Chemical Modification of the Mitochondrial Complex I Inhibitor 1-Trichloromethyl-1,2,3,4-tetrahydro-β-carboline: Synthesis and Evaluation of *N*-Alkanoyl Derivatives §

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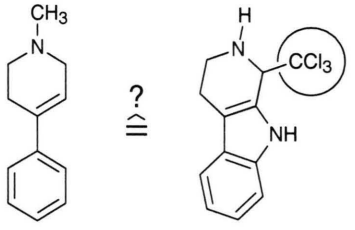
Chloral-Derived Tetrahydro-β-carbolines, 1-Trichloromethyl-1,2,3,4-tetrahydro-β-carboline, Mitochondrial Respiration

Several *N*-alkanoyl derivatives (**4**-**9** and **13**-**16**) of the potent mitochondrial complex I inhibitor TaClo (1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline, **2**) have been synthesized in order to elucidate the role of hydrophobic portion in the inhibitory action. Using rat brain homogenates or submitochondrial particles, the inhibitory effects of these compounds towards NADH-ubiquinone reductase (complex I) activity indeed appeared to correlate quite strongly with their lipophilic character. An X-ray structure analysis, exemplarily performed for *N*-acetyl-TaClo (**4**), revealed the *N*-substituent of such chlorinated agents to be dramatically pushed out of the β-carboline ring 'plane' due to the high steric demand of the huge trichloromethyl group at C-1.

Introduction

Mitochondrial complex I (NADH-ubiquinone reductase) deficiency in dopaminergic neurons is discussed to be one of the pathogenic factors contributing to the progression of cell death in the *substantia nigra* of patients suffering from Parkinson's disease (Lestienne *et al.*, 1990; Schapira *et al.*, 1990; Janetzky *et al.*, 1994; Schapira, 1998). Since exposure to 1-methyl-4-phenylpyridinium ion (MPP^+), the bioactive metabolite of the parkinsonism-inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) turned out to cause a selective reduction of complex I activity (Nicklas *et al.*, 1985; Mizuno *et al.*, 1987), compounds structurally similar to **1**, among them isoquinolines and β-carbolines, have been investigated more closely with respect to their inhibitory potential against mitochondrial respiration (Suzuki *et al.*, 1990; McNaught *et al.*, 1995; Morikawa *et al.*, 1998;



MPTP (**1**) TaClo (**2**)

Fig. 1. Structural analogy of two neurotoxins acting on dopaminergic neurons: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**), a well-established experimental tool in neurosciences, and the highly chlorinated tetrahydro-β-carboline 'TaClo' (**2**), a mammalian alkaloid formed from endogenously present tryptamine ('Ta') and therapeutically administered trichloroacetaldehyde (chloral, 'Clo').

§ "Endogenous Alkaloids in Man", part 36; for part 35 see: Bringmann, Brückner, Mössner, Feineis, Heils & Lesch (2000).

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Albores *et al.*, 1990; Fields *et al.*, 1992; Bringmann *et al.*, 1995; Janetzky *et al.*, 1999; Bringmann *et al.*, 2000).

Our special interest is focussed on 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaClo, **2**) (see Fig. 1), a progressively-acting neurotoxin (for reviews see: Bringmann *et al.*, 1996; Sontag *et al.*, 1996; Bringmann *et al.*, 1998) that was recently found to be formed in trace amounts in humans after intake of the soporific agent, chloral hydrate (Bringmann *et al.*, 1999). The distinct toxic potency of TaClo (**2**) towards dopaminergic (Grote *et al.*, 1995; Rausch *et al.*, 1995) and serotonergic (Gerlach *et al.*, 1998; Bringmann *et al.*, 2000a) neurons appears to be strongly associated with its ability to severely disturb the neuronal energy metabolism. In rat brain homogenates and in submitochondrial particles (SMPs), TaClo was observed to totally block highly selectively mitochondrial electron transport at complex I at a concentration (700 μM) ca. 10-times lower than that of MPP^+ (7.5 mM) (see Table I) (Janetzky *et al.*, 1995; 1999). Nonetheless, although MPP^+ is a rather weak inhibitor in broken mitochondria or SMPs, this pyridinium ion is capable to massively affect complex I in intact mitochondria due to an active accumulation to high concentrations on a millimolar level, finally resulting in a dramatic increase in potency (Nicklas *et al.*, 1985; Ramsay *et al.*, 1986). TaClo (**2**), by contrast, seems not to be actively taken up into mitochondria, presumably due to its lacking positive charge. Indeed, uptake studies on human dopaminergic and serotonergic cells hint at a mainly passive penetration of **2** through cell membranes (Bringmann *et al.*, 2000a), thus giving rise to the assumption that the highly lipophilic character of the trichloromethyl group significantly supports the propagation of TaClo toxicity.

In the present study, we now describe a more detailed investigation on the influence of an additional hydrophobic portion on the inhibitory capacity of chloral-derived tetrahydro-β-carbolines towards mitochondrial respiration. For this purpose, *N*-alkanoyl functions with increasing alkyl chain length (C_1-C_6) or halogenated *N*-acyl groups were introduced into the TaClo molecule by a standard reaction procedure. The compounds thus obtained were tested *in vitro* for inhibitory action on complex I.

Results and Discussion

Synthesis and X-ray structure analysis

As summarized in Tables I and II, a whole series of highly halogenated *N*-acyl compounds (**4-9** and **11-16**) have been synthesized starting from TaClo (**2**) or eleagnine (**10**). All of these tetrahydro-β-carbolines were obtained in good yields (from 51% up to 90%) by performing the derivatization reaction in dichloromethane in the presence of triethylamine using appropriate acid chlorides or anhydrides as reagents.

In addition to the constitutions of these novel agents, as anticipated from the syntheses and the spectroscopic data, the knowledge of steric effects exhibited by the huge 1-trichloromethyl group on the conformation of the tetrahydro-β-carboline ring system was highly desirable. Luckily, acetylation of TaClo (**2**) provided crystalline material of the derivative **4**, which proved to be well-suited for a single-crystal X-ray diffraction analysis. Indeed, the voluminous C(1) substitutent was demonstrated to be the most striking feature about such heterocycles (see Fig. 2(a)): It was found to be largely twisted out of the β-carboline ring 'plane', occupying a pseudo-axial position. The three chlorine atoms at C(14) adopt a perfectly staggered orientation with respect to the C(1)–C(14) bond, leading to a minimization of their steric interactions with C(13) and the *N*-acetyl function. Obviously, also for steric reasons, the tetrahydropyrido moiety is partially planarized with only C(3) and N(2) being located distinctly out of the ring 'plane'.

