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MANZAMINE C CONGENERS WITH MODIFIED AZACYCLIC RINGS: SYNTHESIS AND BIOLOGICAL EVALUATION

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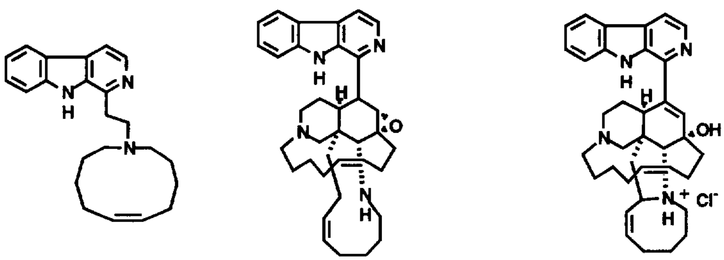
Abstract: Manzamine C congeners with modified azacyclic rings were synthesized using a DPPA-promoted conjunction of the β–carboline-1-acetate salt with various amines as a key reaction. A preliminaly biological evaluation revealed that these analogues retained similar activities as Manzamine C.

Manzamines are a unique family of novel oncolytic marine alkaloids that were first isolated from several Okinawan marine sponges in 1986.[1] Due to their intriguing structural features and their significant biological activities, these alkaloids have attracted considerable interest from both synthetic[2a] and biosynthetic perspectives.[2b,c] The simplest congener, manzamine C (**1**), is a novel *β*–carboline alkaloid which bears an unprecedented azacycloundecene ring.[1b] This simplest manzamine has an antitumor activity equal to that of the more complex congener manzamine B (**2**).[1b] The most complex congener, manzamine A (**3**), has been shown to have the highest biological activity.[1a]



Manzamine C (1) **Manzamine B (2)** **Manzamine A (3)**

We have successfully developed an efficient synthetic route to **1**.[3] We also prepared its geometrical isomer (**4**) and the saturated congener (**5**) to determine the structure-activity relationship.

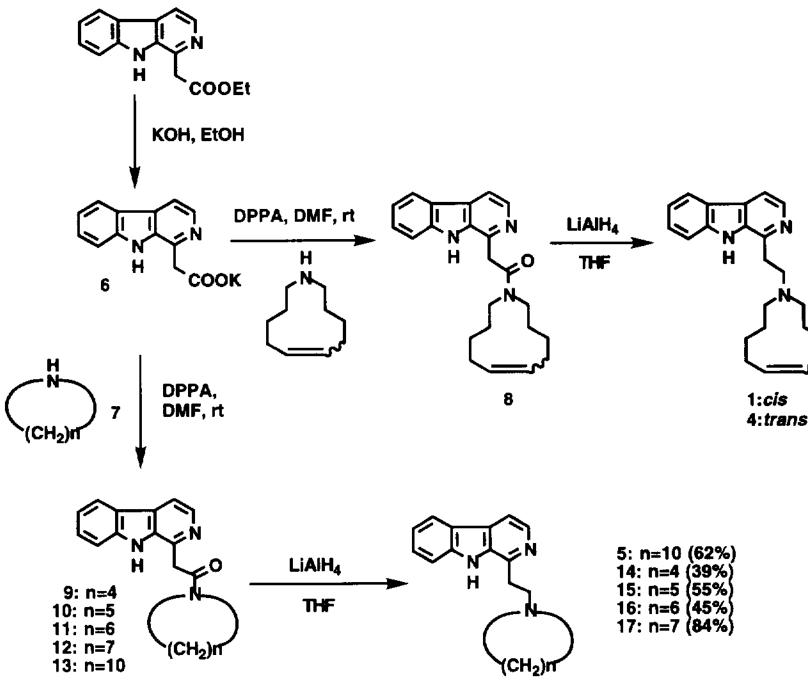
In this report, we describe the preparation and biological evaluation of other congeners with modified azacycles. Our intention is to reveal the role of the azacycloundecene ring in **1** in the observed cytotoxic activity.

Synthesis of the Manzamine C Congeners

The synthesis and biological evaluation of two closely related analogues, i.e. the *trans* geometrical isomer (**4**)[3] and the dihydro (saturated) analog (**5**)[3], could help us to understand the role of the *cis* double-bond in **1**. As novel isomers, we prepared saturated ring analogs with 5-, 6-, 7- and 8-membered rings to clarify the relationship between ring size and activity.

