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ALZHEIMER'S THERAPY: AN APPROACH TO NOVEL MUSCARINIC LIGANDS BASED UPON THE NATURALLY OCCURRING ALKALOID HIMBACINE.

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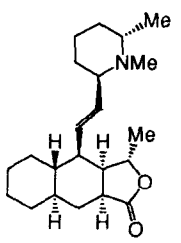
Abstract: The naturally occurring alkaloid himbacine has been found to be a potent muscarinic antagonist that displays selectivity for M2 or M4 receptors. Using himbacine as a lead compound we have begun to explore its structure-activity profile in order to arrive at an analogue for possible use in cholinergic pharmacotherapy.

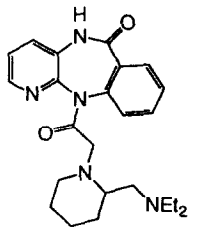
A consistent and early feature of the pathology of Alzheimer's Disease (AD) is the degeneration of the basal forebrain cholinergic system. The loss is usually partial and most muscarinic receptors remain present.[1] Of the attempts at cholinergic pharmacotherapy, the elevation in brain acetylcholine levels with anticholinesterases has had some limited success; however, these efforts may have been limited by short drug half-lives and/or the toxicity of the drugs used.[2] Another approach that should result in elevated synaptic acetylcholine is the blockade of presynaptic inhibitory muscarinic receptors (putative M2 and/or M4 receptors; in the receptor subtype designations, the small letters refer to gene products, while the capital letters refer to pharmacological associations); this was recently demonstrated in animal studies.[3] In order to develop antagonists of the human presynaptic muscarinic receptors for the therapy of AD and other age-related neurodegenerative diseases in which a loss of the cholinergic system occurs we have focussed our chemistry efforts on the natural product himbacine.[4,5] Himbacine together with methoctramine and AF-DX 116 are three antagonists that have been shown to be M2-selective. Himbacine is the most potent of these three, with a K_d value for blocking the cardiac receptor of about 3 nM.[6] In one estimation, himbacine displayed 86-fold selectivity for heart *versus* smooth muscle (putative M3) receptors. AF-DX 116 is considerably less potent than himbacine (about 10-fold), while methoctramine, while being somewhat less potent (about 3-fold), also has additional allosteric effects and direct inotropic effects in the heart.[7]

At heart and brainstem M2 receptors, himbacine bound with K_d values of 6.9 nM and 4.6 nM, respectively. In functional assays for M4 receptors, the inhibition of cAMP levels in rat striatum and in N1E-115 neuroblastoma cells, himbacine's potencies at blocking oxotremorine-M were 5.4 nM and 11.3 nM, respectively. Additionally, himbacine was shown to reverse oxotremorine-M mediated inhibition of evoked acetylcholine release from hippocampal tissue with a K_d value of 8.5 nM, a value consistent with the involvement of M2 or M4 receptors. At the cortical post-synaptic muscarinic receptors involved with phosphoinositide turnover (putative M1 and M3 receptors), himbacine was 21-fold less potent, displaying a K_d value of 175 nM.[8] Himbacine thus appears to be

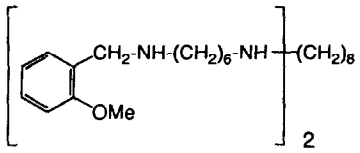
one of the more potent muscarinic antagonists that displays selectivity for M2 or M4 receptors, as compared to M1 or M3 receptors, and for these reasons appears to be an important lead structure in identifying possible new drug candidates for the treatment of Alzheimer's dementia.



