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Bromal-derived Tetrahydro-β-carbolines as Neurotoxic Agents: Chemistry, Impairment of the Dopamine Metabolism, and Inhibitory Effects on Mitochondrial Respiration†

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Abstract—The mammalian alkaloids tryptoline (**1**) and eleagnine (**2**) as well as the highly halogenated (X = F, Cl, Br) tetrahydro-β-carbolines (THβCs) **3**–**5**, structurally similar to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **6**), were found to have a common feature of inducing a severe impairment of the nigrostriatal dopamine metabolism and inhibiting complex I of the mitochondrial respiratory chain highly selectively. Within the series of compounds tested, 1-tribromomethyl-1,2,3,4-tetrahydro-β-carboline ('TaBro', **5**), which was prepared in high yields from the biogenic amine tryptamine ('Ta', **7**) and the unnatural aldehyde bromal ('Bro', **8**) by a Pictet–Spengler cyclization reaction, turned out to be the most potent toxin in vitro and in vivo. As demonstrated by voltammetric measurements on rats, for all the THβCs **1**–**5** investigated, intranigral application of a single dose of 10 μg resulted in a significant reduction of the dopaminergic activity in the striatum, with the strongest effect being observed for TaBro (**5**). Using rat brain homogenates, again **5** ($IC_{50} = 200\,\mu M$) as well as its dehydrohalogenation product **11** ($IC_{50} = 150\,\mu M$) exhibited the most pronounced inhibitory potential on mitochondrial respiration. The halogen-free THβCs **1** and **2** as well as the MPTP metabolite 1-methyl-4-phenylpyridinium ion (MPP^+), by contrast, showed only a moderate inhibition at concentrations in the millimolar range (e.g. for MPP^+: $IC_{50} = 3.5\,mM$). For an elucidation of the role of hydrophobic portion in the inhibitory action against complex I activity, several *N*-acyl derivatives (**15**–**21**) of **5** were synthesized and tested. An X-ray diffraction study on the 3-dimensional structure of trifluoroacetylated highly halogenated THβCs (**12**–**14**) revealed the tetrahydropyrido part to adopt a nearly planarized half-chair conformation. Because of the steric demand of the trihalogenmethyl moiety ($CF_3 < CCl_3 < CBr_3$), the *N*-substituent is dramatically pushed out of that ring 'plane'.

Introduction

The β-carbolines are an interesting class of psychoactive compounds,[1–3] with a structural resemblance to the molecular framework of the well-known dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **6**)[4,5] (Fig. 1), which has become an efficient and widely used experimental tool in neurosciences for the induction of Parkinsonian-type symptoms in rodents and primates. The identification of some tetrahydro-β-carbolines (THβCs) in the human body,[3,6,7] among them the simple formaldehyde- and acetaldehyde-derived compounds tryptoline (**1**) and eleagnine (**2**), respectively, has stimulated increased attention on the putative role of these alkaloid-type heterocycles as causative factors in the pathogenesis of neurodegenerative disorders.[8,9]

We have recently reported the highly lipophilic 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline ('TaClo', **4**)[10–12] (Fig. 1) to be formed in small amounts in elderly

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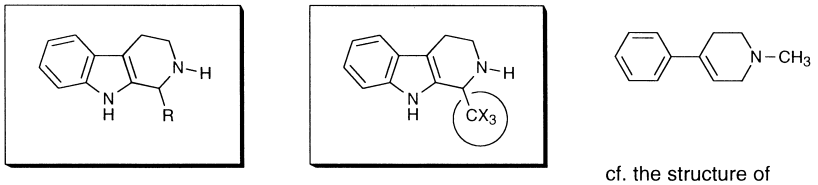


tryptoline (**1**) : R = H
eleagnine (**2**) : R = CH_3

'TaFlu' (**3**) : X = F
'TaClo' (**4**) : X = Cl
'TaBro' (**5**) : X = Br

cf. the structure of MPTP (**6**)

Figure 1. The well-established mammalian alkaloids **1** and **2** as well as the highly halogenated tetrahydro-β-carbolines **3**–**5** in comparison to the structurally closely related dopaminergic neurotoxin MPTP (**6**). We have coined the acronyms 'TaFlu' (**3**), 'TaClo' (**4**) and 'TaBro' (**5**) for this new THβCs because they chemically originate from tryptamine ('Ta') and the aldehydes fluoral ('Flu'), chloral ('Clo'), and bromal ('Bro'), respectively.

patients therapeutically treated with the hypnotic agent, chloral hydrate.[13] This finding is of special importance because **4** was shown to exhibit a pronounced neurotoxicity towards dopaminergic neurons.[11,12,14] The toxic potency of TaClo (**4**), however, seems to be different from that of MPTP (**6**) or its active metabolite 1-methyl-4-phenylpyridinium ion (MPP^+): While MPTP acutely produces a severe and irreversible syndrome similar to Parkinsonism,[15] TaClo is capable of inducing a slowly developing dopaminergic denervation on rats resulting in a Parkinsonian-type diminished locomotion several months after the end of a 7 week treatment.[16,17] This chloral-derived THβC thus appears to be a promising model toxin to study the various stages of a *slowly* progressing neurodegeneration as typical for idiopathic Parkinson's disease. The neurotoxic potential of **4** was confirmed by in vivo pulse voltammetric analysis of the nigrostriatal dopamine metabolism,[18] and by histological as well as biochemical studies[19] on primary mesencephalic cell cultures: these investigations clearly revealed **4** to cause marked disturbances on dopaminergic activity, and to be responsible for morphological changes in neurons such as swelling of axons or loss of dendrites.[20] Furthermore, **4** and related highly chlorinated analogues were found to be potent selective inhibitors of complex I (NADH-ubiquinone reductase) of the mitochondrial respiratory chain, thus giving rise to a significant decrease of neuronal energy production.[19,21,22]

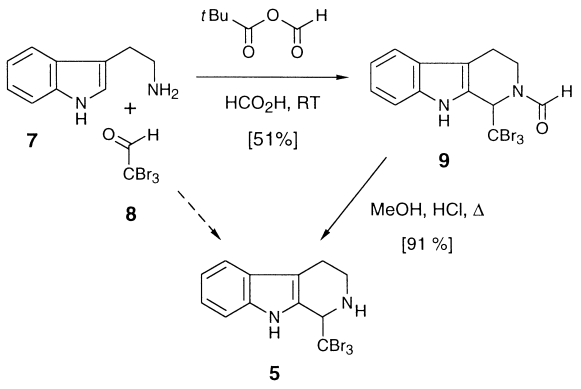
In a previous paper,[18] we have also described the merely synthetic TaClo analogue, 1-tribromomethyl-1,2,3,4-tetrahydro-β-carboline ('TaBro', **5**) to severely affect the dopamine metabolism of the rat after its unilateral injection into the substantia nigra pars compacta (SNpc). Interestingly, the neurotoxic effect was observed to be even more pronounced for this certainly unnatural bromal-derived heterocycle **5** compared with the endogenously occurring agent **4**.

Since a comprehensive structure-neurotoxicity study on highly halogenated THβCs has not been performed so far, we first aimed at a more detailed investigation whether the highly lipophilic (and possibly radical-inducing) trichloromethyl and tribromomethyl substitutents at C1 might be associated with the toxic potential of TaClo (**4**) and TaBro (**5**), respectively, towards the dopaminergic system. For this purpose, we have started to determine the influence of fluorine versus chlorine versus bromine on the neuropharmacological properties of 1-trihalogenmethyl-THβCs in comparison to the halogen-free mammalian alkaloids tryptoline (**1**) and eleagnine (**2**) (Fig. 1). In the present study, the tryptamine-derived heterocycles 'TaFlu' (**3**), 'TaClo' (**4**), and 'TaBro' (**5**) have been evaluated for dopaminergic neurotoxicity in vivo, and for respiratory inhibition in vitro. Furthermore, we report on the synthesis and X-ray diffraction analysis of various highly halogenated THβCs, among them **5** and related *N*-acylated compounds (**15**–**21**), which were tested for their inhibitory effects on complex I activity.

Results and Discussion

Synthesis

The Pictet–Spengler cyclization of tryptamines and reactive aldehydes or α-keto acids is an efficient synthetic pathway for the preparation of THβCs,[3,23] among them the alkaloids tryptoline (**1**)[24] and eleagnine (**2**)[25,26] as well as related heterocycles. Within the series of highly halogenated THβCs, on which we mainly focus in this paper, the fluoral-derived compound TaFlu (**3**) has already been synthesized by Maki et al.,[27] while the 1-trichloromethyl substituted THβC TaClo (**4**) has been introduced by our group.[10] We have established a rational two-step route for the preparation of **4** via its formamide, giving rise to analytically completely pure, crystalline material of **4**, even on a 100 g scale.[11,22] The novel highly brominated TaClo analogue TaBro (**5**) has now been successfully synthesized (see Scheme 1) by an adaption of this procedure: At room temperature, tryptamine (**7**) was condensed with tribromoacetaldehyde (bromal, **8**) in formic acid as the solvent, in the presence of the formylating reagent trimethylacetic formic anhydride,[28] leading to *N*-formyl-TaBro (**9**). A smooth *N*-deformylation of **9** in methanolic hydrochloric acid finally gave the desired target molecule TaBro (**5**) as its colorless hydrochloride salt in an overall yield of 46%, without formation of any by-products. Different from the corresponding chloral-derived THβC **4**, which was shown to be formed rapidly (e.g. in refluxing toluene) within 2 h from both of its precursors tryptamine and chloral,[10] all attempts to obtain **5** directly by carrying out the cyclization reaction of **7** and **8** in aprotic solvents



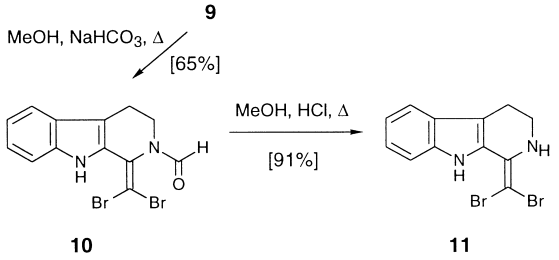
Scheme 1. Bromal-derival tetrahydro-β-carbolines.