Following the general synthetic scheme shown below (**Scheme 1**), four congeners with a smaller azacyclic ring were successfully synthesized. A key step was the diphenyl phosphoroazidate (DPPA)-promoted coupling of the potassium salt of the β-carboline acetate (**6**) with the corresponding cyclic amines (**7**).[3-5] Since the free acid (β-carboline-1-acetic acid) was easily decarboxylated to harman, the potassium salt had to be treated directly with amines. Thus, the 5-membered amide (**9**) was obtained in 81% yield from **6**, while the 6-membered amide (**10**) was obtained in 78% yield. The same reaction sequence gave the 7-membered ring amide (**11**) in quantitative yield and the 8-membered ring amide (**12**) in 89% yield. Reduction of these amides with $LiAlH_4$ in THF gave four novel manzamine C congeners (**14-17**)[5] with smaller saturated azacycles in moderate yields (39~84%).[6]

Scheme 1



Biological Evaluation

Cytotoxic activity assay

Cells were incubated with each sample for 72 h in RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C under 5% CO_2 in air. The viable cell fraction was measured by a modified MTT assay[7,8] and the 50% inhibitory concentration (IC_{50}) value was caluculated by Probit's method.

Cell lines: P388 (mouse leukemia), P388/ADR (multidrug-resistant P388), MKN28, MKN1 (human stomach carcinoma), PC10, PC14 (human lung carcinoma)

Effect of the cis double-bond in the azacycles.

The results of the *in vitro* cytotoxic assay are summarized in **Figure 1**. The most interesting result involved two closely related analogs (**4**, **5**), which were equally or slightly more potent than the natural manzamine C, indicating that the *cis* double-bond in **1** plays no particular role in its cytotoxic activity. We are now performing a conformational analysis of **1** based on MM2 as well as an NOE study to obtain a more clear view of this conformationally unrestricted 11-membered ring system.[9]

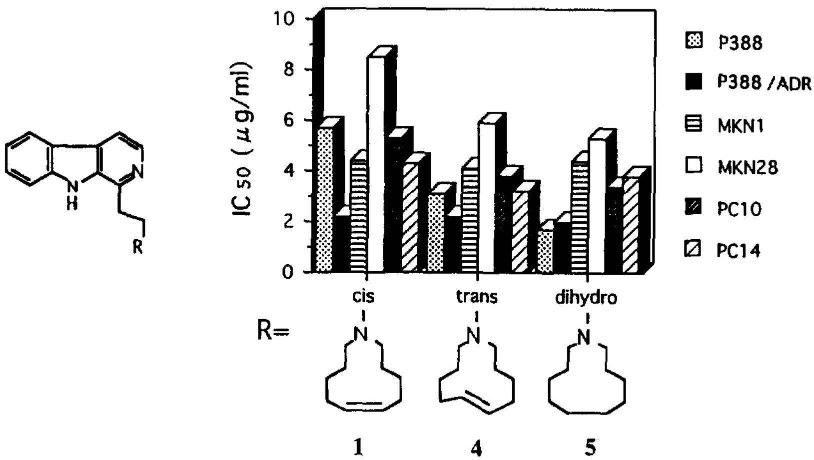


Figure 1 Comparison of *E* and *Z*-Azacycloundecenes and Azacycloundecane Against Various Tumor Cell Lines

Effect of the ring size of the azacycles.

We next focused our attention on the effect of ring size against various tumor cell lines. While the analogs described above (**14-17**) were equally potent towards both P338 and P338/ADR, a slight decline in activity was observed with different cell types. Thus, the 11-membered azacyclic ring is essential for a broad and effective activity against various kinds of tumor cells. (**Figure 2**)

