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Short communication

Beta and gamma carboline derivatives as potential anti-Alzheimer agents: A comparison

Robert Otto [a], Robert Penzis [b], Friedemann Gaube [b], Thomas Winckler [b], Dorothea Appenroth [c], Christian Fleck [c], Christian Tränkle [d], Jochen Lehmann [a], Christoph Enzensperger [e, *]

[a] Pharmaceutical / Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University, Philosophenweg 14, D-07743 Jena, Germany
[b] Pharmaceutical Biology, Institute of Pharmacy, Friedrich-Schiller-University, Semmelweisstr. 10, D-07743 Jena, Germany
[c] Institute for Pharmacology and Toxicology, Friedrich-Schiller-University, Drackendorfer Str. 1, D-07747 Jena, Germany
[d] Pharmaceutical Institute, Rheinische Friedrich-Wilhelms-University, Gerhard-Domagk-Str.3, D-53121 Bonn, Germany
[e] Institute for Organic and Macromolecular Chemistry, Friedrich-Schiller-University, Humboldtstr. 10, D-07743 Jena, Germany

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ABSTRACT

Nine novel β- and γ-carboline derivatives bearing either methyl-, propargyl- or phenethyl-residues at the indole nitrogen were synthesized and tested as potential anti-Alzheimer drugs. Antagonism of recombinantly expressed NMDA receptors, inhibition of cholinesterases, and radical scavenging properties were determined for all compounds. Some were additionally tested *in vivo* for their ability to reverse scopolamine-induced cognitive impairment in an 8-arm radial maze experiment with rats. For the most promising candidates, the interaction with muscarinic M_1 receptors was also investigated. With this set of compounds assays the influence of the scaffold itself and the substituents can be investigated separately. 5-Methyl-γ-carboline (**6**) was the most potent (0.25 μmol/100 g b.w.) compound in the *in vivo* test and might be a good starting point for the development of novel anti-Alzheimer drugs.

1. Introduction

Alzheimer's disease (AD) is a prevalent neurodegenerative disorder with multifactorial causes that requires multi-targeted treatment. Inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) improve cholinergic signaling in the central nervous system and are well established in the therapy of AD to improve cognitive symptoms. AD patients may also benefit from reduction of pathologic glutamate-induced, Ca^{2+}-mediated excitotoxicity by the approved *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine. New drugs that simultaneously affect both cholinergic transmission and reduce glutamate-induced excitotoxicity may further improve AD treatment [1].

β-Carbolines are natural products that are formed from indolylethylamines or tryptophan in a *Pictet-Spengler* reaction. They occur in different plants and animals including humans, and also emerge from fermentation or during the cooking process. It is likely that β-carbolines exert pharmacological effects, because they represent a class of privileged chemical structures with a variety of biological effects [2]. The naturally occurring harmine (**1**) (Fig. 1) is the ancestor of the β-carbolines. It was the first monoamine oxidase (MAO) inhibitor used for the treatment of Parkinson's disease (PD) [3]. More recently, 9-methyl β-carboline (**2**) (Fig. 1) gained certain interest as neuroprotective, neurorestorative, anti-inflammatory and cognitive enhancing drug [4,5]. Moreover, some bivalent β-carboline derivatives have been shown to be highly potent dual inhibitors of acetylcholinesterase and NMDA receptor [6,7]; therefore, they are potential candidates for the treatment of neurodegenerative diseases. The bioavailability of the bivalent β-carbolines investigated earlier [6,7] may be limited due to their high molecular weight and therefore it was the aim of the present work to gain similar bioactivities with smaller molecules by combining promising substituents with the β- and γ-carboline scaffold.

* Corresponding author.
E-mail addresses: robert.otto@uni-jena.de (R. Otto), robert.penzis@uni-jena.de (R. Penzis), Friedemann.gaube@uni-jena.de (F. Gaube), t.winckler@uni-jena.de (T. Winckler), dorothea.appenroth@mti.uni-jena.de (D. Appenroth), christian.fleck@mti.uni-jena.de (C. Fleck), traenkle@uni-bonn.de (C. Tränkle), ch.enzensperger@uni-jena.de (C. Enzensperger).

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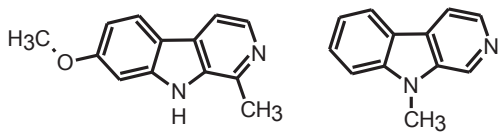


(1) (2)

Fig. 1. Some established β-carboline structures with well-known pharmacological properties.

γ-Carbolines represent a structurally related class where the pyridine nitrogen is shifted by one position compared to β-carbolines. To our knowledge, γ-carbolines do not occur naturally, but certain γ-carbolines are investigated as drug candidates, such as the antihistaminic mebhydrolin (**3**) (Fig. 2) [8]. The γ-carboline derivative flutroline (**4**) (Fig. 2) was used as antipsychotic [9,10]. Serotonin 5-HT6 receptor antagonism, which is associated with Alzheimer's and Parkinson's disease, was also found for some γ-carboline derivatives [11]. The cognition enhancer dimeboline (**5**) (Fig. 2) was extensively tested on working memory and as potential anti-Alzheimer drug [12] (Fig. 2).

In this work we focus on the potential neuroprotective and nootropic effects of a set of β- and γ-carboline derivatives with regard to the scaffold itself and characteristic substitution pattern.

Relying on 9-methyl β-carboline (**2**), we investigated the indole-*N*-methylation on β- and γ-carboline scaffold, yielding **2** and **6**. Secondly, we probed the effect of the propargylamine moiety found in selegiline or rasagiline, which is itself referred to as "neuroprotective and neurorescuing" [13] and is well-known for its peroxynitrite scavenging effect [14] to obtain two resulting β- and γ-congeners **7** and **8**. [15,16].

Finally, we used an arylethylamine residue at the indole-*N* comparable to dimeboline (**5**) because several structure-activity relationships (SARs) on this class revealed that a simple phenethyl residue is superior to the methyl-pyridine residue on the original dimeboline [17]. The two compounds with the phenethyl moiety are **9** and **10**, respectively.

