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N-2 METHYLATED QUATERNARY DERIVATIVES OF β-CARBOLINE-3-CARBOXYLATES INHIBIT ACETYLCHOLINESTERASE *IN VITRO*

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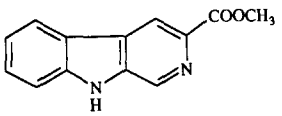
Abstract: Alkyl β-carboline-3-carboxylate derivatives (e.g. β-CCM and β-CCB), known to interact with the central benzodiazepine receptor, were quaternized at the N-2 position using methyl iodide. The products strongly inhibited acetylcholinesterase *in vitro* and displayed affinities for muscarinic receptors in the micromolar range.

The benzodiazepine receptor (BZR) of the central nervous system has for some time been known to mediate some forms of memory processing. Thus, BZR agonists such as diazepam, widely used for their anticonvulsant, anxiolytic and hypnotic properties, also provoke severe anterograde amnesia in humans.[1] The discovery over a decade ago that simple esters of 3-carboxy-β-carbolines (e.g., methyl β-carboline-3-carboxylate, β-CCM, **1**) possess high affinities for the BZR[2] while producing convulsions[3] and anxiety[4] *in vivo* led to the subsequent demonstration that this class of BZR ligands (commonly referred to as inverse agonists) is promnesic in animals.[5] The memory-enhancing effects of β-CCM and other inverse agonists appear to be related to a facilitation of information acquisition. The amnesic and promnesic properties of diazepam and β-CCM, respectively, can be reversed by the BZR antagonist flumazenil, thereby suggesting that the observed mnesic effects are a direct result of a specific interaction of these compounds with the BZR.[5a] There is, however, increasing evidence that the memory effects of BZR ligands may be the result of an indirect influence on the cholinergic system via γ-aminobutyric acid (GABA). This is based on the argument that the pharmacological effects of BZR agonists such as diazepam result from facilitation of neuroinhibitory GABAergic transmission[6] and that GABA agonists (e.g., muscimol)



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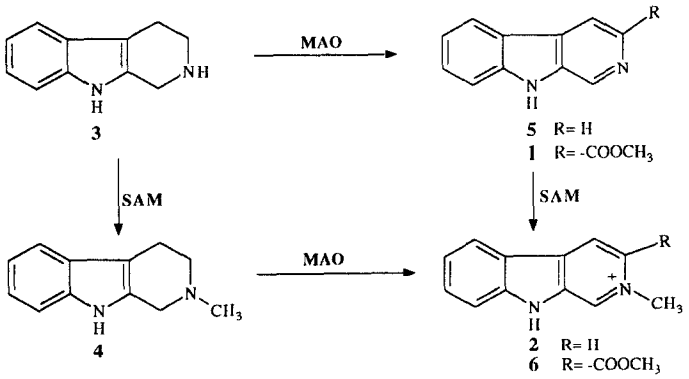
have, in turn, been shown to reduce acetylcholine turnover,[7] high affinity choline uptake[8] as well as acetylcholine output[9] in various parts of the brain. The amnesic effects of diazepam may thus be the result of its facilitation of GABA-mediated cholinergic inhibition. Conversely, the memory-enhancing properties of the β-carboline inverse agonists would then be the consequence of down-regulation of GABAergic transmission with a resulting disinhibition (i.e. stimulation) of the cholinergic pathways.[10,11]

The intervention of the cholinergic system in directing the mnesic properties of BZR ligands has been substantiated by the observation that diazepam, like GABA, reduces cortical acetylcholine turnover[12] and acetylcholine release in the hippocampus[13] while the promnesic β-carbolines have been shown to stimulate cortical high affinity choline uptake[14] as well as acetylcholine release[15] *in vivo*. Colocalization of GABAergic terminals and benzodiazepine binding sites with cholinergic cells has also been demonstrated.[16]

It is in this context that our attention was recently drawn to a report of the isolation of N-2 methylated norharman **2** (Scheme 1) from human cortex in connection with