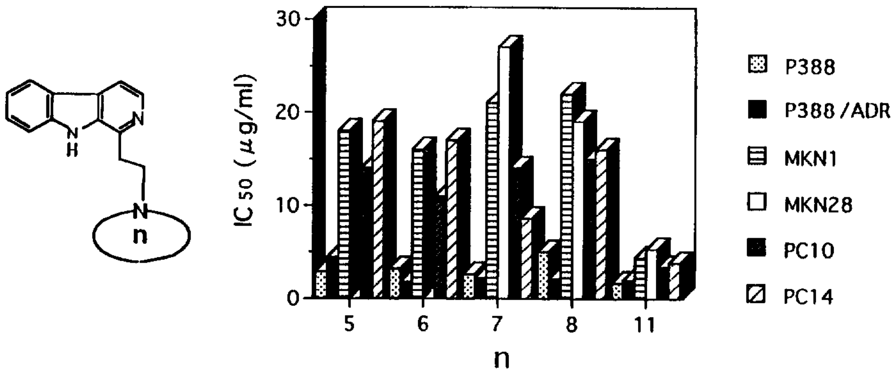


Figure 2 Effect of Ring Size of Azacycles Against Various Tumor Cell Lines

Conclusion

The efficient synthesis and precise biological evaluation of six manzamine C congeners revealed useful information about the structure-activity relationships of the marine alkaloid manzamine C. The results obtained here clearly indicated that the β-carboline moiety plays a primary ıole in the cytotoxic activity of this alkaloid and the attached azacyclic moiety may facilitate these primary interactions to some extent. As has been reported previously, β–carboline can interact with DNA through GC-selective intercalation.[10] Manzamine C (**1**) may act through intercalation by theβ–carboline ring, assisted by the attached azacyclic ring system. To clarify these speculations and to identifiy a more potent and easily accessible analog, we are now focusing on the conformational analysis of this system, especially in comparison with the more complex congeners manzamine A and B. Efforts to synthesize a more water-soluble derivative are now in progress in our laboratory.

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6. Typical experimental procedures and selected spectral data are as follows: **i) Amide formation**: To a solution of **6** (4.0 mmol) in EtOH (15 mL) was added KOH (0.3 g) in H_2O (1 mL). The reaction mixture was stirred at rt until all of the starting material was consumed, and then concentrated *in vacuo*. The residue was further evaporated using a vacuum pump for 2~3 h at rt. To this residue was added DMF (5 mL), **7** (4.0 mmol), DPPA (17.6 mmol) and triethylamine (4.4 mmol). After 20 h of stirring at rt, the reaction mixture was made basic with 10 % NaOH and extracted with AcOEt and benzene (3:1). The organic layer was washed with water and dried over sodium sulfate. The solvent was evaporated to give a residue, which was purified by flash chromatography (silica gel, AcOEt) to yield the amide (**9-13**, 78-100 %). **9**: ^{1}H NMR δ 1.81 (d, 2H, *J*=6.1 Hz), 1.89 (d, 2H, *J*=6.6 Hz), 3.44 (s, 2H), 3.72 (s, 2H), 4.24 (s, 2H), 7.26 (m, 1H), 7.55 (m, 2H), 7.87 (d, 1H, *J*=4.7 Hz), 8.10 (d, 1H, *J*=7.6 Hz), 8.33 (d, 1H, *J*=4.9 Hz), 10.18 (s, 1H); LR-FABMS *m/z* 280 (MH^+, 100). **10**: ^{1}H NMR δ 1.38-1.44 (m, 6H), 1.66 (m, 2H), 3.50 (t, 2H, *J*=6.1 Hz), 3.72 (t, 2H, *J*=6.1 Hz), 7.26 (m, 1H), 7.56 (m, 2H), 7.86 (d, 1H, *J*=5.2 Hz), 8.10 (d, 1H, *J*=7.7 Hz), 8.33 (d, 1H, *J*=5.2 Hz), 10.10 (s, 1H); LR-FABMS *m/z* 294 (MH^+, 100); HR-FABMS Calcd for $C_{18}H_{20}N_3O$ 294.1606, Found 294.1610. **11**: ^{1}H NMR δ 1.38-1.44 (m, 8H), 1.66 (m, 2H), 3.50 (t, 2H, *J*=6.1 Hz), 3.72 (t, 2H *J*=6.1 Hz), 7.26 (m, 1H), 7.56 (m, 2H), 7.86 (d, 1H, *J*=5.2 Hz), 8.10 (d, 1H, *J*=7.7 Hz), 8.33 (d, 1H, *J*=5.2 Hz), 10.10 (s, 1H); LR-FABMS *m/z* 307 (MH^+, 29); HR- FABMS Calcd for $C_{19}H_{21}N_3O$ 307.1684, Found 307.1691. **12**: ^{1}H NMR δ 1.25 (m, 2H), 1.37 (m, 2H), 1.43 (m, 2H), 1.71 (m, 2H), 1.75 (m, 2H), 3.44 (t, 2H, *J*=6.05 Hz), 3.68 (t, 2H, J=6.05 Hz), 4.31 (s, 2H), 7.26 (m, 1 H), 7.55 (m, 2H), 7.87 (d, 1H, *J*=5.0 Hz), 8.09 (d, 1H, *J*=7.69 Hz), 8.33 (d, 1H, *J*=5.22 Hz), 10.07 (s, 1H); LR- FABMS *m/z* 321 (MH^+, 33); HR-FABMS Calcd for $C_{20}H_{23}N_3O$ 321.1841, Found: 321.1839.
ii) LAH reduction: To a cooled and stirred solution of the amide (**9-13**, 1.5 mmol) in THF (40 mL) was added $LiAlH_4$ (11.7 mmol), and the mixture was stirred at rt until almost all of the starting material was consumed (1~4 hr). The mixture was concentrated, the residue was diluted with CH_2Cl_2, and the reaction was quenched by the careful addition of 10 % NaOH. Stirring was continued to obtain a clear organic layer. The mixture was then extracted with CH_2Cl_2 (~200 mL) and dried over anhydrous K_2CO_3. Evaporation of the solvent gave a crude product, which was purified by flash chromatography (silica gel, CH_2Cl_2/AcOEt/MeOH) to give the amines (**14-17**, 39-84%). **14**: ^{1}H NMR δ 2.01 (bs, 4H), 2.80 (bs, 4H), 3.04 (t, 2H, *J*=5.3 Hz), 3.43 (t, 2H, *J*=5.3 Hz), 7.25 (m, 1 H), 7.46 (m, 1H), 7.50 (m, 1H), 7.83 (d, 1H, *J*=5.1 Hz), 8.11 (d, 1H, *J*=7.8 Hz), 8.29 (d, 1H, *J*=5.2 Hz), 12.72 (s, 1H); LR-FABMS *m/z* 266 (MH^+, 100). **15**: ^{1}H NMR δ 1.66 (bs, 2H), 1.85 (t-like, 4H), 2.67 (bs, 4H), 2.83 (t-like, 2H), 3.39 (t-like, 2H), 7.23 (d, 1H, *J*=8.0 Hz), 7.52 (m, 2H,), 7.82 (d, 1H, *J*=5.2 Hz), 8.13 (d, 1H, *J*=8.0 Hz), 8.28 (d, 1H, *J*=5.2 Hz), 12.96 (s, 1H); LR-FABMS *m/z* 280 (MH^+, 100); HR-FABMS Calcd for $C_{18}H_{22}N_3$ 280.1814, Found 280.1808. **16**: ^{1}H NMR δ 1.84 (m, 8H), 2.88 (m, 4H), 2.99 (t, 2H, *J*=5.50 Hz), 3.40