The influence of the big van-der-Waals radius of chlorine (1.8 Å) – in comparison to the small radius of hydrogen (1.0 Å) – on the half-chair conformation of the tetrahydropyrido ring system is best visualized by a joint plot (see Fig. 2(b)) of the crystal structures of **4** and of the eleagnine-derived trifluoroacetamide **11**, on which we reported in a previous paper (Peters *et al.*, 1995). As illustrated in Fig. 2(b), the *N*-substituent of the 1-CCl_3 compound **4** is dramatically pushed upwards compared with the 1-CH_3 analog **11**.

Inhibition of complex I of the mitochondrial respiration

The chloral-derived tetrahydro-β-carboline TaClo (**2**) has emerged as a potent endogenously

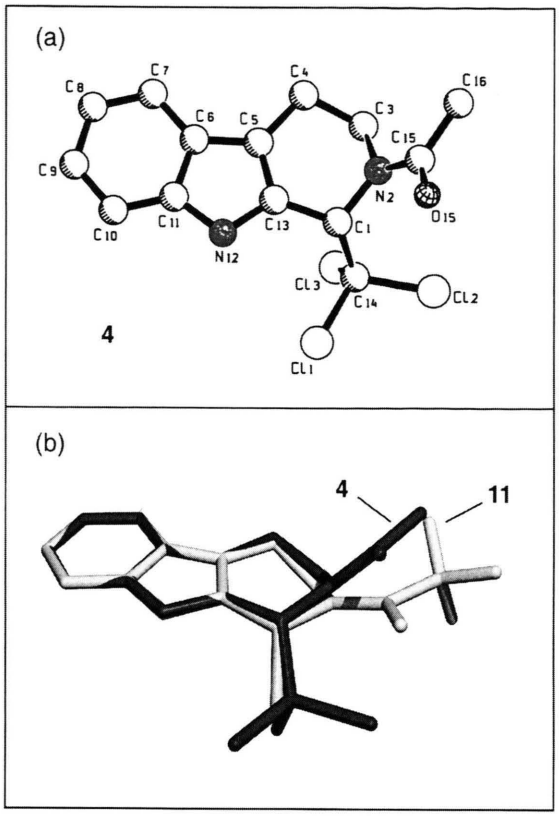


Fig. 2. (a) SCHAKAL plot of the crystal structure of *N*-acetyl-1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (**4**) with a guide of the atomic numbering system adopted in the X-ray investigation. The compound was found to be racemic in the crystal; for presentation, only the enantiomer with the CCl_3-group below the graphical plane, has been chosen, arbitrarily (hydrogen atoms have been omitted for reasons of clarity). – (b) Joint plot of the structures of *N*-trifluoroacetyl-1-methyl-1,2,3,4-tetrahydro-β-carboline (**11**) (light grey) and *N*-acetyl-1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (**4**) (dark grey) in the crystal, matched with respect to the indole part of the molecules (hydrogen atoms have been omitted for reasons of clarity).

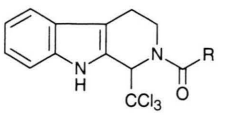
occurring neurotoxin with a strong evidence for inhibition of complex I activity as its presumable mechanism of action (Bringmann *et al.*, 1998; Janetzky *et al.*, 1999). The highly nonpolar character of TaClo is discussed to strongly favor the pronounced inhibitory potential of this agent towards mitochondrial respiration due to the fact that a marked hydrophobicity generally facilitates the passage of an inhibitor through the lipophilic membrane environment to the binding sites of complex I, thus allowing a more facile access to the catalytic proteins (Miyoshi *et al.*, 1998).

As outlined in Table I, we first of all started to investigate the influence of the inhibitory capacities of a series of TaClo-related *N*-alkanoyl derivatives (**3**-**9**) with respect to their lipophilic properties. The ability of these tetrahydro-β-carbolines to enter cells was assessed by measuring their partition coefficients *P* (given as log *P*) in a mixture of cyclohexane/water. As expected, partitioning of **3**-**9** into the organic layer was found to markedly reach higher values step by step, with increasing length of the alkyl chain ($C_1 \rightarrow C_6$) of the acyl function. An approximate 12-fold increase in partitioning was observed between the more polar *N*-formyl derivative **3** and the most unpolar compound *N*-hexanoyl-TaClo (**9**). In comparison to the parent compound TaClo (**2**), however, only a 4-fold enhancement of lipophilicity was observed for **9**.

Incubation experiments using rat brain homogenates or SMPs, in general (except for **8** and **9**), clearly revealed the increasing nonpolar character of these *N*-acylated TaClo derivatives to correlate quite well with their enhanced inhibitory potencies (cp. IC_{100} and IC_{50} values of **3**-**7** in Table I): The more polar TaClo derivatives **3** and **4** (being 3-times and 2-times, respectively, less lipophilic in comparison to **2** as the parent compound) only moderately affected complex I activity (Table I). The heterocycles **5**-**7**, by contrast, turned out to display strong inhibitory effects on the mitochondrial respiration. The concentrations of **5, 6**, or **7** required to achieve a complete inhibition of complex I (IC_{100}) were determined to be approximately 2.5-fold (for **5**) up to 3.5-fold (for **6** or **7**) lower than that of TaClo (**2**), and even ca. 25-fold (for **5**) up to nearly 40-fold (for **6** or **7**) lower than that of the MPTP metabolite MPP^+.

