ORIGINAL PAPER

Benzyl- and dibenzyl tetrahydropyridinylidene ammonium salts with antiplasmodial and antitrypanosomal activity

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Abstract
Several 1-benzyl and 1,3-dibenzyl derivatives of tetrahydropyridinylidene salts with differing electron withdrawing substituents at the aromatic residues have been prepared. In addition, the amine moiety in position 4 was varied. The new compounds were investigated for their antiplasmodial and antitrypanosomal activities as well as for their cytotoxicity. They were characterized using FT-IR, HRMS and NMR spectroscopy. Structure–activity relationships including reported compounds are discussed.

Graphical abstract
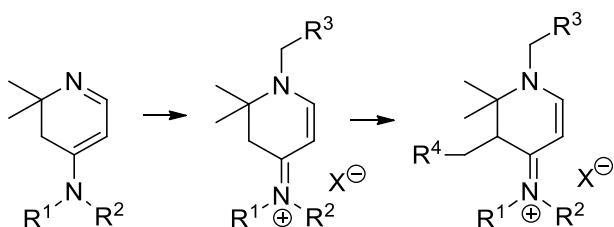
Introduction

Neglected Tropical Diseases (NTDs) and infectious illnesses, such as malaria, tuberculosis and Zika fever, represent a major public health concern in many countries and regions worldwide, especially in developing ones [1]. Most of the drugs available for therapy are toxic and have considerable adverse effects. A considerable number is obsolete, especially with respect to resistance.

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Trypanosoma brucei is one of the protozoan parasites that penetrates the blood–brain barrier causing injury associated with toxic effects of parasite-derived molecules or with immune response against infection. Other protozoan parasites that can cause pathology in the brain tropism include Toxoplasma, Plasmodium, Amoeba and, eventually, other Trypanosomatids such as *T. cruzi* and Leishmania. Together, these parasites affect billions of people worldwide and are responsible for more than 500,000 deaths annually [2]. New drugs against these parasitic protozoa are urgently needed to counteract drug resistance, toxicity and the high cost of commercially available drugs [3].

Recently, we reported about the synthesis of 1-benzyl [4, 5] and 1,3-dibenzyl derivatives of tetrahydropyridinylidene salts (THPS) [6]. Their activities against *Trypanosoma brucei rhodesiense* (*T.b.r.*), the protozoan pathogen of the East African form of sleeping sickness were investigated. Moreover, their activities against the sensitive NF54 strain and the multiresistant K_1 strain of *Plasmodium falciparum*

Scheme 1

2a-2c: R^3 = phenyl
3a-3c: R^3 = 4-CN-phenyl
4a-4c: R^3 = 4-NO$_2$-phenyl
5a-5c: R^3 = 4-Cl-phenyl

a: $R^1 + R^2$ = -(CH$_2$)$_4$-
b: $R^1 + R^2$ = -(CH$_2$)$_5$-
c: $R^1 + R^2$ = -(CH$_2$)$_6$-

9a-9c: R^3 = phenyl
10a, 10b: R^3 = 4-Cl-phenyl
11a: R^3 = 4-NO$_2$-phenyl
12a: R^3 = 4-CN-phenyl

6a: R^3 = phenyl, R^4 = 4-Cl-phenyl
7a-7c: R^3 = phenyl, R^4 = 4-CN-phenyl
8a-8c: R^3 = phenyl, R^4 = 4-NO$_2$-phenyl

(*P.falc.*) were determined [6]. The most promising of these compounds were also investigated for their in vivo activity against *Plasmodium berghei* in a mouse model [4].

Due to the fact that compounds with electron withdrawing groups at the aromatic moiety show better and more selective activities, we introduced such groups in a series of new benzyl- and dibenzyl-THPS in order to reveal structure–activity relationships and to optimize the compounds regarding their activity and cytotoxicity.

Results and discussion

Chemistry

The synthesis of the benzyl-THPS **2–5** starts from bases **1a–1c** [5, 7] by alkylation of the nitrogen atom of the dihydropyridine ring with benzyl halides as described earlier [4, 5]. The obtained compounds **2–5** have differing substitution in ring positions 4 of the piperidine as well as of the phenyl ring. Particularly THPS with electron-withdrawing substituents showed promising activity against protozoan parasites whereas electron-donating substituents like 4-methoxy or 4-alkyl showed moderate potency. Bigger alkyl groups in position 4 causes enhanced cytotoxicity and 3,4,5-trimethoxy compounds have low activity [5, 6].

Therefore, we prepared further analogues with cyano and nitro groups. In addition, the azepane moiety was chosen as a slightly more lipophilic amine component. Compounds **6–8** with an additional benzyl substituent in ring position 3 were afforded by reaction of benzyl-THPS **2–5** with benzyl halides in the presence of potassium carbonate. Analogues **9–12** with identical substitution in ring positions 1 and 3 were obtained by a one pot reaction of **1** with benzyl halides in the presence of potassium carbonate (Scheme 1).

The alkylation at the ring nitrogen in compounds **1** follows a S$_N$2 mechanism. The electron-pair of the ring nitrogen attacks the carbon of the aryl–alkyl halide and leads to **13**, the typical transition state. Detachment of the halide ion causes a migration of double bonds giving the *N*-benzyl-THPS **2–5** (Scheme 2).

The formation of the 1,3-dibenzyl compounds **6–8** starts with a proton abstraction in position 3 of compounds **2–5** to intermediate **14** using potassium carbonate as base. With the aryl–alkyl halide a S$_N$2-type transition state **15** is supposed. By the leaving of the halide ion, 1,3-dibenzyl-THPS **6–8** are formed (Scheme 3).