(e.g. in acetone, tetrahydrofurane, dichloromethane, toluene or formic acid without addition of trimethylacetic formic anhydride) resulted in less favorable yields or failed completely.

Following a similar reaction sequence (see Scheme 2), the 1-dibromomethylene-substituted THβC **11** was also synthesized via its formamide: Thus, the intermediate **10** was obtained from **9** in methanol at reflux by elimination of hydrobromic acid under mild basic conditions. Here as well, similar to the preparation of **5**, the attempt to synthesize **11** directly, by performing the dehydrohalogenation reaction with **5** as the starting material, failed completely, resulting in a multi-component mixture.

X-Ray investigations

In addition to the constitutions of these novel agents, as anticipated from syntheses and spectroscopic data, the knowledge of steric effects exhibited by a trihalogenmethyl group at C1 on the conformation of a tetrahydro-β-carboline ring system was highly desirable. Acetylation of **3**–**5** with trifluoroacetic anhydride provided crystalline material of the respective derivatives **12**–**14**, which proved to be well-suited for single-crystal X-ray diffraction analyses (Fig. 2a–c). These investigations clearly revealed the tetrahydropyrido part to adopt a largely planarized half chair conformation, with only C3 and N2 being located distinctly out of the ring plane (best illustrated in Fig. 2c showing a horizontal view of the molecular structure of **14**). The trihalogenmethyl substituents are twisted out of the β-carboline ring plane, occupying a pseudo-axial position. The three halogen atoms at C14 of all of these THβCs show a perfectly staggered orientation with respect to the C1–C14 bond, thus minimizing their steric interactions with C13 and N2.



Scheme 2. Bromal-derival tetrahydro-β-carbolines.

The influence of the large van der Waals radius of bromine (2.0 Å) or chlorine (1.8 Å) in comparison to the small radius of fluorine (1.4 Å) or hydrogen (1.0 Å) on the conformation of the tetrahydropyrido moiety is best visualized by a matched plot (Fig. 3) of the crystal structures of **12**–**14** (and of the eleagnine-derived trifluoroacetamide, on which we have reported previously[29]). Depending on the degree of steric hindrance due to the steric demand of the respective trihalogenmethyl group ($CF_3 < CCl_3 < CBr_3$) at C1, the substituent at N2 is increasingly pushed upwards: slightly for the halogen-free 1-CH_3 analogue, and finally dramatically for the *N*-trifluoroacetyl moiety of the TaClo and TaBro derivatives **13** and **14**.

Striatal dopamine metabolism in the rat after intranigral application of the THβCs 1–5

With the THβCs **1**–**5** in hand, we could now start investigating their neurotoxic potential. Differential pulse voltammetry using carbon fibre electrodes represents a very sensitive tool for the in vivo investigation of a neurotoxin-induced impairment of the dopaminergic neurotransmission in rat striatum, as previously demonstrated for iron,[30] MPTP, and its metabolite MPP^+.[31] The ability of the THβCs **1**–**5** to disturb neuronal activity in the dopaminergic system (Fig. 4) was determined by assaying in both striata for the extracellular levels of the dopamine metabolite 3,4-dihydroxy-phenylacetic acid (DOPAC), a reliable indicator of dopamine metabolism.

As reported previously,[18] **4** and **5** are able to severely affect the striatal dopaminergic functions. TaClo (**4**) was measured to significantly reduce the DOPAC signal at the lesioned side to about 43% (SEM±10%) of the intact side ($P < 0.05$) 1 week after a single unilateral toxin injection into the right SNpc. The biochemical lesions of the nigrostriatal system found for TaBro (**5**) were distinctly stronger: the DOPAC content in the ipsilateral striatum decreased to ca. 17% (SEM±5.6%) of the contralateral, i.e. intact side ($P < 0.01$). A systemic administration of the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA), which is used for the therapy of Parkinsonism, led to a fully restoration of the DOPAC signal in TaClo-lesioned rats with no differences observable between the striata of both hemispheres (data not shown). By contrast, the TaBro-induced impairment of the dopamine metabolism could not completely be compensated by L-DOPA. The DOPAC content remains significantly reduced to about 49% (SEM±10%).

In our rat intranigral application protocol, the acetaldehyde-derived alkaloid eleagnine (**2**) and the 1-trifluoromethyl substituted THβC TaFlu (**3**) appeared to be about as neurotoxic as TaClo (**4**). Similar to **4**, a

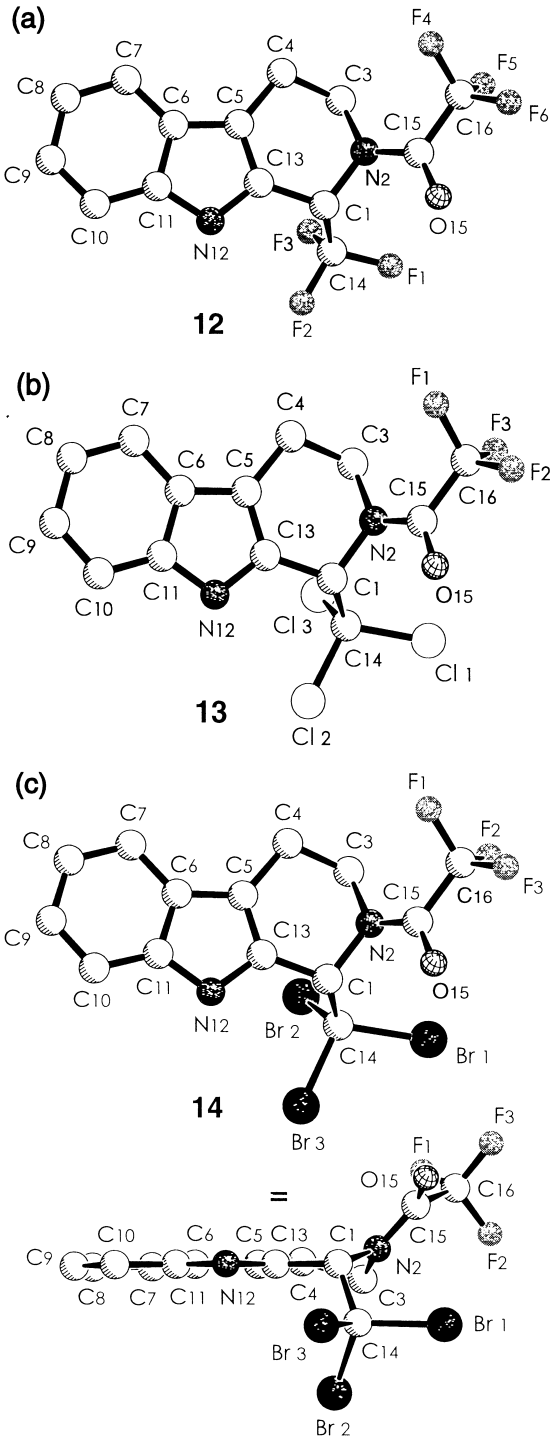


Figure 2. SCHAKAL plots of the crystal structures of the *N*-trifluoroacetyl derivatives **12** (a), **13** (b), and **14** (c) with a guide of the atomic numbering system adopted in the X-ray investigations. The molecule of the bromal-derived THβC **14** is viewed from two directions: peripendicular (top) and horizontal (bottom) to the tetrahydropyrido ring plane. All these compounds are racemic in the crystal; for presentation, only those enantiomers with the CX_3 group (X = F, Cl, Br) below the graphical plane, have been chosen, arbitrarily (hydrogens atoms omitted for reasons of clarity).