Unfortunately, for the two most lipophilic TaClo derivatives **8** and **9** a slight decrease of the inhibitory capacity towards mitochondrial respiration was observed, obviously due to the poor solubility of these compounds in the aqueous test system (with precipitation occurring at concentrations > 300 μM). Thus, although, in general, a high degree of lipophilicity appears to favor the inhibitory potency within this series of chloral-derived tetrahydro-β-carbolines, an improvement of inhibitory action by introducing an unpolar alkanoyl portion

Table I. Inhibitory potential of *N*-alkanoyl derivatives of 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline ('TaClo', **2**) in comparison to the MPTP metabolite MPP^+ on mitochondrial NADH-ubiquinone reductase (complex I) activity in rat brain homogenates or SMPs, and partition coefficients *P* (given as log *P* values) for these compounds in a cyclohexane/water mixture.



3-9

Compd.	R	TLC R_f [a]	log *P* cyclohexane/H_2O [b]	Inhibition of complex I IC_{100} [μM]	IC_{50} [μM]
TaClo (**2**)		0.35	2.37 ± 0.12	700	200
3	H	0.08 / 0.14	1.91 ± 0.04	>>750	500
4[c]	CH_3	0.10	2.03 ± 0.08	IC_{20} = 500 μM	
5	C_2H_5	0.34	2.37 ± 0.02	300	150
6	$CH(CH_3)_2$	0.42	––– [d]	200	>150
7	C_3H_7	0.39	2.53 ± 0.03	200	>150
8[e]	C_4H_9	0.49	2.90 ± 0.07	IC_{25} = 100 μM	
9[e]	C_5H_{11}	0.55	2.97 ± 0.06	IC_{60} = 300 μM	
MPP^+		––– [d]	––– [d]	7500	3500

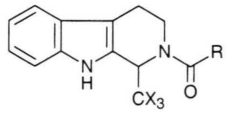
[a] Solvent system used for TLC analysis on silica gel plates: petroleum ether – *tert.*-butyl methyl ether, 2:1.
[b] For the HSCCC method used to determine partition coefficients *P*, refer to the Experimental Section. The data respresent the average value ± S. E. M. of three separate experiments.
[c] The compound precipitated in the test system at a concentration > 550 μM.
[d] Not determined.
[e] The compounds precipitated in the test system at concentrations > 300 μM.

into the TaClo molecule is obviously restricted to molecules with log *P* values < 3.

Another strategy to enhance the lipophilicity of agents, is the replacement of hydrogens by chlorine atoms. For this reason, in a second series of tests, we examined the inhibitory effects of eleagnine (**10**) and TaClo (**2**) derivatives containing a chlorinated *N*-acetyl function *vs.* the inhibitory activity displayed by the more polar *N*-trifluoroacetylated compounds **11** and **13**. At a concentration of 50 μM, the eleagnine-derived trifluoroacetamide **11** failed to affect mitochondrial respiration, while the structurally analogous trichloroacetamide **12** displayed 30% inhibition of complex I activity (see Table II). A similar trend was observed for the inhibitory capacities of the TaClo-related *N*-trifluoro- and *N*-trichloroacetyl derivatives **13** and **16**: At a concentration of 500 μM, **13** exhibited only a weak effect of 20% inhibition towards mitochondrial electron transport. By contrast, a nearly complete inhibition of complex I activity was achieved by **16** as manifest from an IC_{90} value of 500 μM. In comparison to the parent compound **2**, the introduction of a mono- or dichloroacetyl group into the TaClo molecule already resulted in more potent complex I inhibitors (*e.g.*, for **14**: IC_{100} = 500 μM, IC_{50} = 200 μM). The inhibitory activities of **14**–**16** are also distinctly stronger compared with the only weak inhibitory potential of *N*-acetyl-TaClo (**4**) (see Table I), which produces – similar to the trifluoroacetamide **13** – only 20% inhibition at 500 μM. Summarizing, as expected, the (for TaClo additional) introduction of chlorine atoms enhances the lipophilic character (as manifest from the R_f values) of **2** and **10**, and, simultaneously, distinctly favors the inhibitory action of the resulting agents.

The inhibitory effects of all of the TaClo-related *N*-alkanoyl tetrahydro-β-carbolines **3**–**9** and **13**–**16** presented in this paper were found to be highly selective towards complex I of the mitochondrial respiratory chain. The parent compound TaClo (**2**) itself, by contrast, is also able to partially inhibit complex II / III (30% at 500 μM) (Janetzky *et al.*, 1999). As far as we know, also some other TaClo derivatives among them *N*-alkyl compounds possess this inhibitory potential, too (Bringmann *et al.*, 1998). None of these agents was observed to inhibit complex IV.

Table II. Inhibition of mitochondrial respiration by eleagnine (**10**) and its *N*-trihalogenacetyl derivatives **11** and **12** in comparison to the inhibitory effects caused by the (additionally halogenated) *N*-acyl compounds **13**–**16**, which are derived from 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline ('TaClo', **2**).



11-16

Compd.	X	R	TLC R_f [a]	Inhibition of complex I IC_{50} [μM]
Eleagnine (**10**)	H	– – –	0.10	1200
11[b]	H	CF_3	0.31	≤ 50 μM, no inhibition
12[b]	H	CCl_3	0.56	IC_{30} = 50 μM
13[c]	Cl	CF_3	0.42	IC_{20} = 500 μM
14	Cl	CH_2Cl	0.40	200
15	Cl	$CHCl_2$	0.52	>300
16	Cl	CCl_3	0.62	250

[a] Solvent system used for TLC analysis on silica gel plates: petroleum ether – *tert.*-butyl methyl ether, 2:1.
[b] The compounds precipitated in the test system at concentrations > 100 μM.
[c] The compound precipitated in the test system at a concentration > 550 μM.

In conclusion, our findings on chloral-derived heterocycles confirm the lipophilic character of these agents to significantly influence their inhibitory potency: In particular, the highly nonpolar representatives of TaClo-derived *N*-alkanoyl compounds (**5**–**7** and **14**–**16**) turned out to be quite potent mammalian mitochondrial complex I inhibitors. Interestingly, all of these tetrahydro-β-carbolines displayed a markedly more potent inhibition of complex I activity than the MPTP metabolite MPP^+. Although some trends are evident, nonetheless, detailed investigations on the structural requirements of tetrahydro-β-carbolines essential for disturbing mammalian mitochondrial function are still missing. For this reason, current research is now focussing on the evaluation of a broad series of TaClo derivatives, the structures of which have systematically been modified. This work is in progress.

