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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new 1-benzyl tetrahydropyridin-4-ylidene piperidinium salts and their antiplasmodial and antitrypanosomal activities

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Abstract

Our last study revealed the distinct antiplasmodial and antitrypanosomal activities of 1-benzyl tetrahydropyridin-4-ylidene pyrrolidinium salts. Therefore, we prepared a series of new 1-benzyl tetrahydropyridin-4-ylidene ammonium salts with an alternative amino residue and varying substitution patterns at the benzyl group to investigate the influence of these modifications on the biological activities. All new compounds were characterized by spectroscopic methods like ultraviolet, infrared, nuclear magnetic resonance, as well as high- resolution mass spectra. They were tested for their activities against *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* as well as for their cytotoxicity against L6 cells using microplate assays. The results show that the structure of the amino residue as well as the substitution pattern of the benzyl group influences the biological activities distinctly. Physicochemical parameters are calculated and structure–activity relationships are discussed.

Keywords 1-benzyl tetrahydropyridin-4-ylidene ammonium salts · Antiplasmodial activity · Antitrypanosomal activity · Structure–activity relationships

Introduction

Malaria is one of the major global health challenges with 300 million new cases annually. The best regimen for treating *Plasmodium falciparum* induced malaria is based on the combination of antimalarial drugs with artemisinin and its derivatives, which is called artemisinin combination therapy (ACT). These are highly effective drugs that result in rapid clearance of parasites even in severe *P. falciparum* induced malaria patients. However, from the past few years, parasites, which are resistant to ACTs, have begun to emerge in various parts of the world such as Cambodia and Greater Mekong Subregion (Puttappa et al. 2017). Therefore, there is the urgent necessity to develop new compounds with distinct activity against *P. falciparum*.

Trypanosoma brucei is a protozoan parasite of the Trypanosomatidae family, which is responsible for diseases termed sleeping sickness in humans and nagana in domestic animals in Africa (Zufferey et al. 2017). *Trypanosoma brucei gambiense* and *T. b. rhodesiense* are the causative organisms of sleeping sickness, which is invariably fatal if left untreated (Kennedy 2004). The current drugs available for treatment suffer from a number of disadvantages, including toxic side effects, poor clinical efficacy, parenteral administration, and increasing problems with resistance (Khabnadideh et al. 2005). The latest introduced drug is eflornithine but it is unfortunately ineffective against the late stage of the infection with *T. b. rhodesiense* (Agbo et al. 2003). This parasite is still only sensitive to melarsoprol,

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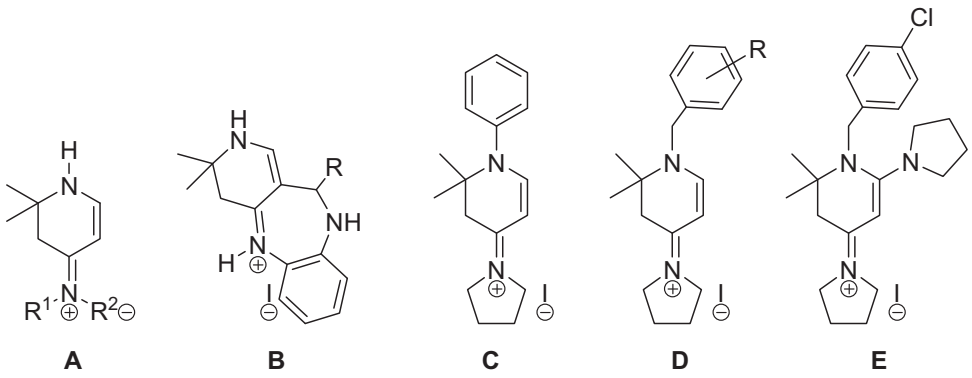


Fig. 1 Structure of readily investigated tetrahydropyridin-4-ylidene ammonium salts

which causes severe side effects. Therefore, it is a great demand to develop new antitrypanosomal drugs.