Scheme 2
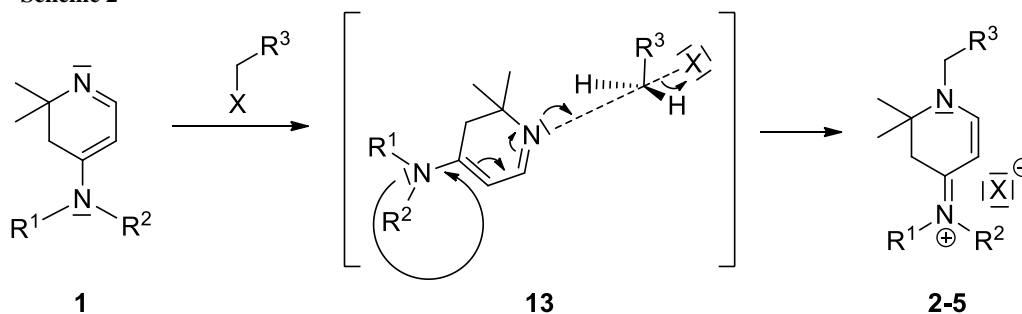
Scheme 3
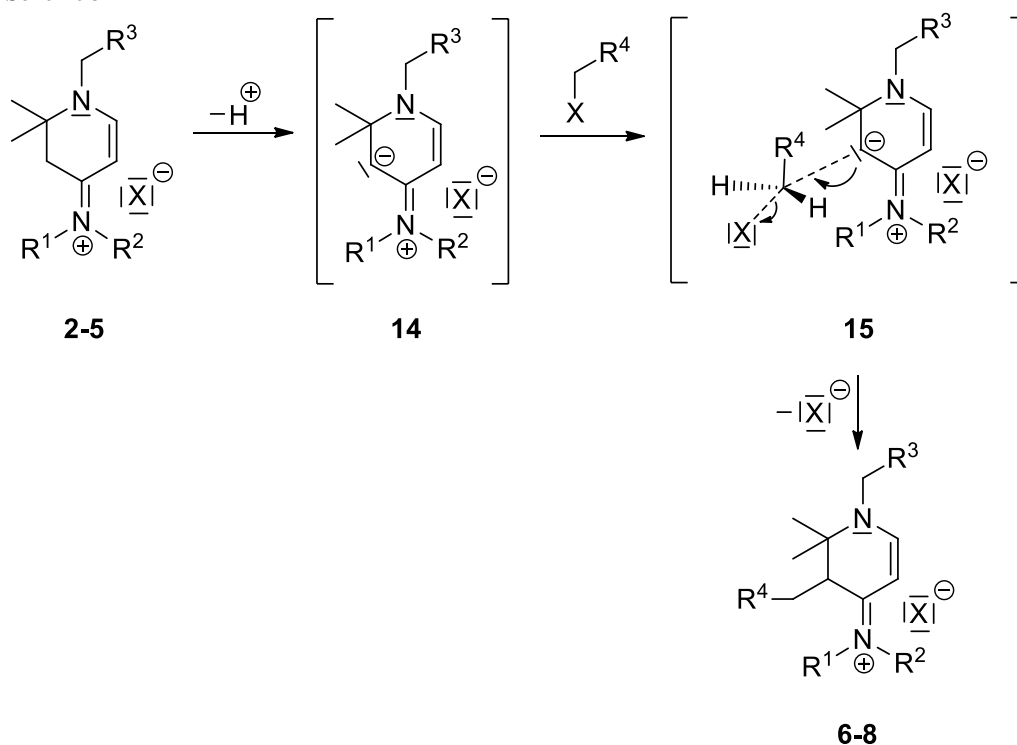
Confirmation of structures

The formation of compounds **2–5** was verified by NMR spectroscopy. The successful alkylation was confirmed by the appearance of resonances of additional protons of the benzyl moiety between 4.77 and 4.95 ppm in ^{1}H NMR spectra. For those protons long range couplings to C-2 and C-6 of the dihydropyridine ring were observed in HMBC spectra to establish connectivity. The additional proton signals of the aromatic protons of the benzyl residues are observed too in ^{1}H NMR spectra. In ^{13}C NMR spectra the signals for the methylene group of the benzyl residues appear at around 53 ppm. The additional signals for the aromatic carbons were detected as well. The results of the HRMS measurements confirmed the proposed structures too. Finally, the structures of compounds **2–5** are also established by a single

X-ray crystal analysis of a reported compound prepared in the same manner [4].

The methylene protons of the additional benzyl group of compounds **6–12** appear as two doubledoublets at around 2.5 ppm ($J = 13$ and 10 Hz) and around 3 ppm ($J = 13$ and 5 Hz). The coupling with the smaller coupling constant is due to the interaction with the remaining proton in position 3, respectively. Furthermore, additional cross-peaks to H-3 in the H,H-COSY spectra, as well as long range couplings to C-4 in the HMBC spectra were observed to verify the position of the substitution. In ^{13}C NMR spectra the signals for the methylene group attached to position 3 appear at around 35 ppm. The signal for C-3 shifted from around 39–45 ppm due to substitution and was detected in DEPT spectra as CH group instead of the former CH_2 group. HRMS measurements confirmed the proposed structures of **6–12** as well.

From a similar reported compound prepared by the same way, a single X-ray structure analysis is described [6].

Antiprotozoal activities

All new THPS were investigated for their activity against chloroquine sensitive *P.falc.* NF54 and *T.b.r.* as well as for their cytotoxicity against L-6 cells using microplate assays. The results are listed in Table 1. For comparative reasons known compounds were included. The most promising compounds with high antiplasmodial activity and high selectivity were additionally tested against *P.falc.* K$_1$.

The majority of compounds **2**–**12** showed weak antitrypanosomal activity (IC_{50} = 4.42–156 µM) and low selectivity (SI = 0.20–37.8). Moderate activity against *T.b.r.* (IC_{50} = 0.51–1.65 µM) was observed for the new 4-nitrobenzyl compounds **8b** and **8c**. In comparison to their more active bis(4-chlorobenzyl) analogues **10a** and **10b** ($IC_{50 L6}$ < 13 µM) they were less toxic (**8b**, **8c**: $IC_{50 L6}$ > 96 µM) and showed improved selectivity (SI = 122–190).

The new monobenzyl substituted compounds **2**–**5** exhibited moderate to good antiplasmodial activity (IC_{50} = 0.019–0.51 µM) against *Plasmodium falciparum* NF54. Their calculated selectivities were eminent

Table 1 Antiprotozoal and cytotoxic activities of compounds **2**–**12** (IC_{50} values in µM)

Cpd	IC_{50}[a]	L-6 cells IC_{50}[a]	P. falc. NF54 SI_{PN}[b]	P. falc. K$_1$ IC_{50}[a]	SI_{PK}[c]	T. b. r IC_{50}[a]	SI_T[d]
2a	12.3	0.13	94.8	–	–	61.6	0.20
2b	140	0.27	517	–	–	1.06	132
2c	193	0.51	378	–	–	13.2	14.6
3a	>267	0.029	9207	0.051	5235	156	1.71
3b	>258	0.25	1032			105	2.46
3c	>249	0.109	2284	–	–	49.8	5.00
4a	>286	0.10	2860	–	–	79.8	3.58
4b	177	0.12	1475	–	–	15.5	11.4
4c	>237	0.085	2788	–	–	8.05	29.4
5a	20.1	0.027	744	–	–	19.7	1.02
5b	116	0.045	2578	–	–	4.42	26.2
5c	1.48	0.019	77.9	–	–	1.79	0.83
6a	89.5	0.017	5265	–	–	0.50	179
7a	>215	0.017	12,647	0.12	1792	11.0	19.5
7b	>209	0.0021	99,524	0.031	6742	2.51	83.3
7c	131	0.0122	10,738	–	–	1.62	80.9
8a	206	0.043	4791	–	–	5.45	37.8
8b	>201	0.010	20,100	0.006	33,500	1.65	122
8c	96.9	0.0059	16,424	–	–	0.51	190
9a	48.8	0.032	1525	0.055	887	0.13	375
9b	24.6	0.015	1640	0.024	1025	0.044	559
9c	7.4	0.0043	1721	–	–	0.13	56.9
10a	12.6	0.0014	9000	0.0016	7875	0.17	74.1
10b	11.1	0.0038	2921	0.00038	29,211	0.14	79.3
11a	>189	0.18	1050			30.1	6.28
12a	>204	0.97	210	–	–	51.7	3.95
Mel	7.78	–	–	–	–	0.0039	1995
Art	451	–	–	0.0034	132,647		
CQ	117	0.007	16,714				
P	0.012						

Mel melarsoprol, *Art* artemisinin, *CQ* chloroquine diphosphate, *P* podophyllotoxin

[a]Values represent the average of four determinations (two determinations of two independent experiments) indicated in µM

[b]Selectivity index for *P. falciparum* NF54 (SI_{PN}), expressed as ratio [IC_{50}(L6)/IC_{50}(*P. falciparum* NF54)]

[c]Selectivity index for *P. falciparum* K$_1$ (SI_{PK}), expressed as ratio [IC_{50}(L6)/IC_{50}(*P. falciparum* K$_1$)]

[d]Selectivity index for *T. b. rhodesiense* (SI_T), expressed as ratio [IC_{50}(L6)/IC_{50}(*T. b. rhodesiense*)]

($SI = 378$–9207) except for compound **5c** ($SI = 77.9$). Compounds **6**–**10** of the bisbenzyl series showed high activity ($IC_{50} = 0.0014$–$0.043\ \mu M$) and outstanding selectivity ($SI = 1525$–$99{,}524$). Only the bis(4-nitrobenzyl) and the bis(4-cyanobenzyl) derivatives **11a** and **12a** were less active ($IC_{50} = 0.18$–$0.97\ \mu M$) and less selective ($SI = 210$–1050).