We investigated the novel compounds in various assays useful to characterize potential anti-Alzheimer drugs. Approved anti-Alzheimer drugs mainly target either the glutamatergic signaling *via* NMDA receptor inhibition, the cholinergic system by inhibiting acetylcholine degradation [18], or reduce oxidative stress [19]. Therefore, we determined the potency of the new compounds to reduce glutamate-induced, Ca^{2+}-mediated excitotoxicity in cell lines expressing NR1-1a/NR2A and NR1-1a/NR2B subunits of the NMDA receptor. In addition, we used cell-free assays to determine the efficacy of the new compounds as AChE and BChE inhibitors and tested them as radical scavengers. Selected compounds were also investigated for interactions with muscarinic M_1 receptors and their ability to reverse scopolamine-induced memory deficits in an 8-arm radial maze model with rats.

2. Chemistry

The main focus of this study was to synthesize and compare β-carboline (9*H*-pyrido[3,4-*b*]indole) and γ-carboline (5*H*-pyrido[4,3-*b*]indole) derivatives with various substituents (Fig. 3). To facilitate the discussion and comparison of these compounds, we use the terms "pyrido-*N*" and "indolo-*N*" to describe the position where substitutions were realized at both carboline scaffolds.

The β-carboline scaffold could be easily obtained in a *Pictet-Spengler*-cyclisation by reacting tryptamine hydrochloride with paraformaldehyde [20]. The formed tetrahydro-β-carboline **(11)** is aromatized as shown in Scheme 1 by catalytic oxidation with Pd/C [6] in toluene to form **12** in 81% yield. The preparation of the analogous tetrahydro-γ-carboline **(13)** follows a classical *Fischer*-indolization by reacting 4-piperidone hydrochloride with phenylhydrazine hydrochloride in ethanol, containing a few drops of aqueous HCl [21]. Afterwards, aromatization was carried out as described for the tetrahydro-β-carboline **(11)** moiety to obtain **14** in a comparable yield. To accomplish indolo-N-alkylation, the fully aromatic carbolines **12** or **14**, respectively, were deprotonated with NaH in DMF and treated with a 1.3-fold molar excess of the appropriate alkyl halides to form **2, 6**–**10** [22]. Methylation at the pyrido-*N* was achieved with a 5-fold molar excess of methyl iodide in acetone, and the resulting quaternary salts **15**–**20** were obtained almost quantitatively. The tetrahydro-derivatives **21** and **22** were obtained by $NaBH_4$ reduction of **19** and **20** in methanol.

3. Pharmacology

In order to characterize and compare the synthesized β- and γ-carbolines in detail, we made use of various pharmacological tests to assess neuroprotective and cognition enhancing properties:

Cholinergic signaling effects were investigated by determination of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition using the *Ellman* assay as described earlier [6] and the effect on muscarinic M_1 receptors by performing equilibrium binding experiments.

Potential neuroprotective properties of the compounds were assessed by the widely used DPPH test which reflects their radical scavenging effects [23,24]. Furthermore the reduction of glutamate-induced excitotoxicity was investigated using a cell-based assay of glutamate-induced, Ca^{2+}-mediated excitotoxicity in mouse fibroblast cell lines L12-G10 and L13-E6 expressing NR1-1a/NR2A and NR1-1a/NR2B subunits of the NMDA receptor, respectively [7].

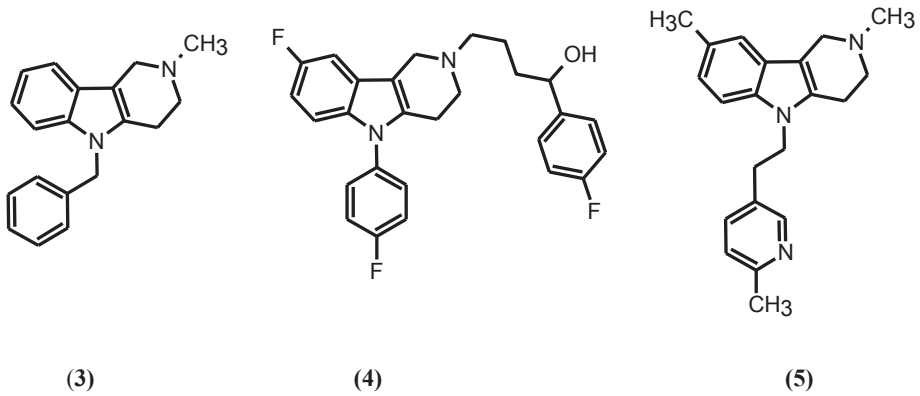
To corroborate our *in vitro* experiments we also performed an *in vivo* test with female Wistar rats (Han:Wist). We used a scopolamine-induced memory deficit test in an 8 arm radial maze which is a common model to evaluate potential anti-Alzheimer drugs on the working memory. If rats were successfully trained to pass the 8 arm radial maze without any error, their working memory is impaired by scopolamine and the number of errors increases. The compounds were tested for their ability to antagonize the scopolamine-induced impairment of the working memory [25].

4. Results & discussion

Nine new indolo-N-substituted carboline derivatives were synthesized and screened for their inhibitory effects on AChE, BChE, NMDA receptors, M_1 receptors as well as their radical scavenging effects in a DPPH test. A preliminary screening was made for all compounds and for active compounds, an IC_{50} was determined. Suitable reference substances as well as the unsubstituted carbolines **12, 14** were included. Finally, we also performed *in vivo* assays with rats in an 8-arm radial maze. The results of the *in vitro* test are shown in Table 1. For a better comparison, data from β-carbolines are shown in grey and the data from the corresponding γ-carbolines are represented in white. Derivatives with the same substitution pattern are shown in sequence.

4.1. Cholinesterase-inhibitory effects

We determined both AChE and BChE inhibition, because it is assumed that BChE inhibition may be even more important for the treatment of AD than AChE inhibition [26], which is because AChE expression is progressively reduced in Alzheimer's patients to reach



(3) (4) (5)

Fig. 2. Some established γ-carboline structures with known pharmacological properties.