Experimental

General

All reagents used were of commercial quality. 3-Morpholinopropanesulfonate was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Organic solvents were dried and distilled prior to use. 1-Trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido-[3,4-*b*]indole hydrochloride (**2**·HCl) was prepared from tryptamine and trichloroacetaldehyde *via* its *N*-formyl derivative **3** as described previously (Bringmann and Hille, 1990; Bringmann *et al.*, 1998). 1-Methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (eleagnine, **10**) and 1-trichloromethyl-2-trifluoroacetyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**13**) were synthesized as reported earlier (Bringmann *et al.*, 2000b).

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 200 or AC 250 spectrometer at 200 MHz (for ^1H NMR), and at 63 MHz (for ^{13}C NMR). Chemical shifts (δ) are reported in parts per million (ppm), and are referenced to internal chloroform (^1H, δ = 7.26 ppm) or acetone (^1H, δ = 2.01 ppm; ^{13}C, 29.85 and 205.9 ppm) in the deuterated solvents. Coupling constants (*J*) are given in Hertz (Hz). Electron impact mass spectral data (electron ionization 70 eV) were obtained on a Finnigan MAT 8200 mass spectrometer. Elemental analyses were performed by the Microanalysis Laboratory of the University of Würzburg (Institute of Inorganic Chemistry) on a Carlo Erba Elemental Analyzer M 1106 apparatus.

General procedure for the preparation of N-acylated tetrahydro-β-carbolines

To a suspension of **2**·HCl (for **4**–**9** and **13**–**16**) or **10** (for **11** and **12**) (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 acylating reagent (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 / petroleum ether provided the acyl derivatives as crystalline material. The data are given below.

2-Acetyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**4**)

Starting from **2**·HCl (100 mg, 0.31 mmol) and acetyl chloride (0.17 ml, 124 mg, 1.23 mmol), the title compound (92 mg, 0.28 mmol, 90% yield) was obtained as colorless crystals, suitable for X-ray structure analysis: mp 215 °C (dec). IR (KBr, cm^{-1}) 3320 (indole NH), 2905, 2895 (CH), 1635 (C=O), 1300, 795 (CCl); ^{1}H NMR (C_3D_6O): δ 2.31 (s, 3 H, CH_3), 2.80–3.00 (m, 2 H, 4-H), 4.12–4.35 (m, 2 H, 3-H), 6.73 (s, 1 H, 1-H), 7.03–7.10 (m, 1 H, 6-H or 7-H), 7.13–7.21 (m, 1 H, 7-H or 6-H), 7.43–7.48 (m, 1 H, 5-H or 8-H), 7.51–7.55 (m, 1 H, 8-H or 5-H); ^{13}C NMR (C_3D_6O): δ 21.78 (C-2′), 21.97 (C-4), 41.52 (C-3), 62.46 (C-1), 102.5 (CCl_3), 107.9, 112.4, 119.1, 120.1, 123.4, 126.7, 127.0, 137.6, 171.0 (C=O); MS (EI, 70eV) *m/z* (rel int) 334 / 332 / 330 (0.1 / 0.4 / 0.4) $[M]^{+\cdot}$, 298 / 296 / 294 (0.7 / 4.9 / 8.2) [M–HCl], 261 / 259 (20 / 64) [M–HCl–Cl], 213 (31) [M–CCl_3], 171 (100) [213 - C_2H_2O]. Anal. calcd. for $C_{14}H_{13}Cl_3N_2O$: C, 50.71; H, 3.95; N, 8.45; found: C, 50.51; H, 4.22; N, 8.66.

2-Monochloroacetyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**14**)

Starting from **2**·HCl (100 mg, 0.31 mmol) and monochloroacetyl chloride (73 µl, 104 mg, 1.23 mmol), the title compound (91 mg, 0.25 mmol, 81% yield) was obtained as colorless crystals: mp 195 °C (dec). IR (KBr, cm^{-1}) 3395 (indole NH), 2910, 2880 (CH), 1660 (C=O), 1300, 805 (CCl); ^{1}H NMR (C_3D_6O): δ 2.92–3.17 (m, 2 H, 4-H), 4.19–4.48 (m, 2 H, 3-H), 4.59 (dd, $^2J_{gem}$ = 26.2 Hz, J = 13.1 Hz, 2 H, CH_2Cl), 6.66 (s, 1 H, 1-H), 7.04–7.11 (m, 1 H, 6-H or 7-H), 7.15–7.22 (m, 1 H, 7-H or 6-H), 7.46 (d, $^3J_{8,7}$ = 8.2 Hz, 1 H, 8-H), 7.54 (d, $^3J_{5,6}$ = 8.0 Hz, 1 H, 5-H), 10.28 (br. s, 1 H, indole NH); ^{13}C NMR (C_3D_6O):

δ 22.03 (C-4), 41.77 (C-3), 63.95 (C-1), 66.50 (CH_2Cl), 101.7 (CCl_3), 112.3, 112.4, 119.2, 120.2, 123.7, 126.0, 126.5, 137.8, 164.8 (C=O); MS (EI, 70eV) *m/z* (rel int) 370 / 368 / 366 / 364 (0.3 / 0.8 / 1.7 / 1.2) $[M]^{+\cdot}$, 334 / 332 / 330 / 328 (0.2 / 2.7 / 7.8 / 8.5) [M–HCl], 297 / 295 / 293 (4.3 / 25 / 41) [M–HCl–Cl], 249 / 247 (34 / 100) [M–CCl_3], 171 (87) [249 / 247 - C_2HClO]. Anal. calcd. for $C_{14}H_{12}Cl_4N_2O$: C, 45.93; H, 3.30; N, 7.65; found: C, 45.68; H, 3.33; N, 7.32.

