ORIGINAL PAPER

Tetrahydropyridinylidene ammonium salts with arylalkyl and diarylalkyl substitution and their antiprotozoal activity
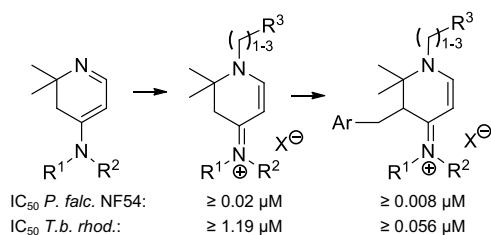
Werner Seebacher[1] · Michael Hoffelner[1] · Ferdinand Belaj[2] · Marcel Kaiser[3] · Pascal Mäser[3] · Robert Saf[4] · Eva-Maria Pferschy-Wenzig[1] · Robert Weis[1]

Abstract

Tetrahydropyridin-4-ylidene salts with benzyl and dibenzyl substitution showed good antiprotozoal activity. This paper reports the synthesis of analogues with longer side chains. They were investigated for their antiprotozoal activities as well as for their cytotoxicity using microplate assays. The most active compounds showed activity against *Trypanosoma brucei rhodesiense* in concentrations < 0.06 μM. A series of compounds was active against *Plasmodium falciparum* NF54 in low nanomolar concentration and exhibited outstanding selectivity. The influence of substitution pattern and chain length on the antiprotozoal potencies were analyzed and structure–activity relationships were given. New compounds were characterized by FT-IR, HRMS, and NMR spectroscopy.

Graphical abstract

| IC$_{50}$ *P. falc.* NF54: | ≥ 0.02 μM | ≥ 0.008 μM |
| IC$_{50}$ *T.b. rhod.*: | ≥ 1.19 μM | ≥ 0.056 μM |

Introduction

Human African trypanosomiasis (HAT), or sleeping sickness, is a severe disease affecting people in the poorest parts of Africa. It is usually fatal without treatment. Conventional treatments require days of intravenous infusion, but a recently developed drug, fexinidazole, is administered orally. Although fexinidazole cures some people, deaths from any cause and treatment failure rates are slightly higher than with conventional treatment. Adverse events were common in both groups [1]. Therefore, there is still need for new drugs with high antitrypanosomal activity and at the same time low toxicity and less side effects.

Globally, there were an estimated 241 million malaria cases in 2020 in 85 malaria endemic countries. These are 14 million cases more compared to 2019 [2]. While more than 3 billion people are at risk of malaria infection globally, antimalarial drugs are their main option for treatment. Antimalarial drug resistance keeps arising periodically and thus threatens the main line of malaria treatment, emphasizing the need to find new alternatives [3]. Since the

✉ Werner Seebacher
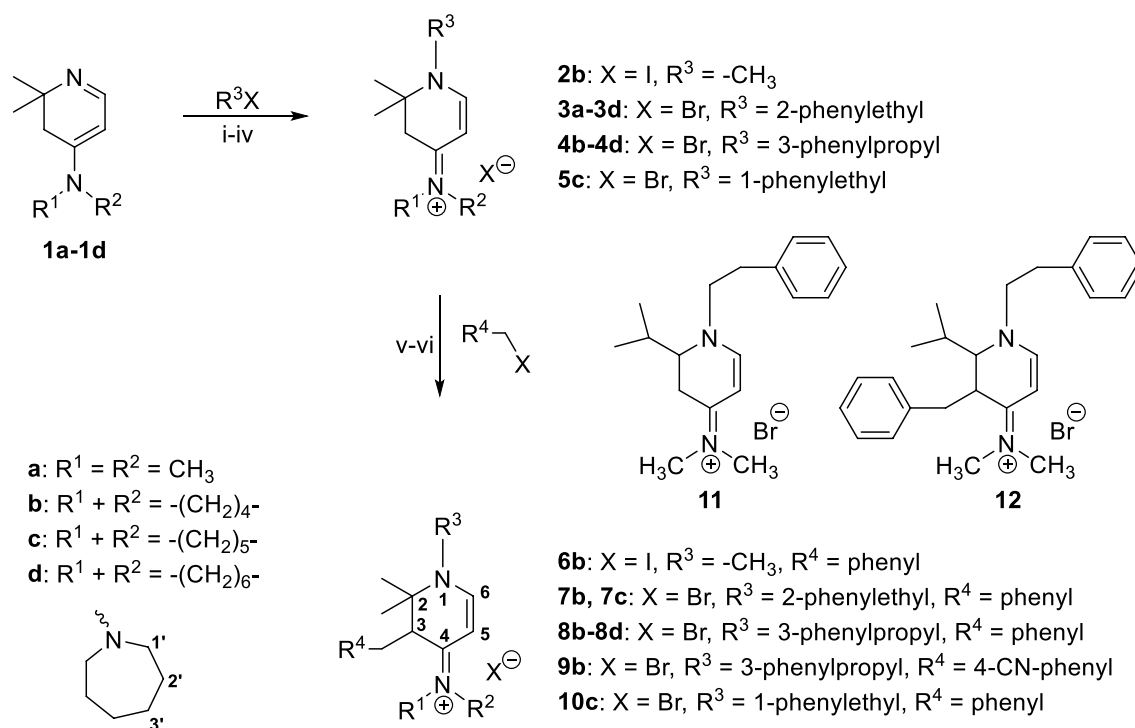we.seebacher@uni-graz.at

1. Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria

2. Institute of Chemistry, University of Graz, Graz, Austria

3. Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland

4. Institute for Chemistry and Technology of Materials (ICTM), Graz University of Technology, Graz, Austria

Scheme 1

2b: X = I, R³ = -CH₃
3a-3d: X = Br, R³ = 2-phenylethyl
4b-4d: X = Br, R³ = 3-phenylpropyl
5c: X = Br, R³ = 1-phenylethyl

11

12

a: R¹ = R² = CH₃
b: R¹ + R² = -(CH₂)₄-
c: R¹ + R² = -(CH₂)₅-
d: R¹ + R² = -(CH₂)₆-

6b: X = I, R³ = -CH₃, R⁴ = phenyl
7b, 7c: X = Br, R³ = 2-phenylethyl, R⁴ = phenyl
8b-8d: X = Br, R³ = 3-phenylpropyl, R⁴ = phenyl
9b: X = Br, R³ = 3-phenylpropyl, R⁴ = 4-CN-phenyl
10c: X = Br, R³ = 1-phenylethyl, R⁴ = phenyl

first-generation malaria vaccine (mosquirix™) demonstrates modest efficacy against malaria illness [4] there is still need for new antiplasmodial compounds.

Recently, we reported the syntheses and the antiplasmodial and antitrypanosomal activities of several *N*-benzyltetrahydropyridinylidene salts (THPS) [5, 6] and of 1,3-dibenzyl THPS [7]. These compounds were generally more active than analogues without aromatic substitution [8]. This paper deals with the synthesis of compounds with differing linkers between the THPS core and the aromatic residue. The effect of the chain length on the antiprotozoal activities is discussed.

