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DESIGN, SYNTHESIS, AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF HIMBACINE DERIVED MUSCARINIC RECEPTOR ANTAGONISTS[†]

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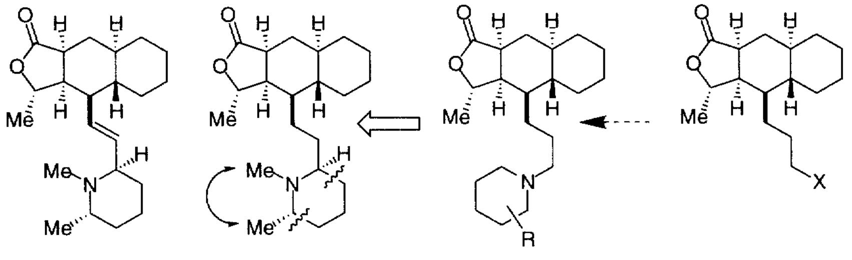
Abstract. A parallel synthesis of racemic himbacine analogs was carried out by *N*-alkylation of various commercially available cyclic amine derivatives with the alkylating agent **4** which bears the tricyclic unit of himbacine. Several of these analogs have potency comparable to that of himbacine, albeit lacking the desired selectivity. Structure-activity relationship studies support the existence of a hydrophobic pocket in the receptor where the piperidine ring of dihydrohimbacine binds.

Alzheimer's disease is a prevalent disorder affecting the elderly population. The early phase of Alzheimer's disease is characterized by a progressive loss of memory and cognitive function that impair activities of daily living.[1] Because of increasing longevity, the prevalence of Alzheimer's disease among the elderly presents a tremendous medical, economic and social problem. The loss of cholinergic innervation in the hippocampus and cerebral cortex coupled with the loss of cholinergic neurons in the basal forebrain is a well defined finding of Alzheimer's disease.[2] While currently available cholinergic pharmacotherapy for Alzheimer's disease addresses this issue by inhibiting acetylcholinesterase which hydrolyzes acetylcholine, possible enhancement of synaptic acetylcholine levels could also be achieved by selectively inhibiting presynaptic



1 Himbacine $K_i = 4.5$ nM (M_1); 48.4 nM (M_2)

2 Dihydrohimbacine $K_i = 4.3$ nM (M_1); 32.2 nM (M_2)

3

4

Figure 1. Strategy for analog design

[†] **This paper is dedicated to the memory of Professor Sir Derek Barton**

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muscarinic receptors (M_2), agonist-induced stimulation of which shuts off acetylcholine release.[3] Since inhibition of post-synaptic M_1 receptors could offset the beneficial effect of presynaptic M_2 receptor inhibition, it is essential that such an agent be selective for M_2 receptors.

Himbacine is a piperidine alkaloid isolated from *Galbulimima baccata* of Magnoliaceae family which is native to New Guinea and northern Queensland of Australia.[4] It is a potent inhibitor of M_2 receptor (K_i = 4.5 nM) with 10-fold selectivity toward M_1 receptors.[5-7] In order to identify a therapeutically useful target, both the potency and selectivity of himbacine need to be optimized through a rigorous structure–activity relationship study. The complex structural features of himbacine render this task difficult. Structure–activity relationship studies reported by Kozikowski et. al. have demonstrated the essential nature of the tricyclic lactone and the *N*-methyl group for potency and selectivity.[6] As part of our efforts directed toward identifying potent and selective M_2 receptor antagonists related to himbacine, we needed a practical synthesis of himbacine analogs. Such a synthesis would proceed through a common advanced intermediate which would serve as a precursor for parallel synthesis of targets. We wish to report here an efficient synthesis of racemic tricyclic intermediate **4** and its use in parallel synthesis to generate himbacine analogs. Several of the simplified synthetic analogs showed potency comparable to that of himbacine.