A selection of promising compounds was also tested against the multiresistant K$_1$ strain of *P.falc.*. The activities of compounds **3a**, **7a**, **7b**, **9a** and **9b** ($IC_{50} = 0.024$–$0.12\ \mu M$) were decreased in comparison to their activities against *Plasmodium falciparum* NF54 ($IC_{50} = 0.0021$–$0.032\ \mu M$) as expected. Therefore, compound **7b**, which was the most selective against the chloroquine sensitive NF54 strain ($SI = 99{,}524$), possessed still high but reduced selectivity against the K1 strain ($SI = 6742$). Interestingly, the activities of the 3-(4-nitrobenzyl) derivative **8b** and its bis(4-chlorobenzyl) analogues **10a** and **10b** showed similar or increased activity against the multiresistant strain. Compounds **8b** and **10a** exhibited activity in the low nanomolar region ($IC_{50} = 0.0016$–$0.006\ \mu M$), whereas **10b** was even active in subnanomolar concentration ($IC_{50} = 0.00038\ \mu M$). The selectivities of compounds **8b** and **10b** against this strain are excellent ($SI = 29{,}211$–$33{,}500$).

Structure–activity relationships

The above stated observation, that the presence of electron withdrawing substituents at the aromatic moiety increase the antiplasmodial activity was confirmed for compounds with only one benzyl residue attached to the ring nitrogen. Furthermore, due to the selection of 4-nitro and 4-cyano substituents, the selectivity was increased significantly due to the much lower cytotoxicity compared with 4-chloro substituted compounds. For the bis-benzyl substituted compounds we observed that the substitution pattern of 4-nitro and 4-cyano substituted benzyl residues in position 3 and at the same time unsubstituted benzyl residues at the ring nitrogen are advantageous for both, antiplasmodial activity and selectivity of compounds. As a result, compounds with outstanding activity and selectivity were yielded. The introduction of 4-nitro and 4-cyano substituted benzyl residues to both positions resulted in lower potent compounds.

The insertion of the bigger azepane ring as amino moiety in position 4 was in general advantageous, often the compounds with this substitution were the most active within their series.

Conclusion

To continue our studies about the antiprotozoal activities of tetrahydropyridinylidene ammonium salts, we prepared 1-substituted benzyl and 1,3-disubstituted dibenzyl derivatives with electron withdrawing substituents at the aromatic moieties, since such substituents were identified to be advantageous for antiplasmodial action. 4-Cyano and 4-nitro compounds show increased antiplasmodial potency and raised selectivity due to less cytotoxicity compared to 4-chloro compounds. In addition to that, the larger azepane ring was introduced as an amino substituent in ring position 4 giving highly active compounds. The most promising of the new compounds is the 1-benzyl-3-(4-nitrobenzyl) derivative **8b** showing low cytotoxicity and antiplasmodial activity against a sensitive and a multiresistant strain of *Plasmodium falciparum* in low nanomolar concentration. The main goal, to improve the biological activity and to decrease cytotoxicity was reached. Physicochemical properties where calculated and a correlation between biological activities and lipophilicity of compounds was detected. Further investigations should provide insight into structure–activity relationships of these compounds and varying substitution pattern on the aromatic moieties.

Experimental

Melting points were obtained on a digital melting point apparatus Electrothermal IA 9200. IR spectra: Bruker Alpha Platinum ATR FT-IR spectrometer (KBr discs). NMR spectra: Bruker Ascend 400, 5 mm tubes, spectra were acquired in CDCl$_3$ containing 0.03% TMS. Chemical shifts were recorded in parts per million (ppm), for ^{1}H spectra TMS (0.00 ppm) was used as internal standard and for ^{13}C spectra the central peak of the CDCl$_3$ signal was used as the internal reference (77.0 ppm). Some spectra were acquired in DMSO-d_6. In this case the central peaks of the DMSO-d_5 signal at 2.49 ppm in ^{1}H spectra and at 39.7 ppm in ^{13}C spectra served as internal reference. Abbreviations: aromatic H, ArH; aromatic C, ArC, quaternary aromatic C, ArC$_q$. Signal multiplicities are abbreviated as follows: s, singlet; d, doublet; dd, doubledoublet; ddd, doubledoubledoublet; dt, doubletriplet; t, triplet; m, multiplet; br, broad. Coupling constants (J) are reported in Hertz (Hz). ^{1}H and ^{13}C resonances were assigned using ^{1}H,^{1}H- and ^{1}H,^{13}C-correlation spectra. ^{1}H and ^{13}C resonances are numbered as given in the formulae. HR-MS: Micromass tofspec 3E spectrometer (MALDI), GCT-Premier, Waters (EI, 70 eV), Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer, Thermo Fisher Scientific (HESI, 3.5 kV). Materials: column chromatography (CC): silica gel 60 (Merck 70–230 mesh, pore-diameter 0.6 nm), aluminium oxide (Alox) basic (Fluka for chromatography, 0.05–0.15 mm, Brockmann activity I, basic); Alox neutral 90 (Merck, 0.063–0.2 mm, activity I, neutral); thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F$_{254}$ 0.2 mm, 200 × 200 mm); TLC plates (Merck, Alox 60

F$_{254}$ neutral, 200 × 200 mm); the substances were detected in UV light at 254 nm. If no stationary phase is mentioned (CC and TLC) the separation took place using silica gel. The preparation of benzyl compounds **2a**, **2b**, **4a**, **4b**, **5a**, **5b** is reported [4, 5] as well as the synthesis of dibenzyl compounds **6a**, **7a**, **8a**, **9a**, **9b**, **10a** and **10b** [6].