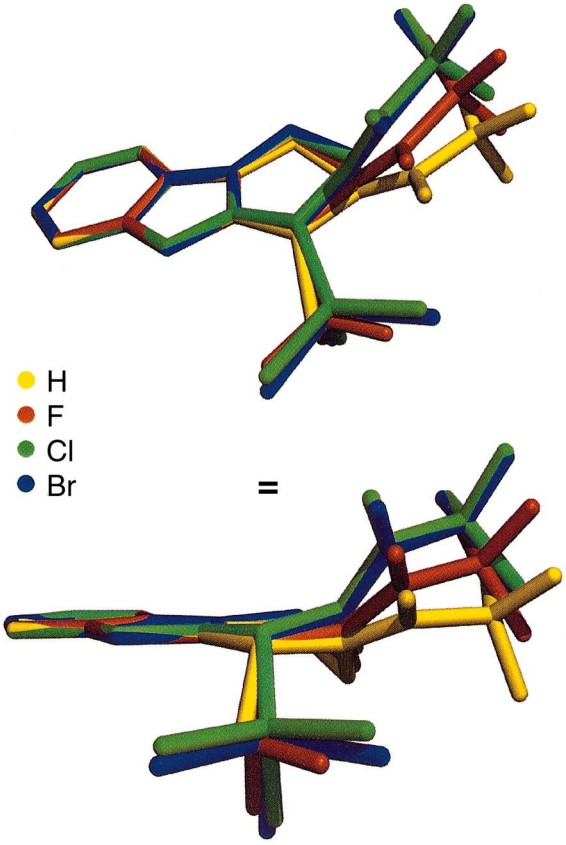


Figure 3. Joint plot of the structures of the eleagnine-derived *N*-trifluoroacetyl derivative (yellow) (cf. lit.[29]), **12** (red-brown), **13** (green), and **14** (blue) in the crystal, matched with respect to the indole part of the molecules (hydrogen atoms omitted for reasons of clarity). The molecules are viewed from two directions: peripendicular (top) and horizontal (bottom) to the tetrahydropyrido ring planes.

unilateral injection of **2** and **3** significantly ($P < 0.05$) decreased the voltammetric DOPAC signal in the ipsilateral striatum to about 38% (SEM±12.0%) for **2**, and to 42% (SEM±12%) for **3**. Interestingly, the 1-unsubstituted THβC **1** exhibited an even more pronounced dopaminergic toxicity than **2**, **3** and **4**. Tryptoline (**1**) reduced the dopamine metabolism to about 24% (SEM±8.5%) of that of the untreated side ($P < 0.01$). As already observed for **4**, intraperitoneal administration of L-DOPA fully restored the DOPAC signal in tryptoline-, eleagnine-and TaFlu-treated rats, thus showing the restitution capacity of the nigrostriatal system after toxin treatment.

Summarizing, severe disturbances of the striatal dopamine metabolism were shown in the rat one week after a single unilateral injection of the THβCs **1**–**5** into the SNpc, with the strongest effect being measured for TaBro (**5**). In a quantitative sense, this highly brominated compound **5** caused nigral lesions that nearly approached the size of the strong impairment of the dopaminergic system produced by the Parkinsonism-inducing model toxin MPTP and its metabolite, MPP^+.[31]

Inhibition of complex I of the mitochondrial respiration

Since the mechanism of nigrostriatal degeneration in idiopathic Parkinson's disease[32] as well as in MPTP-induced Parkinsonism[33] was found to be associated with

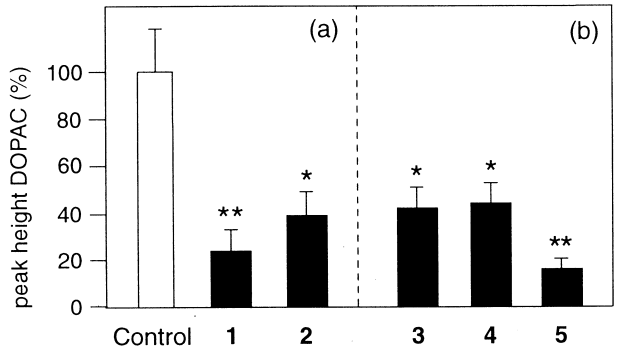


Figure 4. THβC-induced impairment of the striatal dopamine metabolism in the rat demonstrated by the reduction of the DOPAC signal after an unilateral injection of the halogen-free compounds **1** and **2** (a), and the 1-trihalogenmethyl THβCs **3**–**5** (b) into the right SNpc (dose applied: 10 μg THβC in 2 μL of 10% ethanol). After 1 week, extraneuronal DOPAC concentrations (shown as percentage of intact side) in rat striatum were assayed by in vivo pulse voltammetry ($n = 5$–6, mean ±SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, paired Student's *t*-test, lesioned side versus intact side). As clearly shown by control experiments, intranigral application of 2 μL of the solvent ethanol itself did not result in an impairment of the striatal dopamine metabolism of the rat. For further details, see Experimental.

mitochondrial complex I deficiency in dopaminergic neurons, the toxic effects of MPTP-like substances such as β-carboline alkaloids and their analogues have been studied more closely with respect to their inhibitory potential against mitochondrial respiration. Among these compounds, only some representatives of *N*-methylated fully aromatic β-carbolines derived from acetaldehyde (e.g. *N*-methylharmine, *N*-methylharmol) were shown to cause a selective and highly efficient reduction of complex I activity with a magnitude of inhibition quite similar to that of MPP^+, the oxidation product of MPTP (**6**).[34,35] The *N*-methyl derivatives of norharman and harman, however, proved to be nearly ineffective as inhibitors.[34] Interestingly, in a previous study,[22] we obtained similar results for the THβCs tryptoline (**1**) and eleagnine (**2**), which were both demonstrated to only moderately affect complex I activity (cf. Table 1). By contrast, the chloral-derived THβC TaClo (**4**) significantly inhibited complex I of the mitochondrial respiratory chain (cf. Table 1) in rat brain homogenates and submitochondrial particles (SMP), similar to MPP^+, but about 10 times more effectively.[21]

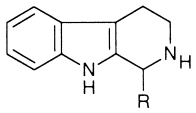
As obvious from Tables 1 and 2, the by far most active compounds within the series of THβCs presented in this paper are the bromal-derived heterocycles. Comparable to TaBro (**5**) itself, which totally inhibited complex I at a concentration similar to that of TaClo (**4**), all of the TaBro derivatives tested, among them **11** (see Scheme 2) and the *N*-acyl derivatives **14**–**21**, were shown to exhibit pronounced inhibitory capacities towards complex I mediated oxidative phosphorylation. The IC_{50} and IC_{100} values of these compounds vary between 650 μM (IC_{100} of **5**) and 150 μM (IC_{50} for **11**). By contrast, like the halogen-free heterocycles **1** and **2**, the fluoral-derived THβC TaFlu (**3**) turned out to display only a weak inhibitory effect on the mitochondrial respiratory chain.

The concentrations of **5** and its derivatives required for a complete inhibition of complex I (IC_{100}) in rat brain homogenates or in SMP were determined to be 10-fold (for **5**) up to nearly 40-fold (e.g. for **17**–**19**) lower than that of MPP^+ (cf. Tables 1 and 2). Nonetheless, it should be mentioned that the high neurotoxic potential of MPP^+ for dopaminergic neurons resides in at least two effects: the induction of a selective inhibition of the mitochondrial respiration, and the energy-driven uptake of MPP^+ into intact mitochondria resulting in an accumulation of this bioactive MPTP metabolite to high concentrations.[36] Numerous β-carboline cations related to **1** or **2**, by contrast, were all shown to have considerably lower affinities for this active transport system than MPP^+.[37] It can be assumed that due to the lacking permanent positive charge (unless by reversible protonation), TaClo (**4**) or TaBro (**5**) will not be actively taken up into mitochondria. Indeed, uptake studies with radiolabeled **4** into human neuroblastoma cells expressing the dopamine transporter, revealed **4** to be transported through the cell membranes mainly passively.[38]

Since the extent of lipophilicity of the THβCs presented in this paper seems to be strongly correlated with their inhibitory capacities against complex I activity, partition coefficients *P* (given as log *P*) were determined to assess the ability of these compounds to enter cells. As expected, partitioning of 1-substituted THβCs into the organic layer markedly increased starting from eleagnine (**2**) and TaFlu (**3**), followed by TaClo (**4**) and TaBro (**5**) (cp. Table 1). An approximate 600- and 300-fold increase in partitioning was observed between the halogen-free compounds **1** and **2**, respectively, and the most lipophilic THβC **5**. In the series of halogenated THβCs, a nearly 25-and 65-fold difference was determined between the partition coefficients of **3** to **4** and **3** to **5**, respectively. Between **2** and **4** there was a two order of magnitude increase (factor: ca. 100) of partitioning into the organic layer, while only a 2.5-fold difference was found between the *P* values of **4** and **5**. Regarding the IC_{50} values of the THβCs **2**–**5** against mitochondrial complex I activity in relation to their 1-octanol/water partition coefficients *P*, it became indeed evident that the extent of the increasing inhibitory potency of **2** to **5** correlates very well with the enhanced lipophilicity of these compounds. Thus, in comparison to the likewise polar and only moderately active eleagnine (**2**), the highly lipophilic TaClo (**4**) and TaBro (**5**) molecules were found to be ca. 5- to 6-times more potent inhibitors.

As mentioned above, a whole series of *N*-acyl derivatives of TaBro (**5**) with increasing alkyl chain length (C_1–C_6) have been synthesized (cp. Table 2) in order to evaluate the influence of an additional hydrophobic portion on the inhibitory potential of highly brominated THβCs towards complex I. Expectedly, maximum partitioning into cyclohexane was achieved with the *N*-hexanoyl compound **21**, but in comparison to the parent compound **5**, only a ca. 3-fold increase of lipophilicity was attained. Interestingly, with alkyl chain lengths smaller than three carbon atoms (compounds **9**, **15**–**17**), no enhancement of lipophilicity was observed

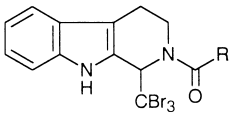
Table 1. Inhibitory potential of halogen-free and highly halogenated THβCs on mitochondrial NADH-ubiquinone reductase (complex I) activity in rat brain homogenates and SMPs, and determination of the lipophilicity of these compounds



Compound	R	log P	log P	log P	Inhibition of complex I	
		cyclohexane/H_2O[a]	1-octanol/H_2O[a]	1-octanol/H_2O[b]	IC_{100} (μM)	IC_{50} (μM)
1	H	—[c]	0.631±0.039	—	IC_{25} = 1000 μM	
2	CH_3	—[c]	0.913±0.026	—	—[c]	1200
3	CF_3	0.985±0.0007	—[c]	1.595±0.007	IC_{25} = 800 μM	
4	CCl_3	2.371±0.122	—[c]	2.981±0.122	700	250
5	CBr_3	2.806±0.071	—[c]	3.416±0.071	650	200
11	$=CBr_2$	—[c]	—[c]	—	350	150
MPP$^+$	—	—[c]	—[c]	—	7500	3500

[a]Log partition coefficients experimentally determined by the HSCCC technique.[48–50] Data represents the average value ±SEM of four separate experiments. Using an alkane/water system instead of the 1-octanol/water mixture to measure the partititon coefficients *P* for the halogenated THβC representatives (see also Table 2) was necessary in order to attain reasonable retention times in relation to the standard anthracene. For further details, see Experimental.
[b]For a systematic comparison of the lipophilicity of the halogen-free compounds **1** and **2** with the data obtained for **3**–**5**, the parameter $I_H = 0.61$,[51] conceived as a measure of the hydrogen-bonding ability of the given solute, was used allowing the interconversion of the *P* values from the cyclohexane/water to the 1-octanol/water system. Log 1-octanol/water partition coefficients were calculated according to the equation: $\log P_{octanol} = \log P_{cyclohexane} + I_H$.
[c]Not determined.

