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CHIRAL REQUIREMENTS FOR TUMOR PROMOTERS: CONFORMATIONS AND ACTIVITY OF BENZOLACTAMS

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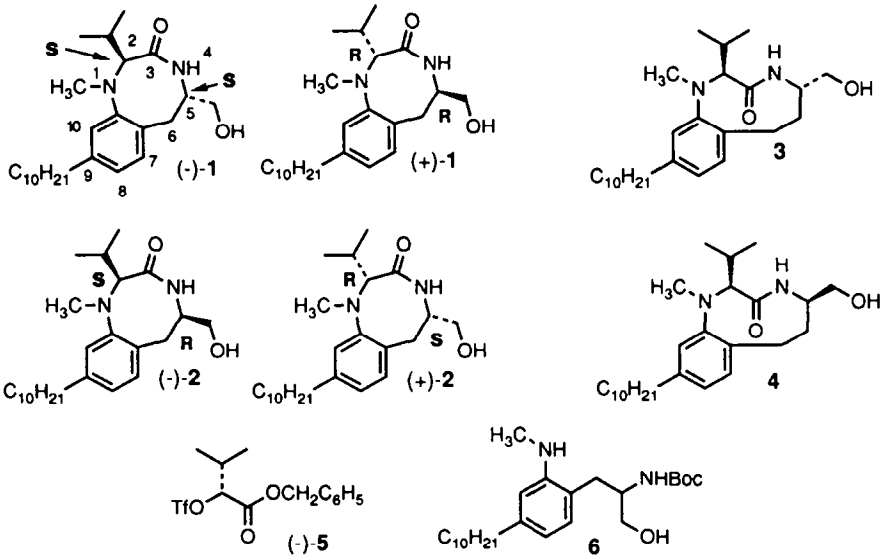
Abstract: (-)-Benzolactam-V8-310 ((-)-**1**), which reproduces the twist conformation and biological activity of teleocidins, its antipode ((+)-**1**), and the epi-benzolactams ((-)-**2** and (+)-**2**) were synthesized as optically pure forms. Structure-activity results indicate that the stereochemistry at C-5 plays a critical role in the binding to the receptor(s).

Teleocidins, typical tumor promoters,[1] exist in an equilibrium in solution between at least two conformational states of the 9-membered lactam ring, the twist and sofa form.[2] The conformational equilibrium is mainly attributed to a *cis-trans* isomerization of the amide bond. Restriction of the equilibrium would be helpful for analyzing the structures and activities of several classes of TPA-type tumor promoters with various skeletal structures.[3] Recently, we have reported the synthesis and biological activity of the twist-restricted racemic benzolactam-V8-310 (**1**) and the sofa-restricted racemic benzolactam-V9-310 (**3**), as well as their epimers (epi-benzolactam-V8-310 (**2**) and epi-benzolactam-V9-310 (**4**)).[4] The potency of these benzolactams for induction of differentiation of human promyelocytic leukemia cells (HL-60), decreased in the order of teleocidin B-4 > **1** > **2** = indolactam-V > **4** > **3**. The strong activity of **1** and lack of activity of **3** clearly indicated that the twist form is close to the active conformation for tumor promoting-activity of teleocidins.[4] Compound **1** was 30 times more potent than **2**, while **2** was almost as active as indolactam-V. Interpretation of the considerable activity of the epimer **2** requires a detailed examination of the conformational situation of **2**.[5] We report here the synthesis and biological activity of optically active **1** and the epimer **2**. Conformational requirements are also discussed on the basis of the structure-activity study.

The optically active benzolactams and epi-benzolactams were synthesized as described for the racemic benzolactams[4] using triflate of benzyl R- or S-α-hydroxyisovalerate (**5**)[6] with N-Boc-2-methylamino-4-decyl phenylalaninol (**6**). The reaction of **6** with R-**5** followed by the procedure reported in the previous paper[4] gave (-)-**1** ($[\alpha]^{25}_D$ = -278.2° c= 0.64, $CHCl_3$) and (-)-**2** ($[\alpha]^{25}_D$ = -140.3° c= 0.75, $CHCl_3$). Similarly, S-**5** was converted into (+)-**1** ($[\alpha]^{25}_D$ = +280.3° c= 0.61, $CHCl_3$) and (+)-**2** ($[\alpha]^{25}_D$ = +137.1° c=0.70, $CHCl_3$).