Tetrahydropyridinylidene ammonium salts **A** (Fig. 1) show in vitro activity against *P. falciparum* and *T. b. rhodesiense* (Seebacher et al. 2015). Several modifications were made to get compounds with higher antiprotozoal activity. The tetrahydropyridinylidene ring was included into a bigger ring system, pyrido-benzodiazepine salts **B** were prepared, but the activity of the so-far highest-active tetrahydropyridinylidene ammonium salt **C**, bearing an aromatic ring at the ring nitrogen, was not reached by these compounds **B** (Seebacher et al. 2017). The insertion of a CH_2 group between the aromatic residue and the nitrogen in compounds **D** turned out to be advantageous and it was shown that the substitution pattern of the aromatic moiety influences the biological activities markedly (Mohsin et al. 2018a, 2018b). A further modification resulted in compound **E** bearing an additional amino residue on the ring; they have even higher antiplasmodial activity but disproportionately high toxicity (Mohsin et al. 2018a). Therefore, we concentrated our efforts on the optimization of compounds of general structure **D**.

Replacement of the pyrrolidine ring by a piperidine ring in position 4 of the 4-chlorobenzyl derivative of typus **D** slightly decreased the antiplasmodial potency. However, its selectivity was much better owing to its fivefold lower cytoxicity. Therefore, we prepared a series of tetrahydropyridin-4-ylidene piperidinium salts bearing benzyl groups with varying substituents.

Materials and methods

Experimental

Melting points were obtained on an Electrothermal IA 9200 melting point apparatus. Infrared (IR) spectra were recorded using a Bruker Alpha Platinum ATR FTIR spectrometer (KBr discs); frequencies are reported in cm^{-1}. Ultraviolet/visible (UV/VIS): Lambda 17 UV/VIS-spectrometer (Perkin Elmer) carried out in CH_3OH solutions. Nuclear magnetic resonance (NMR) spectra: Varian UnityInova 400 (300 K) 5 mm tubes, spectra were acquired in dimethyl sulfoxide (DMSO). Chemical shifts are given in parts per million (ppm), for ^1H spectra the solvent peak (2.49) was used as internal standard and for ^{13}C spectra the central resonance line of the DMSO signal was used as the internal reference (39.7). Abbreviations: ArH, aromatic H; ArC, aromatic C; ArC_q, quaternary aromatic C. Signal multiplicities are abbreviated as follows: s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; dddd double double double doublet; qd quadrupled doublet; t, triplet; q quartet; m, multiplet; sept, septet. Coupling constants (*J*) are reported in Hertz (Hz). ^1H- and ^{13}C-resonances were assigned using $^1H,^1H$-, and $^1H,^{13}C$-correlation spectra (gCOSY, gHSQC, gHMBC optimized on 8 Hz). ^1H- and ^{13}C-resonances are numbered as given in the formulae. HRMS: Micromass tofspec 3E spectrometer (MALDI), GCT-Premier, Waters (EI, 70 eV). Thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F_{254} 0.2 mm, 200 × 200 mm); the compounds were detected in UV light at 254 nm, furthermore they showed deep blue fluorescence at 365 nm. Solvents and reagents were obtained from commercial sources. The preparation of the hydroiodide of **7** is already described (Seebacher et al. 2015). The supplementary material contains ^1H- and ^{13}C NMR spectra as well as high resolution mass spectra of the new compounds.

Syntheses

2,2-Dimethyl-4-piperidino-2,3-dihydropyridin 7

The hydroiodide of **7** (1 g, 3.1 mmol) was stirred with 2 N NaOH (20 mL) for 1 h. After that the solution was extracted exhaustively with dichloromethane. The combined organic layers were dried with sodium sulfate, filtered, and evaporated giving **7** (563 mg, 94%) as greenish oil that was directly used for further reaction steps.

General procedure for the preparation of 10a-t

Compound **7** was dissolved in chloroform and the appropriate benzyl halide was added to the solution. Then the solution was stirred at room temperature. The volume was reduced by evaporation of a part of the solvent and ethyl acetate was added dropwise. The products crystallized overnight. For analytical purposes, the products were recrystallized after treatment with charcoal from chloroform/ethyl acetate or ethanol/acetone. Then they were dried at 100 °C at reduced pressure.

