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6-Hydroxy- and 6-methoxy-β-carbolines as acetyl- and butyrylcholinesterase inhibitors

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Abstract—In the course of studies directed toward the discovery of novel acetyl- and butyrylcholinesterase (AChE and BChE) inhibitors for the treatment of Alzheimer's disease, we focused on β-carbolines (BCs). 6-Oxygenated β-carboline and β-carbolinium derivatives based on the serotonin template were synthesized and tested in vitro for their ability to inhibit AChE and BChE, respectively. Particularly the carbolinium salts, which can be formed by intracerebral methylation out of the tertiary-BC prodrugs, show inhibitory activity levels reaching those of galantamine, physostigmine, and rivastigmine.

The enzyme acetylcholinesterase (AChE) plays an important role in the central nervous system. It is one of the fastest known enzymes and catalyzes the cleavage of acetylcholine in the synaptic cleft after depolarization. Inhibitors of acetylcholinesterase such as galantamine and others are used frequently in the pharmacotherapy of Alzheimer's disease (AD).[1] The less specific butyrylcholinesterase (BChE) has recently got into the focus of research, because BChE concentration stays the same or is even up-regulated while AChE is dramatically down-regulated in the brains of patients suffering from AD.[1]

β-Carbolines (pyrido[3,4-*b*]indoles) were first found in plants and referred to as harman alkaloids because they occur in *Peganum harmala*. In the human organism they may be formed from the condensation of the biogenic amines tryptamine and serotonin with aldehydes or α-keto acids, respectively. In traditional Indian medicine the carboline-containing plant *Desmodium spec.-viz.* is used to treat eye diseases and intestinal malfunctions; the plant's efficacy is probably due to the AChE-inhibiting properties of its alkaloids. Ghosal et al. investigated some tetrahydro- and fully aromatic carboline derivatives, including quaternary salts for ChE inhibitory activity (only AChE was tested in this in vivo assay) and reported that some quaternary compounds—including compound **4a**—were found to be one-sixth as potent as physostigmine.[2]

Furthermore, because endogenous BCs are considered in the literature to induce Parkinson's disease in non-primates, they are discussed as possible causative protoxines in idiopathic Parkinson's disease.[3] Neurotoxic β-carbolinium salts have been found in the lumbar cerebrospinal fluid of patients suffering from Parkinson.[4]

It was subsequently discovered that phenylethanolamine-*N*-methyltransferase exhibits β-carboline 2*N*-methyltransferase activity.[5] If suitable non-neurotoxic tertiary BCs could be identified, they would be bioactivated in vivo to the quaternary ChE-inhibiting BCs, that would in turn be 'locked' in the brain. These compounds could therefore be considered as target-specific prodrugs.

BCs can be regarded as potential anti-AD drugs as well as endogenous tryptamine- and serotonin-derived neurotoxins. To get more insight into their pharmacological profile, we synthesized a series of systematically varied 6-hydroxylated and 6-methoxylated harman and norharman derivatives (**3**, **4**) (Figs. 1 and 2), including the corresponding quaternary *N*-methyl-carbolinium salts. All of these compounds are methoxylated or

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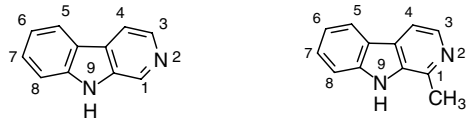
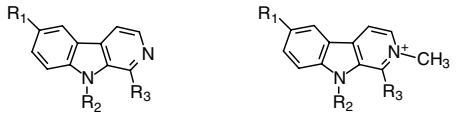


Figure 1. Norharman and harman.



3 a-h **4 a-h**

a: $R_1 = OCH_3$; $R_2 = R_3 = H$ **b**: $R_1 = OCH_3$; $R_2 = CH_3$; $R_3 = H$
c: $R_1 = OCH_3$; $R_2 = H$; $R_3 = CH_3$ **d**: $R_1 = OCH_3$; $R_2 = R_3 = CH_3$
e: $R_1 = OH$; $R_2 = R_3 = H$ **f**: $R_1 = OH$; $R_2 = CH_3$; $R_3 = H$
g: $R_1 = OH$; $R_2 = H$; $R_3 = CH_3$ **h**: $R_1 = OH$; $R_2 = R_3 = CH_3$

Figure 2. Target compounds.