Scheme 1:

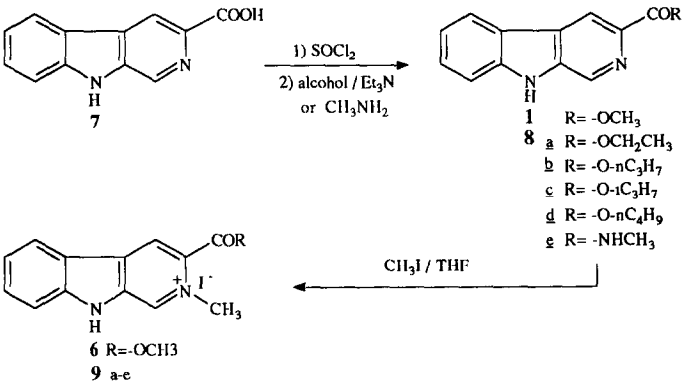


MPTP-type dopaminergic toxicities.[17] Although it has not been ascertained whether compound **2** arises from enzymatic methylation (*via* S-adenosylmethionine or brain N-methyltransferases[18]) of endogenous 1,2,3,4-tetrahydronorharman **3** (to give **4**) prior to MAO-type oxidation or whether **3** is first oxidized to norharman **5** before N-methylation, the presence of the quaternized β-carboline **2** in the brain retained our interest since this compound was reported[19] some time ago to be a fairly potent inhibitor of acetylcholinesterase (AChE) *in vitro* while its precursor **5** was shown to be a much weaker inhibitor of this enzyme.[20] Since AChE inhibitors (e.g., physostigmine) are a well-known class of memory-enhancing agents,[21] it occurred to us that the memory-enhancing properties of β-CCM **1** (and other β-carbolines) may be due, at least partly, to its *in vivo* methylation, to give quaternized derivative **6** and subsequent AChE inhibition. As a first step to investigating this possibility, we decided to synthesize **6** as well as the N-2 methylated derivatives of the pharmacologically well-

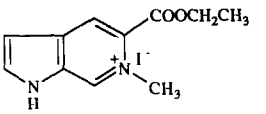
characterized upper homologues of β-CCM in order to determine their AChE inhibitory potencies.

The esters **1**, **8a** (β-CCE), **8b** (β-CCP), **8c**, **8d** (β-CCB) and methylamide **8e** (FG7142) were prepared from β-carboline-3-carboxylic acid **7** *via* published procedures.[22] (Scheme 2). Thus, reaction of **7** with thionyl chloride gave the acid chloride which, upon treatment with the appropriate alcohols (or methylamine) furnished the corresponding esters **1**

Scheme 2:



and **8a-d** (or amide **8e**). These β-carbolines were refluxed for 6-24 h in THF containing excess methyl iodide to provide the desired N-2 methylated quaternary salts **6** and **9a-e**.[23] Compounds **2** and **10** were prepared in similar fashion starting from commercially available norharman **5** or from ethyl 6-azaindole-5-carboxylic acid,[24] respectively.



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These compounds were then tested at two different concentrations (10^{-5} and 10^{-6} M, Table 1) for their ability to inhibit purified electric eel AChE.[25] While the non-quaternized β-carboline **1** did not inhibit AChE to any significant extent at these concentrations, its quaternized derivative **6** produced 43.1% inhibition of this enzyme at 10^{-5} M. Interestingly, this value was substantially the same as that for quaternized N-2-methyl norharman **2**, at first suggesting only a minor role for the C-3 methyl ester function in enzyme inhibition. However, as is clearly evident from Table 1, AChE inhibitory potency progressively increases with the length of the alkyl ester chain, reaching, for the N-butyl derivative **9d**, a value of 88.1% inhibition at 10^{-5} M, double that of the quaternized methyl ester **6**. Butyl ester **9d** is, in fact, as effective an

Table 1.
In vitro AChE inhibitory potencies and benzodiazepine and muscarinic receptor binding affinities