N-(1-Benzyl-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)piperidinium bromide (10a) Compound **7** (563 mg, 2.9 mmol) reacted overnight with benzyl bromide (820 mg, 4.8 mmol) in $CHCl_3$ (3 mL) to 856 mg (80%) of **10a** as yellow crystals; mp (chloroform/ethyl acetate) 207 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.432), 207 (4.159) nm; IR (KBr) ν_{max} 2941, 1608, 1561, 1487, 1477, 1453, 1407, 1364, 1346, 1273, 1243, 1181, 1106, 1019, 761, 751, 708 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.21$ (s, 6 H, $2CH_3$), 1.62 (br, s, 6 H, $(CH_2)_3$), 2.96 (s, 2 H, H-3), 3.69 (s, br, 4 H, $N(CH_2)_2$), 4.77 (s, 2 H, $ArCH_2$), 5.54 (d, $J = 6.6$ Hz, 1 H, H-5), 7.32–7.39 (m, 5 H, ArH), 7.81 (d, $J = 6.6$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta =$ 23.4 (CH_2, $2CH_3$), 25.9, 26.9 ($2CH_2$), 38.7 (C-3), 49.2, 49.3 ($N(CH_2)_2$), 53.5 ($ArCH_2$), 57.4 (C-2), 87.6 (C-5), 127.5, 127.9, 128.9 (ArC), 137.5 (ArC_q), 157.5 (C-6), 163.4 (C-4) ppm; HRMS (MALDI): 283.2174 $C_{19}H_{27}N_2$ [M^+] (calcd: 283.2188).

N-[1-(4-Chlorobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium chloride (10b) Compound **7** (1.04 g, 5.4 mmol) reacted overnight with 4-chlorobenzyl chloride (1.48 g, 9.2 mmol) in $CHCl_3$ (12 mL) to 1.69 g (89%) of **10b** as beige precipitate; mp (chloroform/ethyl acetate) 199 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 349 (4.352), 219 (4.075) nm; IR (KBr) ν_{max} 2941, 2857, 1603, 1553, 1490, 1469, 1405, 1358, 1270, 1239, 1184, 1106, 1014, 792 cm $^{-1}$; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.19$ (s, 6 H, $2CH_3$), 1.63 (br, s, 6 H, $(CH_2)_3$), 2.94 (s, 2 H, H-3), 3.66–3.70 (m, 4 H, $2NCH_2$), 4.75 (s, 2 H, $ArCH_2$), 5.55 (d, $J = 7.3$ Hz, 1 H, H-5), 7.38 (d, $J = 8.4$ Hz, 2 H, ArH), 7.46 (d, $J = 8.4$ Hz, 2 H, ArH), 7.81 (d, $J = 7.0$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.4$ (CH_2, $2CH_3$), 25.9, 26.9 ($2CH_2$), 38.6 (C-3), 49.3 ($N(CH_2)_2$), 52.8 ($ArCH_2$), 57.5 (C-2), 87.8 (C-5), 128.8, 129.5 (ArC), 132.5, 136.8 (ArC_q), 157.8 (C-6), 163.5 (C-4) ppm; HRMS (EI^+): 316.1729 $C_{19}H_{25}ClN_2$ [M-HCl^+] (calcd. 316.1706).

N-[1-(2-Chlorobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium chloride (10c) Compound **7** (1.03 g, 5.4 mmol) reacted overnight with 2-chlorobenzyl chloride (1.47 g, 9.1 mmol) in $CHCl_3$ (12 mL) to 1.59 g (83%) of **10c** as beige precipitate. mp (ethanol/acetone) 215 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 349 (4.349), 212 (4.021) nm; IR (KBr) ν_{max} 2952, 1605, 1554, 1469, 1446, 1401, 1371, 1344, 1277, 1249, 1184, 1114, 770 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.25$ (s, 6 H, $2CH_3$), 1.60 – 1.65 (m, 6 H, $(CH_2)_3$), 3.07 (s, 2 H, H-3), 3.71 (br, s, 4 H, $2NCH_2$), 4.82 (s, 2 H, $ArCH_2$), 5.57 (d, $J = 7.3$ Hz, 1 H, H-5), 7.38–7.52 (m, 4 H, ArH), 7.63 (d, $J = 7.3$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.0$ ($2CH_3$), 23.4, 26.0, 26.9 ($(CH_2)_3$), 38.5 (C-3), 49.3, 49.4 ($N(CH_2)_2$), 51.2 ($ArCH_2$), 57.4 (C-2), 88.0 (C-5), 127.9, 129.9, 130.0 (ArC), 132.3, 134.2 (ArC_q), 157.3 (C-6), 163.6 (C-4) ppm; HRMS (EI^+): 316.1736 $C_{19}H_{25}ClN_2$ [M-HCl^+] (calcd. 316.1706).