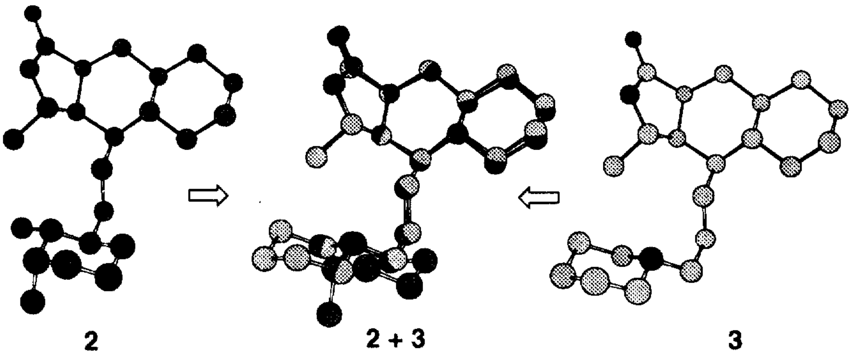


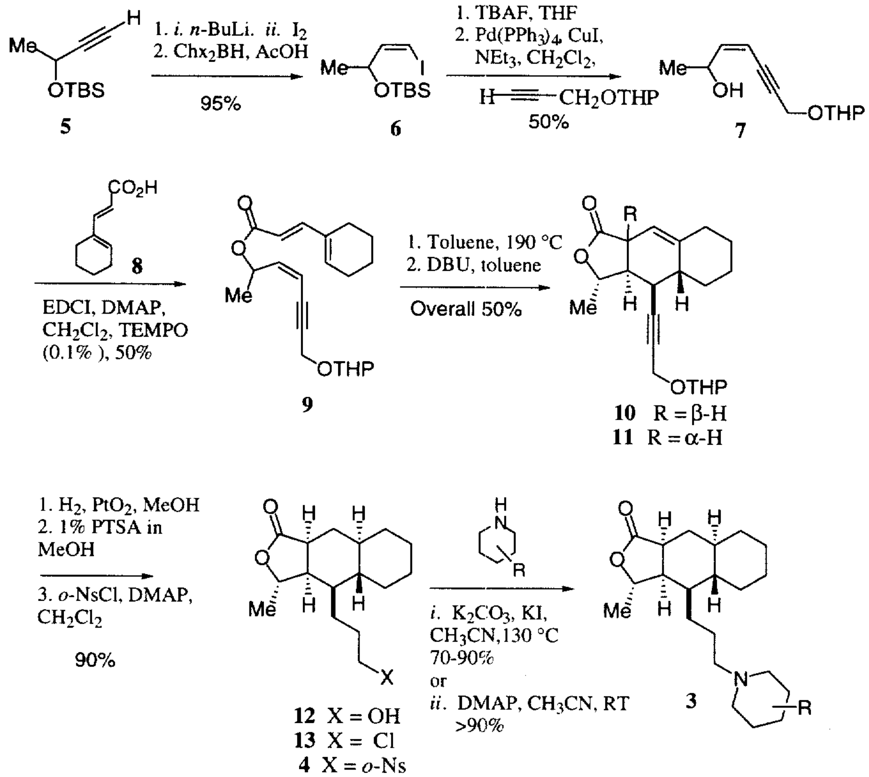
Figure 2. Overlay of low-energy conformations of dihydrohimbacine (**2**) and generic structure **3** (right).

Figure 1 outlines the design strategy for simplified himbacine analogs which was aided by our own finding that dihydrohimbacine has potency and selectivity comparable to that of himbacine in cloned human M_1 and M_2 receptors (Figure 1). Compounds represented by structure **3** can be envisioned to arise by a hypothetical cleavage of the piperidine ring at the indicated sites followed by joining the two methyl groups in a new piperidine ring. These compounds would have their nitrogen atom located at the same relative position to the tricyclic ring as himbacine (4 bonds). Molecular modeling studies[8] of dihydrohimbacine (**2**) and compounds represented by structure **3** indicated a low-energy conformation for each molecule which gave close overlap of the piperidine nitrogen atoms and the adjacent hydrophobic regions. It seemed likely that appropriate substitution of the piperidine ring of **3** might provide additional favorable hydrophobic interactions which could lead to compounds with enhanced potency and selectivity (Figure 2).

Synthesis

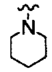
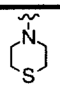
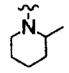
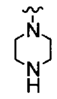
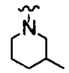
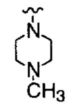
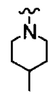
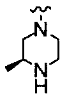
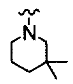
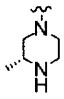
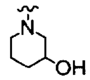
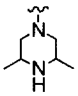
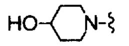
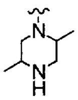
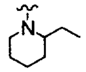
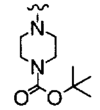
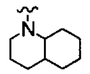
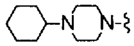
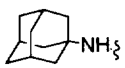
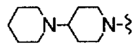
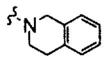
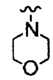
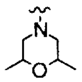
The synthesis of racemic himbacine analogs, based on our total synthesis of himbacine,[9] is outlined in Scheme 1. Commercially available (±)-3-butyn-2-ol (**5**) was converted to the *cis*-vinyl iodide **6** by reduction of the corresponding *O*-TBDMS protected iodoacetylene with dicyclohexylborane.[10] Coupling of *cis*-vinyl iodide **6** with commercially available *O*-THP protected propargyl alcohol took place under Sonogashira conditions[11] after in situ removal of the TBDMS group to yield alcohol **7**. Esterification of alcohol **7** with dienoic acid **8**[9a] yielded the ester **9**, a solution of which in toluene was subjected to intramolecular Diels-Alder reaction at 190 °C to generate the *trans*-lactone **10**.[12]