Results and discussion

The new compounds were prepared starting from bases **1a–1d** of readily prepared THPS [8]. *N*-Alkylations were done using arylalkyl halides in chloroform with or without catalysis of potassium carbonate. The reactions were carried out at room temperature (20–25 °C) or in refluxing chloroform (Scheme 1). The successful reaction to compounds **3–5** was confirmed by NMR spectroscopy: additional proton signals appeared in the aromatic region due to linkage with arylalkyl residues, furthermore the connectivity was verified by cross peaks in HMBC spectra between C-2 and the protons of the methylene group attached to the ring nitrogen. The coupling reaction in position 3 to compounds **6–10** caused the appearance of further aromatic proton resonances of the new benzyl substituent. The

protons of its methylene group showed a long-range coupling to C-3 in HMBC spectra, confirming connectivity.

The racemic compound **10c** gave suitable crystals and therefore was chosen for an X-ray crystal structure analysis. The compound was confirmed as 1-[3-benzyl-2,2-dimethyl-1-(1-phenylethyl)-1,2,3,4-tetrahydropyridin-4-ylidene]-piperidin-1-ium bromide. All atoms lie on general positions. The compound crystalized in the centrosymmetric space group P-3 is a racemate of 3*R*,1*S* and 3*S*,1*R* enantiomers (Fig. 1). The twelve nearest neighbors of the bromide anion are H atoms [Br····H 2.675(12)–3.539(18) Å], the shortest Br····N distance is 4.587(3) Å between Br1 and N21.

All new compounds were tested against the chloroquine sensitive NF54 strain of *Plasmodium falciparum* (*P. falc.*), a causative organism of the most severe malaria form, Malaria tropica. Additionally, the antitrypanosomal activity against *Trypanosoma brucei rhodesiense* (*T. b. rhod.*) the protozoal agent of East African Human Trypanosomiasis was investigated, as well as the cytotoxic properties against L-6 cells (rat skeletal myoblasts). The results are presented in Table 1.

Concerning the antitrypanosomal activity compounds with a single alkylaryl substitutent attached to the ring nitrogen showed low activity (**3–5**: $IC_{50} = 1.19–178$ μM). Among the compounds with substitution in ring positions 1 and 3 the 1-methyl substituted **6b** as well as the 3-(4-cyanobenzyl) substituted **9b** were only weakly active (**6b**, **9b**: $IC_{50} = 1.53–4.25$ μM). However, all compounds with an aryalkyl substituent in
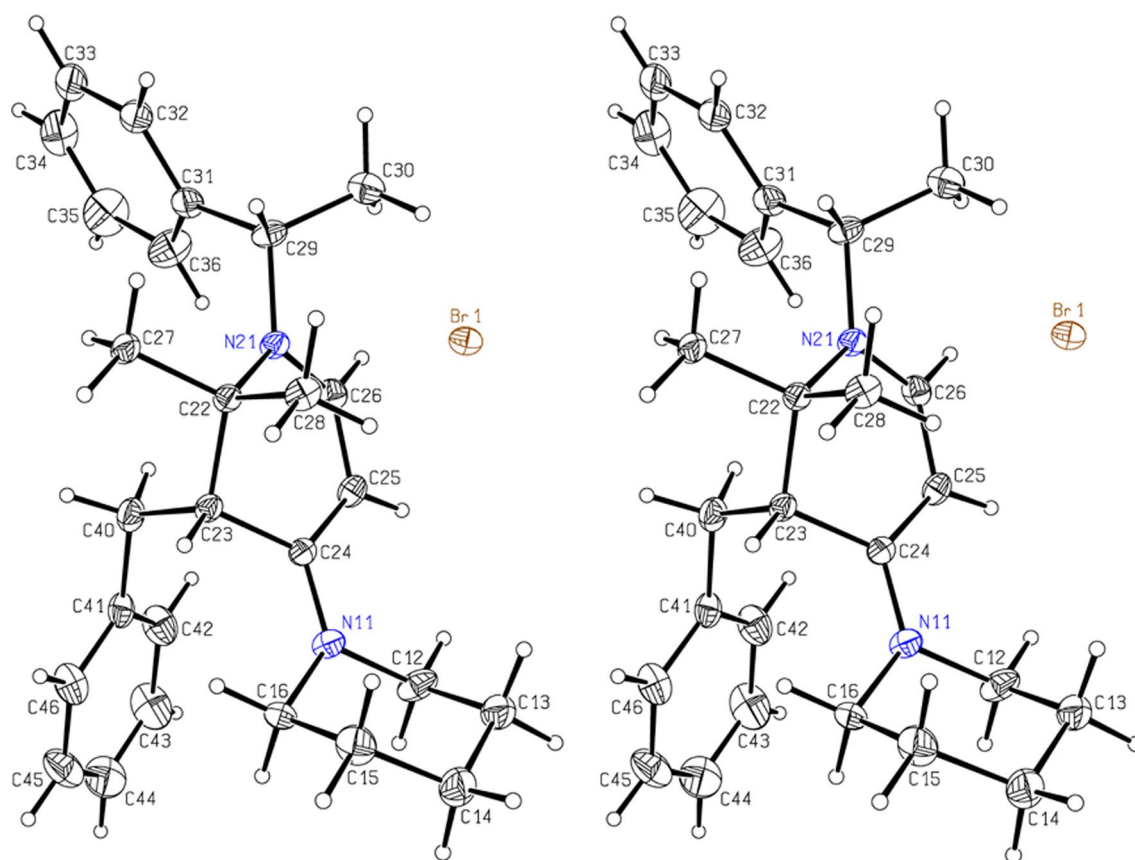
Fig. 1 Stereoscopic ORTEP [9] plot of **10c** showing the atomic numbering scheme. The probability ellipsoids are drawn at the 50% probability level. The H atoms are drawn with arbitrary radii

ring position 1 and an additional benzyl substituent in ring position 3 showed distinct activity against *T. b. rhodesiense* ($IC_{50} = 0.056–0.19$ μM), low cytotoxicity ($IC_{50} = 11.6–203$ μM) and therefore good selectivity ($SI = 157–847$).

The new compounds showed good to excellent antiplasmodial activity against *P. falc.* NF54 ($IC_{50} = 0.008–0.47$ μM). Least activity showed the 1-methyl substituted **6b** as well as compounds with a 1-(1-phenylethyl) or a 1-(2-phenylethyl) group and no substitution in ring position 3 (**6b**, **5c**, **3a**, **3b**: $IC_{50} = 0.26–0.47$ μM). In general, compounds with a phenylpropyl moiety (**4b–4d**: $IC_{50} = 0.020–0.121$ μM) were more active than their phenylethyl analogues (**3b–3d**: $IC_{50} = 0.09–0.30$ μM). An additional benzyl substituent in ring position 3 increased this activity (**7b**, **7c**: $IC_{50} = 0.024–0.039$ μM). Their 3-(3-phenyl-propyl) analogues exhibited the highest antiplasmodial activity (**8b–8d**: $IC_{50} = 0.008–0.0121$ μM). Most of the compounds showed low cytotoxicity and therefore good selectivity. The most promising antiplasmodial compound was **9b** with outstanding selectivity ($SI = 10{,}150$), which results from high activity ($IC_{50} = 0.020$ μM) and very low cytoxicity ($IC_{50} > 203$ μM).

