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Synthesis and Muscarinic M_2 Subtype Antagonistic Activity of Unnatural *ent*-Himbacine and an Enantiomeric Pair of (2′*S*,6′*R*)-Diepihimbacine

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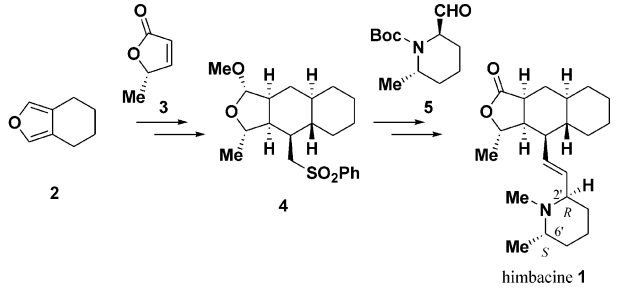
Abstract—The title three compounds *ent*-**1**, **6**, and *ent*-**6** were synthesized by coupling the chiral sulfone **4** or *ent*-**4** with the chiral piperidinaldehyde **5** or *ent*-**5**, which were readily prepared following the synthetic routes previously established by the novel total synthesis of natural himbacine **1**. Their muscarinic M_2 subtype binding affinity was evaluated in comparison to that of **1**, disclosing that the stereochemistry of both the tricyclic moiety and the piperidine part of **1** plays crucial roles in its potent activity.

Himbacine **1** is a piperidine alkaloid isolated from the bark of *Galbulimima baccata*, which is a species of the magnolia family.[1] It has been reported that **1** behaves as a potent antagonist of the muscarinic receptor of the M_2 subtype with a 10–20-fold selectivity toward the M_1 receptor.[2] Accordingly, it is anticipated that **1** is one of the potential candidates or promising lead compounds for the treatment of Alzheimer's disease, because its potent M_2 subtype antagonistic activity can shut down the negative feedback mechanism of presynaptic receptors, thereby increasing the acetylcholine levels in the synapses.[3,4]

Recently, we have reported a novel total synthesis of **1** employing a highly stereoselective intermolecular Diels–Alder reaction.[5] The outline of our synthetic route to **1** is the construction of the tricyclic lactone moiety by means of the intermolecular Diels–Alder reaction of the furan derivative **2** and the chiral furan-2(5*H*)-one **3**, and the subsequent connection of the chiral sulfone **4** with the chiral piperidinaldehyde **5**, according to the Hart's procedure featuring the Julia–Lythgoe olefination reaction (Scheme 1).[6] Our methodology is considered to be more convergent and flexible than other methods so far reported,[7] especially for synthesizing the congeners of **1**, because various structural types of furan, chiral furan-2(5*H*)-one, and aldehyde derivatives can be employed as starting materials or reagents. Therefore, we next focused on elucidating the relationships between the stereochemistry of **1** and its antagonistic activity. We wish to report here on the synthesis of unnatural *ent*-himbacine *ent*-**1** and an enantiomeric pair of (2′*S*,6′*R*)-diepihimbacine **6** and *ent*-**6**, and their binding affinity against the muscarinic receptor of the M_2 subtype (Fig. 1).[8] The latter two congeners **6** and *ent*-**6** were designed with the aim of exploring whether the stereochemistry of the tricyclic moiety or that of the lower piperidine part is crucial for the antagonistic activity.

The synthesis of *ent*-**1**[9] was achieved uneventfully, starting with **2** and *ent*-**3**, and followed by the connection of *ent*-**4**[8] and *ent*-**5**[8] according to the same synthetic



Scheme 1.

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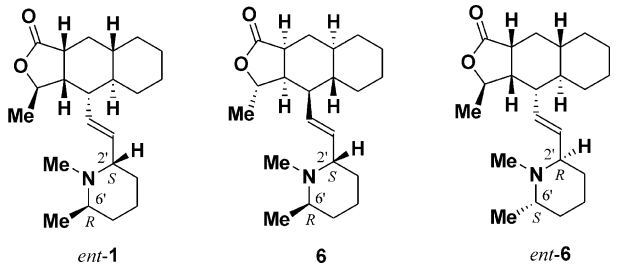
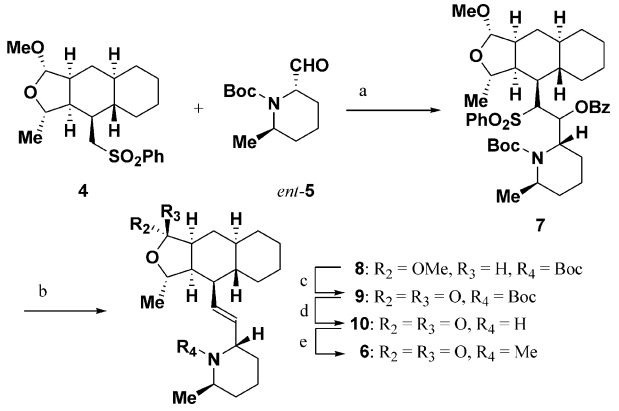


Figure 1.



Scheme 2. (a) (i) *n*BuLi, DME, −78 °C, 2 h; (ii) benzoyl chloride, −78 °C, 0.5 h; (iii) 3-(dimethylamino)propylamine, 0 °C, 0.5 h; (b) 5% Na-Hg, Na_2HPO_4, MeOH, rt, 2.5 h, 33% (four steps) (recovery of **4**: 51%); (c) Jones reagent, acetone, rt, 1 h, 68%; (d) trifluoroacetic acid, CH_2Cl_2, rt, 1.5 h, 91%; (e) 37% HCHO aq, $NaBH_3CN$, CH_3CN, rt, 0.5 h, 73%.