N-(1-Benzyl-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)azepan-1-ium bromide (2c, C$_{20}$H$_{29}$BrN$_2$)

A solution of 1.159 g of **1c** (5.62 mmol) and 1.604 g of benzyl bromide (9.38 mmol) in 25 cm^3 of CHCl$_3$ was stirred at r.t. for 20 h. A part of the solvent was evaporated in vacuo and cooled with an ice bath. Ethyl acetate was added until crystallisation seemed to be complete. The solid was sucked off and dried giving 1.540 g (73%) of **1c** as bright yellow crystals. R_f = 0.31 (CH$_2$Cl$_2$:MeOH = 9:1); m.p.: 180 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.22 (s, 6H, 2CH$_3$), 1.51 (br, s, 4H, H-3′), 1.66–1.76 (m, 4H, H-2′), 2.97 (s, 2H, H-3), 3.73 (td, J = 5.2, 1.8 Hz, 4H, H-1′), 4.77 (s, 2H, ArCH$_2$), 5.43 (d, J = 7.0 Hz, 1H, H-5), 7.30–7.43 (m, 5H, ArH), 7.80 (d, J = 7.0 Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.24 (2CH$_3$), 25.34, 25.45, 25.68 (C-2′, C-3′), 27.98 (C-2′), 38.55 (C-3), 51.34, 51.66 (C-1′), 53.58 (ArCH$_2$), 57.57 (C-2), 87.58 (C-5), 127.58, 127.99, 128.94 (ArC), 137.40 (ArC$_q$), 157.51 (C-6), 164.74 (C-4) ppm; IR (KBr): $\overline{V}$ = 2924, 1558, 1505, 1446, 1397, 1373, 1351, 1231, 1106, 722 cm^{-1}; HRMS (EI$^+$): *m/z* calcd. for C$_{20}$H$_{28}$N$_2$ ([M-HBr]$^+$) 296.2253, found 296.2255.

N-[1-(4-Cyanobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]pyrrolidin-1-ium bromide (3a, C$_{19}$H$_{24}$BrN$_3$)

A solution of 732 mg of **1a** (4.11 mmol) and 1.382 g of 4-bromomethyl benzonitrile (6.98 mmol) in 20 cm^3 of CHCl$_3$ was stirred at r.t. for 4 d. Then ethyl acetate was added with cooling on an ice bath until precipitation seemed complete. The solid was sucked off and dried giving 1.521 g (99%) of **3a** as yellowish crystals. R_f = 0.20 (CH$_2$Cl$_2$:MeOH = 8:1); m.p.: 172 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.21 (s, 6H, 2CH$_3$), 1.93–2.00 (m, 4H, 2CH$_2$), 2.95 (s, 2H, H-3), 3.55 (t, J = 6.2 Hz, 2H, NCH$_2$), 3.67 (t, J = 6.2 Hz, 2H, NCH$_2$), 4.85 (s, 2H, ArCH$_2$), 5.30 (d, J = 7.0 Hz, 1H, H-5), 7.54 (d, J = 8.1 Hz, 2H, ArH), 7.78 (d, J = 7.0 Hz, 1H, H-6), 7.88 (d, J = 8.1 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.50 (2CH$_3$), 24.31, 24.54 (2CH$_2$), 40.30 (C-3), 49.80, 50.00 (2NCH$_2$), 53.10 (ArCH$_2$), 57.46 (C-2), 88.98 (C-5), 110.54 (ArC$_q$), 118.76 (CN), 128.13, 132.78 (ArC), 143.89 (ArC$_q$), 157.66 (C-6), 162.60 (C-4) ppm; IR (KBr): $\overline{V}$ = 2931, 2225, 1607, 1550, 1479, 1441, 1407, 1336, 1272, 1235, 1187, 1106, 999, 969, 860, 831, 766 cm^{-1}; HRMS (EI$^+$): *m/z* calcd. for C$_{19}$H$_{23}$N$_3$ ([M-HBr]$^+$) 293.1892, found 293.1886.

N-[1-(4-Cyanobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidin-1-iumbromide (3b, C$_{20}$H$_{26}$BrN$_3$)

A solution of 1.171 g of **1b** (6.09 mmol) and of 2.05 g 4-bromomethyl benzonitrile (10.4 mmol) in 30 cm^3 of CHCl$_3$ was stirred at r.t. for 5 d. The solvent was evaporated in vacuo and the residue dissolved in CHCl$_3$. The hot solution was treated with charcoal, filtered *and ethyl acetate was added dropwise until first turbidity appeared. Then it was stirred at r.t. until the precipitation seemed to be complete. The precipitate was sucked off and dried giving 1.75 g of **3b** (74%) as yellowish crystals. R_f = 0.22 (CH$_2$Cl$_2$:MeOH = 8:1); m.p.: 133 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.18 (s, 6H, 2CH$_3$), 1.58–1.73 (m, 6H, 3CH$_2$), 2.97 (s, 2H, H-3), 3.67–3.72 (m, 4H, 2NCH$_2$), 4.85 (s, 2H, ArCH$_2$), 5.58 (d, J = 7.0 Hz, 1H, H-5), 7.55 (d, J = 7.3 Hz, 2H, ArH), 7.78 (d, J = 6.2 Hz, 1H, H-6), 7.87 (d, J = 6.6 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.32 (CH$_2$, 2CH$_3$), 25.95, 26.91 (2CH$_2$), 38.62 (C-3), 49.34, 49.40 (2NCH$_2$), 52.99 (ArCH$_2$), 57.56 (C-2), 88.03 (C-5), 110.52 (ArC$_q$), 118.79 (CN), 128.22, 132.75 (ArC), 143.77 (ArC$_q$), 157.97 (C-6), 163.65 (C-4) ppm; IR (KBr): $\overline{V}$ = 2941, 2860, 2225, 1608, 1558, 1468, 1444, 1403, 1353, 1254, 1238, 1186, 1111, 1018, 951, 859, 736 cm^{-1}; HRMS (EI$^+$): *m/z* calcd. for C$_{20}$H$_{25}$N$_3$ ([M-HBr]$^+$) 307.2048, found 307.2054.

N-[1-(4-Cyanobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]azepan-1-ium bromide (3c, C$_{21}$H$_{28}$BrN$_3$)

A solution of 1.763 g of **1c** (8.54 mmol) and 2.798 g of 4-bromomethyl benzonitrile (14.27 mmol) in 45 cm^3 of CHCl$_3$ was stirred at r.t. for 7 d. A part of the solvent was evaporated in vacuo and ethyl acetate was added with stirring and cooling until crystallization seemed to be complete. The precipitate was sucked off and dried giving 3.092 g of **3c** (90%) as bright yellow crystals. R_f = 0.32 (CH$_2$Cl$_2$: MeOH = 10:1); m.p.: 135 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.20 (s, 6H, 2CH$_3$), 1.53 (br, s, 4H, H-3′), 1.64–1.79 (m, 4H, H-2′), 3.01 (s, 2H, H-3), 3.75 (t, J = 6.1 Hz, 4H, H-1 '), 4.89 (s, 2H, ArCH$_2$), 5.48 (d, J = 7.1 Hz, 1H, H-5), 7.57 (d, J = 8.1 Hz, 2H, ArH), 7.82 (d, J = 7.1 Hz, 1H, H-6), 7.88 (d, J = 8.1 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.17 (2CH$_3$), 25.38, 25.41, 25.64 (C-2′, C-3′), 27.88 (C-2′), 38.47 (C-3), 51.46, 51.79 (C-1′), 53.11 (ArCH$_2$), 57.70 (C-2), 88.04 (C-5), 110.61 (ArC$_q$), 118.80 (CN), 128.26, 132.80 (ArC), 143.66 (ArC$_q$), 157.99 (C-6), 165.11 (C-4) ppm; IR (KBr): $\overline{V}$ = 2929, 2227, 1608, 1557, 1401, 1350, 1234, 1184, 1107 cm^{-1}; HRMS (HESI): *m/z* calcd. for C$_{21}$H$_{28}$N$_3$ ([M-Br]$^+$) 322.2282, found 322.2274.