N-[2,2-Dimethyl-1-(4-fluorobenzyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10d) Compound **7** (826 mg, 4.30 mmol) reacted overnight with 4-fluorobenzyl bromide (1.38 g, 7.3 mmol) in $CHCl_3$ (15 mL) to 1.54 g (94%) of **10d** as yellowish precipitate. mp (ethanol/acetone): 183 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 349 (4.408), 206 (4.131) nm; IR (KBr) ν_{max} 2940, 1603, 1559, 1508, 1488, 1454, 1403, 1364, 1346, 1274, 1237, 1222, 1181, 1158, 1104, 1009, 759 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.21$ (s, 6 H, $2CH_3$), 1.61 (br, s, 6 H, $(CH_2)_3$), 2.96 (s, 2 H, H-3), 3.69 (br, s, 4 H, $2NCH_2$), 4.77 (s, 2 H, $ArCH_2$), 5.54 (d, $J = 7.0$ Hz, 1 H, H-5), 7.19 – 7.24 (m, 2 H, ArH), 7.40–7.44 (m, 2 H, ArH), 7.84 (d, $J = 7.0$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.4$ (CH_2, $2CH_3$), 25.9, 26.9 ($2CH_2$), 38.7 (C-3), 49.2, 49.3 ($N(CH_2)_2$), 52.7 ($ArCH_2$), 57.5 (C-2), 87.7 (C-5), 115.7 (d, $^2J(C,F) = 21.4$ Hz, ArC), 129.8 (d, $^3J(C,F) = 8.0$ Hz, ArC), 133.7 (d, $^4J(C,F) = 3.1$ Hz, ArC_q), 157.5 (C-6), 161.8 (d, $^1J(C,F) = 244.1$ Hz, ArC_q), 163.4 (C-4) ppm; HRMS (EI^+): 300.2008 $C_{19}H_{25}FN_2$ [M-HBr^+] (calcd. 300.2002).

N-[1-(4-Bromobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10e) Compound **7** (932 mg, 4.85 mmol) reacted overnight with 4-bromobenzyl bromide (2.06 g, 8.24 mmol) in $CHCl_3$ (15 mL) to 1.93 g (90%) of **10e** as yellowish crystals. mp (ethanol/acetone) 243 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.374), 205 (4.132) nm; IR (KBr) ν_{max} 2941, 2856, 1613, 1547, 1469, 1439, 1404, 1370, 1357, 1342, 1276, 1248, 1184, 1112, 1009, 813 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.19$ (s, 6 H, $2CH_3$), 1.62 (br, s, 6 H, $(CH_2)_3$), 2.96 (s, 2 H, H-3), 3.67 – 3.70 (m, 4 H, $2NCH_2$), 4.75 (s, 2 H, $ArCH_2$), 5.55 (d, $J = 7.3$ Hz, 1 H, H-5), 7.33 (d, $J = 8.4$ Hz, 2 H, ArH), 7.58 (d, $J = 8.4$ Hz, 2 H, ArH), 7.82 (d, $J = 7.0$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.4$ (CH_2, $2CH_3$), 25.9, 26.9 ($2CH_2$), 38.7 (C-3), 49.4 ($N(CH_2)_2$), 52.8 ($ArCH_2$), 57.5 (C-2), 87.8 (C-5), 129.8, 131.8 (ArC), 121.0,

137.2 (ArC_q), 157.7 (C-6), 163.5 (C-4) ppm; HRMS (EI^+): 360.1172 $C_{19}H_{25}BrN_2$ [M-HBr^+] (calcd. 360.1201).

