ^1H NMR spectrum of analogue **3** was very similar to that of **1** (Fig. 3 and see Supplementary data Table S1).[17] The



Scheme 3. Synthesis of analogue **3**.

^{1}H NMR spectrum of analogue **3** in chloroform-*d* at 300 K demonstrated that it consisted of two conformers in a 91:9 ratio. In the NOESY spectrum, the major conformer (population 91%) showed NOE correlations between D-Ala-1 H_α/Ala-2 NH, D-Ala-1 H_α/Ala-4 $H_{3\beta}$, D-Ala-1 $H_{3\beta}$/Ala-2 NH, D-Ala-1 NH/Tyr-6 H_α, Ala-2 H_α/Tyr-3 NMe, Tyr-3 H_α/Tyr-3 NMe, Tyr-3 H_α/Ala-4 NH, Ala-4 H_α/Tyr-5 NMe, Ala-4 $H_{3\beta}$/Tyr-5 NMe, and Tyr-5 H_α/Tyr-6 H_α (see Supplementary data Fig. S1). These correlations are characteristic of the major conformer of peptide **1** in solution, adopting a *cis* amide configuration between Tyr-5 and Tyr-6 and a *trans* amide configuration at the other five amide bonds and thus adopting the peptide backbone conformation as depicted in the crystal structure of RA-VII (**1**) in Figure 2.[8] Accordingly, the structure of the major conformer of **3** was proved to be almost identical to that of the corresponding conformer of **1** in solution, as expected. The structure of the minor conformer (population 9%) could not be determined due to weak signal intensities.

RA-VII (**1**) and analogue **3** were evaluated for their cytotoxic activity against human promyelocytic leukemia HL-60 cells and human colon carcinoma HCT-116 cells.[18] Their IC_{50} values on HL-60 cells were 0.0025 and 0.018 μg/mL, respectively, and those on HCT-116 cells were 0.0033 and 0.017 μg/mL, respectively. These results indicated that as RA-VII (**1**) and its more electron-rich aza-analogue **3**, which is 5–7 times less cytotoxic than **1**, possess almost the same 3D structures, enhancement of the electron density of the phenyl rings in Tyr-5 and Tyr-6 may reduce the cytotoxic activity of this series of peptides.

Acknowledgment

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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.2013.10.033.