2-Dichloroacetyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**15**)

Starting from **2**·HCl (200 mg, 0.62 mmol) and dichloroacetyl chloride (93 µl, 143 mg, 0.97 mmol), the title compound (243 mg, 0.61 mmol, 88% yield) was obtained as colorless crystals: mp 243 °C (dec). IR (KBr, cm^{-1}) 3295 (indole NH), 3040, 2990, 2940, 2850 (CH), 1660 (C=O), 1440, 1420, 1040, 750; ^{1}H NMR (C_3D_6O): δ 2.97–3.20 (m, 2 H, 4-H), 4.25–4.38 (m, 2 H, 3-H), 6.45 (s, 1 H, 1-H), 7.08–7.11 (dt, $^3J_{6,7}$ = 7.6 Hz, $^4J_{6,8}$ = 1.1 Hz, 1 H, 6-H), 7.19 (dt, $^3J_{7,6}$ = 7.6 Hz, $^4J_{7,5}$ = 1.2 Hz, 1 H, 7-H), 7.21 (s, 1 H, $CHCl_2$), 7.47 (d, $^3J_{8,7}$ = 8.2 Hz, 1 H, 8-H), 7.55 (d, $^3J_{5,6}$ = 7.6 Hz, 1 H, 5-H), 10.3 (br. s, 1 H, indole NH); ^{13}C NMR (C_3D_6O): δ 22.03 (C-4), 41.78 (C-3), 63.95 (C-1), 66.50 (C-2′), 101.7 (CCl_3), 107.9, 112.3, 112.4, 119.2, 120.2, 123.7, 126.1, 126,5, 137.8, 164.8 (C=O); MS (EI, 70eV) *m/z* (rel int) 404 / 402 / 400 / 398 (0.8 / 2.2 / 3.6 / 2.0) $[M]^{+\cdot}$, 368 / 366 / 364 / 362 (1.0 / 4.0 / 7.9 / 6.2) [M–HCl], 333 / 331 / 229 / 227 (1.0 / 6.7 / 19 / 22) [M–HCl–Cl], 286 / 284 / 282 (10 / 62 / 100) [M–CCl_3], 169 (45) [M–CCl_3–$C_2H_2Cl_2O$]. Anal. calcd. for $C_{14}H_{11}Cl_5N_2O$: C, 41.98; H, 2.76; N, 6.99; found: C, 41.68; H, 2.65; N, 6.80.

2-Trichloroacetyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**16**)

Starting from **2**·HCl (100 mg, 0.31 mmol) and trichloroacetyl chloride (0.11 ml, 167 mg, 0.92 mmol), the title compound (81 mg, 0.23 mmol, 75% yield) was obtained as a beige-colored amorphous solid: mp 96–97 °C (dec). IR (KBr, cm^{-1}) 3415 (indole NH), 2910 CH), 1670 (C=O), 1300, 805 (CCl); ^{1}H NMR (C_3D_6O): δ 2.80–3.00 (m, 2 H, 4-H), 4.38 (ddd, $^2J_{gem}$ = 15.0 Hz, $^3J_{3a,4a}$ = 11.6 Hz, $^3J_{3a,4e}$ = 4.9 Hz, 1 H, 3-H), 4.89–4.98 (m, 1 H, 3-H), 6.73 (s, 1 H, 1H), 7.06–7.13 (m, 1 H, 6-H or 7-H), 7.17–7.25 (m, 1 H, 7-H or 6-H), 7.49 (d, $^3J_{8,7}$ = 7.3 Hz, 1 H, 8-H), 7.56 (d, $^3J_{5,6}$ = 7.9 Hz, 1 H, 5-H), 10.39 (br. s, 1 H, indole NH); ^{13}C NMR (C_3D_6O): δ 22.34 (C-4), 43.66 (C-3), 50.39 (C-1), 94.56 ($COCCl_3$), 104.7 (CCl_3), 107.7, 112.2, 119.0, 120.2, 122.6, 127.8, 135.4, 137.8, 160.3 (C=O); MS (EI, 70eV) *m/z* (rel int) 440 / 438 / 436 / 434 / 432 (0.6 / 2.5 / 6.9 / 8.2 / 4.0) $[M]^{+\cdot}$, 404 / 402 / 400 / 398 / 396 (0.2 / 1.4 / 4.5 / 7.4 / 4.3) [M–HCl], 369 / 367 / 365 / 363 / 361 (0.2 / 2.4 / 8.5 / 16 / 13) [M–HCl–Cl], 333 / 331 /

329 / 327 (1.1 / 6.8 / 21 / 21) [M - 3 Cl], 321 / 319 / 317 / 315 (3.2 / 31 / 98 / 100) [M−CCl_3], 169 (12) [M−CCl_3−C_2HCl_3O]. Anal. calcd. for $C_{14}H_{10}Cl_6N_2O$: C, 38.66; H, 2.32; N, 6.44; found: C, 38.33; H, 2.10; N, 6.32.

2-Propanoyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**5**)

Starting from **2**·HCl (2.00 g, 6.14 mmol) and propionyl chloride (1.61 ml, 1.70 g, 18.4 mmol), the title compound (1.91 g, 5.53 mmol, 90% yield) was obtained as colorless crystals: mp 197 °C. IR (KBr, cm^{-1}) 3310 (indole NH), 3040, 2960, 2920, 2815 (CH), 1635 (C=O), 1440, 1400 (CH), 1200, 1180 (CN), 800 (CCl); ^1H NMR ($CDCl_3$): δ 1.24 (t, 3 H, 3′-CH_3), 2.58 (dq, J_{gem} = 48.4 Hz, J = 7.3 Hz, 2 H, 2′-CH_2), 2.84–2.95 (m, 2 H, 4-H), 4.12–4.19 (m, 2 H, 3-H), 6.74 (s, 1 H, 1-H), 7.11–7.17 (m, 1 H, 6-H or 7-H), 7.22–7.28 (m, 1 H, 6-H or 7-H, overlay by solvent), 7.38–7.42 (m, 1 H, 5- or 8-H), 7.50–7.54 (m, 1 H, 5-H or 8-H), 8.48 (br. s, 1H, indole NH); ^{13}C NMR (C_3D_6O): δ 9.59 (C-3′), 22.03 (C-4), 27.14 (C-2′), 40.49 (C-3), 62.60 (C-1), 102.6 (CCl_3), 107.8, 112.4, 119.1, 120.0, 123.3, 126.7, 127.1, 137.6, 174.2 (C=O); MS (EI, 70eV) *m/z* (rel int) 348 / 346 / 344 (0.1 / 0.2 / 0.1) $[M]^{+\cdot}$, 312 / 310 / 308 (0.8 / 6.0 / 7.3) [M−HCl], 275 / 273 (25 / 74) [M−HCl−Cl], 227 (23) [M−CCl_3], 171 (100) [227-C_3H_4O]. Anal. calcd. for $C_{15}H_{15}Cl_3N_2O$: C, 52.12; H, 4.38; N, 8.11; found: C, 52.27; H, 4.42; N, 7.95.