N-[1-(3,4-Dichlorobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium chloride (10f) Compound **7** (1.03 g, 5.4 mmol) reacted overnight with 3,4-dichlorobenzyl chloride (1.77 g, 9.1 mmol) in $CHCl_3$ (12 mL) to 1.60 g (76%) of **10f** as white precipitate. mp (ethanol/acetone) 239 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.463), 205 (4.348) nm; IR (KBr) ν_{max} 2946, 1602, 1554, 1468, 1451, 1401, 1354, 1271, 1241, 1181, 1107 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.20$ (s, 6 H, $2CH_3$), 1.63–1.64 (m, 6 H, $(CH_2)_3$), 2.97 (s, 2 H, H-3), 3.67 – 3.71 (m, 4 H, $2NCH_2$), 4.80 (s, 2 H, $ArCH_2$), 5.57 (d, $J = 7.0$ Hz, 1 H, H-5), 7.38 (d, $J = 8.1$ Hz, 1 H, ArH), 7.64 – 7.66 (m, 2 H, ArH), 7.89 (d, $J = 7.3$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.4$ (CH_2, $2CH_3$), 26.0, 26.9 ($2CH_2$), 38.6 (C-3), 49.3 ($N(CH_2)_2$), 52.2 ($ArCH_2$), 57.6 (C-2), 88.0 (C-5), 128.0, 129.6, 130.5 (ArC), 131.0, 131.5, 139.1 (ArC_q), 157.9 (C-6), 163.6 (C-4) ppm; HRMS (EI^+): 350.1349 $C_{19}H_{24}Cl_2N_2$ [M-HCl^+] (calcd. 350.1317).

N-[1-(2,4-Dichlorobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10g) Compound **7** (110 mg, 0.57 mmol) reacted overnight with 2,4-dichlorobenzyl bromide (231 mg, 0.96 mmol) in $CHCl_3$ (8 mL) to 175 g (71%) of **10g** as greenish precipitate. mp (acetone) 208 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.476) nm; IR (KBr) ν_{max} 2951, 1611, 1544, 1470, 1407, 1344, 1279, 1250, 1177, 1114, 1044, 952, 863 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.25$ (s, 6 H, $2CH_3$), 1.66 (br, s, 6 H, $(CH_2)_3$), 3.06 (s, 2 H, H-3), 3.71 (br, s, 4 H, $2NCH_2$), 4.78 (s, 2 H, $ArCH_2$), 5.57 (d, $J = 7.3$ Hz, 1 H, H-5), 7.47 – 7.52 (m, 2 H, ArH), 7.59 (d, $J = 7.0$ Hz, 1 H, H-6), 7.69 (s, 1 H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 22.9$ ($2CH_3$), 23.3, 25.9, 26.9 ($3CH_2$), 38.6 (C-3), 49.4 ($N(CH_2)_2$), 50.7 ($ArCH_2$), 57.5 (C-2), 88.2 (C-5), 128.0, 129.3, 131.3 (ArC), 133.3, 133.5, 133.6 (ArC_q), 157.3 (C-6), 163.7 (C-4) ppm; HRMS (EI^+): 350.1327 $C_{19}H_{24}Cl_2N_2$ [M-HBr^+] (calcd. 350.1317).

N-[2,2-Dimethyl-1-(4-methoxybenzyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium chloride (10h) Compound **7** (1.22 g, 6.35 mmol) reacted overnight with 4-methoxybenzyl chloride (1.67 g, 10.7 mmol) in $CHCl_3$ (10 mL) to 224 mg (10%) of **10h** as yellowish precipitate. mp (acetone/ethyl acetate) 107 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.429), 224 (4.095) nm; IR (KBr) ν_{max} 2938, 1611, 1549, 1513, 1458, 1410, 1356, 1343, 1276, 1248, 1175, 1109, 1019, 815 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.24$ (s, 6 H, $2CH_3$), 1.63–1.68 (m, 6 H, $(CH_2)_3$), 2.95 (s, 2 H, H-3), 3.67 – 3.71 (m, 4 H, $2NCH_2$), 3.76 (s, 3 H, OCH_3), 4.70 (s, 2 H, $ArCH_2$), 5.52 (d, $J = 7.3$ Hz, 1 H, H-5), 6.96 (d, $J = 8.4$ Hz, 2 H, ArH), 7.31 (d, $J = 8.4$ Hz, 2 H, ArH), 7.83 (d, $J = 7.3$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.4$ (CH_2, $2CH_3$), 25.8, 26.9 ($2CH_2$), 38.7 (C-3), 49.1, 49.2 ($N(CH_2)_2$), 53.0 ($ArCH_2$), 55.3 (OCH_3), 57.3 (C-2), 87.4 (C-5), 114.3, 129.3 (ArC), 128.8, 159.0 (ArC_q), 157.1 (C-6), 163.2 (C-4) ppm; HRMS (EI^+): 312.2204 $C_{20}H_{28}N_2O$ [M-HCl^+] (calcd. 312.2202).