Scheme 1



In situ treatment of *trans*-lactone **10** with DBU effected complete epimerization to the *cis*-lactone **11** which, after chromatographic purification, was obtained in an overall yield of 50% from alcohol **7**. Catalytic hydrogenation followed by *O*-deprotection under acidic conditions yielded the saturated alcohol **12** which, upon treatment with *o*-nitrobenzenesulfonyl chloride, gave the nosylate **4** along with a small quantity of the corresponding chloride (**13**). Chromatographic purification of **4** followed by reaction with various commercially available heterocyclic secondary amines gave the target compounds presented in Table 1.

Table 1. Binding data for himbacine analogs

Entry	Basic unit	K_i (nM) M_1	K_i (nM) M_2	Entry	Basic unit	K_i (nM) M_1	K_i (nM) M_2
3a		427	352	3n		179	189
3b		399	275	3o		>420	>42
3c		376	312	3p		579	595
3d		554	283	3q		581	>500
3e		416	224	3r		857	>500
3f		1533	148	3s		1361	>500
3g		>900	>280	3t		353	570
3h		341	209	3u		445	485
3i		38	13	3v		464	520
3j		136	26	3w		312	650
3k		304	184				
3l		222	51				
3m		601	376				

Binding Assay[13]

Human muscarinic receptor constructs were transfected into CHO-K1 cells by the calcium phosphate precipitation method. Colonies were selected for G418 resistance and tested for receptor expression by radioligand binding to *l*-quinuclidinyl [*phenyl*-4-^{3}H]benzilate ([^{3}H] QNB). Saturation experiments were performed using 5-2500 pM [^{3}H] QNB in 10 mM potassium phosphate buffer, pH 7.4, to determine the K_d of

[^{3}H] QNB for each receptor subtype. Competition binding experiments were performed using 180 pM [^{3}H] QNB and 10–50 mg of membrane protein. All binding experiments were performed in the presence of 1% DMSO and 0.4% methylcellulose. Nonspecific binding was defined by 0.5 mM atropine. After equilibrium was reached (120–min incubation at room temperature), bound and free radioactivity were separated by filtration using Whatman GF-C filters. K_i values were derived from IC_{50} values using the Cheng–Prusoff equation.

Results and Discussion

In our assays himbacine and dihydrohimbacine gave K_i values of 4.5 nM and 4.3 nM, respectively, against cloned human M_2 receptors (Figure 1). The M_1 and M_2 binding constants for the synthetic derivatives are given in Table 1. The simple piperidine derivatives (**3a–3h**) showed only marginal affinity for the muscarinic receptors. The bicyclic derivatives (**3i** and **3j**) were more promising. In fact, the perhydroquinoline analog **3i**, which was obtained as an inseparable mixture of four diastereomers in approximately equal amounts, gave a K_i value of 13 nM, which is comparable to that of himbacine. The adamantyl derivative **3j** was also quite potent (K_i = 26 nM). The morpholine derivative **3l** was somewhat potent; however, the corresponding dimethylmorpholine derivative **3m** and the thiomorpholine derivative **3n** were much less active. The piperazine derivatives (**3o–3w**) showed uniformly poor affinity.

These observations suggest the presence of a hydrophobic pocket near the region where the piperidine ring of himbacine binds. Introduction of dibasic amines and other polar functionalities reduces the potency. The drastic variation of affinity with minor structural perturbations of the piperidine ring (e.g., himbacine vs. compound **3a**) suggests a specific shape requirement which, if gainfully exploited, has the potential to produce greater potency and selectivity.

In summary, we have designed a practical synthetic route to racemic himbacine analogs by *N*-alkylation of the tricyclic intermediate **4**. Utilizing this approach we have identified several potent muscarinic antagonists some of which have M_2 affinity comparable to that of himbacine. However, the goal of identifying a muscarinic antagonist with M_2 specificity still remains elusive and it is an ongoing area of investigation in our laboratories.

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