N-[2,2-Dimethyl-1-(4-nitrobenzyl)-1,2,3,4-tetrahydropyridin-4-ylidene]azepan-1-ium bromide (4c, C$_{20}$H$_{28}$BrN$_3$O$_2$)

A solution of 1.073 g of **1c** (5.2 mmol) and 1.876 g of 4-nitrobenzyl bromide (8.68 mmol) in 25 cm^3 of CHCl$_3$ was stirred at r.t. for 20 h. A part of the solvent was evaporated in

vacuo and ethyl acetate was added with stirring and cooling until crystallization was completed. The solid was sucked off giving 1.692 g of **4c** (77%) as a yellow powder which still contained $CHCl_3$ after rigorous drying. Therefore, it was dissolved in CH_2Cl_2 and the solvent was evaporated in vacuo. After drying in vacuo a yellow foam without any traces of $CHCl_3$ was yielded. $R_f = 0.35$ (CH_2Cl_2: MeOH = 9:1); ^1H NMR (DMSO-d_6, 400 MHz): δ = 1.21 (s, 6H, 2CH$_3$), 1.53 (br, s, 4H, H-3′), 1.62–1.82 (m, 4H, H-2′), 3.02 (s, 2H, H-3), 3.76 (t, J = 6.1 Hz, 4H, H-1′), 4.95 (s, 2H, ArCH$_2$), 5.50 (d, J = 7.1 Hz, 1H, H-5), 7.65 (d, J = 8.3 Hz, 2H, ArH), 7.86 (d, J = 7.1 Hz, 1H, H-6), 8.25 (d, J = 8.3 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.16 (2CH$_3$), 25.39, 25.41, 25.64 (C-2′, C-3′), 27.87 (C-2′), 38.49 (C-3), 51.49, 51.82 (C-1′), 52.93 (ArCH$_2$), 57.75 (C-2), 88.13 (C-5), 123.96, 128.54 (ArC), 145.81, 147.13 (ArC$_q$), 158.04 (C-6), 165.16 (C-4) ppm; IR (KBr): $\overline{V}$ = 2930, 1609, 1559, 1519, 1398, 1343, 1106 cm^{-1}; HRMS (HESI): m/z calcd. for $C_{20}H_{28}N_3O_2$ ([M-Br]$^+$) 342.2182, found 342.2171.

N-[1-(4-Chlorobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]azepan-1-ium chloride (5c, $C_{20}H_{28}Cl_2N_2$)

A solution of 200 mg of **1c** (0.96 mmol) and 310 mg of 4-chlorobenzyl bromide (1.93 mmol) in 1.5 cm^3 of $CHCl_3$ was stirred at r.t. for 3 d. The solvent was evaporated and the residue dissolved in hot ethanol, treated with charcoal and filtered. The solvent was removed in vacuo and the pure product precipitated from a mixture of $CHCl_3$ and ethyl acetate. It was sucked off and dried giving 320 mg of **5c** (91%) as grey precipitate. For analytical purposes it was dissolved in $CHCl_3$ and ethyl acetate was added. The product crystallized overnight in form of grey platelets. $R_f = 0.13$ (CH_2Cl_2:MeOH = 8:1); m.p.: 132 °C; ^1H NMR (CDCl$_3$, 400 MHz): δ = 1.21 (s, 6H, 2CH$_3$), 1.51 (br, s, 4H, H-3′), 1.67–1.74 (m, 4H, H-2′), 2.98 (s, 2H, H-3), 3.71–3.75 (m, 4H, H-1′), 4.79 (s, 2H, ArCH$_2$), 5.43 (d, J = 7.0 Hz, 1H, H-5), 7.40 (d, J = 8.4 Hz, 2H, ArH), 7.45 (d, J = 8.4 Hz, 2H, ArH), 7.88 (d, J = 7.0 Hz, 1H, H-6) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 23.21 (2CH$_3$), 25.34, 25.42, 25.65 (C-2′, C-3′), 27.92 (C-2′), 38.54 (C-3), 51.35, 51.67 (C-1′), 52.81 (ArCH$_2$), 57.61 (C-2), 87.72 (C-5), 128.80, 129.51 (ArC), 132.49, 136.63 (ArC$_q$), 157.70 (C-6), 164.82 (C-4) ppm; IR (KBr): $\overline{V}$ = 3426, 2926, 1557, 1445, 1401, 1237, 1178, 1106, 759 cm^{-1}; HRMS (EI$^+$): m/z calcd. for $C_{20}H_{27}ClN_2$ ([M-HCl]$^+$) 330.1863, found 330.1852.

(3*RS*)-(±)-*N*-[1-Benzyl-3-(4-cyanobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidin-1-ium bromide (7b, $C_{27}H_{32}BrN_3$)

A mixture of 1.007 g **2b** (2.77 mmol) and 654 mg of 4-bromomethyl benzonitrile (3.33 mmol) in 50 cm^3 of $CHCl_3$ was refluxed overnight in the presence of 3.2 g K_2CO_3 (23.2 mmol). Charcoal was added to the mixture and then it was heated until it boiled. It was filtered and the

solvent was evaporated in vacuo. The residue was dissolved in acetone and ethyl acetate was added until the mixture got turbid. Upon stirring at r.t. a precipitate was formed which was sucked off and was recrystallized from acetone, giving **7b** as beige crystals containing acetone. Therefore, it was dissolved in CH_2Cl_2 and the solvent was evaporated in vacuo. After drying in vacuo 243 mg (18%) of a solvent-free beige foam was yielded. $R_f = 0.36$ (CH_2Cl_2:MeOH = 8:1); ^1H NMR (DMSO-d_6, 400 MHz): δ = 1.06 (br, s, 4H, CH$_3$, CH$_2$), 1.16–1.32 (m, 1H, CH$_2$), 1.34–1.49 (m, 5H, CH$_3$, CH$_2$), 1.59–1.62 (m, 2H, CH$_2$), 2.36 (br, dt, J = 13.0, 2.7 Hz, 1H, NCH$_2$), 2.49–2.54 (m, 1H, ArCH$_2$CH), 3.04 (dd, J = 13.2, 5.1 Hz, 1H, ArCH$_2$CH), 3.09 (br, t, J = 10.3 Hz, 1H, NCH$_2$), 3.46–3.56 (m, 2H, H-3, NCH$_2$), 3.84 (br, d, J = 12.8 Hz, 1H, NCH$_2$), 4.72 (d, J = 15.4 Hz, 1H, ArCH$_2$N), 4.84 (d, J = 15.4 Hz, 1H, ArCH$_2$N), 5.54 (d, J = 6.6 Hz, 1H, H-5), 7.33–7.44 (m, 7H, ArH), 7.74 (d, J = 8.1 Hz, 2H, ArH), 7.87 (d, J = 7.0 Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.30, 22.13 (2CH$_3$), 22.83, 25.94, 27.11 (3CH$_2$), 34.71 (ArCH$_2$CH), 44.94 (C-3), 49.18, 49.51 (N(CH$_2$)$_2$), 53.86 (ArCH$_2$N), 60.77 (C-2), 86.60 (C-5), 109.89 (ArC$_q$), 118.97 (CN), 128.11, 128.15, 128.93, 130.82, 132.19 (ArC), 137.05, 143.35 (ArC$_q$), 156.14 (C-6), 167.12 (C-4) ppm; IR (KBr): $\overline{V}$ = 2950, 2222, 1604, 1557, 1479, 1453, 1397, 1349, 1298, 1231, 1162, 1097, 1017, 947, 829, 754, 719 cm^{-1}; HRMS (EI$^+$): m/z calcd. for $C_{27}H_{31}N_3$ ([M-HBr]$^+$) 397.2518, found 397.2535.