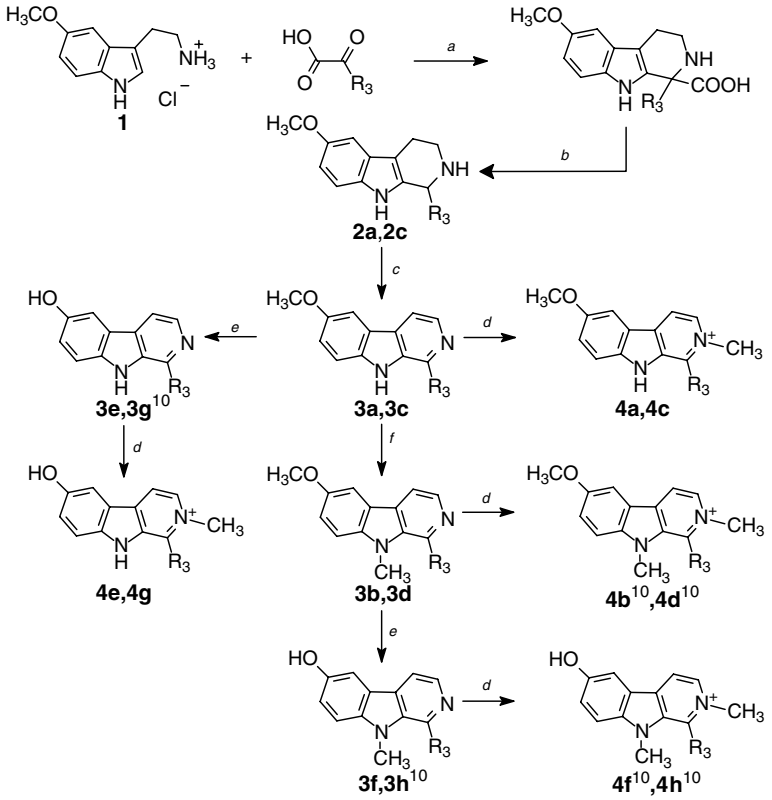
hydroxylated, respectively, in position 6 and methylated or non-methylated in position 9 (Fig. 2).

6-Methoxy-tryptamine (**1**) was cyclized via the *Pictet-Spengler* reaction[6,7] using glyoxylic acid for the norharman and pyruvic acid for the harman derivatives. Aromatization of compounds **2a** ($R_3 = H$) and **2b** ($R_3 = CH_3$) by Pd/C in boiling cumene yielded **3a** and **3c**. The indole-N atom was methylated using methyl iodide after deprotonation with sodium hydride. Methylation of the pyridine-*N* was achieved by having the carbolines react directly with methyl iodide in acetone or methanol. A mixture of acetic acid and aqueous hydrogen bromide (1:1) was used to convert the methoxy group in position 6 into a hydroxy group. Further details are given in Scheme 1.

In an initial pharmacological evaluation, the inhibitory activities at AChE and BChE were measured in vitro in order to get information about their ability to influence cognitive functions. The anti-AD drug galantamine, physostigmine, and all target compounds synthesized, respectively, were tested for inhibition of AChE and BChE activity levels using the Ellman assay.[8,9] This colorimetric assay is based on chromophores generated in situ and formed after enzymatic cleavage of acetyl- and butyrylthiocholine and reaction of the resulting thiocholine with Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid).

It may not be an advantage for a cholinesterase inhibitor (ChEI) to be selective for AChE (like galantamine); on the contrary, a balance between AChE and BChE may result in higher efficacy.[1]

Test solutions with $c = 10^{-5}$ μM of the tertiary amine salts **3a**–**c**, **3e**–**h**,[10] the tetrahydro-β-carboline derivatives **2a**,**b**, and the quaternary salt **4d**,[10] respectively, were prepared and tested in triplicate. All of them showed less



Scheme 1. Reagents and conditions: (a) for norharmans with $R_3 = H$: H_2O, KOH, 1 h, rt; for harmans with $R_3 = CH_3$: methanol/H_2O (1:1), 12 h, rt; (b) HCl, 15–30 min, 100 °C; (c) Pd/C (10%Pd), cumene, 10–15 h, reflux; (d) acetone, MeI, 2–4 h, rt; (e) HBr/CH_3COOH (1:1), 2–4 h, 130 °C; (f) i—NaH, DMF, 0 °C → rt; ii—MeI.