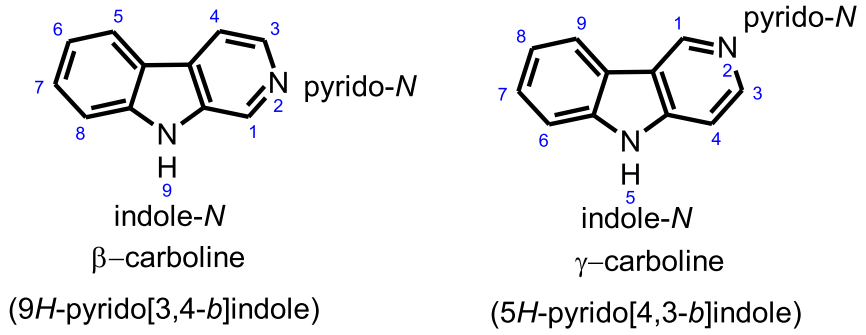


Fig. 3. Comparison of the β-carboline (9*H*-pyrido[3,4-*b*]indole) and γ-carboline (5*H*-pyrido[4,3-*b*]indole).

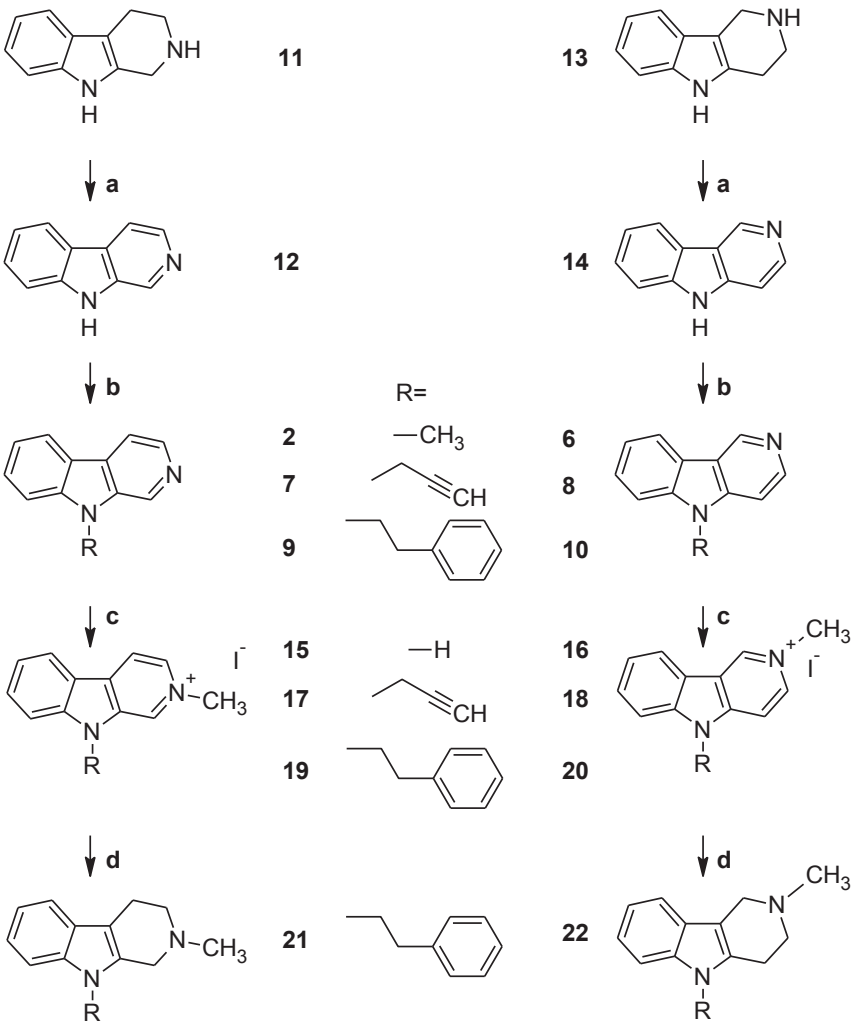
10–15% compared to normal values [27]. In a previous study we found that indolo-N-linked, homobivalent β-carboline derivatives are potent inhibitors of both AChE and BChE, while monomeric β-carbolines were rather ineffective [7]. Thus, it seemed not surprising that the plain β-carboline **12** had no effects on cholinesterases (AChE and BChE IC_{50}: >100 μM) (Table 1). However, the corresponding γ-carboline **14** showed moderate cholinesterase inhibition with IC_{50} value of 19.8 μM and 1.2 μM for AChE and BChE, respectively. This considerable cholinesterase inhibitory effect of γ-carbolines can be modulated by substitution at the indolo-*N*. The bulky phenethyl residue in compound **10** leads to a 10-fold increase of the AChE inhibition and a 2-fold increase at BChE compared to **14**. In contrast, the propargyl group in **8** resulted in a loss of activity. The smallest substituent (**2**) caused the smallest effect on the activity compared to the unsubstituted **14**.

These findings could be well transferred to the β-carboline moiety with one exception. As mentioned above, **12** had no activity whereas compound **2** showed a slight inhibition at least at the BChE. The bulky residues in compounds **9** and **7** mediated an increase in affinity. The propargyl substitution of β-carboline **12** enhances the inhibitory potency distinctly which is the contrary to our findings with γ-carbolines.

Overall, bulky, lipophilic residues at the indole-*N* led to higher inhibitory effects and a slight prevalence for BChE over AChE could be observed for γ-carboline derivatives. Quaternization mostly increases the cholinesterase-inhibitory potency, most pronounced for compound **19**, where nanomolar IC_{50} values were achieved. The reason for this finding might be that a permanently charged nitrogen is also present in the endogenous substrate, acetylcholine. For partially reduced derivatives **21** and **22** which have the methyl group, but lack a permanent charge, there is a nearly complete loss of AChE inhibition, however BChE is still inhibited in a micromolar range. The obvious shift in AChE-inhibitory potency suggests a more pronounced effect of a permanent charge to the activity of AChE than BChE. Compared to the "gold standard" galantamine, compounds **19** and **20** can easily compete in terms of inhibitory potency. Compound **19** represents the so far most potent monovalent carboline derivative.