(3*RS*)-(±)-*N*-[1-Benzyl-3-(4-cyanobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]azepan-1-ium bromide (7c, $C_{28}H_{34}BrN_3$)

A mixture of 1 g of **2c** (2.65 mmol) and 623 mg of 4-bromomethyl benzonitrile (3.18 mmol) in 55 cm^3 of $CHCl_3$ was refluxed overnight in the presence of 3.392 g of K_2CO_3 (24.54 mmol). It was cooled down to r.t., then 55 cm^3 of $CHCl_3$ were added and it was treated with charcoal and filtered. The solvent was evaporated in vacuo giving a brown resin. It was dissolved in acetone. Upon addition of a few drops of ethyl acetate crystallization occurred giving brown crystals which were sucked off and dried. The crystals contained acetone. The solid was dissolved in CH_2Cl_2 and evaporated in vacuo giving 172 mg **7c** (13%) as brown foam. $R_f = 0.89$ (CH_2Cl_2:MeOH = 10:1); ^1H NMR (DMSO-d_6, 400 MHz): δ = 1.10 (s, 3H, CH$_3$), 1.17–1.62 (m, 7H, H-2′, H-3′), 1.41 (s, 3H, CH$_3$), 1.66–1.81 (m, 1H, H-2′), 2.22–2.46 (m, 1H, H-1′), 2.55 (dd, J = 13.1, 10.3 Hz, 1H, ArCH$_2$CH), 3.06 (dd, J = 12.9, 5.3 Hz, 1H, ArCH$_2$CH), 3.16 (ddd, J = 13.8, 10.2, 3.9 Hz, 1H, H-1′), 3.24–3.38 (m, 2H, H-1′, H-3), 3.78 (dt, J = 14.1, 4.3 Hz, 1H, H-1′), 4.72 (d, J = 15.5 Hz, 1H, ArCH$_2$N), 4.83 (d, J = 15.5 Hz, 1H, ArCH$_2$N), 5.44 (d, J = 6.9 Hz, 1H, H-5), 7.33–7.57 (m, 7H, ArH), 7.75 (d, J = 8.1 Hz, 2H, ArH), 7.83 (d, J = 6.9 Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.55, 22.18 (2CH$_3$), 24.73, 26.02, 29.13 (C-2′, C-3′), 34.82 (ArCH$_2$CH), 45.49

(C-3), 50.97, 51.25 (C-1′), 54.02 (ArCH$_2$N), 60.97 (C-2), 87.33 (C-5), 110.01 (ArC$_q$), 119.06 (CN), 128.18, 128.27, 129.04, 130.85, 132.15 (ArC), 136.99, 143.37 (ArC$_q$), 156.40 (C-6), 167.92 (C-4) ppm; IR (KBr): $\bar{V}$ = 2934, 2223, 1557, 1453, 1401, 1352, 1231, 1094, 763 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{28}$H$_{33}$N$_3$ ([M-HBr]$^+$) 411.2675, found 411.2689.

(3RS)-(±)-N-[1-Benzyl-2,2-dimethyl-3-(4-nitrobenzyl)-1,2,-3,4-tetrahydropyridin-4-ylidene]piperidin-1-ium bromide (8b, C$_{26}$H$_{32}$BrN$_3$O$_2$) A mixture of 1048 mg of **2b** (2.88 mmol) and 749 mg of 4-nitrobenzyl bromide (3.47 mmol) in 50 cm^3 of CHCl$_3$ was refluxed in the presence of 3.24 g of K$_2$CO$_3$ (23.54 mmol) overnight. It was treated with charcoal filtered and the solvent evaporated in vacuo. The residue was dissolved with acetone and ethyl acetate was added until the mixture got turbid. Upon stirring at r.t. a precipitate was formed which was sucked off and recrystallized from acetone, giving **8b** as beige crystals containing acetone. Therefore, the crystals were dissolved in CH$_2$Cl$_2$ and the solvent was evaporated to yield 282 mg of **8b** (20%) as beige foam. R_f = 0.24 (CH$_2$Cl$_2$:MeOH = 8:1); ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.07 (s, 3H, CH$_3$), 1.11–1.24 (m, 2H, CH$_2$), 1.36–1.42 (m, 2H, CH$_2$), 1.46 (s, 3H, CH$_3$), 1.50–1.61 (m, 2H, CH$_2$), 2.34 (ddd, J = 13.2, 10.2, 2.9 Hz, 1H, NCH$_2$), 2.57 (dd, J = 12.6, 11.0 Hz, 1H, ArCH$_2$CH), 3.05 (br, t, J = 10.3 Hz, 1H, NCH$_2$), 3.10 (dd, J = 12.6, 5.1 Hz, 1H, ArCH$_2$CH), 3.53–3.57 (m, 2H, H-3, NCH$_2$), 3.86–3.89 (m, 1H, NCH$_2$), 4.73 (d, J = 15.6 Hz, 1H, ArCH$_2$N), 4.85 (d, J = 15.6 Hz, 1H, ArCH$_2$N), 5.55 (d, J = 7.0 Hz, 1H, H-5), 7.32–7.45 (m, 7H, ArH), 7.88 (d, J = 7.0 Hz, 1H, H-6), 8.13 (d, J = 8.4 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.30, 22.12 (2CH$_3$), 22.79, 26.00, 27.31 (3CH$_2$), 34.41 (ArCH$_2$CH), 44.93 (C-3), 49.16, 49.56 (2NCH$_2$), 53.87 (ArCH$_2$N), 60.76 (C-2), 86.62 (C-5), 123.33, 128.12, 128.15, 128.93, 131.03 (ArC), 137.03, 145.57, 146.67 (ArC$_q$), 156.16 (C-6), 166.94 (C-4) ppm; IR (KBr): $\bar{V}$ = 2940, 1603, 1556, 1517, 1452, 1396, 1345, 1234, 1164, 1108, 1016, 859, 703 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{26}$H$_{31}$N$_3$O$_2$ ([M-HBr]$^+$) 417.2416, found 417.2428.