The benzolactams were evaluated in a standard [^{3}H]TPA competitive binding assay to the protein kinase C regulatory domain.[7] Only, the (-)-isomer of **1** (1000 fold excess), but not (+)-**1**, showed significant inhibition of [^{3}H]TPA binding (59 %). In the case of epi-benzolactams (**2**), the (+)-isomer of **2** (1000 fold) showed 18 % inhibition. The binding was not inhibited in the presence of 1000 fold excess of (+)-**1** or (-)-**2**. The TPA-type tumor promoters are known to induce growth inhibition, cell adhesion and differentiation of HL-60 cells.[8,9]

The benzolactam (-)-**1** caused a pronounced growth inhibition and monocytic differentiation of HL-60 cells to monocyte-like cells at the concentration of 10^{-8} M. The epi-benzolactam (+)-**2** was similarly effective at the concentration of $5x10^{-7}$ M. On the contrary, the enantiomers (+)-**1** and (-)-**2** proved to be inactive below the concentration of 10^{-5} M. The results indicate that the stereochemistry at C-5 plays an important role in the appearance of the biological activity, and the preferred absolute stereochemistry is S.



The simplest and most plausible assumption is that the stable conformations, the twist form of (-)-**1** and the r-cis-sofa form of (+)-**2**, interact with the macromolecular receptor without conformational conversion. The relative atomic positions of the amide bond and hydroxymethyl group of the r-cis-sofa form of (+)-**2** (Figure 1.**B**) are possibly arranged similarly to those of the twist form of (-)-**1** (Figure 1.**A**), in spite of the difference of the C-2 stereochemistry. The distinction between the twist (**A**) and the r-cis-sofa (**B**) is in the position of N-1 and the dihedral angles between the amide and aromatic ring planes. The difference of activity between (-)-**1** and (+)-**2** should be interpreted in terms of the difference in overall shapes of the molecules. Although the benzolactams and epi-benzolactams each showed a single set of signals in ^{1}H-NMR even at -70°C, it is impossible to eliminate the equilibrium without *cis-trans* isomerization because of the low energy barrier between the conformers.[10] The other conformations were observed in solution as well as in the crystals in the case of indolactams (i.e., in the case of indolactam-G, the fold form).[11] From the viewpoint of the conformational conversion, it is possible that the active conformation of (+)-**2** is also the twist form, since the conversion of the r-cis-sofa form of (+)-**2** to the twist form does not involve a high energy barrier. We can not rule out the possibility that the fold form is the active conformation, since conversion of the most stable conformations of (-)-**1** and (+)-**2** to the fold forms is possible.[12]

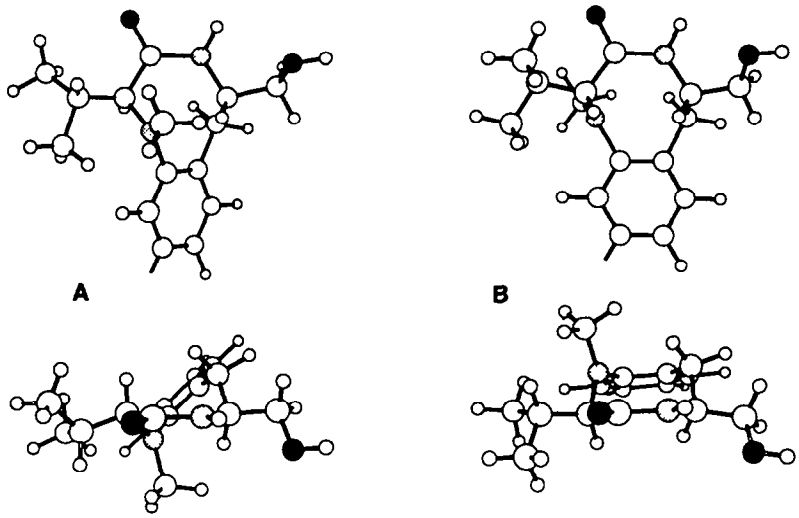


Figure 1. Stable Conformations of (-)-**1** (**A**: twist form) and (+)-**2** (**B**: r-cis-sofa form)
Upper: Views from the face of the amide plane. Lower: Views from the side of the amide plane.
The alkyl side chain is omitted for the sake of clarity.