Himbacine



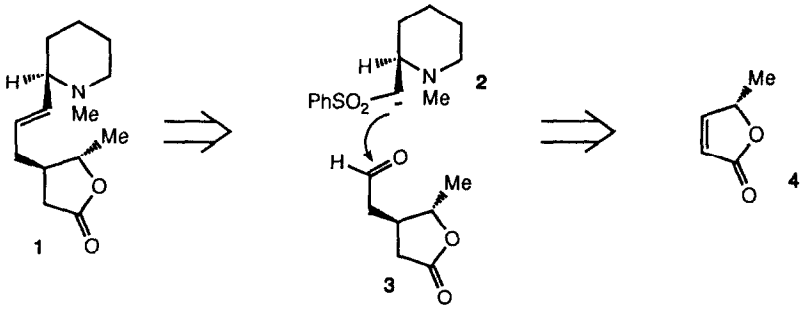
AF-DX 116



Methoctramine

Our desired goal is the development of novel himbacine analogues with high potency (better than 1 nM) and selectivity (better than 100-fold) for the M2 and M4 muscarinic receptor subtypes, which are likely to be localized at presynaptic sites. Such analogues could be used to block residual presynaptic muscarinic receptors to enhance the release of acetylcholine, and used in combination with a potent, long-lasting anticholinesterase (e.g., huperzine A),[9] which would protect the released neurotransmitter from degradation.

In beginning our SAR studies, we chose to focus our efforts on the synthesis of a highly simplified himbacine analogue, 1, which embodies only its more hydrophilic elements, i.e, the γ-lactone ring connected via an olefinic appendage to a piperidine ring. The selection of this particular analogue for synthesis was made from the standpoint not only of synthetic expediency, but also

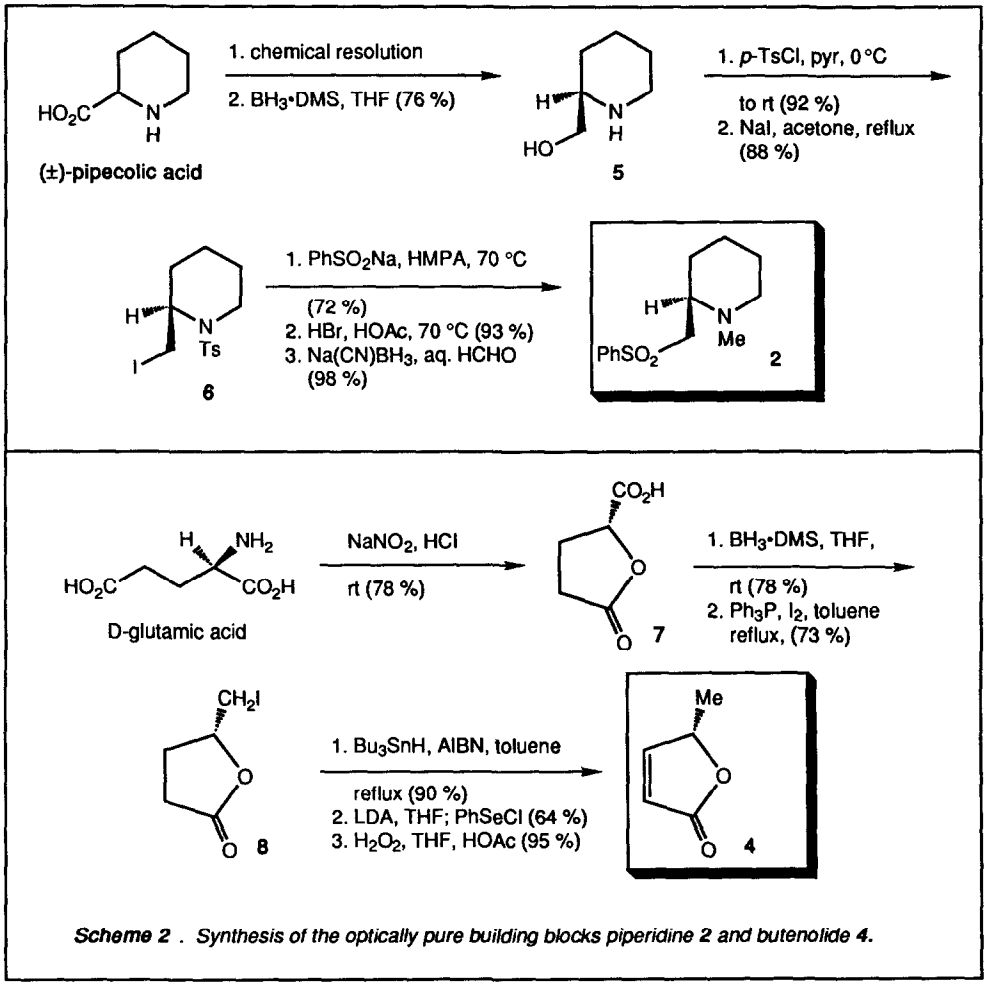


Scheme 1. *A retrosynthetic analysis of the highly simplified himbacine analogue 1 .*

from our desire to explore the contribution of the hydrophobic decalin ring system to receptor affinity together with subtype selectivity. The importance of the decalin unit to rigidification of the himbacine structure would also fall within the purview of this investigation.