Conclusion

Several new tetrahydropyridinylidene salts with aryl alkyl moieties attached to the ring nitrogen and to position 3 of the tetrahydropyridine core have been prepared and investigated for their antiplasmodial and antitrypanosomal activities as well as for their cytotoxicity using microplate assays. Compounds with aryalkyl substitution in ring position 1 and an additional benzyl substituent in ring position 3 showed good antitrypanosomal activity and selectivity. The antiplasmodial activity was strongly influenced by the length of the alkyl spacer between the ring nitrogen and the aromatic moiety. Compounds with propylene spacers were distinctly more active than those with ethylene and methylene chains. The most promising compound showed activity in low nanomolar concentration and outstanding selectivity. Further investigations seem to be worth-while.

Table 1 Antiprotozoal and cytotoxic activities of compounds **3–12** (IC$_{50}$ values in μM)

Cpd	L-6 cells	P. falc. NF54		T. b. rhod	
	IC$_{50}$[a]	IC$_{50}$[a]	SI$_{PN}$[b]	IC$_{50}$[a]	SI$_T$[c]
3a	191	0.46	415	178	1.07
3b	155	0.30	517	7.21	21.5
3c	142	0.09	1578	1.97	72.1
3d	72.6	0.061	1190	1.19	61.0
4b	27.0	0.040	680	15.0	1.80
4c	16.5	0.020	825	1.61	10.2
4d	169	0.121	1397	17.7	9.55
5c	30.4	0.26	117	1.47	20.7
6b	140	0.47	298	4.25	32.9
7b	57.6	0.024	2400	0.068	847
7c	88.5	0.039	2269	0.18	492
8b	26.8	0.008	3309	0.171	157
8c	11.6	0.008	1398	0.058	200
8d	13.0	0.012	1074	0.17	74.5
9b	> 203	0.020	10,150	1.53	133
10c	41.5	0.047	883	0.056	741
12	29.9	0.018	1661	0.19	157
Mel[d]	7.78	–	–	0.004	1995
CQ[e]	117	0.007	16,714		
P[f]	0.012				

[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 *T. b. rhodesiense* (SI$_T$), expressed as ratio [IC$_{50}$(L6)/IC$_{50}$(*T. b. rhodesiense*)]

[d]*Mel* melarsoprol

[e]*CQ* chloroquine diphosphate

[f]*P* podophyllotoxin

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 Avance Neo 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$_3$ peak 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 solvent 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; 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 compounds **2b**, **3b**, and **3c** was reported [10] as well as the synthesis of **6b**, **11**, and **12** [11].

***N,N*,2,2-Tetramethyl-1-(2-phenylethyl)-1,2,3,4-tetrahydropyridin-4-iminium bromide (3a, C$_{17}$H$_{25}$BrN$_2$)** To a solution of 1.617 g of **1a** (10.6 mmol) in 30 cm^3 of CHCl$_3$ 3 g of K$_2$CO$_3$ (21.7 mmol) were added. 5.19 g of 2-phenylethyl bromide (28.03 mmol) dissolved in 10 cm^3 of CHCl$_3$ were added dropwise. The mixture was stirred for 23 days at r.t. under an atmosphere of argon. Then it was filtered and the solvent evaporated in vacuo giving a resin which was dissolved in CHCl$_3$. Ethyl acetate was added until the solution became turbid, upon stirring at r.t.. The crude product precipitated as brown solid. It was sucked off, dissolved in dichloromethane, treated with charcoal, filtered, and the solvent was evaporated. The product was again crystallized from CHCl$_3$/ethyl acetate. Yield: 2.697 g of **3a** (75%) as light brown powder. R_f = 0.20 (CH$_2$Cl$_2$:MeOH = 8:1); m.p.: 183 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.32 (s, 6H, 2CH$_3$), 2.91 (s, 2H, H-3), 2.92 (t, *J* = 7.8 Hz, 2H, ArCH$_2$), 3.15 (s, 3H, NCH$_3$), 3.25 (s, 3H, NCH$_3$), 3.68 (t, *J* = 7.8 Hz, 2H, ArCH$_2$C*H*$_2$N), 5.21 (d, *J* = 7.0 Hz, 1H, H-5), 7.21–7.31 (m, 5H, ArH), 7.56 (d, *J* = 7.0 Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 23.29 (2CH$_3$), 36.40 (ArCH$_2$), 38.88 (C-3), 40.64, 40.93 (2NCH$_3$), 50.96 (ArCH$_2$*C*H$_2$N), 57.04 (C-2), 87.46 (C-5), 126.69, 128.55, 129.21 (ArC), 137.86 (ArC$_q$), 156.67 (C-6), 164.87 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 2938, 1575, 1504, 1456, 1394, 1351, 1318, 1234, 1173, 1126, 1107, 757, 714 cm^{-1}; HRMS (EI$^+$): *m/z* calcd. for C$_{17}$H$_{24}$N$_2$ ([M-HBr]$^+$) 256.1939, found 256.1939.

***N*-[2,2-Dimethyl-1-(2-phenylethyl)-1,2,3,4-tetrahydropyridin-4-ylidene]azepan-1-ium bromide (3d, C$_{21}$H$_{31}$BrN$_2$)** To a solution of 1 g of **1d** (4.85 mmol) in 14 cm^3 of CHCl$_3$ 2.37 mg (12.8 mmol) of 2-phenylethyl bromide was added and the mixture stirred for 34 days at r.t.. then 70 cm^3 of

CHCl$_3$ were added and the mixture was treated with charcoal and filtered. The solvent was concentrated in vacuo and ethyl acetate was added until crystallization seemed to be complete. Yield: 967 mg (51%) of **3d** as yellow crystals. $R_f = 0.88$ (CH$_2$Cl$_2$: MeOH = 9:1); m.p.: 161 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.34$ (s, 6H, 2CH$_3$), 1.50 (br, s, 4H, H-3′), 1.61–1.75 (m, 4H, H-2′), 2.93 (br, 4H, H-3, ArCH$_2$), 3.61–3.78 (m, 6H, ArCH$_2$CH_2N, H-1′), 5.30 (d, $J = 7.0$ Hz, 1H, H-5), 7.19–7.36 (m, 5H, ArH), 7.55 (d, $J = 7.0$ Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.10$ (2CH$_3$), 25.28, 25.44, 25.67 (C-2′, C-3′), 28.10 (C-2′), 36.48 (ArCH$_2$), 38.40 (C-3), 51.12 (ArCH$_2$CH_2N), 51.21, 51.54 (C-1′), 57.16 (C-2), 87.20 (C-5), 126.76, 128.60, 129.23 (ArC), 137.90 (ArC$_q$), 156.87 (C-6), 164.20 (C-4) ppm; IR (KBr): $\bar{v} = 2930$, 1552, 1453, 1408, 1354, 1184, 1102, 770 cm^{-1}; HRMS (HESI): m/z calcd. for C$_{21}$H$_{31}$N$_2$ (M$^+$) 311.2487, found 311.2477.