Scheme employed in the preparation of **1**. Next, in order to produce **6**, the Julia–Lythgoe coupling reaction of **4** and *ent*-**5** was examined. However, being different from the cases for the synthesis of **1** and *ent*-**1**, the starting material **4** was fully recovered when the reaction was quenched by adding water. It seems likely that the retro-aldol type reaction might occur during quenching with water, probably due to the decreased stability of the in situ formed lithium aldolate or the aldol type product. After much experimentation, it was finally found that quenching the reaction of **4** and *ent*-**5** by the addition of excess benzoyl chloride successfully gave the desired benzoates **7** as a diastereomeric mixture, after removal of the excess benzoyl chloride with 3-(dimethylamino)-propylamine (Scheme 2). Without separation, the reaction mixture was directly subjected to reductive elimination, giving rise to (*E*)-olefin **8** in a 33% yield from **4**, along with a 51% recovery of **4**. According to the same synthetic procedure as for **1**, **8** was converted to the target compound **6**[9] in a three step sequence involving oxidation of the hemiacetal moiety, deprotection of the *N*-Boc group, and reductive *N*-methylation. The enantiomer of **6**, *ent*-**6**,[9] was also synthesized employing *ent*-**4** and **5** in the same manner as described for the synthesis of **6**.

With the synthetic targets *ent*-**1**, **6**, and *ent*-**6** in hand, their muscarinic M_1 and M_2 subtype binding affinity assay was evaluated.[10] The results are shown in Table 1 along with that of **1**. Being different from **1**, all the tested compounds *ent*-**1**, **6**, and *ent*-**6** were found to show very weak binding affinity with the same level against the muscarinic M_1 and M_2 subtype. Accordingly, it appears evident that the stereochemistry of both the tricyclic moiety and the piperidine part of **1** plays important roles for its strong muscarinic M_2 binding affinity.

Table 1. In vitro muscarinic M_1 and M_2 receptors binding affinity of novel himbacine congeners

Entry	Compd	$-\log K_i$	
		M_1 (cortex)	M_2 (brainstem)
1	**1**	7.2	8.0
2	*ent*-**1**	6.0	6.1
3	**6**	6.5	6.7
4	*ent*-**6**	6.5	6.7

In conclusion, we have succeeded in synthesizing the novel himbacine congeners *ent*-**1**, **6**, and *ent*-**6** by employing the synthetic Scheme which was previously established by our total synthesis of natural **1**. From the results of the muscarinic M_2 subtype binding affinity assay of these congeners, it was discovered that the stereochemistry of both the tricyclic moiety and the piperidine part of **1** is crucial for its potent antagonistic activity.

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8. The starting materials, **4**, *ent*-**4**, **5**, and *ent*-**5** were synthesized from the optically pure starting materials, respectively, according to our reported procedures.[5] The specific rotations of **4**, *ent*-**4**, **5**, and *ent*-**5** are as follows: **4**, $[\alpha]_D^{20}$ +104° (*c* 0.35, $CHCl_3$) [lit.[7b] $[\alpha]_D^{20}$ +100° (*c* 0.35, $CHCl_3$)]; *ent*-**4**, $[\alpha]_D^{22}$ −104° (*c* 0.35, $CHCl_3$); **5**, $[\alpha]_D^{26}$ +120° (*c* 1.03, $CHCl_3$) [lit.[7b] $[\alpha]_D^{20}$ +122° (*c* 0.96, $CHCl_3$)]; *ent*-**5**, $[\alpha]_D^{22}$ −128° (*c* 0.82, $CHCl_3$).

9. The physical data on *ent*-**1**, **6**, and *ent*-**6** are as follows: *ent*-**1**, mp 128–130 °C, $[\alpha]_D^{23}$ −59° (*c* 0.29, $CHCl_3$) [**1**,[5] mp 127–128 °C, $[\alpha]_D^{24}$ +55° (*c* 0.21, $CHCl_3$)]; **6**, mp 136–138 °C, $[\alpha]_D^{24}$ +6.6° (*c* 0.10, $CHCl_3$); *ent*-**6**, mp 135–137 °C, $[\alpha]_D^{21}$ −7.0° (*c* 0.40, $CHCl_3$). All compounds were characterized by ^1H and ^{13}C NMR, IR, and Mass spectroscopic methods.

10. Binding assay: the receptor binding analysis for the muscarinic receptor of the M_1 and M_2 subtypes was performed using homogenates of the cerebral cortex and brainstem of a rat, respectively. The radioligands used were [^{3}H]-pirenzepine for the cerebral cortex and [^{3}H]-quinuclidinyl benzilate (QNB) for the brainstem, respectively. The homogenates were incubated in a 50 mM Tris–buffer (pH 7.4) at 25 °C for 90 min, and rapidly filtrated on Whatman GF-B filters. The radioactivities were counted using a liquid scintillation counter. Nonspecific binding was defined in the presence of 2 μM atropine. The test compounds were dissolved in DMSO and diluted with buffer to final concentrations. The competition binding experiments were performed in the presence of less than 0.1% DMSO, which did not affect the specific binding. The equilibrium dissociation constants (K_i) were calculated using the Cheng–Prusoff equation, $K_i = IC_{50}/(1 + L/K_d)$, where L and K_d were the concentration and the dissociation constant of radioligand, respectively. The K_d values were determined by a Scatchard analysis.