4.2. Inhibition of glutamate-induced excitotoxicity

In a previous study we found that indolo-N-linked, homobivalent β-carboline derivatives with a pyrido-*N*-methylated, quaternary nitrogen were not only potent AChE and BChE inhibitors, but also effective inhibitors of the glutamate-induced, Ca^{2+}-mediated excitotoxicity in cell lines expressing NR1-1a/NR2A and NR1-1a/NR2B subunits of the NMDA receptor [7]. This NMDA receptor-antagonistic property of the quaternary bivalent β-carboline was comparable to the approved drug memantine, but, interestingly, the activity of the compound was completely lost upon partial hydration of the pyridine ring or abstinence of the pyrido-*N*-methyl group. Similarly, we found here that only the pyrido-*N*-quaternary compound **19** showed considerable activity as an NMDA receptor antagonist (Table 1). Compound **19** is the first monovalent β-carboline that we found active in this assay set-up. Also, we have seen here for the first time that for carboline derivatives bivalency is not a mandatory feature for high NMDA-receptor-inhibitory activity. Remarkably compound **19** has an approximately 7-fold higher activity on receptors containing the NR2B subunit than the NR2A subunit (IC_{50} values 5.1 μM vs. 34.8 μM). It is worth mentioning that this activity was drastically reduced in the analogous γ-carboline, compound **20** (Table 1). Thus, compared to the previously described indolo-N-linked, homobivalent β-carboline, compound **19** has a similar activity on NR2B-containing NMDA receptors and is



Scheme 1. Synthesis of indolo-N-substituted carboline derivatives: *Reagents and conditions*: a) 10% Pd/C, toluene, 5 h, reflux; b) 1.NaH, DMF, 0 °C, 1 h, 2. alkyl halide, 0 °C → rt, 1–24 h; c) Methyl iodide, acetone, 72 h, rt; d) $NaBH_4$, methanol, 0 °C → rt, 1 h.

considerably smaller, which may positively affect its bioavailability after oral application. Yet it seems that only quaternary, permanently charged β-carbolines are effective NMDA receptor antagonists. So it remains an open question whether such compounds would be able to cross the blood brain barrier and enter the CNS to mediate neuroprotective effects. Although this seems unlikely, it may also be considered that such compounds would be actively transported to the CNS *via* transporter proteins. This question remains to be addressed in future studies.

4.3. In vivo radial maze test

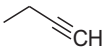
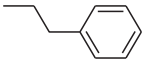
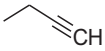
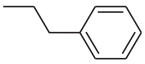
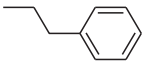
The design of the *in vivo* study and the selection of the investigated compounds should give an overview on the influence of the substituents and the backbone scaffold. To assess the effects of substituents on β- and γ-carbolines, we selected the six following compounds **2, 6**–**10**. The investigated dose was divided into halves until the lowest effective concentration was found. Besides **9**, all investigated carbolines are able to compensate the scopolamine-induced memory impairment significantly. Obviously a bulky substituent at the indolo-*N* position decreases the effect, which contradicts the structure activity relationship we observed for the choline esterase inhibition. However, concerning the scaffold we also found the γ-carbolines are almost twice as potent as β-carbolines.

As given in Table 2, the γ-carboline **6** represents the most potent derivative with an active range between 0.25 and 1 μmol per 100 g b.w. In this test every compound seems to have an active range, while higher and lower dosage leads to a loss of activity. Compound **6** shows on one side the lowest active dose and on the other side the widest range of activity at all.

4.4. M_1 receptor antagonism

Since the findings from the *in vivo* experiments do not correlate with the results of choline esterase inhibition test we investigated whether the outcome of this radial maze test is related to muscarinic M_1 receptors. It was studied whether the compounds **2**, **6**, **9** and **10** interfered with the specific equilibrium binding of the muscarinic inverse agonist [^{3}H] *N*-methyl-scopolaminium bromide (NMS) at muscarinic acetylcholine receptors of the M_1 subtype (Fig. 4). Changes introduced into the β-carboline **2**, i.e. either switching to a γ-carboline structure as in **6**, as well as a replacement of the methyl group at the indolo-*N* in **2** by a phenethyl group as in **9**, both increased significantly and to a similar extent the maximum inhibitory effect and the $\log IC_{0.5}$ concentration

Table 1
Cholinesterase and NMDA receptor inhibitory activities of compounds tested in this study.