Table 1. AChE/BChE inhibition results of β-carbolines[a] (R_1, R_2, R_3 as defined in Fig. 2)

Compound	R_1	R_2	R_3	AChE inhibition (μM) IC_{50} (pIC_{50} ± SEM)	BChE inhibition (μM) IC_{50}(pIC_{50} ± SEM)	Selectivity IC_{50}(BChE)/IC_{50}(AChE)
Galantamine				0.6 (6.197 ± 0.052)	8.4 (5.076 ± 0.033)	14.0
Physostigmine				0.5 (6.307 ± 0.112)	1.2 (5.934 ± 0.084)	2.4
3d	OCH_3	CH_3	CH_3	11.8 (4.929 ± 0.100)	17.4 (4.758 ± 0.081)	1.5
4a	OCH_3	H	H	2.2 (5.656 ± 0.073)	17.5 (4.758 ± 0.366)	8.0
4b	OCH_3	CH_3	H	1.3 (5.885 ± 0.090)	20.9 (4.679 ± 0.087)	16.1
4c	OCH_3	H	CH_3	0.8 (6.122 ± 0.030)	1.2 (5.959 ± 0.062)	1.5
4e	OH	H	H	4.2 (5.377 ± 0.082)	8.7 (5.059 ± 0.085)	2.1
4f	OH	CH_3	H	1.8 (5.740 ± 0.050)	32.2 (4.492 ± 0.057)	17.9
4g	OH	H	CH_3	1.0 (6.007 ± 0.051)	1.6 (5.789 ± 0.068)	1.6
4h	OH	CH_3	CH_3	1.9 (5.722 ± 0.133)	2.7 (5.571 ± 0.051)	1.4

[a] Values are means of three independent experiments.

than 50% inhibition at 10^{-5} M, indicating IC_{50} values >10 μM. To measure the exact IC_{50} values of these low activity compounds would have afforded higher concentrations which were not possible due to poor solubility in water.

In general, the quaternary β-carbolinium salts were found to be potent inhibitors whose activity[10] levels reached those of the reference drugs (Table 1). No relationship between the inhibitory activity of the carbdinium salts at AChE and methylation in position 9 was observed. Interestingly, there was also no prominent difference in inhibitory activity for 6-methoxylated compared to 6-hydroxylated compounds.

Quaternary harmanium salts (i.e., with an additional methylation in position 1, but not at the indole-N) showed increased inhibitory activity levels by a factor of three (**4c** to **4a** and **4g** to **4e**). The highest activity was found for the 6-methoxy-harmanium salt **4c** with IC_{50} = 0.8 μM for AChE and IC_{50} = 1.2 μM for BChE.

Regarding the selectivity profiles for the cholinesterases: some preference for AChE was observed but selectivity was not prominent, with the exception of the 9-methylated (indole-N-methylated) norharmanium derivatives: the non-9-methylated harmanium salts **4a** and **4c** showed mixed activity profiles, whereas compounds **4b** and **4f** exhibited a considerable selectivity toward AChE, indicating that indole-N methylation leads to AChE selectivity. Therefore, most BCs tested show improved activity profiles in the light of the decreased AChE in AD patients.[1]

The activity increase between class of quaternary compounds, **4**, with tertiary compounds **3** might be well explained by the resemblance of **4** to the natural substrate acetylcholine.

In conclusion, a series of β-carbolines and β-carbolinium salts were synthesized and their inhibitory activity levels at AChE and BChE were measured in vitro. All of the carbolinium salts showed moderate to high activity levels in the ChEs reaching those of physostigmine, galantamine, and rivastigmine (IC_{50} (AChE) = 48 μM; IC_{50} (BChE) = 54 μM[1]). In contrast to the tertiary compounds, which can penetrate the blood–brain barrier, the quaternary compounds show strong levels of inhibitory activity. Therefore tertiary compounds might act as pro-drugs for the quaternary BCs that are formed in vivo in the brain, representing a novel and target-specific approach for the therapy of AD. Additional high levels of activity at BChE might be a further advantage of these compounds due to the increasing relevance of this enzyme in later stages of AD.[1]