(3RS)-(±)-N-[1-Benzyl-2,2-dimethyl-3-(4-nitrobenzyl)-1,2,-3,4-tetrahydropyridin-4-ylidene]azepan-1-ium bromide (8c, C$_{27}$H$_{34}$BrN$_3$O$_2$) A mixture of 1.2 g of **2c** (3.2 mmol) and 824 mg of 4-nitrobenzyl bromide (3.8 mmol) in 66 cm^3 CHCl$_3$ was stirred for 60 d at r.t. in the presence of 4.095 g of K$_2$CO$_3$ (29.6 mmol). 66 cm^3 of CHCl$_3$ were added and it was treated with charcoal and filtered. The solvent was evaporated in vacuo and the residue was dissolved in MeOH, diluted with water and was put into a separatory funnel. The aqueous layer was extracted three times with ether. The combined ethereal layers were discarded and the aqueous layer was extracted five times with CH$_2$Cl$_2$. The organic layer

was treated with anhydrous Na$_2$SO$_4$ and filtered. The solvents were evaporated in vacuo and the residue crystallized from ethyl acetate giving a brown powder which was dissolved in CH$_2$Cl$_2$ and evaporated in vacuo giving 113 mg of **8c** (0.22 mmol, 7%) as a brown foam. R_f = 0.34 (CH$_2$Cl$_2$: MeOH = 10:1); ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.10 (s, 3H, CH$_3$), 1.20–1.79 (m, 8H, H-2′, H-3′), 1.43 (s, 3H, CH$_3$), 2.29–2.40 (m, 1H, H-1′), 2.56–2.67 (m, 1H, ArCH$_2$CH), 3.08–3.19 (m, 2H, H-1′, ArCH$_2$CH), 3.27–3.40 (m, 2H, H-1′, H-3), 3.69 – 3.82 (m, 1H, H-1′), 4.73 (d, J = 15.5 Hz, 1H, ArCH$_2$N), 4.83 (d, J = 15.4 Hz, 1H, ArCH$_2$N), 5.44 (d, J = 7.1 Hz, 1H, H-5), 7.31–7.47 (m, 7H, ArH), 7.85 (d, J = 6.9 Hz, 1H, H-6), 8.13 (d, J = 8.3 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.54, 22.14 (2CH$_3$), 24.68, 26.00, 29.18 (C-2′, C-3′), 34.50 (ArCH$_2$CH), 45.50 (C-3), 50.92, 51.25 (C-1′), 54.01 (ArCH$_2$N), 60.95 (C-2), 87.35 (C-5), 123.28, 128.18, 128.25, 129.01, 131.07 (ArC), 136.91, 145.57, 146.73 (ArC$_q$), 156.38 (C-6), 167.75 (C-4) ppm; IR (KBr): $\bar{V}$ = 2927, 1717, 1556, 1519, 1455, 1396, 1345, 1108 cm^{-1}; HRMS (HESI): m/z calcd. for C$_{27}$H$_{34}$N$_3$O$_2$ ([M-Br]$^+$) 432.2651, found 432.2639.

(3RS)-(±)-N-(1,3-Dibenzyl-2,2-dimethyl-1,2,3,4-tetrahydro-pyridin-4-ylidene)azepan-1-ium bromide (9c, C$_{27}$H$_{35}$BrN$_2$) A mixture of 1 g of **1c** (2.65 mmol) and of 544 mg benzyl bromide (3.18 mmol) in 55 cm^3 of CHCl$_3$ was refluxed overnight in the presence of 3.392 g of K$_2$CO$_3$ (24.54 mmol). It was cooled down to r.t. and 55 cm^3 of CHCl$_3$ were added and then treated with charcoal and filtered. The solvent was evaporated in vacuo giving a residue which was dissolved in acetone. Crystallization gave a brown powder which was sucked off. The solid was dissolved in CH$_2$Cl$_2$ and evaporated in vacuo giving 417 mg of **9c** (56%) as brownish foam. R_f = 0.93 (CH$_2$Cl$_2$: MeOH = 9:1); ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 1.24 (s, 3H, CH$_3$), 1.30–1.62 (m, 7H, H-2′, H-3′), 1.67 (s, 3H, CH$_3$), 1.80–1.91 (m, 1H, H-2′), 2.16 (ddd, J = 14.0, 8.9, 4.5 Hz, 1H, H-1′), 2.56 (dd, J = 12.8, 10.9 Hz, 1H, ArCH$_2$CH), 3.06 (ddd, J = 13.6, 9.3, 3.6 Hz, 1H, H-1′), 3.15 (dd, J = 12.8, 5.1 Hz, 1H, ArCH$_2$CH), 3.50 (dd, J = 10.9, 5.1 Hz, 1H, H-3), 3.64 (dt, J = 14.4, 5.1 Hz, 1H, H-1′), 3.73 (dt, J = 14.4, 5.0 Hz, 1H, H-1′), 4.72 (d, J = 15.2 Hz, 1H, ArCH$_2$N), 4.82 (d, J = 15.2 Hz, 1H, ArCH$_2$N), 5.45 (d, J = 6.9 Hz, 1H, H-5), 7.23–7.39 (m, 10H, ArH), 8.05 (d, J = 6.9 Hz, 1H, H-6) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 21.98, 22.80 (2CH$_3$), 25.10, 25.16, 26.48, 29.18 (C-2′, C-3′), 35.12 (ArCH$_2$CH), 47.11 (C-3), 51.22, 51.71 (C-1′), 54.79 (ArCH$_2$N), 61.22 (C-2), 87.52 (C-5), 127.09, 128.06, 128.28, 128.40, 128.98, 129.66 (ArC), 135.27, 136.43 (ArC$_q$), 155.87 (C-6), 168.65 (C-4) ppm; IR (KBr): $\bar{V}$ = 2926, 1555, 1403, 1351, 1242, 1153, 1096, 763 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{27}$H$_{34}$N$_2$ ([M-HBr]$^+$) 386.2722, found 386.2740.

(3RS)-(±)-N-[2,2-Dimethyl-1,3-bis(4-nitrobenzyl)-1,2,3,4-tetrahydropyridin-4-ylidene]pyrrolidin-1-ium bromide (11a, $C_{25}H_{29}BrN_4O_4$) To a solution of 1 g of **1a** (5.64 mmol) in 25 cm^3 of $CHCl_3$ 2.03 g of 4-nitrobenzyl bromide (9.4 mmol) were added. It was stirred at r.t. for two days and ethyl acetate was added to the already turbid solution. The formed precipitate was sucked off giving 1.98 g (89%) of the monosubstituted product **4a**. 950 mg of **4a** (2.41 mmol) and 627 mg of 4-nitrobenzyl bromide (2.9 mmol) in 40 cm^3 of $CHCl_3$ were refluxed overnight in the presence of 2.7 g of K_2CO_3 (19.5 mmol). Then 100 cm^3 of $CHCl_3$ were added and the mixture was treated with charcoal and filtered. The solvent was evaporated in vacuo and the residue was dissolved in acetone and ethyl acetate was added until the solution got turbid. Upon stirring on an ice bath, a precipitate was formed which was sucked off and washed with ethyl acetate. It was recrystallized three times from acetone giving bright brown needles containing acetone. They were sucked off and dissolved in CH_2Cl_2. The solvent was evaporated to yield 120 mg of **11a** (4%) as a brownish foam. $R_f = 0.13$ (CH_2Cl_2:MeOH = 8:1); ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 10.17$ (s, 3H, CH_3), 1.22–1.30 (m, 1H, CH_2), 1.34 (s, 3H, CH_3), 1.56–1.69 (m, 2H, CH_2), 1.82 (quin, $J = 6.1$ Hz, 1H, NCH_2), 2.26 (quin, $J = 6.5$ Hz, 1H, NCH_2), 2.69 (dd, $J = 12.1$, 10.6 Hz, 1H, ArCH_2CH), 3.20 (dd, $J = 12.8$, 5.1 Hz, 1H, ArCH_2CH), 3.33–3.43 (m, 2H, H-3, NCH_2), 3.50–3.62 (m, 2H, NCH_2), 4.90 (d, $J = 16.1$ Hz, 1H, ArCH$_2$N), 5.05 (d, $J = 16.5$ Hz, 1H, ArCH$_2$N), 5.34 (d, $J = 7.0$ Hz, 1H, H-5), 7.48 (d, $J = 8.4$ Hz, 2H, ArH), 7.76 (d, $J = 8.1$ Hz, 2H, ArH), 8.00 (d, $J = 7.0$ Hz, 1H, H-6), 8.14 (d, $J = 8.4$ Hz, 2H, ArH), 8.27 (d, $J = 8.4$ Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 21.12$, 22.35 (2CH_3), 23.81, 24.36 (2CH_2), 34.28 (ArCH$_2$CH), 47.49 (C-3), 50.01, 50.17 (2NCH_2), 53.20 (ArCH$_2$N), 61.32 (C-2), 88.43 (C-5), 123.40, 124.05, 129.19, 131.06 (ArC), 145.31, 145.95, 146.66, 147.26 (ArC$_q$), 156.62 (C-6), 166.06 (C-4) ppm; IR (KBr): $\bar{V} = 2940$, 1606, 1560, 1518, 1449, 1394, 1346, 1230, 1164, 1108, 859, 752 cm^{-1}; HRMS (EI$^+$): *m/z* calcd. for $C_{24}H_{25}N_4O_4$ ([M-HBr-CH$_3$]$^+$) 433.1876, found 433.1887.