	Indolo-*N* residue (R1)	Cpd	Cholinesterase		NMDA receptors			
			AChE	BChE	NR1-1a/NR2A		NR1-1a/NR2B	
			IC_{50} μM (pIC_{50} ± SEM)		Excitotoxicity [%][c] at			
					10 μM	50 μM	10 μM	50 μM
Pyridine- derivatives	H	**12**	>100	>100	93.7 ± 19.7	88.2 ± 18.7	100.8 ± 11.9	75.6 ± 6.2
		14	19.8 (4.7 ± 0.048)	1.2 (5.9 ± 0.046)	119.4 ± 25.2	100.6 ± 34.0	86.3 ± 16.0	73.7 ± 10.3
	—CH_3	**2**	>100	46.9 (4.7 ± 0.238)	99.8 ± 6.4	90.3 ± 12.3	98.1 ± 8.4	77.8 ± 5.1
		6	14.7 (4.8 ± 0.036)	6.9 (5.2 ± 0.059)	105.0 ± 10.2	104.5 ± 15.6	104.0 ± 12.2	92.5 ± 7.6
		**7**	2.0 (5.7 ± 0.105)	7.2 (5.1 ± 0.068)	98.1 ± 7.4	101.1 ± 3.3	98.0 ± 21.7	110.1 ± 15.7
		8	>100	6.9 (5.2 ± 0.056)	115.6 ± 11.6	113.5 ± 24.1	100.3 ± 10.9	82.4 ± 6.5
		**9**	4.2 (5.4 ± 0.053)	3.8 (5.4 ± 0.111)	87.2 ± 9.0	236.2 ± 68.0	83.7 ± 18.3	389.4 ± 131.5
		10	1.6 (5.8 ± 0.065)	0.7 (6.2 ± 0.026)	96.0 ± 32.5	137.5 ± 55.8	43.3 ± 16.7	147.1 ± 114.1
Pyridinium - quats	H	**15**	6.7 (5.2 ± 0.467	0.8 (6.1 ± 0.068)	101.6 ± 5.7	101.2 ± 7.7	114.4 ± 8.0	110.9 ± 7.1
		16	3.2 (5.5 ± 0.373)	0.9 (6.05 ± 0.239)	103.1 ± 1.7	104.6 ± 3.7	110.6 ± 9.1	104.0 ± 5.1
		**17**	3.2 (5.5 ± 0.022)	2.7 (5.6 ± 0.043)	97.0 ± 22.9	95.5 ± 18.6	105.0 ± 10.0	102.1 ± 9.3
		18	2.7 (5.6 ± 0.039)	5.2 (5.3 ± 0.018)	92.9 ± 15.7	92.6 ± 10.0	105.4 ± 10.2	106.2 ± 16.9
		**19**	0.6 (6.2 ± 0.092)	0.6 (6.2 ± 0.046)	69.6 ± 9.4 IC_{50} = 34.8 ± 1.8 μM	23.4 ± 10.8	28.3 ± 10.5 IC_{50} = 5.1 ± 2.5 μM	0.4 ± 4.1
		20	5.2 (5.2 ± 0.029)	0.9 (6.0 ± 0.010)	80.3 ± 9.0 IC_{50} = 39.6 ± 8.2 μM	47.4 ± 8.6	93.8 ± 11.2 IC_{50} = 29.4 ± 2.3 μM	37.9 ± 12.2
N-methyl-tetra-hydro-pyridine		**21**	>100	7.9 (5.1 ± 0.361)	108.9 ± 12.6 IC_{50} > 100 μM	90.5 ± 15.8	94.7 ± 18.4 IC_{50} > 100 μM	71.5 ± 12.2
		22	97.3 (4.0 ± 0.372)	3.6 (5.5 ± 0.511)	114.5 ± 17.0 IC_{50} > 100 μM	83.0 ± 20.8	104.6 ± 18.4 IC_{50} > 100 μM	62.5 ± 17.0
Memantine			>100	>100	IC_{50} = 4.9 ± 2.5 μM		IC_{50} = 5.6 ± 2.4 μM	
Galantamine			0.85 (6.1 ± 0.088)	11.13 (5.0 ± 0.141)	96.8 ± 7.5[a,b]		93.2 ± 8.4[a,b]	

[a] values taken from Ref. [7].
[b] determined at 25 μM.
[c] glutamate-induced excitotoxicity; 0% and 100% is defined as values obtained in the presence of 100 μM ketamine or absence of ketamine [5].

necessary to induce a half maximum effect on [^{3}H]NMS binding ($P < 0.05$, one-way ANOVA with Tukey's post test). Remarkably, applying both changes simultaneously as in **10** significantly lowered the bottom plateau of specific radioligand binding to zero compared with **6** and **9** and significantly increased the log$IC_{0.5}$ concentration back to a value not different from that of **2** ($P < 0.05$, one-way ANOVA with Tukey's post test). Obviously, in **10**, the γ-position of the nitrogen and its substitution with a space demanding phenethyl moiety favors its interaction with muscarinic M_1 receptors (Fig. 4). In the presence of **10** the slope of the inhibition curve was significantly lower than unity which may point to a deviation from competitive mass action behavior of the compound (cf. Table 3). A compilation of the parameters collected in the binding experiments is given in Table 3.

4.5. Radical scavenging ability – DPPH assay

The radical scavenging ability, where the reduction of the stable free 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) is measured, was determined at pH 5 to 6.5 and without pH adjustment [28,29]. The test was carried out as a prescreening where the concentration of the tested compounds was 4 times the concentration of DPPH. All in all some structures showed slight activity but none could be regarded as active, so no IC_{50} values were determined. The procedure and the data are given in the Supporting information.

5. Conclusion

In our studies we probed the potential antidementive properties of a set of carboline derivatives. Therefore we synthesized 8 β-carboline and γ-carboline derivatives, with different residues, relying on drugs known for their neuroprotective properties.

We tested these compounds for their ability to inhibit choline esterase and NMDA receptor subtypes and tested them as radical scavengers. Additionally we tested 6 different β- and γ-couples in an *in vivo* rat model to test whether they are able to reverse scopolamine-induced cognitive impairment and investigated their interaction with muscarinic M1 receptors for some compounds. The data are shown in Table 1.

For the choline esterase inhibitory effects, most compounds show stronger effects for butyryl-choline esterase (BChE) than for acetyl-choline esterase (AChE). This might a therapeutic advantage for the treatment of AD, because AChE activity is strongly decreased in AD patients whereas BChE is unaffected. Therefore it is more promising to target BChE than AChE [27]. In some cases a clear selectivity is present. Compounds **2, 8** and **21** show for AChE IC_{50} values below the detectable limit (>100 μM) whereas the BChE inhibitory potency is two- (compound **2**) to 14-fold (compound **8**) stronger. Additionally all fully aromatic and not quaternary γ-carboline derivatives show noticeably higher BChE values compared to their respective β-carboline congeners.

Table 2
Reduction of errors in the radial maze test by compounds **2, 6**–**10**.

Concentration of test compound [per 100 g b.w.]	Number of errors					
	9	**10**	**7**	**8**	**2**	**6**
Control (no test compound)	6.4	6.4	6.7	7.7	7.9	7.1
0.125 μmol					n.d.	7.0
0.25 μmol	7.5			4.0	6.0	3.7*
0.5 μmol	4.7	7.3	5.3	1.9*	1.2*	2.3*
1 μmol	9.0	2.0*	2.4*	5.2	4.3	2.3*
2 μmol		3.3	8.9		n.d.	3.8

*Significant reduction of errors compared to control ($p < 0.05$); n.d. not determined; grey is the lowest dose which provides a significant reduction of errors.