Acknowledgments

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10. **6-Methoxy-2,9-dimethyl-9*H*-β-carbolin-2-ium iodide** (**4b**): mp 304 °C; IR (KBr, selected lines) 3004, 1645, 1524, 1217, 810 cm^{-1}. ^1H NMR (DMSO-d_6) δ 9.59 (s, 1H, 1-H), 8.78–8.74 (d, *J* = 6.6 Hz, 1H, 3-H), 8.61–8.57 (dd, *J* = 1.1, 6.6 Hz, 1H, 4-H), 8.06–8.05 (d, *J* = 2.4 Hz, 1H, 5-H), 7.87–7.82 (dd, *J* = 0.5, 9.2 Hz, 1H, 8-H), 7.55–7.49 (dd, *J* = 2.6, 9.0 Hz, 1H, 7-H), 4.47 (s, 3H, N(2)-CH_3), 4.06 (s, 3H, N(9)-CH_3), 3.90 (s, 3H, OCH_3). ^{13}C NMR (DMSO) 154.8 (C6), 139.8 (C9a), 135.9 (C8a), 132.4 (C4), 130.6 (C4a),

129.5 (C3), 122.8 (C1), 119.3 (C4b), 117.4 (C7), 112.3 (C8), 104.1 (C5), 55.8 (OCH_3), 47.7 (N(2)-CH_3), 30.3 (N(9)-CH_3). Anal. ($C_{14}H_{15}IN_2O$) C, H, N.

9-Methyl-9*H*-β-carbolin-6-ol (**3f**): mp 250 °C; IR (KBr, selected lines) 3424, 2932, 1573, 1199-1053, 810, 617 cm^{-1}. ^1H NMR (DMSO-d_6) δ 8.93–8.92 (d, J = 0.9 Hz, 1H, 1-H), 8.28–8.26 (d, J = 5.1 Hz, 1H, 3-H), 7.99–7.95 (dd, J = 1.1, 5.2 Hz, 1H, 4-H), 7.55–7.53 (dd, J = 0.6, 2.4 Hz, 1H, 5-H,) 7.50–7.45 (dd, J = 0.5, 8.8 Hz, 1H, 8-H), 7.17–7.11 (dd, J = 2.4, 8.8 Hz, 1H, 7-H), 3.89 (s, 3H, N(2)-CH_3). ^{13}C NMR (DMSO) 151.0 (C6), 137.4 (C9a), 135.6 (C8a), 132.3 (C3), 126.6 (C1), 123.5 (C4b), 120.7 (C7), 118.0 (C4a), 114.4 (C4), 110.5 (C8), 105.9 (C6), 29.2 (N(9)-CH_3). HRMS Calcd for $C_{12}H_{11}N_2O$ $[M+H]^+$: 199.0871 199.0880.

6-Hydroxy-2,9-dimethyl-9*H*-β-carbolin-2-ium iodide (**4f**)—**exemplary procedure**: A solution of 10 mmol of **2a** in 100 mL of cumene was treated with 2% Pd/C (10%) and refluxed for 10–15 h under N_2. Thereafter the reaction mixture was treated with 20 mL of ethanol, heated again and the hot mixture filtered off the catalyst, washed with 150 mL of hot ethanol. The solvent was removed under reduced pressure and the residue was recrystallized from toluene. Product **3a** (5 mmol) was dissolved in DMF and the mixture cooled to 0 °C, after which 6.75 mmol of NaH was added. The mixture was allowed to reach room temperature and stirred for an hour until evolution of hydrogen stopped. The mixture was cooled to −10 °C, 5 mmol of MeI was added and the conditions were retained for 15 min. Thereafter, the mixture was allowed to reach rt and stirring was continued for 12 h. The mixture was then treated with 85 mL of water and **3b** was extracted with chloroform. A mixture of 1.5 mmol of **3b** and 20 mL HBr/acetic acid (1:1) was stirred for 2–4 h at 130 °C, then evaporated to dryness to yield **3f**. Eight millimole of MeI was added to a solution of 0.5 mmol of **3f** in 20 mL of acetone. The product **4f** was filtered off and dried in vacuo. mp 258 °C; IR (KBr, selected lines) 3172, 1645, 1621,1518, 1362, 1211, 816, 774, 599 cm^{-1}. ^1H NMR (DMSO-d_6) δ 9.69 (s, 1H, OH), 9.54 (s, 1H, 1-H), 8.69–8.67 (d, J = 6.4 Hz, 1H, 3-H), 8.55–8.52 (d, J = 6.5 Hz, 1H, 4-H), 7.74 (s, 1H, 8-H), 7.70 (d, J = 1.7 Hz, 1H, 5-H), 7.40–7.35 (dd, J = 2.5, 8.9 Hz, 1H, 7-H), 4.46 (s, 3H, N(2)-CH_3), 4.03 (s, 3H, OCH_3). ^{13}C NMR (DMSO) 152.6 (C6), 139.0 (C9a), 135.9 (C8a), 132.1 (C4), 130.3 (C1), 129.2 (C3), 122.6 (C4a), 119.6 (C7), 117.4 (C4b), 111.9 (C8), 106.4 (C5), 47.6 (N(2)-CH_3), 30.2 (N(9)-CH_3). Anal. ($C_{13}H_{13}IN_2O$) C, H, N.