The importance of structural simplification to our overall objectives cannot be overestimated, for the rapidity of synthetic execution is fundamental to the development of a thoroughly encompassing SAR study. However, it must also to be appreciated that synthetic expediency may come at the expense of structural features which are key to the molecule's more desirable pharmacological as well as pharmacokinetic parameters.

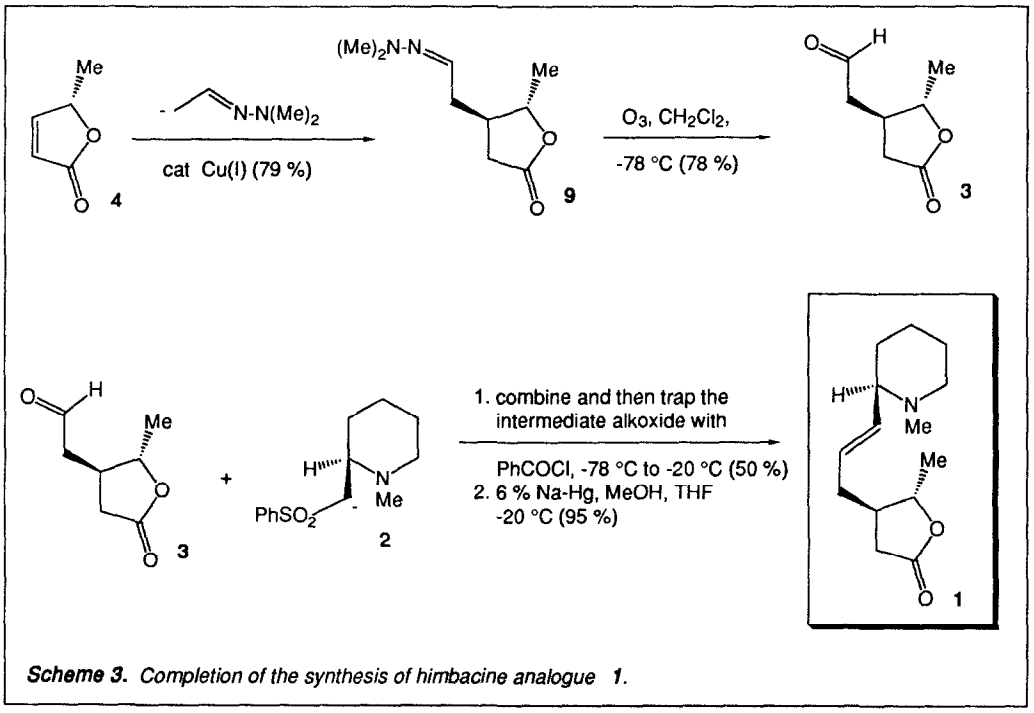
A retrosynthetic dissection of 1 is shown in Scheme 1. In order to avoid problems of diastereomer formation, it was important to have access to both the piperidine and lactone components in optically pure form and, of course, in a minimum number of synthetic steps. Thus, as shown in Scheme 2 the piperidine portion was constructed from pipecolic acid through a sequence of steps involving an initial chemical resolution[10] followed by reduction, tosylation, and iodide



Scheme 2 . Synthesis of the optically pure building blocks piperidine 2 and butenolide 4.

formation to give **6**. Next the iodide **6** was reacted with sodium benzenesulfinate to yield a sulfone, which after desulfonylation[11] was subjected to a reductive methylation reaction to afford the desired piperidinyl sulfone **2** ($[\alpha]_D^{25}$ = +28.5 °, $CHCl_3$, c = 9 mg/mL).