Table 2. Inhibitory effects of *N*-acyl derivatives of the bromal-derived THβC **5** on complex I of the mitochondrial respiration, and partition coefficients *P* (given as log *P* values) for these compounds in a cyclohexane/water mixture



Compound	R	log *P*	Inhibition of complex I	
		cyclohexane/H_2O[a]	IC_{100} (μM)	IC_{50} (μM)
9	H	2.137±0.039	350–400	200
14	CF_3	—[b]	150	—[b]
15	CH_3	2.205±0.015	—[c]	—[c]
16	C_2H_5	2.596±0.073	350	>200
17	C_3H_7	2.724±0.010	200	>150
18	$CH(CH_3)_2$	—[b]	200	>150
19	$C(CH_3)_3$	—[b]	200	>150
20	C_4H_9	3.112±0.053	400	250
21	C_5H_{11}	3.252±0.028	200	150

[a]For the HSCCC method used to determine partition coefficients *P* refer to Experimental. Data represents the average value ±SEM of three separate experiments.
[b]Not determined.
[c]The compound was not sufficiently soluble in the test system.

relative to **5**. Obviously, due to this effect, the IC_{100} and IC_{50} values of the *N*-acyl compounds **9** and **12**–**21** are nearly identical. In general, an increase of inhibitory activity (cf. IC_{100} values of **9** and **12**–**21**) by a factor of up to 3 as compared to **5** was observed for all of the TaBro derivatives.

Outlook

The mode of action of TaClo (**4**) and TaBro (**5**) on the dopaminergic system is as yet unknown. It might be ascribed to either the strong inhibition of complex I of the mitochondrial respiratory chain (see section above), or to the generation of reactive oxygen species, such as hydroxyl radicals. Indeed, a progressive increase in hydroxyl radical formation in the striatum was recently shown after a systemic adminstration of TaClo to rats.[39] The increase of lipophilicity and—simultaneously—neurotoxicity of a THβC molecule by introducing a large trichloro- or tribromomethyl group at C1, may explain the ability of **4** or **5** to stimulate damaging processes on a more long-term basis presumably triggered by a slow and continuous uptake of these agents.[16–18] Current research is now focussing on a more detailed investigation concerning the role of highly halogenated THβCs in the manifestation of a slowly developing Parkinsonian-type neurodegeneration.

Experimental

General methods

Melting points (uncorrected) were determined on a Reichert-Jung Thermovar hot-stage apparatus. Infrared spectra (IR) were obtained on a Perkin–Elmer Model 1420 spectrophotometer. KBr refers to a potassium bromide disk for infrared spectra. Proton and Carbon-13 spectra were recorded on a Bruker AC 250 spectrometer at 250 MHz (for ^{1}H NMR), and at 63 MHz (for ^{13}C NMR). Chemical shifts (δ) are reported in parts per million (ppm) with the proton or carbon-13 signals of chloroform (^{1}H, δ = 7.26 ppm), methanol (^{1}H, δ = 3.33 ppm; ^{13}C, δ = 49.02 ppm), acetone (^{1}H, δ = 2.01 ppm; ^{13}C, δ = 29.85 and 205.9 ppm), or dimethylsulfoxide (^{13}C, δ = 39.43 ppm) in the deuterated solvents as internal reference. Coupling constants (*J*) are given in Hertz (Hz). The following abbreviations are used: s (singlet), d (doublet), dd (double doublet), t (triplet), m_c

(central multiplet). Electron impact mass spectral data were obtained on either a Finnigan MAT 8200 or a Finnigan MAT 90 mass spectrometer. The peaks listed are those arising from the molecular ion $[M^+]^\bullet$, those attributable to loss of certain fragments (M^+ minus a fragment), and some other prominent peaks. Elemental analyses were conducted by the Microanalysis Laboratory of the University of Würzburg (Institute of Inorganic Chemistry) on a Carlo Erba Elemental Analyzer M 1106 apparatus. Where organic solvents are noted as part of the elemental analysis, they were seen in the ^{1}H NMR spectrum in proper amounts.

Materials

All reagents used were of commercial quality. Organic solvents were dried and distilled prior to use. Tryptamine hydrochloride was purchased from Sigma (Deisenhofen, Germany), tribromoacetaldeyhde (bromal) from Merck (Darmstadt, Germany), and trifluoroacetaldehyde ethyl hemiacetal from Janssen Chimica (Beerse, Belgium). 1,2,3,4-Tetrahydro-9*H*-pyrido[3,4-*b*]-indole (**1**) was obtained from Aldrich (Milwaukee, WI, USA). 1-Trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido-[3,4-*b*]indole hydrochloride (**4**·HCl) was prepared from tryptamine and trichloroacetaldehyde, as described previously.[10,11] 2-Hexanoyl-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*] indole (**21**) was synthesized as reported earlier.[40] Reactions were monitored by thin-layer chromatography (TLC) on aluminum plates coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany). Column chromatography was performed on Merck silica gel (0.063–0.200 mm).

1-Methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (2). The title compound was synthesized according to a published procedure,[25] which was slightly modified: A solution of tryptamine hydrochloride (5.0 g, 25.4 mmol) and pyruvic acid sodium salt (17.4 g, 158.5 mmol) in 400 mL of 2 M sodium acetate buffer (pH = 5.2) was stirred at 37 °C for 4 d. 1-Methyl-1-carboxy-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole was obtained as a crude crystalline precipitate, which was filtered off and washed with ice cold water (40 mL). For decarboxylation, this material was then refluxed in 6 N HCl (150 mL) for 45 min, with intensive stirring. After cooling, the strongly acidic solution was adjusted to pH = 12 by addition of 1 M NaOH, and then extracted with ethyl acetate (3×150 mL). The combined layers were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Crystallization from ethyl acetate afforded **2** (3.0 g, 16.1 mmol, 63% yield) as beige-colored crystals: mp 180 °C (lit.[41,42] 179–180 °C). The spectroscopic data are in agreement with those reported in the literature.[26,43]

1-Trifluoromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]-indole hydrochloride (3·HCl). The title compound was synthesized according to a published procedure,[27] which was slightly modified: To a solution of tryptamine hydrochloride (2.0 g, 10.2 mmol) in water (50 mL), trifluoroacetaldehyde ethyl hemiacetal (4.41 g, 30.6 mmol) was added dropwise. The reaction mixture was stirred at 60 °C for 12 h, and then allowed to cool to room temperature affording a colorless crude solid. Crystallization from methanol gave **3**·HCl (2.72 g, 9.83 mmol, 96% yield) as white crystals: mp 205–207 °C (dec) (lit.[28] 147–149 °C, free base). IR (KBr, cm^{-1}) 3380 (indole NH), 2880, 2580, 2560 (CH), 1560 (NH), 1190 (CF); ^{1}H NMR (CD_3OD): δ 3.17 (t, $^3J_{3,4}$ = 6.11 Hz, 2H, 4-H), 3.58–3.82 (m, 2H, 3-H), 5.78 (q, $^3J_{1,F}$ = 6.72 Hz, 1H, 1-H), 7.08–7.14 (m, 1H, 6- or 7-H), 7.20–7.27 (m, 1H, 7- or 6-H), 7.41–7.45 (m, 1H, 5- or 8-H), 7.54–7.58 (m, 1H, 8- or 5-H); ^{13}C NMR (CD_3OD): δ 18.0 (C-4), 42.0 (C-3), 53.9 (q, $^2J_{C,F}$ = 34 Hz, C-1), 110.6, 112.5, 119.2, 119.4, 120.7, 123.3 (q, $^1J_{C,F}$ = 282 Hz, CF_3), 124,4, 126,4, 138.6; EI–MS *m*/*z* (rel int) 240 (53) $[M^+]^\bullet$, 171 (100) $[M^+ - CF_3]$. Anal. calcd for $C_{12}H_{11}F_3N_2HCl$: C, 52.09; H, 4.37; N, 10.12; found: C, 51.76; H, 4.31; N, 9.92.