(t, 2H, *J*=5.50 Hz), 7.26 (m, 1H), 7.54 (m, 2H), 7.84 (d, 1H, *J*=5.49 Hz), 8.13 (d, 1H, *J*=7.88 Hz), 8.27 (d, 1H, *J*=5.31 Hz), 12.70 (s, 1H); LR-FABMS *m/z* 293 (M^+, 4); HR-FABMS Calcd for $C_{19}H_{23}N_3$ 293.1892, Found 293.1900. **17**: ^1H NMR δ 1.81 (m, 10H), 2.92 (m, 4H), 3.05 (t, 2H, *J*=5.50 Hz), 3.44 (t, 2H, *J*=5.50 Hz), 7.26 (m, 1H), 7.53 (m, 2H), 7.84 (d, 1H, *J*=5.31 Hz), 8.12 (d, 1H, *J*=7.87 Hz), 8.28 (d, 1 H, *J*=5.31 Hz), 12.70 (s, 1H); HR-FABMS Calcd for $C_{20}H_{25}N_3$ 307.2051, Found 307.2037.

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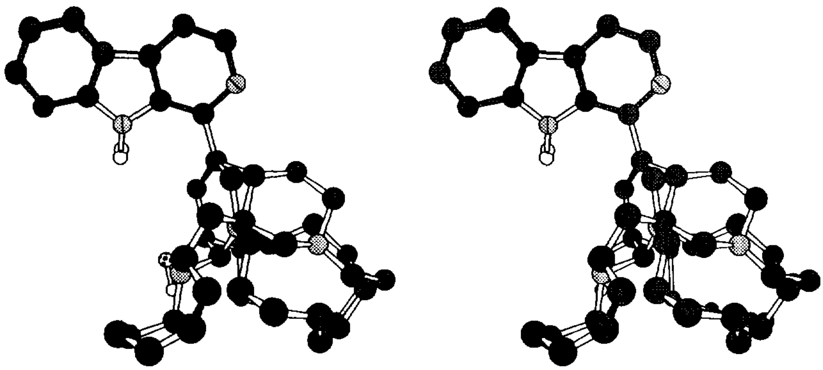

Figure 3 Stereoview of an Overlay of the X-Ray Crystal Structures of Manzamine A[1a] and C.[1b]

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