2-(2′-Methylpropanoyl)-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**6**)

Starting from **2**·HCl (2.00 g, 6.14 mmol) and isobutyryl chloride (1.94 ml, 1.96 g, 18.4 mmol), the title compound (1.77 g, 4.92 mmol, 80% yield) was obtained as colorless crystals: mp 181 °C. IR (KBr, cm^{-1}) 3250 (indole NH), 3020, 2940, 2900, 2880, 2820 (CH), 1620 (C=O), 1430, 1400 (CH), 1200 (CN), 800, 785 (CCl); ^1H NMR ($CDCl_3$): δ 1.02 [dd, J_{gem} = 21.7 Hz, J = 6.7 Hz, 6 H, $CH(CH_3)_2$], 2.86–2.95 (m, 2 H, 4-H), 3.00 [sept, 1 H, $CH(CH_3)_2$], 4.14–4.26 (m, 2 H, 3-H), 6.80 (s, 1 H, 1-H), 7.11–7.17 (m, 1 H, 6-H or 7-H), 7.22–7.28 (m, 1 H, 6-H or 7-H, overlay by solvent), 7.38–7.42 (m, 1 H, 5-H or 8-H), 7.51–7.55 (m, 1 H, 5-H or 8-H), 8.51 (br. s, 1H, indole NH); ^{13}C NMR (C_3D_6O): δ 19.40 (C-3′), 20.23 (C-3′), 22.60 (C-4), 31.09 (C-2′), 40.49 (C-3), 63.37 (C-1), 102.7 (CCl_3), 108.2, 112.4, 119.1, 120.0, 123.3, 126.7, 127.1, 137.6, 177.4 (C=O); MS (EI, 70eV) *m/z* (rel int) 360 / 358 (0.1 / 0.3) $[M]^{+\cdot}$, 326 / 324 / 322 (1.0 / 4.5 / 8.6) [M−HCl], 289 / 287 (33 / 94) [M−HCl−Cl], 241 (15) [M−CCl_3], 171 (100) [241 - C_4H_6O]. Anal. calcd. for $C_{16}H_{17}Cl_3N_2O$: C, 53.42; H, 4.77; N, 7.79; found: C, 53.64; H, 4.81; N, 7.58.

2-Butanoyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**7**)

Starting from **2**·HCl (2.00 g, 6.14 mmol) and butyryl chloride (1.92 ml, 1.96 g, 18.4 mmol), the title compound (1.83 g, 5.09 mmol, 83% yield) was obtained as colorless crystals: mp 159 °C. IR (KBr, cm^{-1}) 3320 (indole NH), 3020, 2940, 2880, 2830 (CH), 1635 (C=O), 1440, 1410, 1200, 1180 (CN), 820, 805 (CCl); ^1H NMR ($CDCl_3$): δ 1.02 (t, 3 H, 4′-CH_3), 1.76 (m, 2 H, 3′-CH_2), 2.38–2.67 (m, 2 H, 2′-CH_2), 2.85–2.96 (m, 2 H, 4-H), 4.14–4.19 (m, 2 H, 3-H), 6.75 (s, 1 H, 1-H), 7.11–7.17 (m, 1 H, 6-H or 7-H), 7.22–7.28 (m, 1 H, 6-H or 7-H, overlay by solvent), 7.38–7.42 (m, 1 H, 5-H or 8-H), 7.51–7.54 (m, 1 H, 5-H or 8-H), 8.49 (br. s, 1H, indole NH); ^{13}C NMR (C_3D_6O): δ 13.97 (C-4′), 19.08 (C-3′), 22.10 (C-4), 35.70 (C-2′), 40.61 (C-3), 62.46 (C-1), 102.6 (CCl_3), 107.4, 112.3, 119.1, 120.0, 123.3, 126.7, 127.1, 137.6, 173.4 (C=O); MS (EI, 70eV) *m/z* (rel int) 360 / 358 (0.31 / 0.4) $[M]^{+\cdot}$, 326 / 324 / 322 (1.3 / 6.3 / 9.5) [M−HCl], 289 / 287 (34 / 95) [M−HCl−Cl], 241 (24) [M−CCl_3], 171 (100) [227 - C_4H_6O]. Anal. calcd. for $C_{16}H_{175}Cl_3N_2O$: C, 53.42; H, 4.77; N, 7.79; found: C, 53.57; H, 4.62; N, 7.62.

2-Pentanoyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**8**)

Starting from **2**·HCl (2.00 g, 6.14 mmol) and valeroyl chloride (2.24 ml, 2.22 g, 18.4 mmol), the title compound (1.73 g, 4.63 mmol, 75% yield) was obtained as colorless crystals: mp 140 °C (dec). IR (KBr, cm^{-1}) 3230 (indole NH), 3040, 3020, 2940, 2920, 2840 (CH), 1620 (C=O), 1440, 1400 (CH), 820, 800 (CCl); ^1H NMR ($CDCl_3$): δ 0.96 (t, 3 H, 5′-CH_3), 1.42 (sext, 2 H, 4′-CH_2), 1.64–1.77 (m, 2 H, 3′-CH_2), 2.40–2.68 (m, 2 H, 2′-CH_2), 2.84–2.96 (m, 2 H, 4-H), 4.14–4.19 (m, 2 H, 3-H), 6.75 (s, 1 H, 1-H), 7.11–7.17 (m, 1 H, 6-H or 7-H), 7.22–7.28 (m, 1 H, 6-H or 7-H, overlay by solvent),

7.38–7.42 (m, 1 H, 5-H or 8-H), 7.51–7.54 (m, 1 H, 5-H or 8-H), 8.49 (br. s, 1 H, indole NH); ^{13}C NMR (C_3D_6O): δ 14.05 (C-5′), 22.16 (C-4′), 22.94 (C-4), 27.92 (C-3′), 33.58 (C-2′), 40.68 (C-3), 62.51 (C-1), 102.6 (CCl_3), 108.0, 112.4, 119.1, 120.1, 123.3, 126.7, 127.1, 137.6, 173.6 (C=O); MS (EI, 70eV) *m/z* (rel int) 372 (0.1) $[M]^{+\cdot}$, 340 / 338 / 336 (0.4 / 3.5 / 5.2) [M–HCl], 303 / 301 (26 / 78) [M–HCl–Cl], 255 (15) [M–CCl_3], 171 (100) [255 - C_5H_8O]. Anal. calcd. for $C_{17}H_{19}Cl_3N_2O$: C, 54.63; H, 5.13; N, 7.49; found: C, 54.40; H, 5.10; N, 7.67.