2-Formyl-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (9). A solution of tryptamine hydrochloride (11.9 mg, 60.7 mmol) and tribromoacetaldehyde (9.9 mL, 26.7 g, 95.3 mmol) in formic acid (20 mL) was stirred at room temperature for 5 h. Subsequently, trimethylacetic formic anhydride[28] (9.93 g, 68.7 mmol) was added to the solution, and stirring was continued for 6 h. The reaction mixture was then poured onto ice. The solid thus obtained was filtered off and washed with *tert*-butyl methyl ether (30 mL). Crystallization from methanol yielded **9** (14.6 g, 32.6 mmol, 51% yield) as colorless crystals, mp 176 °C (dec). IR (KBr, cm^{-1}) 3410 (indole NH), 2940, 2870, 2820 (CH), 1650 (C=O), 1440 (CH), 745 (1,2-disubstitution); ^{1}H NMR (C_3D_6O): occurrence of two distinct rotational isomers A and B, ratio 1.4:1. Isomer A: δ 2.83-3.00 (m, 2H, 4-H, overlay by isomer B), 3.82–4.20 (m, 1H, 3-H), 5.93 (s, 1H, 1-H), 7.07–7.22 (m, 2H, 6-H, 7-H, overlay by isomer B), 7.45–7.52 (m, 2H, 5-H, 8-H, overlay by isomer B), 8.44 (s, 1H, NC*H*O), 10.3 (br. s, 1H, indole NH, overlay by isomer B). Isomer B: δ 2.83–3.00 (m, 2H, 4-H, overlay by isomer A), 4.35–4.82 (m, 1H, 3-H), 6.48 (s, 1H, 1-H), 7.07–7.22 (m, 2H, 6-H, 7-H, overlay by isomer A), 7.45–7.52 (m, 2H, 5-H, 8-H, overlay by isomer A), 8.65 (s, 1H, NC*H*O), 10.3 (br. s, 1H, indole NH, overlay by isomer A); ^{13}C NMR [$(CD_3)_2SO$]: isomer A: δ 20.3 (C-4), 33.5 (C-3), 61.8 (C-1), 110.4, 110.9 (CBr_3), 112.0, 119.0, 122.3, 125.3, 126.5, 136.6, 163.1 (CHO). Isomer B: δ 21.1 (C-4), 39.2 (C-3), 67.7 (C-1), 110.4, 110.9 (CBr_3), 112.0, 118.3, 119.0, 122.4, 125.3, 126.5, 136.7, 164.4 (CHO); EI–MS *m*/*z* (rel int) 454/452/450/448 (0.5/1.6/1.6/0.5) $[M^+]^\bullet$, 372/370/368 (0.2/0.5/0.2) $[M^+ - HBr]$, 291/289 (16/18) $[M^+ - HBr\text{-}Br]$, 199 (100) $[M^+ - CBr_3]$, 170 (5) [199-CHO]. Anal. calcd for $C_{13}H_{11}Br_3N_2O$: C, 34.62; H, 2.46; N, 6.21; found: C, 34.56; H, 2.45; N, 6.27.

1-Tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]-indole hydrochloride (5·HCl). To a solution of **9** (7.03 g, 15.7 mmol) in methanol (20 mL), concentrated HCl (2.8 mL) was added dropwise with intensive stirring at room temperature. The reaction mixture was then heated at reflux for 7 h. After cooling, the solvent was evaporated under reduced pressure affording a crude colorless solid. Crystallization from methanol-ether gave **5**·HCl (6.45 g, 14.2 mmol, 91% yield) as an amorphous solid: mp 162 °C (dec). IR (KBr, cm^{-1}) 3430

(indole NH), 2910 (CH), 1550 (NH), 1450 (CH_2), 750 (1,2-disubstitution); ^{1}H NMR (C_3D_6O): δ 3.10–3.16 (m, 2H, 4-H), 3.43–3.54 (m, 1H, 3-H), 3.87–3.96 (m, 2H, 3-H), 6.02 (s, 1H, 1-H), 7.08 (dt, $^3J_{6,7}$ = 7.5 Hz, $^5J_{6,8}$ = 1.2 Hz, 1H, 6-H), 7.21 (dt, $^3J_{7,6}$ = 7.6 Hz, $^5J_{7,5}$ = 1.2 Hz, 1H, 7-H), 7.48 (d, $^3J_{8,7}$ = 7.9 Hz, 1H, 8-H), 7.53 (d, $^3J_{5,6}$ = 7.9 Hz, 1H, 5-H); ^{13}C NMR (CD_3OD): δ 19.5 (C-4), 45.0 (C-3), 69.2 (C-3), 112.9, 113.3 (CBr_3), 119.3, 121.0, 124,7, 125.1, 126.5, 138.6; EI–MS *m/z* (rel int) 426/424/422/420 (0.2/0.7/0.7/0.2) [M^+]•, 344/342/340 (0.3/0.9/0.5) [M^+−HBr], 263/261 (25/26) [M^+−HBr−Br], 171 (100) [M^+−CBr_3]. Anal. calcd for $C_{12}H_{11}Br_3N_2HCl$: C, 31.37; H, 2.63; N, 6.09; found: C, 31.53; H, 2.89; N, 6.00.

2-Formyl-1-dibromomethylene-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (10). A suspension of **9** (5.02 g, 11.2 mmol) and sodium hydrogen carbonate (3.90 g, 32.8 mmol) in methanol (300 mL) was refluxed for 5 h. Concentration of the solvent yielded the title compound as a crude precipitate, which was filtered off. Crystallization from methanol afforded **10** (4.03 g, 10.9 mmol, 97% yield) as colorless crystals: mp 192 °C (dec). IR (KBr, cm^{-1}) 3460 (indole NH), 2920, 2880 (CH), 1670 (C=O), 1500 (NH), 1450 (CH), 750 (1,2-disubstitution); ^{1}H NMR (CD_3OD): occurrence of two distinct rotational isomers A and B, ratio 3.6:1. δ 3.21–3.69 (m, 2H, 4-H), 4.10–4.28 (m, 2H, 3-H), 7.08–7.38 (m, 2H, 6-H or 7-H), 7.49–7.70 (m, 2H, 5-H or 8-H), 8.19 (s, 1H, C*H*O, isomer A), 8.36 (s, 1H, C*H*O, isomer B); ^{13}C NMR [$(CD_3)_2SO$]: δ 23.1 (C-4), 44.8 (C-3), 107.5 (C-1), 112.7, 114.2 (CBr_2), 115.4, 118.8, 118.9, 119.8, 123.8, 124.0, 125.0, 126.2, 137.1, 160.6 (CHO, isomer A), 163.4 (CHO, isomer B); EI–MS *m/z* (rel int) 372/370/368 (1.6/3.2/1.5) [M^+]•, 291/289 (99/100) [M^+-Br], 210 (14) [M^+−2 Br], 181 (57) [210-CHO]. Anal. calcd for $C_{13}H_{10}Br_2N_2O$: C, 42.19; H, 2.72; N, 7.57; found: C, 42.10; H, 2.73; N, 7.46.

1-Dibromomethylene-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]-indole hydrochloride (11·HCl). Concentrated HCl (2.8 mL) was added to a solution of **10** (1.00 g, 2.70 mmol) in methanol (20 mL). The mixture was refluxed for 8 h. The orange-colored residue obtained after removal of the solvent was purified by column chromatography on silica gel (eluent: dichloromethane-methanol, 95:5). Saturated methanolic HCl was added to the combined fractions. After evaporation to dryness, the crude product was recrystallized from dichloromethane-diethyl ether affording **11**·HCl (417 mg, 1.22 mmol, 45% yield) as a red amorphous solid: mp 163 °C (dec). IR (KBr, cm^{-1}) 3420 (indole NH), 2920 (CH), 1620 (C=C), 1540 (aryl C=C), 750 (1,2-disubstitution); ^{1}H NMR (CD_3OD): δ 3.17–3.63 (m, 2H, 4-H), 4.03–4.25 (m, 2H, 3-H), 7.16–7.60 (m, 2H, 6-H, 7-H), 7.71–7.83 (m, 2H, 5-H, 8-H); ^{13}C NMR (CD_3 OD): δ 20.1 (C-4), 44.6 (C-3), 107.7 (C-1), 114.6, 114.7 (CBr_2), 123.2, 123.7, 123.8, 125.9, 131.0, 132,2, 144.3; EI–MS *m/z* (rel int) 344/342/340 (4.3/8.5/4.2) [M^+]•, 263/261 (12/14) [M^+−Br], 182 (48) [M^+−2 Br], 154 (100) [182-CH_2N]. Anal. calcd for $C_{12}H_{10}Br_2N_2HCl$: C, 38.08; H, 2.93; N, 7.40; found: C, 38.32; H, 3.11; N, 7.45.

General procedure for the preparation of *N*-acylated compounds (12–21). To a suspension of **3**·HCl (for **12**), **4**·HCl (for **13**), or **5**·HCl (for **14**–**21**) (1 mol equiv) in dry dichloromethane (0.5 mmol dissolved in 15 mL), triethylamine (4 mol equiv) was added. The mixture was treated dropwise with the respective activated acid derivative (3 mol equiv) at 0 °C, then stirred at room temperature for 3 h, and extracted with water (3×). The combined organic phases were dried over anhydrous $MgSO_4$, filtered off, and concentrated in vacuo. Purification of the respective residues by crystallization from methanol-ether provided the acyl derivatives **12**–**21** as crystalline material. The data are given below.