N-[2,2-Dimethyl-1-(3-phenylpropyl)-1,2,3,4-tetrahydropyridin-4-ylidene]pyrrolidin-1-ium bromide (4b, C$_{20}$H$_{29}$BrN$_2$) To a solution of 1.152 g of **1b** (6.46 mmol) in 17 cm^3 of CHCl$_3$, 1.683 g K$_2$CO$_3$ (12.18 mmol) and 2.734 g of 3-phenylpropyl bromide (13.73 mmol) were added. The suspension was stirred for 18 days at r.t.. then 17 cm^3 of CHCl$_3$ were added and the mixture treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin, which was recrystallized from CHCl$_3$/ethyl acetate. Yield: 1.706 g of **4b** (70%) as yellow crystals. $R_f = 0.31$ (CH$_2$Cl$_2$:MeOH = 9:1); m.p.: 153 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.31$ (s, 6H, 2CH$_3$), 1.83–2.00 (m, 6H, ArCH$_2$CH_2CH$_2$N, 2CH$_2$), 2.60 (t, $J = 7.9$ Hz, 2H, ArCH_2CH$_2$CH$_2$N), 2.90 (s, 2H, H-3), 3.43–3.52 (m, 4H, ArCH$_2$CH$_2$CH_2N, NCH$_2$), 3.66 (t, $J = 6.4$ Hz, 2H, NCH$_2$), 5.15 (d, $J = 6.8$ Hz, 1H, H-5), 7.14–7.32 (m, 5H, ArH), 7.67 (d, $J = 6.8$ Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.45$ (2CH$_3$), 24.32, 24.58 (2CH$_2$), 32.13, 32.19 (ArCH$_2$CH_2CH$_2$N, ArCH_2CH$_2$CH$_2$N), 40.12 (C-3), 49.52, 49.70, 49.86 (ArCH$_2$CH$_2$CH_2N, 2NCH$_2$), 56.93 (C-2), 88.08 (C-5), 126.12, 128.41, 128.54 (ArC), 141.19 (ArC$_q$), 156.56 (C-6), 161.83 (C-4) ppm; IR (KBr): $\bar{v} = 2943$, 1608, 1563, 1454, 1410, 1372, 1348, 1328, 1300, 1178, 1103, 767 cm^{-1}; HRMS (HESI): m/z calcd. for C$_{20}$H$_{29}$N$_2$ (M$^+$) 297.2331, found 297.2320.

N-[2,2-Dimethyl-1-(3-phenylpropyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidin-1-ium bromide (4c, C$_{21}$H$_{31}$BrN$_2$) To a solution of 2.269 g of **1c** (11.80 mmol) in 33 cm^3 of CHCl$_3$ 3.294 g (23.83 mmol) of K$_2$CO$_3$ and 6.202 g of 3-phenylpropyl bromide (31.15 mmol) were added. The suspension was stirred for 18 days at r.t.. Then 33 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 recrystallized from CHCl$_3$/ethyl acetate. Yield: 2.484 g of **4c** (54%) as yellow crystals. $R_f = 0.90$ (CH$_2$Cl$_2$:MeOH = 4:1);

m.p.: 138 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.29$ (s, 6H, 2CH$_3$), 1.60–1.66 (m, 6H, 3CH$_2$), 1.91 (quin, $J = 7.7$ Hz, 2H, ArCH$_2$CH_2CH$_2$N), 2.61 (t, $J = 7.9$ Hz, 2H, ArCH_2CH$_2$CH$_2$N), 2.90 (s, 2H, H-3), 3.49 (t, $J = 7.5$ Hz, 2H, ArCH$_2$CH$_2$CH_2N), 3.65 (br, s, 4H, 2NCH$_2$), 5.43 (d, $J = 7.1$ Hz, 1H, H-5), 7.16–7.30 (m, 5H, ArH), 7.67 (d, $J = 7.1$ Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.33$ (2CH$_3$), 23.42 (CH$_2$), 25.86, 26.97 (2CH$_2$), 32.10 (ArCH$_2$CH_2CH$_2$N), 32.15 (ArCH_2CH$_2$CH$_2$N), 38.47 (C-3), 49.05, 49.11 (2NCH$_2$), 49.86 (ArCH$_2$CH$_2$CH_2N), 57.00 (C-2), 87.26 (C-5), 126.13, 128.43, 128.55 (ArC), 141.20 (ArC$_q$), 156.93 (C-6), 162.88 (C-4) ppm; IR (KBr): $\bar{v} = 2936$, 1552, 1408, 1357, 1264, 1016, 761 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{21}$H$_{30}$N$_2$ ([M-HBr]$^+$) 310.2409, found 310.2400; calcd. for C$_{20}$H$_{27}$N$_2$ ([M-HBr-CH$_3$]$^+$) 295.2174, found 295.2166.

N-[2,2-Dimethyl-1-(3-phenylpropyl)-1,2,3,4-tetrahydropyridin-4-ylidene]azepan-1-ium bromide (4d, C$_{22}$H$_{33}$BrN$_2$) To a solution of 1.43 g of **1d** (6.93 mmol) in 20 cm^3 of CHCl$_3$ 3.64 g of 3-phenylpropyl bromide (18.3 mmol) were added. The mixture was stirred for 18 days at r.t. Then 30 cm^3 of CHCl$_3$ were added and the solution was treated with charcoal and filtered. The solvent was evaporated in vacuo giving a residue which was dissolved in MeOH, diluted with water and put into a separatory funnel. The aqueous layer was extracted three times with diethyl ether. The combined organic layers were discarded and the aqueous layer was extracted five times with CH$_2$Cl$_2$. The organic layer was dried with Na$_2$SO$_4$ and filtered. The solvent was evaporated in vacuo giving a resin which was recrystallized by stirring for 30 days with ethyl acetate. Yield: 435 mg of **4d** (15%) as yellow crystals. $R_f = 0.48$ (CH$_2$Cl$_2$:MeOH = 10:1); m.p.: 120 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.30$ (s, 6H, 2CH$_3$), 1.45–1.54 (m, 4H, H-3′), 1.62–1.76 (m, 4H, H-2′), 1.87–1.95 (m, 2H, ArCH$_2$CH_2CH$_2$N), 2.61 (br, t, $J = 7.9$ Hz, 2H, ArCH_2CH$_2$CH$_2$N), 2.92 (s, 2H, H-3), 3.49 (br, t, $J = 7.6$ Hz, 2H, ArCH$_2$CH$_2$CH_2N), 3.70 (quin, $J = 6.0$ Hz, 4H, H-1′), 5.32 (d, $J = 7.0$ Hz, 1H, H-5), 7.16–7.30 (m, 5H, ArH), 7.68 (d, $J = 7.0$ Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.11$ (2CH$_3$), 25.29, 25.43, 25.67 (C-2′, C-3′), 28.07 (C-2′), 32.07, 32.13 (ArCH$_2$CH$_2$CH$_2$N, ArCH$_2$CH$_2$CH$_2$N), 38.34 (C-3), 49.90 (ArCH$_2$CH$_2$CH_2N), 51.14, 51.50 (C-1′), 57.10 (C-2), 87.18 (C-5), 126.10, 128.41, 128.52 (ArC), 141.17 (ArC$_q$), 156.86 (C-6), 164.18 (C-4) ppm; IR (KBr): $\bar{v} = 2930$, 1543, 1451, 1407, 1369, 1356, 1339, 1284, 1187, 1165, 1106, 761 cm^{-1}; HRMS (HESI): m/z calcd. for C$_{22}$H$_{33}$N$_2$ (M$^+$) 325.2644, found 325.2635.