2-Hexanoyl-1-trichloromethyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**9**)

Starting from **2**·HCl (2.00 g, 6.14 mmol) and hexanoyl chloride (3.40 ml, 2.48 g, 24.6 mmol), the title compound (1.66 g, 4.28 mmol, 70% yield) was obtained as colorless crystals: mp 159 °C. IR (KBr, cm^{-1}) 3290 (indole NH), 3040, 3020, 2940, 2910, 2830 (CH), 1635 (C=O), 1445, 1400 (CH), 820, 805 (CCl); ^{1}H NMR ($CDCl_3$): δ 0.92 (m_c, 3 H, 6′-CH_3), 1.37 (sext, 4 H, 5- and 4′-CH_2), 1.69–1.80 (m, 2 H, 3′-CH_2), 2.40–2.68 (m, 2 H, 2′-CH_2), 2.84–2.95 (m, 2 H, 4-H), 4.13–4.18 (m, 2 H, 3-H), 6.75 (s, 1 H, 1-H), 7.11–7.17 (m, 1 H, 6-H or 7-H), 7.22–7.28 (m, 1 H, 6-H or 7-H, overlay by solvent), 7.39–7.42 (m, 1 H, 5-H or 8-H), 7.51–7.54 (m, 1 H, 5-H or 8-H), 8.49 (br. s, 1 H, indole NH); ^{13}C NMR (C_3D_6O): δ 14.16 (C-6′), 22.18 (C-4), 23.03 (C-5′), 25.48 (C-4′), 32.09 (C-3′), 33.82 (C-2′), 40.68 (C-3), 62.53 (C-1), 102.6 (CCl_3), 107.9, 112.4, 119.1, 120.1, 123.4, 126.7, 127.1, 137.6 (C=O); MS (EI, 70eV) *m/z* (rel int) 388 / 386 (0.1 / 0.1) $[M]^{+\cdot}$, 354 / 352 / 350 (0.7 / 3.6 / 5.7) [M–HCl], 317 / 315 (30 / 94) [M–HCl–Cl], 269 (14) [M–CCl_3], 171 (100) [269 - $C_6H_{10}O$]. Anal. calcd. for $C_{18}H_{21}Cl_3N_2O$: C, 55.75; H, 5.47; N, 7.23; found: C, 55.84; H, 5.47; N, 7.35.

1-Methyl-2-trifluoroacetyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**11**)

Starting from **10** (500 mg, 2.68 mmol) and trifluoroacetic anhydride (0.95 ml, 1.41 g, 6.70 mmol), the title compound (387 mg, 1.37 mmol, 51% yield) was obtained as pale yellow crystals: mp 208 °C (dec). IR (KBr, cm^{-1}) 3310 (indole NH), 1665 (C=O), 1190, 1170, 1140 (CN, CF); ^{1}H NMR (C_3D_6O): occurrence of two distinct rotational isomers A and B, ratio 8:1. – Isomer A: δ 1.57 (d, 3 H, CH_3,), 2.78–3.01 (m, 2 H, 4-H, overlay by isomer B), 3.50–3.63 (m, 1 H, 3-H, overlay by isomer B), 4.21–4.28 (m, 1H, 3-H), 5.69 (q, 1 H, 1-H), 7.10- 7.25 (m, 2 H, 6-H and 7-H, overly by isomer B), 7.32–7.37 (m, 1 H, 5-H or 8-H, overlay by isomer B), 7.46–7.52 (m, 1 H, 5-H or 8-H, overlay by isomer B), 8.00 (s, 1 H, indole NH). Isomer B: δ 1.66 (d, 3 H, CH_3,), 2.78–3.01 (m, 1 H, 4-H, overlay by isomer A), 3.23–3.34 (m, 1 H, 4-H), 3.50–3.63 (m, 1 H, 3-H, overlay by isomer A), 4.80–4.87 (m, 1 H, 3-H), 5.24 (q, 1 H, 1-H), 7.10- 7.25 (m, 2 H, 6-H and 7-H, overly by isomer A), 7.32–7.37 (m, 1 H, 5-H or 8-H, overlay by isomer A), 7.46–7.52 (m, 1 H, 5-H or 8-H, overlay by isomer A), 7.87 (s, 1 H, indole NH); ^{13}C NMR (C_3D_6O): δ 18.7 (CH_3, isomer A), 21.06 (CH_3, isomer B), 21.27 (C-4, isomer B), 22.63 (C-4, isomer A), 38.18 (C-3, isomer B), 40.84 (CF_3, 2.86 Hz, 3.81 Hz), 40.93 (C-3, isomer A), 44.15 (C-1, isomer B), 48.18 (C-1, isomer A), 107.3, 111.8, 118.7, 119.8, 122.3, 127.3, 134.4, 137.4; MS (EI, 70eV) *m/z* (rel int) 282 (52) $[M]^{+\cdot}$, 267 (100) [M–CH_3], 169 (27) [267 - C_2HF_3O]. Anal. calcd. for $C_{14}H_{13}F_3N_2O$: C, 59.57; H, 4.64; N, 9.92; found: C, 59.53; H, 4.74; N, 9.72.