2-Trifluoroacetyl-1-trifluoromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (12). Starting from **3**·HCl (840 mg, 3.05 mmol) and trifluoroacetic anhydride (1.30 mL, 1.95 g, 9.07 mmol), the title compound (950 mg, 2.83 mmol, 94% yield) was obtained as colorless crystals, suitable for X-ray structure analysis: mp 211–214 °C (dec). IR (KBr, cm^{-1}) 3340 (indole NH), 3060, 2920, 2840 (CH), 1680 (C=O), 1260 (CF). ^{1}H NMR (CD_3OD): δ 2.86–3.05 (m, 2H, 4-H), 3.78–3.89 (m, 1H, 3-H), 4.36–4.42 (m, 1H, 3-H), 6.22–6.30 (q, $^3J_{1,F}$ = 7.32 Hz, 1H, 1-H), 7.03–7.09 (m, 1H, 6- or 7-H), 7.15–7.21 (m, 1H, 7- or 6-H), 7.38–7.41 (m, 1H, 5 or 8-H), 7.48–7.52 (m, 1H, 8-or 5-H); ^{13}C NMR (C_3D_6O): δ 22.4 (C-4), 43.4 (C-3), 52.3 (q, $^2J_{C,F}$ = 34 Hz, C-1), 110.8, 112.8, 115.3 (CF_3), 119.7, 120.8, 124.1, 124.2 (q, $^1J_{C,F}$ = 280 Hz, 1-CF_3), 127.0, 138.5; EI–MS *m/z* (rel int) 336 (64) [M^+]•, 316 (76) [M^+−HF], 267 (100) [M^+−CF_3], 169 (29) [267-$COCF_3$-H]. Anal. calcd for $C_{14}H_{10}F_6N_2O$: C, 50.01; H, 2.99; N, 8.33; found: C, 49.72; H, 2.70; N, 8.46.

1-Trichloromethyl-2-trifluoroacetyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (13). Starting from **4**·HCl (1.00 g, 3.07 mmol) and trifluoroacetic anhydride (1.08 mL, 1.61 g, 7.68 mmol), the title compound (1.01 g, 2.62 mmol, 85% yield) was obtained as colorless crystals, suitable for X-ray structure analysis: mp 231 °C (dec). IR (KBr, cm^{-1}) 3350 (indole NH), 3045, 2940, 2900, 2840 (CH), 1675 (C=O), 820, 800 (CCl). ^{1}H NMR ($CDCl_3$): δ 2.97–3.02 (m, 2H, 4-H), 4.28–4.33 (m, 2H, 3-H), 6.57 (s, 1H, 1-H), 7.15–7.21 (m, 1H, 6- or 7-H), 7.26–7.32 (m, 1H, 6-or 7-H, overlay by solvent), 7.40–7.44 (m, 1H, 5-or 8-H), 7.53–7.57 (m, 1H, 5-or 8-H), 8.45 (br. s, 1H, indole NH); ^{13}C NMR (C_3D_6O): δ 22.3 (C-4), 41.6 (C-3), 64.3 (C-1), 109.0 (CCl_3), 112.2, 112.5, 115.0 (CF_3), 119.3, 120.4, 123.9, 125.3, 126.4, 137.9; EI–MS *m/z* (rel int) 390/388/386/384 (0.2/1.1/3.4/3.8) [M^+]•, 352/350/348 (0.5/2.3/3.0) [M^+−HCl], 315/313 (6.8/3.0) [M^+−HCl-Cl], 267 (100) [M^+−CCl_3], 169 (21) [267-$COCF_3$-H]. Anal. calcd for $C_{14}H_{10}Cl_3F_3N_2O$: C, 43.61; H, 2.61; N, 7.27; found: C, 43.55; H, 2.63; N, 7.14.

1-Tribromomethyl-2-trifluoroacetyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (14). Starting from **5**·HCl (100 mg, 0.22 mmol) and trifluoroacetic anhydride (90 mL, 134 mg, 0.63 mmol), the title compound (108 mg, 0.21 mmol, 96% yield) was obtained as pale yellow crystals, suitable for X-ray structure analysis: mp 163 °C

(dec). IR (KBr, cm^{-1}) 3450 (indole NH), 2920 (CH), 1680 (C=O). ^1H NMR (C_3D_6O): δ 2.92–3.13 (m, 2H, 4-H), 4.31–4.59 (m, 2H, 3-H), 6.70 (s, 1H, 1-H), 7.15 (dt, $^3J_{6,7}=7.95$ Hz, $^5J_{6,8}=0.9$ Hz, 1H, 6-H), 7.29 (dt, $^3J_{7,6}=7.95$ Hz, $^5J_{7,5}=1.3$ Hz, 1H, 7-H), 7.60 (d, $^3J_{8,7}=8.1$ Hz, 1H, 8-H), 7.66 (d, $^3J_{5,6}=7.8$ Hz, 1H, 5-H); ^{13}C NMR (C_3D_6O): δ 22.7 (C-4), 41.2 (C-3), 65.8 (C-1), 112.0 (CBr_3), 113.0, 115.3 (CF_3), 119.6, 120.7, 124.1, 126.6, 127.1; EI–MS *m/z* (rel int) 522/520/518/516 (0.6/1.9/2.0/0.5) $[M^+]^\bullet$, 359/357 (79/71) $[M^+-HBr$- Br], 267 (100) $[M^+-CBr_3]$, 169 (14) [267-$COCF_3$-H]. Anal. calcd for $C_{14}H_{10}Br_3F_3N_2O$: C, 32.39; H, 1.94; N, 5.39; found: C, 32.44; H, 2.01; N, 5.31.

2-Acetyl-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (15). Starting from **5**·HCl (100 mg, 0.22 mmol) and acetyl chloride (66 mL, 72 mg, 0.92 mmol), the title compound (99 mg, 0.21 mmol, 98% yield) was obtained as colorless crystals: mp 165 °C (dec). IR (KBr, cm^{-1}) 3430 (indole NH), 2910, 2890 (CH), 1660 (C=O). ^1H NMR (C_3D_6O): δ 2.29 (s, 3H, CH_3), 2.91–2.96 (m, 2H, 4-H), 4.28–4.43 (m, 2H, 3-H), 6.84 (s, 1H, 1-H), 7.06 (dt, $^3J_{6,7}=6.9$ Hz, $^5J_{6,8}=1.1$. Hz, 1H, 6-H), 7.20 (dt, $^3J_{7,6}=6.9$ Hz, $^5J_{7,5}=1.1$ Hz, 1H, 7-H), 7.48 (d, $^3J_{8,7}=8.1$ Hz, 1H, 8-H), 7.53 (d, $^3J_{5,6}=7.6$ Hz, 1H, 5-H); ^{13}C NMR (CD_3OD): δ 22.8 (CH_3), 23.0 (C-4), 41.8 (C-3), 64.8 (C-1), 108.8 (CBr_3), 113.4, 120.0, 124.3, 139.1, 171.3 (C=O); EI–MS *m/z* (rel int) 468/466/464/462 (0.2/0.4/0.4/0.2) $[M^+]^\bullet$, 386/384/382 (0.8/1.7/0.8) $[M^+-HBr]$, 305/303 (89/82) $[M^+-HBr$-Br], 213 (66) $[M^+-CBr_3]$, 171 (100) [213-C_2H_2O]. Anal. calcd for $C_{14}H_{13}Br_3N_2O$: C, 36.16; H, 2.88; N, 6.02; found: C, 35.92; H, 2.86; N, 5.98.

2-Propanoyl-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (16). Starting from **5**·HCl (200 mg, 0.44 mmol) and propionyl chloride (120 mL, 125 mg, 1.35 mmol), the title compound (192 mg, 0.40 mmol, 91% yield) was obtained as colorless crystals: mp 168 °C (dec). IR (KBr, cm^{-1}) 3300 (indole NH), 3030, 2920, 2890, 2820 (CH), 1640 (C=O). ^1H NMR (C_3D_6O): δ 1.15 (t, 3H, 3′-CH_3), 2.65 (m, 2H, 2′-CH_2), 2.89–2.93 (m, 2H, 4-H), 4.29–4.32 (m, 2H, 3-H), 6.88 (s, 1H, 1-H), 7.06 (dt, $^3J_{6,7}=7.5$ Hz, $^5J_{6,8}=1.2$ Hz, 1H, 6-H), 7.18 (dt, $^3J_{7,6}=7.6$ Hz, $^5J_{7,5}=1.2$ Hz, 1H, 7-H), 7.48 (d, $^3J_{8,7}=7.9$ Hz, 1H, 8-H), 7.53 (d, $^3J_{5,6}=7.9$ Hz, 1H, 5-H); ^{13}C NMR (C_3D_6O): δ 9.7 (C-3′), 27.2 (C-4), 39.9 (C-2′), 49.3 (C-3), 64.2 (C-1), 112.3 (CBr_3), 112.6, 119.2, 120.2, 123.4, 126.8, 128.7, 137.6, 174.1 (C=O); EI–MS *m/z* (rel int) 400/398/396 (0.2/0.4/0.2) $[M^+-HBr]$, 319/317 (89/92) $[M^+-HBr$- Br], 227 (39) $[M^+-CBr_3]$, 171 (100) [227-C_3H_4O]. Anal. calcd for $C_{15}H_{15}Br_3N_2O$: C, 37.57; H, 3.15; N, 5.84; found: C, 37.64; H, 3.11; N, 6.03.