(1RS)-(±)-N-[2,2-Dimethyl-1-(1-phenylethyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidin-1-ium bromide (5c, C$_{20}$H$_{29}$BrN$_2$) To a solution of 2.269 g of **1c** (11.80 mmol)

in 50 cm^3 of CHCl$_3$ 3.647 g of (1-bromoethyl)benzene (19.71 mmol) were added. The solution was stirred at r.t. for 3 days and then diluted with CHCl$_3$, treated with charcoal and filtered. The solvent was evaporated in vacuo and the residue recrystallized from CHCl$_3$/ethyl acetate. Yield: 2.702 g of **5c** (61%) as incarnadine crystals. For analytical purposes the substance was recrystallized from acetone. $R_f = 0.37$ (CH$_2$Cl$_2$:MeOH = 9:1); m.p.: 156 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.16 (s, 3H, CH$_3$), 1.38 (s, 3H, CH$_3$), 1.59–1.64 (m, 9H, ArCHCH_3, 3CH$_2$), 2.89 (d, J = 16.8 Hz, 1H, H-3), 2.97 (d, J = 17.2 Hz, 1H, H-3), 3.65–3.67 (m, 4H, 2NCH$_2$), 5.14 (q, J = 7.0 Hz, 1H, ArCHCH$_3$), 5.49 (d, J = 7.3 Hz, 1H, H-5), 7.30–7.41 (m, 5H, ArH), 7.85 (d, J = 7.3 Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ = 22.92 (CH$_3$), 23.30 (CH$_2$), 23.39 (ArCHCH$_3$), 24.23 (CH$_3$), 25.90, 26.91 (2CH$_2$), 38.94 (C-3), 49.18 (2NCH$_2$), 56.32 (ArCHCH$_3$), 58.40 (C-2), 87.68 (C-5), 126.56, 127.84, 128.98 (ArC), 142.37 (ArC$_q$), 153.94 (C-6), 162.81 (C-4) ppm; IR (KBr): $\bar{v}$ = 2934, 1547, 1461, 1404, 1306, 1266, 1183, 1119, 1017, 769 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{20}$H$_{28}$N$_2$ ([M-HBr]$^+$) 296.2253, found 296.2242; calcd. for C$_{19}$H$_{25}$N$_2$ ([M-HBr-CH$_3$]$^+$) 281.2018, found 281.2009.

(3RS)-(±)-N-[3-Benzyl-2,2-dimethyl-1-(2-phenylethyl)-1,-2,3,4-tetrahydropyridin-4-ylidene]pyrrolidin-1-ium bromide (7b, C$_{26}$H$_{33}$BrN$_2$) To a mixture of 1 g of **3b** (2.75 mmol) and 3.527 g (25.52 mmol) of K$_2$CO$_3$ in 24 cm^3 of CHCl$_3$ a solution of 565 mg of benzyl bromide (3.30 mmol) in 24 cm^3 of CHCl$_3$ was added dropwise via a dropping funnel over a period of 1 h with stirring and cooling. Then it was stirred for 67 days at r.t.. After that it was diluted with 48 cm^3 of CHCl$_3$, treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin. It was recrystallized from ethyl acetate/ethanol. Yield: 736 mg of **7b** (59%) as a yellow solid. For analytical purposes, the salt was dissolved in methanol, diluted with water and put into a separatory funnel. The aqueous layer was extracted 3 times with diethyl ether. The combined ethereal layers were discarded and the aqueous layer was extracted 5 times with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$) and filtered. The solvent was evaporated in vacuo and the residue was recrystallized from acetone. $R_f = 0.40$ (CH$_2$Cl$_2$:CH$_3$OH = 9:1); m.p.: 114 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.12 (quin, J = 6.6 Hz, 1H, CH$_2$), 1.20 (s, 3H, CH$_3$), 1.43–1.65 (m, 2H, CH$_2$), 1.61 (s, 3H, CH$_3$), 1.76 (quin, J = 6.6 Hz, 1H, CH$_2$), 2.08 (quin, J = 6.5 Hz, 1H, NCH$_2$), 2.35 (dd, J = 12.1, 9.9 Hz, 1H, ArCH$_2$), 3.00 (br, t, J = 7.6 Hz, 2H, ArCH$_2$CH$_2$N), 3.05–3.14 (m, 2H, H-3, ArCH$_2$), 3.20 (quin, J = 6.7 Hz, 1H, NCH$_2$), 3.36–3.46 (m, 2H, NCH$_2$), 3.67 (quin, J = 7.5 Hz, 1H, ArCH$_2$CH_2N), 3.79 (quin, J = 7.5 Hz, 1H, ArCH$_2$CH_2N), 5.12 (d, J = 6.8 Hz, 1H, H-5), 7.13 (d, J = 7.0 Hz, 2H, ArH), 7.21–7.37 (m,

8H, ArH), 7.59 (d, J = 6.8 Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.55, 21.68 (2CH$_3$), 23.77, 24.33 (2CH$_2$), 34.53 (ArCH$_2$), 36.33 (ArCH$_2$CH$_2$N), 48.34 (C-3), 49.58, 49.67 (2NCH$_2$), 50.98 (ArCH$_2$CH$_2$N), 60.33 (C-2), 87.54 (C-5), 126.80, 127.09, 128.46, 128.62, 129.29, 129.63 (ArC), 137.65, 137.95 (ArC$_q$), 155.20 (C-6), 165.97 (C-4) ppm; IR (KBr): $\bar{v}$ = 2977, 2917, 1567, 1473, 1455, 1405, 1353, 1284, 1157, 756, 703 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{26}$H$_{32}$N$_2$ ([M-HBr]$^+$) 372.2566, found 372.2581.

(3RS)-(±)-N-[3-Benzyl-2,2-dimethyl-1-(2-phenylethyl)-1,-2,3,4-tetrahydropyridin-4-ylidene]piperidin-1-ium bromide (7c, C$_{27}$H$_{35}$BrN$_2$) To a mixture of 1 g of **3c** (2.65 mmol) and 3.4 g of K$_2$CO$_3$ (24.6 mmol) in 47 cm^3 of CHCl$_3$ 544 mg of benzyl bromide (3.18 mmol) were added. The suspension was refluxed overnight at 80 °C. Then it was diluted with 47 cm^3 of CHCl$_3$, treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin. It was dissolved in CHCl$_3$, treated again with charcoal, filtered and evaporated to dryness giving a brown foam. This residue was recrystallized twice from cyclohexane/acetone. Yield: 121 mg of **7c** (10%) as a light brown solid. $R_f = 0.61$ (CH$_2$Cl$_2$:CH$_3$OH = 10:1); m.p.: 168 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.84–0.94 (m, 1H, CH$_2$), 1.13–1.23 (m, 1H, CH$_2$), 1.16 (s, 3H, CH$_3$), 1.27–1.54 (m, 4H, 2CH$_2$), 1.62 (s, 3H, CH$_3$), 2.23–2.32 (m, 2H, ArCH$_2$, NCH$_2$), 2.98–3.08 (m, 4H, ArCH_2CH$_2$N, ArCH$_2$, NCH$_2$), 3.33–3.41 (m, 2H, H-3, NCH$_2$), 3.62–3.82 (m, 3H, NCH$_2$, ArCH$_2$CH_2N), 5.41 (d, J = 6.8 Hz, 1H, H-5), 7.09 (d, J = 7.0 Hz, 2H, ArH), 7.22–7.36 (m, 8H, ArH), 7.52 (d, J = 6.8 Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.52, 21.58 (2CH$_3$), 22.91, 25.74, 26.61 (3CH$_2$), 34.66 (ArCH$_2$), 36.37 (ArCH$_2$CH$_2$N), 45.47 (C-3), 49.00, 49.25 (2NCH$_2$), 50.84 (ArCH$_2$CH$_2$N), 60.16 (C-2), 86.23 (C-5), 126.77, 127.00, 128.41, 128.59, 129.29, 129.75 (ArC), 137.36, 137.97 (ArC$_q$), 155.34 (C-6), 167.51 (C-4) ppm; IR (KBr): $\bar{v}$ = 2922, 1563, 1474, 1454, 1329, 1268, 1258, 1167, 1015, 770, 761, 750, 705 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{27}$H$_{34}$N$_2$ ([M-HBr]$^+$) 386.2722, found 386.2683; calcd. for C$_{26}$H$_{31}$N$_2$ ([M-HBr-CH$_3$]$^+$) 371.2487, found 371.2482.