The optically pure butenolide **4** was acquired from D-glutamic acid by a sequence of fairly well precedented steps[12] involving lactone formation, carboxyl group reduction, iodide formation,[13] tin hydride reduction, and then selenylation/selenoxide elimination. From **4**, it was now necessary to append a functionalized two carbon unit to allow coupling to the piperidine ring via the Julia-Lythgoe procedure (Scheme 3).[14] While efforts were made to add allylcuprate reagents to **4**, such attempts led only to products of 1,2-addition. On the other hand, we found that the N,N-dimethylhydrazone of acetaldehyde[15] added smoothly to this butenolide under the influence of Cu(I) catalysis. Next, the hydrazone was cleaved by ozone treatment to afford the aldehyde **3**, and then the coupling reaction between **2** and **3** was carried out in the standard manner to afford analogue 1, which while conformationally more mobile than himbacine is still replete in all of its heteroatoms. *It is of interest to note here that no evidence of the decomposition of the anion of* ***2*** *through a β-elimination mechanism was evident, although this process was observed in the case of the N-tosyl derivative.* All spectral data obtained for 1 were fully consistent with the structure depicted.[16]



Scheme 3. *Completion of the synthesis of himbacine analogue* ***1.***

The effect of the present modification of the himbacine structure on the potency and selectivity for muscarinic receptor subtypes was determined in binding assays. The rat brainstem

was used as a source of M2 receptors, while CHO-K1 cells transfected with the hm1 or the hm3 receptor sequences were used as sources of these receptor proteins. The M1 and M3 receptors, which in the brain couple to phosphoinositide turnover, are localized predominantly post-synaptically, while cortical or hippocampal presynaptic muscarinic receptors may be of the M2 subtype. Analogue **1** bound to brainstem M2 receptors with a K_d value of 1.21 μM, about 260-fold less potently than himbacine at this receptor. The removal of the decalin ring also reduced selectivity for the M2 receptor, as **1** bound to the cloned hm1 and hm3 receptors with K_d values of 9.7 μM and 4.4 μM, respectively. Himbacine is about 20-fold selective for the M2 receptor, with respect to the M1 receptor; analogue **1** is only 8-fold selective. While himbacine is about 14-fold selective for the M2 receptor, with respect to the hm3 receptor, analogue **1** is only about 3.6-fold selective in the same comparison.

While molecular modeling studies are currently being conducted to examine **1** and other himbacine analogues in order to glean information of value in selecting additional analogues for synthesis, it is likely that the energetically costly reorientation in the conformation of **1** which may be required for binding to the M2 or M4 receptors together with the loss of the hydrophobic binding component of himbacine's decalin moiety contribute to its poor binding. It is further apparent that himbacine's decalin moiety plays an important role in conferring M2 selectivity to this drug.

Work is now in progress to examine the activity of other himbacine analogues which are structurally simpler than the parent structure in terms of their stereochemical content, but which embody elements crucial to imparting both hydrophobicity as well as rigidity to these products. The information disclosed herein does serve to further underscore the importance of natural products as lead structures in the quest for therapeutic agents for the treatment of neurodegenerative diseases.

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have input the actual x-ray structural coordinates reported for this compound,[5] and verified that the stereochemistry denoted in the x-ray paper is correct.

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16. $[\alpha]_D^{25}$ = -2.7 ° ($CHCl_3$, c = 1.5 mg/ml); IR 2933, 1777, 1456, 1261 cm^{-1}; H NMR ($CDCl_3$) δ 5.97 (dt, *J* = 16, 8 Hz, 1H), 5.53 (dd, *J* = 16 Hz, 9H), 4.50 (m, 1H), 3.6-3.4 (m, 2H), 3.00 (br t, *J* = 12 Hz), 2.9-2.7 (m, 4H, contains s for $N\text{-}CH_3$), 2.5-2.3 (m, 3H), 2.3-2.2 (m, 1H), 2.0-1.8 (m, 3H), 1.8-1.6 (m, 3H); ^{13}C NMR (D_2O) δ 183.46, 138.81, 129.39, 86.28, 70.48, 58.11, 43.95, 43.55, 37.28, 36.98, 33.15, 25.74, 23.93, 21.44; MS (FD) M^+ = 237.6.