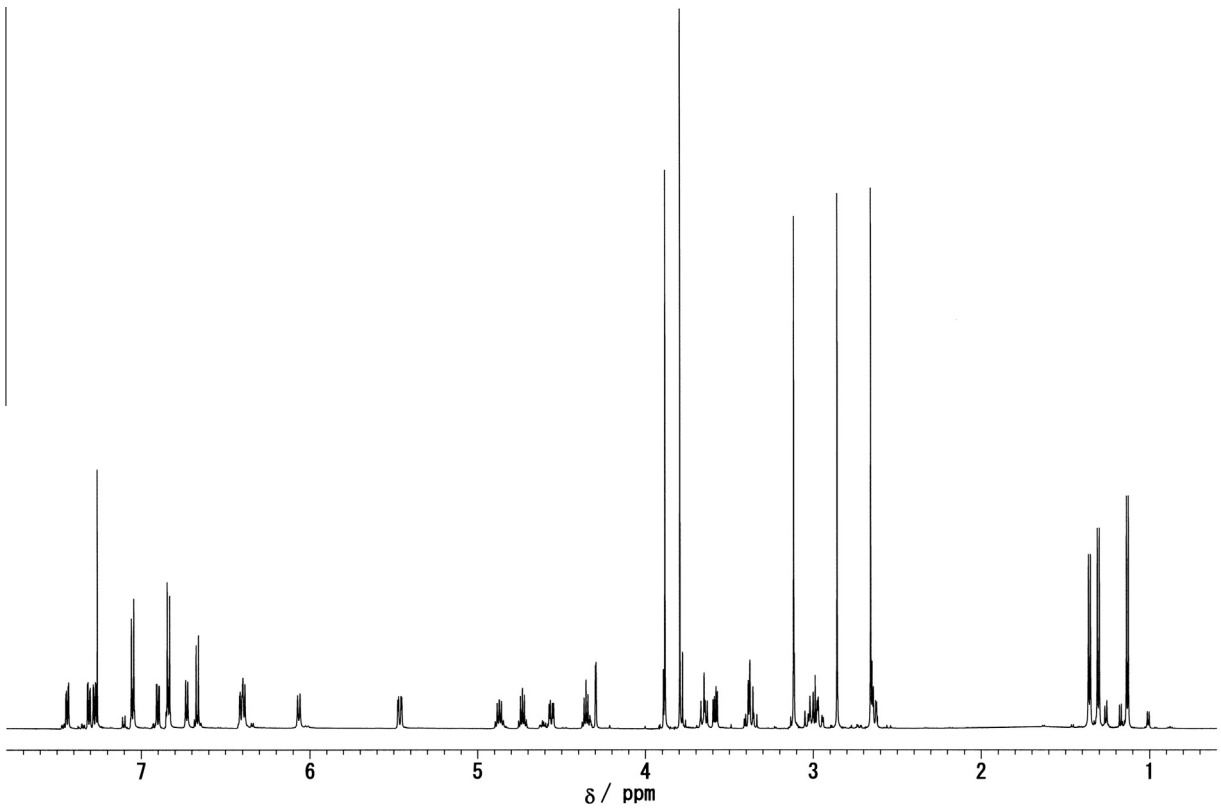

Figure 3. ^{1}H NMR (600 MHz) spectrum of analogue **3**.

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14. The software used for this calculation was MacroModel ver. 7.0 (Schrödinger Inc.). The Monte Carlo (MC) search was configured using the 'automatic setup' routine in MacroModel with the amide configuration between Tyr-5 and Tyr-6 fixed as *cis* and the other amide configurations in the macrocycle fixed as *trans*. The calculation consisted of 50,000 MC steps with 1000 iterations per step using the MMFFs force field and the PR conjugate gradient with no solvation.
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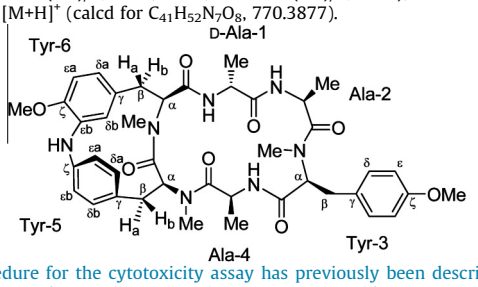
17. *Data of* ***3***: Colorless, amorphous solid; $[\alpha]_D^{25}$ −189 (MeOH, *c* 0.12); IR (film) ν_{max} 3308, 2926, 1660, 1632, 1514 cm^{-1}; NMR (600 MHz for ^1H and 150 MHz for ^{13}C, chloroform-*d*, 300 K, major conformer) D-Ala-1 (α, δ_H 4.36/δ_C 47.9; β, δ_H 1.31 (3H)/δ_C 20.7; C=O, δ_C 172.2; NH, δ_H 6.39), Ala-2 (α, δ_H 4.87/δ_C 44.5; β, δ_H 1.36 (3H)/δ_C 16.7; C=O, δ_C 172.5; NH, δ_H 6.07), Tyr-3 (α, δ_H 3.59/δ_C 68.4; βa,b, δ_H 3.39, 3.36/δ_C 32.7; γ, δ_C 130.8; δ, δ_H 7.05 (2H)/δ_C 130.2 (2C); ε, δ_H 6.84 (2H)/δ_C 114.0 (2C); ζ, δ_C 158.4; C=O, δ_C 168.0; NMe, δ_H 2.86 (3H)/δ_C 39.7; OMe, δ_H 3.80 (3H)/δ_C 55.3), Ala-4 (α, δ_H 4.73/δ_C 46.4; β, δ_H 1.13 (3H)/δ_C 18.5; C=O, δ_C 171.7; NH, δ_H 6.73), Tyr-5 (α, δ_H 5.47/δ_C 54.1; βa,b, δ_H 2.64, 3.65/δ_C 37.0; γ, δ_C 136.1; δa, δ_H 7.28/δ_C 132.7; δb, δ_H 7.44/δ_C 130.7; εa, δ_H 6.90/δ_C 130.4; εb, δ_H 7.31/δ_C 132.1; ζ, δ_C 145.0; C=O, δ_C 169.2; NMe, δ_H 3.12 (3H)/δ_C 30.5), Tyr-6 (α, δ_H 4.56/δ_C 57.4; βa,b, δ_H 3.02, 2.96/δ_C 36.1; γ, δ_C 128.1; δa, δ_H 6.41/δ_C 118.5; δb, δ_H 4.30/δ_C 112.3; εa, δ_H 6.67/δ_C 110.0; εb, δ_C 142.3; ζ, δ_C 145.8; C=O, δ_C 170.9; NMe, δ_H 2.66 (3H)/δ_C 29.3; OMe, δ_H 3.88 (3H)/δ_C 55.5); HR-ESI-MS: *m*/*z* 770.3892 $[M+H]^+$ (calcd for $C_{41}H_{52}N_7O_8$, 770.3877).



18. The procedure for the cytotoxicity assay has previously been described, see: Inaba, Y.; Hasuda, T.; Hitotsuyanagi, Y.; Aoyagi, Y.; Fujikawa, N.; Onozaki, A.; Watanabe, A.; Kinoshita, T.; Takeya, K. *J. Nat. Prod.* **2013**, *76*, 1085.