(3RS)-(±)-N-(3-Benzyl-2,2-dimethyl-1-(3-phenylpropyl)-1,2,3,4-tetrahydropyridin-4-ylidene]pyrrolidin-1-ium bromide (8b, C$_{27}$H$_{35}$BrN$_2$) To a mixture of 800 mg of **8b** (2.12 mmol) and 2.721 g of K$_2$CO$_3$ (19.69 mmol) in 17 cm^3 of CHCl$_3$ a solution of 443 mg of benzyl bromide (2.59 mmol) in 21 cm^3 of CHCl$_3$ was added. The mixture was stirred for 26 days at r.t.. Then it was diluted with 38 cm^3 of CHCl$_3$, treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin, which was recrystallized from ethyl acetate/ethanol. The yellowish powder contained ethyl acetate which was not removable. Therefore, it was dissolved in CH$_2$Cl$_2$ and evaporated repeatedly to yield the

product as solvent-free foam. Yield: 547 mg of **8b** (55%). $R_f = 0.44$ (CH$_2$Cl$_2$:MeOH = 9:1); ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.13$ (quin, $J = 6.3$ Hz, 1H, CH$_2$), 1.21 (s, 3H, CH$_3$), 1.48–1.63 (m, 5H, CH$_2$, CH$_3$), 1.78 (quin, $J = 6.4$ Hz, 1H, CH$_2$), 1.91–2.00 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$N), 2.08 (quin, $J = 6.6$ Hz, 1H, NCH$_2$), 2.43 (br, t, $J = 13.0$ Hz, 1H, ArCH$_2$), 2.60–2.67 (m, 2H, ArC*H*$_2$CH$_2$CH$_2$N), 3.08–3.16 (m, 2H, H-3, ArCH$_2$), 3.23 (quin, $J = 6.8$ Hz, 1H, NCH$_2$), 3.39–3.60 (m, 4H, ArCH$_2$CH$_2$C*H*$_2$N, NCH$_2$), 5.16 (d, $J = 6.7$ Hz, 1H, H-5), 7.15–7.32 (m, 10H, ArH), 7.67 (d, $J = 6.7$ Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 21.66$ (2CH$_3$), 23.79, 24.35 (2CH$_2$), 32.30, 32.32 (ArC*H*$_2$CH$_2$CH$_2$N, ArCH$_2$C*H*$_2$CH$_2$N), 34.62 (ArCH$_2$), 48.31 (C-3), 49.56, 49.63 (2NCH$_2$), 50.15 (ArCH$_2$CH$_2$C*H*$_2$N), 60.19 (C-2), 87.49 (C-5), 126.16, 127.09, 128.44, 128.57, 129.67 (ArC), 137.65, 141.16 (ArC$_q$), 155.28 (C-6), 165.93 (C-4) ppm; IR (KBr): $\bar{v} = 2922$, 1607, 1559, 1493, 1474, 1452, 1400, 1338, 1285, 1162, 756, 702 cm^{-1}; HRMS (HESI): *m/z* calcd. for C$_{27}$H$_{35}$N$_2$ (M$^+$) 387.2800, found 387.2786.

(3*RS*)-(±)-*N*-[3-Benzyl-2,2-dimethyl-1-(3-phenylpropyl)-1,-2,3,4-tetrahydropyridin-4-ylidene]piperidin-1-ium bromide (8c, C$_{28}$H$_{37}$BrN$_2$) To a mixture of 1 g of **4c** (2.55 mmol) and of 3.277 g of K$_2$CO$_3$ (23.71 mmol) in 20 cm^3 ofCHCl$_3$ a solution of 524 mg of benzyl bromide (3.06 mmol) in 25 cm^3 of CHCl$_3$ was added. The mixture was stirred for 75 days at r.t.. Then it was diluted with 45 cm^3 of CHCl$_3$, treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin, which was recrystallized from ethyl acetate/ethanol. The salt was dissolved in methanol, diluted with water and put into a separatory funnel. The aqueous layer was extracted 3 times with diethyl ether. The combined ethereal layers were discarded and the aqueous layer was extracted 5 times with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$) and filtered. The solvent was evaporated in vacuo. Yield: 141 mg of **8c** (11%) as off-white foam. $R_f = 0.51$ (CH$_2$Cl$_2$:MeOH = 9:1); ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 0.87$–0.90 (m, 1H, CH$_2$), 1.10–1.23 (m, 1H, CH$_2$), 1.17 (s, 3H, CH$_3$), 1.24–1.36 (m, 1H, CH$_2$), 1.37–1.43 (m, 1H, CH$_2$), 1.44–1.52 (m, 2H, CH$_2$), 1.57 (s, 3H, CH$_3$), 1.91–2.00 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$N), 2.26 (ddd, $J = 13.2$, 9.6, 3.2 Hz, 1H, NCH$_2$), 2.35 (dd, $J = 12.9$, 10.8 Hz, 1H, ArCH$_2$), 2.61–2.65 (m, 2H, ArC*H*$_2$CH$_2$CH$_2$N), 3.04–3.12 (m, 2H, NCH$_2$, ArCH$_2$), 3.38–3.59 (m, 4H, H-3, ArCH$_2$CH$_2$C*H*$_2$N, NCH$_2$), 3.73–3.77 (m, 1H, NCH$_2$), 5.44 (d, $J = 6.8$ Hz, 1H, H-5), 7.13–7.31 (m, 10H, ArH), 7.65 (d, $J = 6.8$ Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 21.57$, 21.66 (2CH$_3$), 22.93, 25.73, 26.61 (3CH$_2$), 32.30 (ArC*H*$_2$CH$_2$CH$_2$N, ArCH$_2$C*H*$_2$CH$_2$N), 34.73 (ArCH$_2$), 45.45 (C-3), 48.98, 49.19 (2NCH$_2$), 50.00 (ArCH$_2$CH$_2$C*H*$_2$N), 60.01 (C-2), 86.19 (C-5), 126.15, 127.02, 128.41, 128.44, 128.57, 129.81 (ArC), 137.37, 141.17 (ArC$_q$), 155.40 (C-6), 167.48 (C-4) ppm; IR (KBr): $\bar{v} = 2933$, 1568, 1474, 1450,

1392, 754, 738, 703 cm^{-1}; HRMS (EI$^+$): *m/z* calcd. for C$_{28}$H$_{36}$N$_2$ ([M-HBr]$^+$) 400.2878, found 400.2884.