6-Methoxy-1,2,9-trimethyl-9*H*-β-carbolin-2-ium iodide (**4d**): mp 319 °C; IR (KBr, selected lines) 3040, 1609, 1488, 1434, 1229, 1036, 810, 765, 624 cm^{-1}. ^1H NMR (DMSO-d_6) δ 8.60 (s, 2H, 3-H, 4-H), 8.00-7.99 (d, J = 2.5 Hz, 1H, 5-H), 7.89-7.85 (d, J = 9.2 Hz, 1H, 8-H), 7.50-7.45 (dd, J = 2.6, 9.2 Hz, 1H, 7-H), 4.35 (s, 3H, N(2)-CH_3), 4.25 (s, 3H, N(9)-CH_3), 3.89 (s, 3H, OCH_3), 3.22 (s, 3H, C(1)-CH_3). ^{13}C NMR (DMSO) 158.6 (C6), 158.0 (C9a), 154.8 (C1), 134.2 (C8a), 122.6 (C4a), 119.0 (C4), 117.4 (C3), 115.1 (C4b), 112.8 (C7), 112.5 (C8), 103.5 (C5), 55.7 (OCH_3), 45.5 (N(2)-CH_3), 33.5 (N(9)-CH_3), 16.3 (C(1)-CH_3). Anal. ($C_{15}H_{17}IN_2O$) C, H, N.

6-Hydroxy-1,9-dimethyl-9*H*-β-carbolin-2-ium bromide (**3h**): mp 320 °C; IR (KBr, selected lines) 3556-3412, 2932, 1621, 1579, 1476, 1374, 1205, 813, 618 cm^{-1}. ^1H NMR (DMSO-d_6) δ 9.16 (s, 1H, OH), 8.10–8.08 (d, J = 5.3 Hz, 1H, 3-H), 7.84–7.81 (dd, J = 0.5, 5.2 Hz, 1H, 4-H), 7.51–7.47 (m, 2H, 5-H, 8-H), 7.13–7.07 (dd, J = 2.4, 9.0 Hz, 1H, 7-H), 4.08 (s, 3H, N(9)-CH_3), 2.97 (s, 3H, C(1)-CH_3). ^{13}C NMR (DMSO) 151.1 (C6), 141.9 (C9a), 136.7 (C1), 136.1 (C8a), 135.8 (C4), 127.2 (C3), 121.0 (C7), 118.1 (C4a), 112.9 (C4b), 110.9 (C8), 105.9 (C5), 32.2 (N(9)-CH_3), 23.3 (C(1)-CH_3). HRMS Calcd for $C_{13}H_{13}N_2O$: M 213.1028. Found M 213.0985.

6-Hydroxy-1,2,9-trimethyl-9*H*-β-carbolin-2-ium iodide (**4 h**): mp 320 °C; IR (KBr, selected lines) cm^{-1} 3148, 1513, 1482, 1338, 1217, 810, 635.^1H NMR (DMSO-d_6) δ 9.67 (s, 2H, OH), 8.56–8.53 (d, J = 6.6 Hz, 1H, 3-H), 8.52–8.49 (d, J = 6.6 Hz, 1H, 4-H), 7.76–7.72 (d, J = 9.1 Hz, 1H, 8-H), 7.65–7.64 (d, J = 2.2 Hz, 5-H), 7.36–7.31 (d, J = 2.4, 9.0 Hz, 1H, 7-H), 4.34 (s, 3H, N(2)-CH_3), 4.19 (s, 3H, N(9)-CH_3), 3.19 (s, 3H, CCH_3). ^{13}C NMR (DMSO) 152.5 (C6), 141.4 (C1), 139.5 (C9a), 135.1 (C8a), 133.9 (C4), 130.8 (C3), 122.4 (C7), 119.3 (C4a), 115.1 (C4b), 112.1 (C8), 105.7 (C5) 45.4 (N(2)-CH_3), 33.4 (N(9)-CH_3), 16.3 (C(1)-CH_3). Anal. ($C_{14}H_{15}BrN_2O$) C, H, N.