2-Butanoyl-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (17). Starting from **5**·HCl (200 mg, 0.44 mmol) and *n*-butyryl chloride (145 mL, 148 mg, 1.39 mmol), the title compound (189 mg, 0.38 mmol, 87% yield) was obtained as colorless crystals: mp 148 °C (dec). IR (KBr, cm^{-1}) 3300 (indole NH), 3030, 2940, 2910, 2850, 2820 (CH), 1645 (C=O). ^1H NMR (C_3D_6O): δ 0.98 (t, 3H, 4′-CH_3), 1.63–1.78 (m, 2H, 3′-CH_2), 2.47–2.89 (m, 2H, 2′-CH_2), 2.89–2.95 (m, 2H, 4-H), 4.31–4.33 (m, 2H, 3-H), 6.89 (s, 1H, 1-H), 7.06 (dt, $^3J_{6,7}=7.5$ Hz, $^5J_{6,8}=1.2$ Hz, 1H, 6-H), 7.18 (dt, $^3J_{7,6}=7.6$ Hz, $^5J_{7,5}=1.2$ Hz, 1H, 7-H), 7.48 (d, $^3J_{8,7}=7.9$ Hz, 1H, 8-H), 7.53 (d, $^3J_{5,6}=7.9$ Hz, 1H, 5-H); ^{13}C NMR (C_3D_6O): δ 14.2 (C-4′), 19.2 (C-3′), 22.4 (C-4), 35.9 (C-2′), 40.2 (C-3), 64.1 (C-1), 107.9, 112.3 (CBr_3), 112.6, 119.2, 120.2, 123.4, 126.7, 128.8, 137.6, 173.3 (C=O); EI–MS *m/z* (rel int) 414/412/410 (1.2/2.6/1.0) $[M^+-HBr]$, 344/342/340 (7.7/14/7.6) $[M^+-HBr$-$C_4H_6O]$, 333/331 (97/97) $[M^+-HBr$-Br], 253 (19) $[M^+-3$ Br], 241 (28) $[M^+-CBr_3]$, 171 (100) [241-C_4H_6O]. Anal. calcd for $C_{16}H_{17}Br_3N_2O$: C, 38.98; H, 3.48; N, 5.68; found: C, 38.88; H, 3.40; N, 5.64.

2-(2′-Methylpropanoyl)-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (18). Starting from **5**·HCl (200 mg, 0.44 mmol) and isobutyryl chloride (150 mL, 151 mg, 1.42 mmol), the title compound (193 mg, 0.39 mmol, 89% yield) was obtained as colorless crystals: mp 171 °C (dec). IR (KBr, cm^{-1}) 3310 (indole NH), 3050, 2970, 2900, 2870 (CH), 1645 (C=O). ^1H NMR (C_3D_6O): δ 1.12 [dd, $J_{gem}=21.7$ Hz, $J=6.7$ Hz, 6H, CH(CH_3)$_2$), 2.89–2.95 (m, 2H, 4-H), 3.17 [sept, 1H, C$\underline{H}$(CH_3)$_2$], 4.35–4.40 (m, 2H, 3-H), 6.93 (s, 1H, 1-H), 7.06 (dt, $^3J_{6,7}=7.5$ Hz, $^5J_{6,8}=0.9$ Hz, 1H, 6-H), 7.18 (dt, $^3J_{7,6}=7.6$ Hz, $^5J_{7,5}=1.2$ Hz, 1H, 7-H), 7.49 (d, $^3J_{8,7}=8.8$ Hz, 1H, 8-H), 7.51 (d, $^3J_{5,6}=7.9$ Hz, 1H, 5-H); ^{13}C NMR (C_3D_6O): δ 19.6 (C-3′), 20.5 (C-3′), 22.8 (C-4), 31.2 (C-2′), 40.0 (C-3), 63.9 (C-1), 112.1 (CBr_3), 112.6, 119.2, 120.2, 123.4, 126.8, 128.7, 137.6, 177.1 (C=O); EI–MS *m/z* (rel int) 414/412/410 (0.4/0.8/0.4) $[M^+-HBr]$, 344/342/340 (7.2/15/7.5) $[M^+-HBr$-$C_4H_6O]$, 333/331 (99/99) $[M^+-HBr$-Br], 253 (31) $[M^+-3$ Br], 241 (23) $[M^+-CBr_3]$, 182 (16) $[M^+-3$ Br-$C_4H_6O]$. 171 (100) [241-C_4H_6O]. Anal. calcd for $C_{16}H_{17}Br_3N_2O$: C, 38.98; H, 3.48; N, 5.68; found: C, 38.84; H, 3.52; N, 5.83.

2-(2′,2′-Dimethylpropanoyl)-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (19). Starting from **5**·HCl (100 mg, 0.22 mmol) and pivaloyl chloride (55 mL, 79 mg, 0.65 mmol), the title compound (81 mg, 0.14 mmol, 63% yield) was obtained as colorless amorphous solid: mp 151 °C (dec). ^1H NMR (C_3D_6O): δ 1.39 [s, 9H, C(CH_3)$_3$], 2.91–2.94 (m, 2H, 4-H), 4.30–4.62 (m, 2H, 3-H), 7.01 (s, 1H, 1-H), 7.05 (dt, $^3J_{6,7}=6.9$ Hz, $^5J_{6,8}=1.2$ Hz, 1H, 6-H), 7.16 (dt, $^3J_{7,6}=6.9$ Hz, $^5J_{7,5}=1.2$ Hz, 1H, 7-H), 7.48 (d, $^3J_{8,7}=8.1$ Hz, 1H, 8-H), 7.53 (d, $^3J_{5,6}=7.8$ Hz, 1H, 5-H); ^{13}C NMR (C_3D_6O): δ 23.5 (C-3), 29.4 [C(CH_3)$_3$], 40.7 [C(CH_3)$_3$], 41.9 (C-4), 65.4 (C-1), 112.4 (CBr_3), 113.3, 119.9, 120.9, 124.1, 127.3, 129.4, 138.3, 178.4 (C=O); EI–MS *m/z* (rel int) 428/426/424 (0.5/1.1/0.5) $[M^+-HBr]$, 347/345 (49/50) $[M^+-HBr$-Br], 255 (100) $[M^+-CBr_3]$, 171 (15) [255-C_5H_8O]. Anal. calcd for $C_{17}H_{19}Br_3N_2O\cdot 1/3\ C_4H_{10}O$: C, 41.38; H, 4.23; N, 5.27; found: C, 41.41; H, 4.33; N, 5.08.

2-Pentanoyl-1-tribromomethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (20). Starting from **5**·HCl (200 mg, 0.44 mmol) and valeroyl chloride (160 mL, 159 mg, 1.31 mmol), the title compound (187 mg, 0.37 mmol, 84% yield) was obtained as yellow crystals: mp 157 °C (dec). IR (KBr, cm^{-1}) 3430 (indole NH), 2910, 2890 (CH), 1660 (C=O). ^1H NMR (C_3D_6O): δ 0.95 (t, 3 H, 5′-CH_3),

1.34-1.49 (m, 2H, 4′-CH_2), 1.63-1.82 (m, 2H, 3′-CH_2), 2.49-2.76 (m, 2H, 2′-CH_2), 2.89-2.97 (m, 2H, 4-H), 4.31-4.34 (m, 2H, 3-H), 6.89 (s, 1H, 1-H), 7.06 (dt, $^3J_{6,7}=7.5$ Hz, $^5J_{6,8}=1.2$ Hz, 1H, 6-H), 7.18 (dt, $^3J_{7,6}=7.6$ Hz, $^5J_{7,5}=1.2$ Hz, 1H, 7-H), 7.48 (d, $^3J_{8,7}=7.9$ Hz, 1H, 8-H), 7.53 (d, $^3J_{5,6}=7.9$ Hz, 1H, 5-H); ^{13}C NMR (C_3D_6O): δ 14.2 (C-5′), 22.4 (C-4′), 23.1 (C-4), 28.0 (C-3′), 33.7 (C-2′), 40.1 (C-3), 64.1 (C-1), 107.9, 112.3 (CBr_3), 112.6, 119.2, 120.2, 123.4, 126.8, 128.8, 137.6, 173.4 (C=O); EI–MS *m/z* (rel int) 428/426/424 (0.8/2.0/0.8) [M^+-HBr], 347/345 (90/92) [M^+-HBr- Br], 267 (22) [M^+-3 Br], 255 (22) [M^+-CBr_3], 171 (100) [255-C_5H_8O]. Anal. calcd for $C_{17}H_{19}Br_3N_2O$: C, 40.27; H, 3.78; N, 5.52; found: C, 40.24; H, 3.68; N, 5.73.

Single-crystal X-ray diffraction analysis of 12, 13 and 14. Unless stated otherwise, all measurements of diffraction intensities were performed on a Siemens *P*4 diffractometer with an incident beam graphite monochromator (Mo-K_α radiation, $\lambda=0.71073$ Å) in ω-scan mode in the range of $1.75^\circ<\theta<27.5^\circ$.[44] The structures were solved by direct phase determination and refined by full-matrix anisotropic least-squares with the aid of the program SHELXTL-PLUS.[45] All non-hydrogen atoms were refined anisotropically. The hydrogen positions were calculated using a riding model and were considered fixed with isotropic thermal parameters in all refinements. Software used to prepare material for publication: SCHAKAL 88.[46]

Crystal data for 12. The crystal chosen for X-ray investigations was a clear colorless prism with the approximate dimensions 0.50×0.60×0.60 mm. $C_{14}H_{10}F_6N_2O$ (336.24 g mol^{-1}) crystallizes in the monoclinic system, space group $P2_1/n$, with $a=9.0197$ (4), $b=12.036$ (1), $c=12.944$ (1) Å, $\beta=98.043$ (4)°, $V=1391.4$ (1) Å^3, $Z=4$, μ (Mo-K_α) = 0.16 mm^{-1}, and $D_{calcd}=1.605$ g cm^{-3}. The unit cell parameters were determined by least-squares refinement using 60 centered reflections within $25.3^\circ<\theta<29.9^\circ$. A total of 3558 reflections were collected in ω-scan mode to $2\theta_{max}=55^\circ$ (*h*: 0→11, *k*: 0→15, *l*:−16→16) of which 3197 were unique with $I>2\sigma(I)$. In refinements, weights were used according to the scheme $w=1/[\sigma^2(F_o)]$. The refinement converged to the final agreement factors $R=0.051$, and $R_w=0.055$, for 213 parameters and 2633 observed reflections with $F>3\sigma(F)$; data-to-parameter ratio being 12.36. The electron density of the largest difference peak was found to be 0.23 eÅ^{-3}, while that of the largest difference hole was 0.20 eÅ^{-3}. In the crystals of **12**, the molecules are connected by intermolecular hydrogen bonds H(12)···O(15) [2.1 Å] to form a zigzag chain parallel to [001].