(3*RS*)-(±)-*N*-[3-Benzyl-2,2-dimethyl-1-(3-phenylpropyl)-1,-2,3,4-tetrahydropyridin-4-ylidene]azepan-1-ium bromide (8d, C$_{29}$H$_{39}$BrN$_2$) To a mixture of 1 g of **4d** (2.47 mmol) and 3.166 g of K$_2$CO$_3$ (22.91 mmol) in 55 cm^3 of CHCl$_3$ 508 mg of benzyl bromide (2.97 mmol) were added. The reaction mixture was stirred for 5 days at 50 °C. Then it was cooled down to r.t. and 55 cm^3 of CHCl$_3$ were added. It was treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin. It was dissolved in methanol, diluted with water and transferred into a separatory funnel. The aqueous layer was extracted 3 times with diethyl ether. The combined ethereal layers were discarded and the aqueous layer was extracted 5 times with CH$_2$Cl$_2$. The combined organic layers were dried (Na$_2$SO$_4$) and filtered. The solvent was evaporated in vacuo giving a resin, which was recrystallized repeatedly from ethyl acetate. Yield: 389 mg of **8d** (32%) as green crystals. $R_f = 0.47$ (CH$_2$Cl$_2$:MeOH = 10:1); m.p.: 142 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.20$ (s, 3H, CH$_3$), 1.24–1.76 (m, 8H, H-2′, H-3′), 1.55 (s, 3H, CH$_3$), 1.90–2.03 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$N), 2.12–2.23 (m, 1H, H-1′), 2.41 (dd, $J = 12.9$, 10.4, 1H, ArCH$_2$), 2.63 (br, t, $J = 8.0$ Hz, 2H, ArC*H*$_2$CH$_2$CH$_2$N), 3.03–3.16 (m, 2H, H-1′, ArCH$_2$), 3.23 (dd, $J = 10.6$, 5.0 Hz, 1H, H-1′), 3.32–3.40 (m, 1H, H-3), 3.43–3.59 (m, 2H, ArCH$_2$CH$_2$C*H*$_2$N), 3.70–3.80 (m, 1H, H-1′), 5.33 (d, $J = 6.9$ Hz, 1H, H-5), 7.14–7.31 (m, 10H, ArH), 7.64 (d, $J = 6.9$ Hz, 1H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 21.53$, 21.84 (2CH$_3$), 24.76, 24.84, 26.12, 28.84 (C-2′, C-3′), 32.24, 32.29 (ArC*H*$_2$CH$_2$CH$_2$N, ArCH$_2$C*H*$_2$CH$_2$N), 34.84 (ArCH$_2$), 46.03 (C-3), 50.06 (ArCH$_2$CH$_2$C*H*$_2$N), 50.62, 50.98 (C-1′), 60.18 (C-2), 86.87 (C-5), 126.15, 127.07, 128.28, 128.44, 128.56, 129.71 (ArC), 137.32, 141.14 (ArC$_q$), 155.57 (C-6), 168.21 (C-4) ppm; IR (KBr): $\bar{v} = 2930$, 1561, 1493, 1452, 1391, 1350, 1163, 1141, 1103, 753, 704 cm^{-1}; HRMS (HESI): *m/z* calcd. for C$_{29}$H$_{39}$N$_2$ (M$^+$) 415.3113, found 415.3102.

(3*RS*)-(±)-1-[3-(4-Cyanobenzyl)-2,2-dimethyl-1-(3-phenyl propyl)-1,2,3,4-tetrahydropyridin-4-ylidene]pyrrolidin-1-ium bromide (9b, C$_{28}$H$_{34}$BrN$_3$) To a mixture of 777 mg of **4b** (2.06 mmol) and 2.649 g of K$_2$CO$_3$ (19.17 mmol) in 17 cm^3 of CHCl$_3$ a solution of 492 mg of benzyl bromide (2.88 mmol) in 21 cm^3 of CHCl$_3$ was added. The mixture was stirred for 27 days at r.t.. Then it was diluted with 38 cm^3 of CHCl$_3$, treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin, which was recrystallized from ethyl acetate. Yield: 0.458 g of **9b** (45%) as a pale yellow powder containing ethyl acetate. Therefore, it was dissolved in CH$_2$Cl$_2$ and evaporated repeatedly to yield a pure foam for further analysis. $R_f = 0.46$ (CH$_2$Cl$_2$:MeOH = 9:1); ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.14$–1.31 (m, 4H,

CH$_2$, CH$_3$), 1.52–1.69 (m, 5H, CH$_2$, CH$_3$), 1.80 (quin, J = 6.5 Hz, 1H, CH$_2$), 1.89–2.02 (m, 2H, ArCH$_2$C*H$_2$*CH$_2$N), 2.24 (quin, J = 6.5 Hz, 1H, NCH$_2$), 2.55 (dd, J = 11.9, 9.1 Hz, 1H, ArCH$_2$), 2.59–2.69 (m, 2H, ArC*H$_2$*CH$_2$CH$_2$N), 3.17–3.28 (m, 3H, 3-H, NCH$_2$, ArCH$_2$), 3.39–3.58 (m, 4H, ArCH$_2$CH$_2$C*H$_2$*N, NCH$_2$), 5.16 (d, J = 6.7 Hz, 1H, 5-H), 7.16–7.31 (m, 5H, ArH), 7.40 (d, J = 7.8 Hz, 2H, ArH), 7.68 (d, J = 6.8 Hz, 1H, 6-H), 7.76 (d, J = 7.8 Hz, 2H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 21.64, 21.68 (2CH$_3$), 23.84, 24.40 (2CH$_2$), 32.22, 32.27 (ArC*H$_2$*CH$_2$CH$_2$N, ArCH$_2$C*H$_2$*CH$_2$N), 34.38 (ArCH$_2$), 47.53 (C-3), 49.60, 49.82 (2NCH$_2$), 50.23 (ArCH$_2$CH$_2$C*H$_2$*N), 60.29 (C-2), 87.62 (C-5), 109.87 (ArC$_q$), 118.91 (CN), 126.15, 128.43, 128.56, 130.82, 132.24 (ArC), 141.13, 143.80 (ArC$_q$), 155.54 (C-6), 165.28 (C-4) ppm; IR (KBr): $\bar{v}$ = 2925, 2226, 1607, 1558, 1452, 1399, 1333, 1163, 755 cm^{-1}; HRMS (HESI): m/z calcd. for C$_{28}$H$_{34}$N$_3$ (M$^+$) 412.2753, found 412.2742.