		% Inhibition of AChE[a] (m ± SD ; n = 3)		BZR binding affinity[b]	Muscarinic receptor binding affinity[b]
No°	R	10^{-6} M	10^{-5} M	Ki (μM)	Ki (μM)
1	CO_2CH_3	NA[c]	NA	0.003	NA
2	H	7.6 ± 0.7	41.2 ± 2.4	NA	NA
6	CO_2CH_3	7.2 ± 2.5	43.1 ± 1.9	1.4	3.1
9a	$CO_2C_2H_5$	13.7 ± 2.5	60.9 ± 1.2	3.2	0.7
9b	$CO_2C_3H_7$	34.9 ± 1.9	80.1 ± 0.7	0.6	1.0
9c	CO_2 i-C_3H_7	14.0 ± 2.5	62.0 ± 1.6	4.1	0.5
9d	$CO_2C_4H_9$	46.0 ± 0.6	88.0 ± 0.5	4.6	0.5
9e	$CONHCH_3$	1.3 ± 3.9	7.4 ± 1.1	0.6	27.0
10		4.0 ± 4.8	17.0 ± 1.5	NA	NA
Pyridostigmine		59.0 ± 1.0	93.0 ± 1.9	NA	NA

[a] Determined colorimetrically by the method of Ellman[25] using purified electric eel AChE. Values are the average of three determinations for each concentration. [b] Competitive binding assays were performed *in vitro* on rat brain membranes using standard techniques by displacement of ^{3}H-flunitrazepam (for BZR) and ^{3}H-QNB (for muscarinic receptors). Values are the average of three determinations and the maximum variance was 6%. [c] No significant activity observed at 10^{-3} M.

AChE inhibitor as pyridostigmine used as a control in these assays. Enzyme inhibition is sensitive to steric bulk at the C-3 position since the isopropyl ester **9c** is a weaker inhibitor (62.0% at 10^{-5} M) than its n-propyl and n-butyl analogues (80.1 and 88%, respectively).

Two results indicate that inhibition of AChE by the N-2 quaternized β-carboline esters is the result of specific interactions of these molecules with the enzyme and not simply the result of a non-specific recognition of their acetylcholine-like quaternary ammonium center.[26] Firstly, replacement of the methyl ester function of **6** by a methyl amide function (i.e., **9e**), gives a practically inactive compound. Secondly, removal of the aromatic A-ring of the quaternized β-carboline esters, as in azaindole **10**, also greatly reduces inhibitory potency (17.0% inhibition for **10** versus 60.9% inhibition for the ethyl ester **9a**).

We also investigated the effect of quaternization on the binding of these β-carbolines to benzodiazepine and muscarinic receptors (Table 1). While quaternization of the 3-carboxy-β-carboline derivatives severely reduces their affinity for the BZR by 2-3 orders of magnitude relative to β-CCM **1**, a non-negligible affinity for muscarinic receptors is produced by this chemical transformation. Interestingly, the quaternized norharman derivative **2** displays no affinity for the muscarinic receptor though it does inhibit AChE. The presence of a carboxylate

function is thus necessary to assure binding of these molecules to the muscarinic receptor, as is the aromatic A-ring (evident from the absence of binding in compound **10**). Moreover, as in the case of AChE inhibition, a long alkyl ester chain appears to favor muscarinic receptor binding (compare methyl ester **6** (K_i = 3.1 μM) to butyl ester **9d** (K_i = 0.5 μM)).

The quaternary N-2-methyl-β-carboline-3-carboxylates may thus be considered a new class of cholinergic agents, and in particular, of AChE inhibitors. Though the memory enhancing effects reported for the parent β-carbolines (i.e. of types **1** and **8**) may be partly the result of AChE inhibition by their putative N-2 methylated metabolites (i.e. of types **6** and **9**), confirmation of such a hypothesis must await the isolation and identification of such molecules in the brain following systemic administration of their non-methylated precursors. The effects of these new AChE inhibitors on memory and learning are currently being investigated.

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