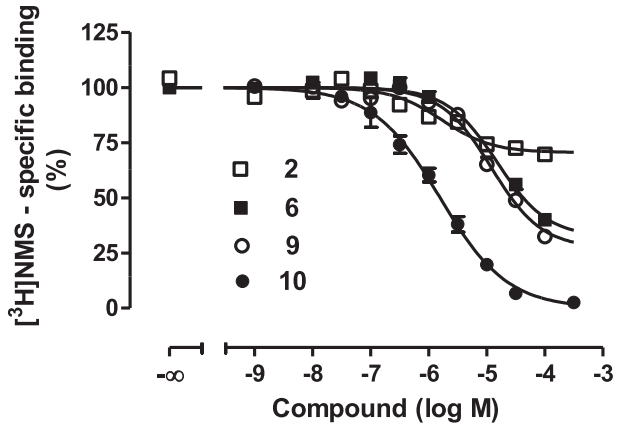


Fig. 4. Inhibition of specific orthosteric [^{3}H]NMS (0.2 nM) equilibrium binding by the indicated compounds at M_1 receptors. Curves were fitted individually using Prism 5.03 for Windows (GraphPadTM Software, Inc., San Diego, U.S.A.) by applying a built-in standard four parameter logistic equation. Data are the means ± S.E.M. of three to four experiments performed as triplicate determinations. For details refer to pharmacological procedures (see Supplementary information).

Table 3
Parameters characterizing the interaction of the test compounds with the inverse orthosteric agonist [^{3}H]NMS at muscarinic M_1 receptors in equilibrium binding experiments.

Cpd	[^{3}H]NMS-equilibrium binding		
	$logIC_{0.5}$	Bottom plateau	*n* slope
2	−5.77 ± 0.28[b]	71 ± 4[c]	−0.78 ± 0.46
6	−4.82 ± 0.14[b]	32 ± 7[c]	−0.80 ± 0.28
9	−4.92 ± 0.11[b]	27 ± 7[c]	−0.99 ± 0.23
10	−5.80 ± 0.04	0	−0.75 ± 0.04[a]

$logIC_{0.5}$: log concentration of the test compound that reduces the specific equilibrium binding of [^{3}H]NMS to muscarinic M_1 receptors in the absence of a test compound by 50% (=inflection point of the curve); bottom: lower plateau of the curve; n: slope factor of the curve. The data shown are mean values ± S.E.M. of three to four experiments carried out in triplicate.
[a] Value is different from unity (*F-test*, $P < 0.05$).
[b] $logIC_{0.5}$ values were read from curves with *n* fixed to −1.
[c] Value is significantly different from zero (*F*-test, $P < 0.05$). For further details see Supporting information.

The influence of the substitution pattern at the indolo-*N* is much harder to detect. Phenethyl- (**10, 9**) and propargyl (**8, 7**) derivatives seem to be slightly more potent than methyl- (**6, 2**) or H (**14, 12**).

The NMDA antagonistic properties are not very pronounced for the regular indolo-*N* alkylated derivatives, but for quaternary derivatives **19** and **20** μM IC_{50} values are present. However, the quaternary 2,9-dimethyl-β-carbolinium ion is known for its neurotoxic potential by inhibiting complex 1 of the respiratory chain [30]. Therefore we didn't synthesize and test this particular compound and also did not test the quaternary derivatives (**15, 16, 17, 18, 19, 20**) *in vivo*. We also excluded **21** and **22** from our *in vivo* experiments, because these tetrahydro-derivatives didn't show promising *in vitro* effects.

In our *in vivo* rat model we also found all γ-carboline derivatives are more potent than their β-carboline congeners. The reversal of scopolamine-induced memory impairment in an 8-arm radial maze experiment is a common model for cognition enhancing drugs. In our studies we investigated the reduction of errors compared to controls and reduced the dose from 2 to 0.125 μmol until we found the lowest effective dose. (See Table 2) In this experiment we observed a bell-shaped relation between dose and error reduction. This means that we have a most effective dose and higher and lower doses are less effective. We suppose that on higher concentrations other targets might be addressed, resulting in this effect. In the correct dose, all investigated β–γ-couples (**9, 10, 7, 8** and **2, 6**) are able to reduce the scopolamine effect to some extent. The phenethyl- β-carboline (**9**) displays the lowest efficacy, reducing the errors from 6.4 (control) to 4.7 errors at 0.5 μmol. The respective phenethyl-γ-carboline (**10**) displays a significant reduction to 2.0 errors at 1 μmol concentration. For the propargyl couples **7** and **8** and the methyl couples **2** and **8** the γ-carboline derivatives are effective in lower concentrations than their β-congeners. The methyl γ-derivative **6** is clearly the most potent compound amongst all tested carbolines, reducing the number of errors from 7.1 (control) to 3.7 at 0.25 μmol per 100 g b.w..

This effect could not be confirmed in equilibrium binding experiments applying an orthosteric radioligand labelling the site of the endogenous neurotransmitter acetylcholine in muscarinic M1 receptors. Instead, we found that the phenethyl-γ-carboline (**10**) showed a considerably stronger reduction and inhibitory potency with regard to [^{3}H]NMS specific binding compared to the effect of the respective methyl-γ-carboline.

(**6**) (See Table 3). However, we also found that γ-carbolines are more potent than their β-isomers.

Unfortunately, the results from the radical scavenging properties (DPPH assay) were hard to interpret and do not draw a clear picture, neither in buffer, nor in acetic acid. Therefore we do not want to include any of these data into this discussion.

Taken the results together, we found that γ-carboline derivatives are more potent than the β-carboline derivatives in inhibiting choline esterases. In muscarinic M_1 receptors this holds true for the derivatives under study containing a large substituent at the indolo-*N* (**9, 10**) but not for the *N*-methylated derivative (**2, 6**). Finally, γ-carboline derivatives are significantly more potent than their β-carboline counterparts with respect to the effective dose and error reduction of rats in the 8-arm radial maze experiment with scopolamine pretreated rats. Our results render the γ-carboline derivatives, especially derivative **6** as highly attractive candidate for the development of novel compounds against neurodegenerative conditions like Alzheimer's or Parkinson's disease. The neuroprotective properties deserve further investigation regarding neuronal differentiation, outgrowth of dendrites and expression of neurotrophic factors and are being actually investigated in more detail.