(3*RS*,1*SR*)-*N*-(3-Benzyl-2,2-dimethyl-1-(1-phenylethyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidin-1-ium bromide (10c, C$_{27}$H$_{35}$BrN$_2$) To a mixture of 1 g of **5c** (2.65 mmol) and 3.397 g of K$_2$CO$_3$ (24.58 mmol) in 25 cm^3 of CHCl$_3$ a solution of 544 mg benzyl bromide (3.18 mmol) in 25 cm^3 of CHCl$_3$ was added. The mixture was stirred for 27 days at r.t.. Then it was diluted with 50 cm^3 of CHCl$_3$, treated with charcoal and filtered. The solvent was evaporated in vacuo giving a resin. It was dissolved in a small amount of acetone and cyclohexane was added. The liquid phase was decanted and the resinous precipitate was recrystallized from ethyl acetate/ethanol. The brown solid was recrystallized from acetone. Yield: 85 mg of **10c** (7%) as green crystals. R_f = 0.50 (CH$_2$Cl$_2$:CH$_3$OH = 9:1); m.p.: 249 °C; ^{1}H NMR (CDCl$_3$, 400 MHz): δ = 1.11 (br, s, 1H, CH$_2$), 1.36 (br, s, 2H, CH$_2$), 1.47 (s, 3H, CH$_3$), 1.53 (br, s, 1H, CH$_2$), 1.63 (br, s, 2H, CH$_2$), 1.73–1.75 (m, 6H, CH$_3$, ArCHC*H$_3$*), 2.13–2.23 (m, 1H, NCH$_2$), 2.33 (t, J = 12.1 Hz, 1H, ArCH$_2$), 3.03–3.08 (m, 2H, NCH$_2$, ArCH$_2$), 3.70 (br, s, 2H, 3-H, NCH$_2$), 3.85–3.88 (m, 1H, NCH$_2$), 4.99 (q, J = 7.0 Hz 1H, ArC*H*CH$_3$), 5.64 (d, J = 7.0 Hz, 1H, 5-H), 7.19–7.42 (m, 10H, ArH), 7.81 (d, J = 7.3 Hz, 1H, 6-H) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 22.29 (CH$_3$), 23.02 (CH$_2$), 23.10 (CH$_3$), 23.75 (ArCHC*H$_3$*), 25.96, 26.84 (2CH$_2$), 35.40 (ArCH$_2$), 46.70 (C-3), 49.69, 50.28 (2NCH$_2$), 56.91 (Ar*C*HCH$_3$), 62.25 (C-2), 87.36 (C-5), 126.14, 127.08, 128.11, 128.43, 129.18, 129.82 (ArC), 136.49, 140.94 (ArC$_q$), 151.90 (C-6), 167.62 (C-4) ppm; IR (KBr): $\bar{v}$ = 2931, 1560, 1475, 1454, 1312, 1274, 1252, 1168, 1121, 1021, 752, 703 cm^{-1}; HRMS (EI$^+$): m/z calcd. for C$_{27}$H$_{34}$N$_2$ ([M−HBr]$^+$) 386.2722, found 386.2741.

Crystal structure determination of 10c

All the measurements were performed using monochromatized Mo K$_\alpha$ radiation at 100 K: C$_{27}$H$_{35}$N$_2$$^+Br^-$, M_r

467.48, trigonal, space group P-3, $a = b$ = 19.1533(9) Å, c = 11.8151(6) Å, V = 3753.7(4) Å^3, Z = 6, d_{calc} = 1.241 g cm^{-3}, μ = 1.658 mm^{-1}. A total of 29,545 reflections were collected (Θ_{max} = 28.0°), from which 6060 were unique (R$_{int}$ = 0.0564), with 5377 having $I > 2\sigma(I)$. The structure was solved by direct methods (SHELXS-97) [12] and refined by full-matrix least-squares techniques against F^2 (SHELXL-2014/6) [13]. Since rhombohedral obverse / reverse twinning was detected an appropriate twin matrix (− 1 0 0 / 0 − 1 0 / 0 0 1) was applied and a scale factor was refined [0.4774(8)] between the two unequal components lowering R1 from 0.2079 to 0.0287 (!). The non-hydrogen atoms were refined with anisotropic displacement parameters without any constraints. The positions of the H atoms of the asymmetric C atoms were taken from a difference Fourier map, the C–H distances were fixed to 1.00 Å, and these H atoms were refined with individual displacement parameters without any constraints to the bond angles. The H atoms of the CH$_2$ groups were refined with common isotropic displacement parameters for the H atoms of the same group and idealized geometry with approximately tetrahedral angles and C–H distances of 0.99 Å. The H atoms H25 and H26 of the double bond were taken from a difference Fourier map, the C–H distances were fixed to 0.95 Å and a common isotropic displacement parameter was refined for these H atoms. The H atoms of the phenyl rings were put at the external bisectors of the C–C–C angles at C–H distances of 0.95 Å and common isotropic displacement parameters were refined for the H atoms of the same ring. The H atoms of the methyl groups were refined with common isotropic displacement parameters for the H atoms of the same group and idealized geometries with tetrahedral angles, enabling rotations around the C–C bonds, and C–H distances of 0.98 Å. For 301 parameters final R indices of R1 = 0.0287 and wR^2 = 0.0657 (GOF = 1.021) were obtained. The largest peak in a difference Fourier map was 0.489 e Å^{-3}. The final atomic parameters, as well as bond lengths and angles are deposited at the Cambridge Crystallographic Data Centre (CCDC 2141590).

In vitro assays

The in vitro growth inhibition assay of *Plasmodium falciparum NF54* and the in vitro growth inhibition assay of *Trypanosoma b. rhodesiense,* as well as the assay for the determination of cytotoxicity against L6-cells were performed as described earlier [14].

References

1. Lutje V, Probyn K, Seixas J, Bergman H, Villanueva G (2021) Cochrane Database Syst Rev 12:CD015374
2. WHO (2021) World Malaria report 28 December 2021 https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021
3. Chhibber-Goel J, Yogavel M, Sharma A (2021) Protein Sci 30:1793
4. Laurens MB (2020) Hum Vaccines Immunother 16:480
5. Mohsin N-ul-A, Seebacher W, Faist J, Hochegger P, Kaiser M, Mäser P, Belaj F, Saf R, Kretschmer N Alajlani M, Turek I, Brantner A, Bauer R, Bucar F, Weis R (2018) Eur J Med Chem 143:97
6. Mohsin N-ul-A, Seebacher W, Hochegger P, Faist J, Saf R, Kaiser M Mäser P, Weis R (2019) Med Chem Res 28:742
7. Petritsch M, Seebacher W, Mohsin N-ul-A, Dolensky J, Hochegger P, Kaiser M, Mäser P, Belaj F, Saf R, Kretschmer N, Alajlani M, Brantner A, Bauer R, Schühly W, Weis R (2021) Eur J Med Chem 210:112969
8. Seebacher W, Faist J, Belaj F, Saf R, Kaiser M, Brun R, Weis R (2015) Monatsh Chem 146:1299
9. Johnson CK (1965) ORTEP. Report ORNL-3794. Oak Ridge National Laboratory, Tennessee
10. Seebacher W, Mohsin N-ul-A, Dolensky J, Hochegger P, Kaiser M, Mäser P, Saf R, Schuster D, Temml V, Weis R (2021) Monatsh Chem 152:1347
11. Hoffelner M, Seebacher W, Usama H, Leitner A, Weis R, Saf R (2021) Tetrahedron 90:132189
12. Sheldrick GM (2008) Acta Cryst A64:112
13. Sheldrick GM (2015) Acta Cryst C17:3
14. Mohsin N-ul-A, Seebacher W, Faist J, Hochegger P, Kaiser M, Mäser P, Saf R, Weis R (2018) Monatsh Chem 149:99

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.