Crystal data for 13. The crystal chosen for X-ray investigations was a clear colorless prism with the approximate dimensions 0.45×0.50×0.30 mm. Data were collected on a Siemens R3m/V four-circle diffractometer using graphite monochromatic Mo-K_α radiation. $C_{14}H_{10}Cl_3F_3N_2O$ (385.60 g mol^{-1}) crystallizes in the monoclinic system, space group $P2_1/a$, with $a=12.091$ (1), $b=12.125$ (1), $c=10.860$ (1) Å, $\beta=98.72$ (1)°, $V=1573.6$ (3) Å^3, $Z=4$, μ(Mo-K_α) = 0.62 mm^{-1}, and $D_{calcd}=1.627$ g cm^{-3}. The unit cell parameters were determined by least-squares refinement using 22 centered reflections within $15.3^\circ<\theta<27.5^\circ$. A total of 3973 reflections were collected in ω-scan mode to $2\theta_{max}=55^\circ$ (*h* : 0→15, *k* : 0→15, *l* :-14→13) of which 3792 were unique with $I>2\sigma(I)$. In refinements, weights were used according to the scheme $w=1/[\sigma^2(F_o)]$. The refinement converged to the final agreement factors $R=0.044$, and $R_w=0.041$, for 213 parameters and 2642 observed reflections with $F>3\sigma(F)$; data-to-parameter ratio being 12.40. The electron density of the largest difference peak was found to be 0.28 eÅ^{-3}, while that of the largest difference hole was 0.24 eÅ^{-3}. In the crystals of **13**, the molecules are connected by intermolecular hydrogen bonds H(12)···O(15) [2.2 Å] to form a zigzag chain parallel to [100].

Crystal data for 14. The crystal chosen for X-ray investigations was a clear pale yellow prism with the approximate dimensions 0.30×0.40×0.15 mm. $C_{14}H_{10}Br_3F_3N_2O$ (518.97 g mol^{-1}) crystallizes in the monoclinic system, space group $P2_1/c$, with $a=10.695$ (9), $b=12.278$ (9), $c=12.766$ (9) Å, $\beta=105.216$ (6)°, $V=1617.2$ (2) Å^3, $Z=4$, μ(Mo-K_α) = 7.52 mm^{-1}, and $D_{calcd}=2.132$ g cm^{-3}. The unit cell parameters were determined by least-squares refinement using 60 centered reflections within $16.7^\circ<\theta<30.0^\circ$. A total of 4093 reflections were collected in ω-scan mode to $2\theta_{max}=55^\circ$ (*h* : 0→13, *k* : 0→15, *l* :-16→16) of which 3715 were unique with $I>2\sigma(I)$. In refinements, weights were used according to the scheme $w=1/[\sigma^2(F_o)]$. The refinement converged to the final agreement factors $R=0.052$, and $R_w=0.050$, for 213 parameters and 2814 observed reflections with $F>3\sigma(F)$; data-to-parameter ratio being 13.21. The electron density of the largest difference peak was found to be 0.65 eÅ^{-3}, while that of the largest difference hole was 0.60 eÅ^{-3}. In the crystals of **14**, the molecules are connected by intermolecular hydrogen bonds H(12)···O(15) [2.4 Å] to form a zigzag chain parallel to [010].

Tables of bond distances and angles, atomic coordinates, and anisotropic thermal parameters for **12** (CCDC 118762), **13** (CCDC 118764), and **14** (CCDC 118763) have been deposited with the Cambridge Crystallographic Data Centre.[47]

1-Octanol/water partition coefficients

Partition coefficients were determined at room temperature by high speed counter current chromatography (HSCCC) on a centrifugal partition apparatus (P.C., Potomac, MD, USA), equipped with analytical coil No. 14 (volume 78 mL, I.D. 1.7 mm). The chromatograph was connected to two P 700 LC pumps (Latek, Eppelheim, Germany). Pump A was used for the delivery of the mobile phase (1-octanol or cyclohexane), and pump B for the delivery of the stationary phase (0.01 M solution of 3-morpholinopropanesulfonate, pH 7.4). Both, the organic and the aqueous phases were mutually saturated. Concentrated or saturated solutions of each THβC tested were prepared in the mobile phase and introduced to the chromatograph via a 0.5-mL sample loop (Merck, Darmstadt, Germany). The THβCs were

detected at 254 nm using an SPD-6AV UV detector (Shimadzu, Kyoto, Japan). The chromatograms were recorded with a Model SP 4290 integrator (Spectra Physics, San Jose, CA, USA). Measurements were performed using operation conditions described in literature,[48–50] but modified in the following manner: The rotating coil was first filled with the mobile phase, and then the desired volume of the stationary phase was added to the coil by displacing the mobile phase. The installed coil was used with a volume ratio of mobile and stationary phase of ca. 1:2. The flow-rate of the eluent (setting 3 mL/min) was measured to be 2.5 mL/min for the 1-octanol/water system, and 2.15 mL/min for the cyclohexane/water system. The speed of rotation was set to 900 rpm. The highly lipophilic anthracene was taken as the non-retained compound to assess the column dead time, t_0. The 1-octanol/water partition coefficient P of a given THβC (at pH 7.4) was calculated according to the equation $\log P = \log [V_s/(V_r - V_0)]$, with V_0 being the dead volume ($V_0 = \text{flow} \times t_0$), V_s being the volume of the stationary phase, and V_r the retention volume of the sample ($V_r = \text{flow} \times t_r$, with t_r being the retention time of the sample).

In vivo pulse voltammetry after intranigral application of the THβCs 1–5. Adult male Han-Wistar rats (body weight: 280–320 g) were housed with free access to food and water on a 12/12 light/dark cycle (room temperature: 23 °C, humidity: 60%) for a period of 6 weeks. The animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.) prior to the stereotactic implantation of a glass micro cannula attached to a Hamilton syringe into the right substantia nigra pars compacta (SNpc) using coordinates according to Paxinos and Watson[52] (from interaural zero: anterior = 4.0 mm, lateral = 1.9 mm, vertical = 2.0 mm). Intranigral injection (for THβCs: 10 μg dissolved in 2 μL of 10% ethanol; control experiment: 2 μL of 10% ethanol) was performed over a period of 10 min. The cannula was subsequently removed 2.5 min after the end of the toxin application, and the skin was immediately closed with sutures. The rats were then single housed with food and water ad libitum. One week after toxin treatment, carbon fibre microelectrodes were implanted into the striata of both hemispheres (from interaural line: anterior = +8.5 mm, lateral = ± 3.5 mm, vertical = +4.5 mm)[52] in anaesthetized rats (400 mg/kg of chloral hydrate, i.p.). Subsequently, 40 min after the implantation procedure, the extracellular levels of 3,4-dihydroxyphenylacetic acid (DOPAC) were assayed as a measure of the dopamine metabolism by pulse voltammetry as described previously.[30,31,53] The voltammetric peak 2 at +100 mV was obtained by oxidation of the catechol moiety present in dopamine and DOPAC. The pharmacological characterization of this peak with the monoamine oxidase inhibitor pargyline (75 mg/kg, i.p.) showed a complete reduction of the signal, indicating the measurement of DOPAC.[53,54] Recordings were performed every 8 min for about 3 h. For a measurement of the restitution capacity of the dopaminergic system, the dopamine precursor L-dihydroxyphenylalanine (L-DOPA) was intraperitoneally administered (dose: 100 mg/kg) 40 min after the beginning of the voltammetric registration. The data were expressed as mean (%)±SEM of the peak height of the catechol signal recorded at the contralateral side. The paired Student's *t*-test was used to test the statistical significance. After the completion of the voltammetric measurements, the rats were perfused with 4% buffered formaldehyde solution. Brain slices (20 μm) were performed and stained with eosin in order to verify the exact position of the glass micro cannula and the carbon fibre electrodes.

Inhibition of mitochondrial respiration enzymes

Rat brain homogenates and submitochondrial particles (SMPs) from isolated rat liver mitochondria were prepared as described previously.[21] Enzyme activities in rat brain homogenates and SMPs were assayed in a final volume of 500 μL at 30 °C using a Kontron Uvicon 943 spectrophotometer. Mitochondrial respiration on SMPs and intact mitochondria were measured polarographically using a Clark-type oxygen electrode (Rank Brothers, England) at 30 °C with 0.25 mg protein/mL. The IC_{50} respectively IC_{100} values, defined as the concentrations required to inhibit NADH-ubiquinone reductase activity (complex I; EC 1.6.99.3) by 50% respectively 100% were determined after 5 min of preincubation with the inhibitors. NADH-linked oxidation was measured by adding NADH to a concentration of 0.28 mM. All toxins were dissolved in methanol. The total volume of the organic solvent did not exceed 5% (v/v). At this concentration, methanol did not significantly affect the enzymes tested. Protein concentrations were determined according to the method of Lowry et al.[55] using fat-free bovine serum albumin as standard.

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