6. Experimental protocols

6.1. General information/methods

Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus. ^{1}H and ^{13}C NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 and 63 MHz) and Advance 400 spectrometer (400 and 100 MHz), respectively. MS data were determined by

GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific) Elemental analyses were performed on a Hereaus Vario EL III apparatus (Elementar Analysensysteme GmbH, Germany) at the Institute of Organic Chemistry, University of Jena, Germany, and results were within (0.4% of the theoretical values. Furthermore, all compounds were checked by TLC and showed single spots. Taken together, both features ensure purities ≥95%. TLC was performed on silica gel F254 plates (Merck). High resolution mass spectrometry (HRMS) data were determined on a TSQ QuantumAMspectrometer (Therma Electron Corporation).

The preparation of the following derivatives was described previously: Tetrahydro-γ-carboline **(13)** [21], Tetrahydro-β-carboline **(11)** [20], γ-Carboline **(14)** [31], β-Carboline **(12)** [6], 9-(2-Phenethyl)-β-carboline **(9)** [32], 5-Methyl-γ-carboline **(6)** [33], 9-Methyl-β-carboline **(2)** [5], 2-Methyl-γ-carbolinium iodide **(16)** [31], 2-Methyl-β-carbolinium iodide **(15)** [7].

γ-Carboline **(14)** is a known and described compound [31], but our synthetic route *via* PC/C catalyzed oxidation of the respective tetrahydro-derivative **(13)** was not described so far.

The analytical data of these known compounds were as described in the given literature. All β-carboline derivatives were prepared by a *Pictet-Spengler* reaction of tryptamine and para-formaldehyde in phosphate buffer (according to reference [20]), whereas the γ-carboline congeners were prepared according *Fischer Indole* reaction of phenylhydrazine HCl and piperidin-4-one HCl × H_2O leading to the corresponding tetrahydro derivatives **11** and **13** [21]. Dehydration of these tetrahydro-derivatives to the respective fully aromatic derivatives **12** and **14** was accomplished with palladium on charcoal (10%) in refluxing toluene, instead of cumene which is commonly used for this type of reaction [34]. The reaction time in refluxing toluene turned out to be equal to that of cumene and evaporation of the solvent is much easier.

6.2. Synthesis of indolo-N-alkyl-carboline derivatives (**2**, **6**–**10**): General procedure 1

A solution of 4 mmol carboline **(12 or 14)** and 4.4 mmol of NaH in DMF was stirred at room temperature for 30 min. After addition of 4.4 mmol of corresponding alkyl halide the solution was stirred for another 4–16 h. The conversion was monitored by TLC and GC/MS. When no further reaction was observed the solution was poured into iced water extracted with dichloromethane and purified by column chromatography and was subsequently converted into its HCl salt in 2-propanol with ethereal HCl.

6.3. Synthesis of the pyrido-N-quaternary salts (**15**–**20**): general procedure 2

A 10-fold molar excess of methyl iodide was added to a solution of the respective carboline derivative **(7–10, 12 or 14)** in acetone at 0 °C. The mixture was vigorously stirred at room temperature for 72 h. The precipitated solids were isolated by filtration, washed with Et_2O and dried *in vacuo*.

6.4. Synthesis of the partially reduced compounds (**21**, **22**): general procedure 3

A stirred solution of 2 mmol quaternary carbolinium iodide **(19, 20)** in 50 mL of methanol at 0 °C was treated with 20 mmol $NaBH_4$. The mixture was stirred for 2–4 h at room temperature, the organic phase was carefully evaporated, and 10 mL of water was added. The aqueous phase was then extracted with dichloromethane. The organic phase was dried over $MgSO_4$, evaporated, and dried *in vacuo*.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.048.