(3RS)-(±)-N-[1,3-Bis(4-cyanobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]pyrrolidin-1-ium bromide (12a, $C_{27}H_{29}BrN_4$) A mixture of 1.615 g of **1a** (9.06 mmol) and 3.919 g of 4-bromomethyl benzonitrile (19.99 mmol) in 50 cm^3 of $CHCl_3$ was stirred for 6 d at r.t. in the presence of 4.673 g of K_2CO_3 (33.81 mmol). The reaction mixture was treated with charcoal, filtered and the solvent was evaporated in vacuo. The residue was dissolved in acetone and ethyl acetate was added until the first turbidity appeared. While stirring at r.t. and then on an ice-bath a precipitate was formed which was sucked of and recrystallized from acetone giving a mixture of bis- and monosubstituted products as white powder. In order to complete the

reaction, the mixture was dissolved in 35 cm^3 of $CHCl_3$ and refluxed overnight in the presence of 2.215 g of K_2CO_3 (16.03 mmol) and 996 mg 4-bromomethyl benzonitrile (5.08 mmol). Then 15 cm^3 of $CHCl_3$ were added and the mixture was treated with charcoal and filtered. The solvent was evaporated in vacuo and the residue was dissolved in acetone. Then ethyl acetate was added until the first turbidity appeared. While stirring at r.t. and then on an ice-bath a precipitate was formed which was sucked of, recrystallized from acetone and dried giving 0.817 g of **12a** (18%) as white powder containing acetone. Therefore, it was dissolved in $CHCl_3$ and the solvent was evaporated to yield a white foam. $R_f = 0.34$ (CH_2Cl_2:MeOH = 9: 1); ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.12$ (s, 3H, CH_3), 1.25 (quin, $J = 6.3$ Hz, 1H, CH_2), 1.29 (s, 3H, CH_3), 1.57–1.73 (m, 2H, CH_2), 1.78–1.86 (m, 1H, CH_2), 2.24 (quin, $J = 6.5$ Hz, 1H, NCH_2), 2.62 (dd, $J = 12.9$, 9.9 Hz, 1H, ArCH_2CH), 3.07 (dd, $J = 12.9$, 5.4 Hz, 1H, ArCH_2CH), 3.16 (dd, $J = 10.1$, 5.5 Hz, 1H, H-3), 3.28–3.36 (m, 1H, NCH_2), 3.47–3.55 (m, 2H, NCH_2), 4.79 (d, $J = 16.1$ Hz, 1H, ArCH$_2$N), 4.91 (d, $J = 16.1$ Hz, 1H, ArCH$_2$N), 5.30 (d, $J = 6.8$ Hz, 1H, H-5), 7.37 (d, $J = 8.0$ Hz, 2H, ArH), 7.64 (d, $J = 8.0$ Hz, 2H, ArH), 7.77 (d, $J = 8.0$ Hz, 2H, ArH), 7.87 (d, $J = 6.8$ Hz, 1H, H-6), 7.91 (d, $J = 8.0$ Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 21.14$, 22.36 (2CH_3), 23.84, 24.37 (2CH_2), 34.51 (ArCH$_2$CH), 47.54 (C-3), 49.96, 50.06 (2NCH_2), 53.49 (ArCH$_2$N), 61.24 (C-2), 88.34 (C-5), 109.92, 110.86 (ArC$_q$), 118.78, 118.89 (CN), 128.89, 130.78, 132.29, 132.91 (ArC), 143.21, 143.72 (ArC$_q$), 156.58 (C-6), 166.06 (C-4) ppm; IR (KBr): $\bar{V} = 2925$, 2227, 1608, 1558, 1448, 1395, 1333, 1230, 1163 cm^{-1}; HRMS (HESI): *m/z* calcd. for $C_{27}H_{29}N_4$ ([M-Br]$^+$) 409.2392, found 409.2380.

In vitro growth inhibition assay of *Plasmodium falciparum* NF54

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay [8, 9], using the drug sensitive NF54 strain (Schipol Airport, The Netherlands, [10]) and the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/cm^3 and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/dm^3), NaHCO$_3$ (2.1 g/dm^3), neomycin (100 U/cm^3), Albumax^R (5 g/dm^3) and washed human red cells A$^+$ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven threefold dilution steps covering a range from 100 to 0.002 μg/cm^3 were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, 93% N_2. After 48 h 50 mm^3 of ^{3}H-hypoxanthine (= 0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with

a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells were transferred onto a glass fibre filter and then washed with distilled water. The dried filters were inserted into a plastic foil with 10 cm³ of scintillation fluid and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression [11] using Microsoft Excel. Chloroquine was used as control.

In vitro growth inhibition assay of *Trypanosoma b. rhodesiense*

Minimum Essential Medium (50 mm³) supplemented according to [12] with 25 mM HEPES, 1 g/dm³ additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of eleven threefold dilution steps covering a range from 100 to 0.002 μg/cm³ were prepared. Then 4×10^3 bloodstream forms of *T. b. rhodesiense* (STIB 900) in 50 mm³ was added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 70 h. 10 mm³ Alamar Blue (resazurin, 12.5 mg in 100 cm³ double-distilled water) was then added to each well and incubation continued for a further 2–4 h [13]. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression [11] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA). Melarsoprol was used as control.

Cytotoxicity against L6-cells

Assays were performed in 96-well microtiter plates, each well containing 100 mm³ of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L-6 cells (a primary cell line derived from rat skeletal myoblasts) [14, 15]. Serial drug dilutions of eleven threefold dilution steps covering a range from 100 to 0.002 μg/cm³ were prepared. After 70 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 mm³ of Alamar Blue was then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression [11] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation,

Sunnyvale, CA, USA). Podophyllotoxin (Sigma P4405) was used as control.

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