1-Methyl-2-trichloroacetyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole (**12**)

Starting from **10** (50 mg, 0.27 mmol) and trichloroacetyl chloride (90 µl, 146 mg, 0.81 mmol), the title compound (73 mg, 0.22 mmol, 82% yield) was obtained as pale brown needles: mp 205 °C (dec). IR (KBr, cm^{-1}) 3370 (indole NH), 1650 (C=O), 1300, 805 (CCl); ^{1}H NMR (C_3D_6O): δ 1.59 (d, ^3J = 6.1 Hz, 3 H, CH_3), 2.86–2.92 (m, 1 H, 4-H_a), 2.93–3.11 (m, 1 H, 4-H_e), 3.61–3.72 (m, 1 H, 3-H_a), 4.79 (d, J = 12.8 Hz, 1 H, 3-H_e), 5.55–5.65 (m, 1 H, 1-H), 6.98–7.06 (m, 1 H, 6-H or 7-H), 7.07–7.14 (m, 1 H, 7-H or 6-H), 7.36 (d, $^3J_{8,7}$ = 7.6 Hz, 1 H, 8-H), 7.46 (d, $^3J_{5,6}$ = 7.6 Hz, 1 H, 5-H), 10.12 (br. s, 1 H, indole NH); ^{13}C NMR (C_3D_6O): δ 18.42 (CH_3), 21.87 (C-4), 43.22 (C-3), 49.91 (C-1), 94.11 (CCl_3), 111.7, 118.6, 119.7, 122.2, 127.4, 129.3, 134.9, 137.3, 159.9 (C=O); MS (EI, 70eV) *m/z* (rel int) 336 / 334 / 332 / 330 (0.2 / 1.8 / 5.1 / 5.2) $[M]^{+\cdot}$, 321 / 319 / 317 / 315 (0.2 / 1.6 / 3.4 / 3.6) [M–CH_3], 297 / 295 (64 / 100) [M–Cl], 259 (16) [M–HCl–Cl], 169 (12) [M–CH_3–C_2HCl_3O]. Anal. calcd. for $C_{14}H_{13}Cl_3N_2O$: C, 50.71; H, 3.95; N, 8.45; found: C, 50.90; H, 3.82; N, 8.17.

Single-crystal X-ray diffraction analysis of **4**

The crystal chosen for X-ray investigations was a colorless prism with the approximate dimensions 0.35 × 0.40 × 0.45 mm. Data were collected on a Siemens *P*4 diffractometer using graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å) in ω-scan mode in the range of 1.75° < θ < 27.5° (Siemens, 1996). $C_{14}H_{13}Cl_3N_2O$ (331.63 g mol^{-1}) crystallizes in the orthorhombic system, space group *Pbca*, with a = 14.450 (1), b = 12.309 (9), c = 16.544 (1) Å, V = 2942.5 (4) Å^3, Z = 8, μ(Mo-$K\alpha$) = 0.62 mm^{-1}, and D_{calcd} = 1.496 g cm^{-3}. Unit cell parameters were determined by least-squares refinement using 61 centered reflections within 7.3° < θ < 17.5°. A total of 4672 reflections were collected in ω-scan mode to $2\theta_{max}$ = 55° (h : -1 → 16, k : -18 → 1, l : -21 → 1) of which 3347 were unique with $I > 2\sigma$. The structure was solved by direct phase determination and refined by full-matrix anisotropic least-squares with the aid of the program SHELXTL-PLUS (Sheldrick, 1990). In refinements, weights were used according to the scheme $w = 1/[\sigma^2(F_o)]$. The refinement converged to the final agreement factors R = 0.065, and R_w = 0.062, for 181 parameters and 2390 observed reflections with $F > 3\sigma(F)$; data-to-parameter ratio being 13.20. The electron density of the largest difference peak was found to be 0.43 eÅ^{-3}, while that of the largest difference hole was 0.58 eÅ^{-3}. 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. In the crystals of **4**, the molecules are connected by intermolecular hydrogen bonds H(12)···O(15) [2.07 Å] to form a zigzag chain parallel to [010]. Atomic coordinates and further crystallographic details have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2, 1EZ, UK [fax: (+Int) 44–1223 336 033; E-mail: deposit@ccdc.com.ac.uk]. A complete listing of the atomic coordinates for **4** can be obtained free of charge, on request, on quoting the depository number CCDC-135165, the names of the authors and the journal citation. Software used to prepare material for publication: SCHAKAL 88 (Keller, 1990).

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 delivery of the mobile phase (cyclohexane), and pump B for 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 tetrahydro-β-carboline tested were prepared in the mobile phase and introduced onto the chromatograph *via* a 0.5-ml sample loop (Merck, Darmstadt, Germany). The tetrahydro-β-carbolines 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). Employing the highly lipophilic anthracene as a non-retained compound for assessing the column dead time (t_0), measurements were performed using operation conditions as described elsewhere (Slacanin *et al.*, 1989; El Tayar *et al.*, 1989; Vallat *et al.*, 1990), but modified in the following manner: The rotating coil was first filled with cyclohexane, and the desired volume of the stationary phase was then added to the coil 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 2.15 ml/min. The speed of rotation was set to 900 rpm.

Inhibition of mitochondrial respiration enzymes

Rat brain homogenates and submitochondrial particles (SMPs) from isolated rat liver mitochondria were prepared as described previously (Janetzky *et al.*, 1995). 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. The IC_{50} resp. IC_{100} values, defined as the concentrations required to inhibit NADH-ubiquinone reductase activity (complex I; EC 1.6.99.3) by 50% resp. 100% were determined after 5 min of preincubation with

the inhibitors. The toxins were dissolved in methanol or dimethylsulfoxide. The total volume of the organic solvent did not exceed 5% (v/v). At this concentration, methanol or dimethylsulfoxide did not significantly affect the enzymes tested.

As described earlier (Janetzky *et al.*, 1995), TaClo (**2**) is capable to inhibit malate/glutamate-induced oxygen consumption in intact mitochondria at concentrations equal to those needed for inhibition of complex I in SMP (for comparison: MPP^+ inhibits the respiratory chain at concentrations of 50–100 μM, and is accumulated a hundredfold in intact mitochondria). The potency of the TaClo-related analogs **3**–**9** and **13**–**16** to affect intact mitochondria has not been examined so far.

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