References

[1] L. Frolich, J. Fox, F. Padberg, K. Maurer, H.J. Moller, H. Hampel, Targets of antidementive therapy: drugs with a specific pharmacological mechanism of action, Curr. Pharm. Des. 10 (2004) 223–229.
[2] K.M. Brummond, J.R. Goodell, M.G. Laporte, L. Wang, X.Q. Xie, Synthesis and in silico screening of a library of beta-carboline-containing compounds, Beilstein J. Org. Chem. 8 (2012) 1048–1058.
[3] J.R. Sanchez-Ramos, Banisterine and Parkinson's disease, Clin. Neuropharmacol. 14 (1991) 391–402.
[4] M. Gruss, D. Appenroth, A. Flubacher, C. Enzensperger, J. Bock, C. Fleck, G. Gille, K. Braun, 9-Methyl-beta-carboline-induced cognitive enhancement is associated with elevated hippocampal dopamine levels and dendritic and synaptic proliferation, J. Neurochem. 121 (2012) 924–931.
[5] H. Rommelspacher, Preparation of 9-alkyl-β-Carbolines for the Treatment of Neurodegenerative Illnesses, 2008. Germany.
[6] Y. Schott, M. Decker, H. Rommelspacher, J. Lehmann, 6-Hydroxy- and 6-methoxy-beta-carbolines as acetyl- and butyrylcholinesterase inhibitors, Bioorg. Med. Chem. Lett. 16 (2006) 5840–5843.
[7] Y. Rook, K.U. Schmidtke, F. Gaube, D. Schepmann, B. Wunsch, J. Heilmann, J. Lehmann, T. Winckler, Bivalent beta-carbolines as potential multitarget anti-Alzheimer agents, J. Med. Chem. 53 (2010) 3611–3617.
[8] D.A. Kharkevich, Pharmacology of diazoline, Farmakol. Toksikol. 20 (1957) 46–51.
[9] A.A. Kurland, A. Nagaraju, T.E. Hanlon, Flutroline in the treatment of the schizophrenic patient, J. Clin. Pharmacol. 22 (1982) 441–449.
[10] C.A. Harbert, J.J. Plattner, W.M. Welch, A. Weissman, B.K. Koe, Neuroleptic activity in 5-aryltetrahydro-gamma-carbolines, J. Med. Chem. 23 (1980) 635–643.
[11] A.J. Henderson, P.R. Guzzo, A. Ghosh, J. Kaur, J.M. Koo, K. Nacro, S. Panduga, R. Pathak, B. Shimpukade, V. Tan, K. Xiang, J.D. Wierschke, M.L. Isherwood, Epiminocyclohepta[b]indole analogs as 5-HT6 antagonists, Bioorg. Med. Chem. Lett. 22 (2012) 1494–1498.
[12] S.J. Webster, C.A. Wilson, C.H. Lee, E.G. Mohler, A.V. Terry Jr., J.J. Buccafusco, The acute effects of dimebolin, a potential Alzheimer's disease treatment, on working memory in rhesus monkeys, Br. J. Pharmacol. 164 (2011) 970–978.
[13] M.B. Youdim, Multi target neuroprotective and neurorestorative anti-Parkinson and anti-Alzheimer drugs ladostigil and m30 derived from rasagiline, Exp. Neurobiol. 22 (2013) 1–10.
[14] S. Dragoni, V. Porcari, M. Travagli, D. Castagnolo, M. Valoti, Antioxidant properties of propargylamine derivatives: assessment of their ability to scavenge peroxynitrite, J. Pharm. Pharmacol. 58 (2006) 561–565.
[15] W. Maruyama, Y. Akao, M.C. Carrillo, K. Kitani, M.B. Youdium, M. Naoi, Neuroprotection by propargylamines in Parkinson's disease: suppression of apoptosis and induction of prosurvival genes, Neurotoxicol. Teratol. 24 (2002) 675–682.
[16] L. Kupershmidt, T. Amit, O. Bar-Am, O. Weinreb, M.B. Youdim, Multi-target, neuroprotective and neurorestorative M30 improves cognitive impairment and reduces Alzheimer's-like Neuropathology and Age-related Alterations in mice, Mol. Neurobiol. 46 (1) (2012) 217–220.
[17] A.V. Ivachtchenko, E.B. Frolov, O.D. Mitkin, S.E. Tkachenko, I.M. Okun, A.V. Khvat, Synthesis and biological activity of 5-styryl and 5-phenethyl-substituted 2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indoles, Bioorg. Med. Chem. Lett. 20 (2010) 78–82.
[18] N.I.o.A. (NIA), Alzheimer's Disease Medications – Fact Sheet, 2008. http://www.bu.edu/alzresearch/files/pdf/ADEARMedicationsFactSheet3.pdf.
[19] G. Saxena, S.P. Singh, R. Agrawal, C. Nath, Effect of donepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice, Eur. J. Pharmacol. 581 (2008) 283–289.
[20] Y.X. Li, S.R. Prakash, Synthesis of C-14-labeled novel IKK inhibitor: 2-[C-14]-N-(6-chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridinecarboxamide, J. Label. Compd. Rad. 48 (2005) 323–330.
[21] A. Bridoux, L. Goossens, R. Houssin, J.P. Henichart, Synthesis of 8-substituted tetrahydro-gamma-carbolines, J. Heterocycl. Chem. 43 (2006) 571–578.
[22] R.H. Cao, Q. Chen, X.R. Hou, H.S. Chen, H.J. Guan, Y. Ma, W.L. Peng, A.L. Xu, Synthesis, acute toxicities, and antitumor effects of novel 9-substituted beta-carboline derivatives, Bioorg. Med. Chem. 12 (2004) 4613–4623.
[23] P. Molyneux, The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, J. Sci. Technol. 26 (2004) 211–219.
[24] M.S. Blois, Antioxidant determinations by the Use of a stable free radical, Nature 181 (1958) 1199–1200.
[25] L. Fang, D. Appenroth, M. Decker, M. Kiehntopf, A. Lupp, S. Peng, C. Fleck, Y. Zhang, J. Lehmann, NO-donating tacrine hybrid compounds improve scopolamine-induced cognition impairment and show less hepatotoxicity, J. Med. Chem. 51 (2008) 7666–7669.
[26] E. Giacobini, Cholinesterase inhibitors: new roles and therapeutic alternatives, Pharm. Res. 50 (2004) 433–440.

[27] E.K. Perry, R.H. Perry, G. Blessed, B.E. Tomlinson, Changes in brain cholinesterases in senile dementia of Alzheimer type, Neuropathol. Appl. Neurobiol. 4 (1978) 273–277.
[28] A.M. Schaible, H. Traber, V. Temml, S.M. Noha, R. Filosa, A. Peduto, C. Weinigel, D. Barz, D. Schuster, O. Werz, Potent inhibition of human 5-lipoxygenase and microsomal prostaglandin E-2 synthase-1 by the anti-carcinogenic and anti-inflammatory agent embelin, Biochem. Pharmacol. 86 (2013) 476–486.
[29] J. Bauer, A. Koeberle, F. Dehm, F. Pollastro, G. Appendino, H. Northoff, A. Rossi, L. Sautebin, O. Werz, Arzanol, a prenylated heterodimeric phloroglucinyl pyrone, inhibits eicosanoid biosynthesis and exhibits anti-inflammatory efficacy *in vivo*, Biochem. Pharmacol. 81 (2011) 259–268.
[30] J. Hamann, H. Rommelspacher, A. Storch, H. Reichmann, G. Gille, Neurotoxic mechanisms of 2,9-dimethyl-beta-carbolinium ion in primary dopaminergic culture, J. Neurochem. 98 (2006) 1185–1199.
[31] G. Van Baelen, S. Hostyn, L. Dhooghe, P. Tapolcsanyi, P. Matyus, G. Lemiere, R. Dommisse, M. Kaiser, R. Brun, P. Cos, L. Maes, G. Hajos, Z. Riedl, I. Nagy, B.U. Maes, L. Pieters, Structure-activity relationship of antiparasitic and cytotoxic indoloquinoline alkaloids, and their tricyclic and bicyclic analogues, Bioorg. Med. Chem. 17 (2009) 7209–7217.
[32] G. Cerbai, G.F. Dipaco, Synthesis of Aryl- and Diaryl-alkyl derivatives of Norharman, Farm. Sci. 18 (1963) 721–731.
[33] K. Eiter, M. Nagy, Zur Konstitution des Calycanthins .2. Die Synthese der Abbaubase C12H10N2, Monatsh. Chem. 79 (1948) 17–21.
[34] D. Soerens, J. Sandrin, F. Ungemach, P. Mockry, G.S. Wu, E. Yamanaka, L. Hutchins, M. DiPierro, J.M. Cook, Study of the Pictet-Spengler reaction in aprotic media: synthesis of the p-galactosidase inhibitor, pyridindolol 1c, J. Org. Chem. 44 (1978) 535–545.