Therefore, we examined all possible ring conformations of these benzolactams by high temperature molecular dynamics (HTMD) calculations.[13] From the 100 picosecond MD calculation at 3000K using the AMBER program,[14] 2000 structures of every 0.05 picosecond were subjected to energy minimization and were classified into 10 ring conformations in a similar manner to the case of indolactams.[15] Among the 10 conformations, six structures have a *cis*-amide bond and the remaining four have a *trans*-amide bond. In the case of (-)-**1**, the energy difference between the most stable twist conformation and the sofa form which was the most stable among the *trans*-amide conformations was more than 6 kcal/mol. The six *cis*-amide conformations involving the twist form may be candidates for the active conformation, because conversions among the *cis*-amide conformations proceed through relatively low energy barriers (<10 kcal/mol) in contrast to the high barriers between *cis-trans* conversion (>20 kcal.mol). Among the six *cis*-amide conformations of (-)-**1**, the solution conformation (the twist form) coincides with the most stable conformation, but the energy differences between the twist form and the three other conformations (the cis-sofa, fold and r-cis-sofa forms) are within 1.6 kcal/mol. On the other hand, in the case of (+)-**2**, the energy differences between the most stable r-cis-sofa form and the next most stable conformations (the twist, fold and r-twist forms) are greater than 4.5 kcal/mol. The remarkable stability of the r-cis-sofa form of (+)-**2**, and the difference of the activity between (-)-**1** and (+)-**2** suggested that the two compounds interact with the macromolecular receptor as twist form and r-cis-sofa form, respectively.

Thus, the synthesis of four stereoisomers of benzolactams has indicated the importance of the absolute stereochemistry and the twist conformation of benzolactam for the activity. Synthesis of molecules in which the conformation is fixed (strictly restricted) or molecules having different skeletons with rigid conformations might allow us to identify the active conformation on the receptor. The present findings should be helpful in the design of such molecules and the analysis of the active conformation in which teleocidins bind to the receptors.

References and Notes

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4. Ohno, M., Endo, Y., Hirano, M., Itai, A., Shudo, K., *Tetrahedron Lett.* in press.
5. The conformational structures of the epi-benzolactams **2** and **4** were deduced from ^{1}H-NMR spectral data and nuclear Overhauser effect (NOE) experiments. The two epi-benzolactams were each proved to exist in a single conformational state in solution. The spectral data of **2** and **4** were similar to those of epi-indolactam-V, which exists predominantly in the cis-sofa form (r-cis-sofa form for the enantiomer) in solution as well as in the crystalline state.[10] The r-cis-sofa form of the 2S,5S-isomer of **2** (shown in Figure. 1) and the cis-sofa form of the enantiomer, 2R,5R-isomer, of **2** are characterized by a *cis* amide bond facing the outside of the molecule, the planes of the amide and aromatic ring being parallel. In both **2** and **4**, NOE enhancements (above 15 %) between the H-2 and H-5 protons confirmed the cis-sofa structure. The NOE enhancements might also be observed if the conformation were the fold form. However, the absence of high field shift of the 4-NH signal (6.00 ppm) is consistent with the cis-sofa form.
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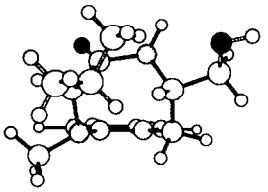
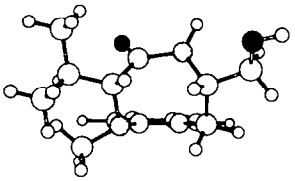
12. The relative atomic positions of the amide bond and hydroxymethyl group of the fold form of (+)-**2** are close to those of the fold form of (-)-**1**. The figures shown below are possible fold conformations of (-)-**1** (left) and (+)-**2** (right), seen from the side of the benzene ring. In the NOE experiment with (-)-**1**, a very weak enhancement of one methyl signal on the isopropyl group was observed on irradiation of H-5. This result could not be interpreted in terms of the single twist structure. A rapid flip between the twist and the fold form should exist (the later in a minor amount), leading to NOE transfer between the two conformations. In the case of (+)-**2**, a conversion of the r-cis-sofa form to the fold form is not observed.



(-)-1 (fold form)



(+)-2 (fold form)

13. The 10 ring conformations (S1-S10) were defined in terms of the dihedral angles between the amide bond and the aromatic rings, as shown in Kawai, T., Ichinose, T., Endo, Y., Shudo, K., Itai, A., *J. Med. Chem.* **1992**, *25*, 2248-2253.
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15. Detailed results of the molecular dynamics study of benzolactams and epi-benzolactams will be published.

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