N-[2,2-Dimethyl-1-(4-methylbenzyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10i) Compound **7** (1.03 g, 5.4 mmol) reacted overnight with 4-methylbenzyl bromide (1.68 g, 9.1 mmol) in $CHCl_3$ (12 mL) to 1.90 g (93%) of **10i** as yellow precipitate. mp (ethanol/acetone) 213 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.397), 210 (4.069) nm; IR (KBr) ν_{max} 2937, 1609, 1561, 1478, 1457, 1402, 1355, 1276, 1234, 1183, 1104, 1019 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.21$ (s, 6 H, $2CH_3$), 1.61 (br, s, 6 H, $(CH_2)_3$), 2.28 (s, 3 H, $ArCH_3$), 2.94 (s, 2 H, H-3), 3.68 (br, s, 4 H, $2NCH_2$), 4.71 (s, 2 H, $ArCH_2$), 5.52 (d, $J = 7.3$ Hz, 1 H, H-5), 7.19 (d, $J = 8.1$ Hz, 2 H, ArH), 7.24 (d, $J = 8.1$ Hz, 2 H, ArH), 7.79 (d, $J = 7.3$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 20.9$ ($ArCH_3$), 23.4 (CH_2, $2CH_3$), 25.9, 26.9 ($2CH_2$), 38.7 (C-3), 49.2, 49.3 ($N(CH_2)_2$), 53.3 ($ArCH_2$), 57.4 (C-2), 87.5 (C-5), 127.6, 129.4 (ArC), 134.3, 137.2 (ArC_q), 157.3 (C-6), 163.3 (C-4) ppm; HRMS (EI^+): 296.2273 $C_{20}H_{28}N_2$ [M-HBr^+] (calcd. 296.2253).

N-[1-(4-Isopropylbenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10j) Compound **7** (912 mg, 4.75 mmol) reacted overnight with 4-(isopropyl) benzyl bromide (1.70 g, 8.0 mmol) in $CHCl_3$ (10 mL) to 604 mg (31%) of **10j** as greenish solid. mp (acetone) 150 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 352 (4.388) nm; IR (KBr) ν_{max} 2943, 1614, 1552, 1448, 1407, 1356, 1273, 1237, 1185, 1103, 1016 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta =$ 1.18 (d, $J = 7.0$ Hz, 6 H, $CH(CH_3)_2$), 1.22 (s, 6 H, $2CH_3$), 1.62 (br, s, 6 H, $(CH_2)_3$), 2.87 (sept, $J = 7.0$ Hz, 1 H, $CH(CH_3)_2$), 2.95 (s, 2 H, H-3), 3.69 (br, s, 4 H, $2NCH_2$), 4.71 (s, 2 H, $ArCH_2$), 5.52 (d, $J = 7.0$ Hz, 1 H, H-5), 7.23 – 7.28 (m, 4 H, ArH), 7.78 (d, $J = 7.0$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.3$ (CH_2), 23.4 ($2CH_3$), 24.0 ($CH(CH_3)_2$), 25.9, 26.9 ($2CH_2$), 33.2 ($CH(CH_3)_2$), 38.7 (C-3), 49.2 ($N(CH_2)_2$), 53.2 ($ArCH_2$), 57.4 (C-2), 87.5 (C-5), 126.8, 127.6 (ArC), 134.6, 148.1 (ArC_q), 157.3 (C-6), 163.3 (C-4) ppm; HRMS (EI^+): 324.2584 $C_{22}H_{32}N_2$ [M-HBr^+] (calcd. 324.2566).

N-[1-(4-Tert-butylbenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10k) Compound **7** (773 mg, 4.02 mmol) reacted overnight with 4-(*tert*-butyl) benzyl bromide (932 mg, 4.1 mmol) in $CHCl_3$ (12 mL) to 1.58 g (94%) of **10k** as yellowish precipitate. mp (ethanol/acetone) 176 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.456), 207

(4.417) nm; IR (KBr) ν_{max} 2941, 1614, 1552, 1471, 1448, 1407, 1380, 1356, 1342, 1269, 1248, 1237, 1185, 1100 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.22$ (s, 6 H, $2CH_3$), 1.26 (s, 9 H, $3CH_3$), 1.62 (br, s, 6 H, $(CH_2)_3$), 2.95 (s, 2 H, H-3), 3.68 (br, s, 4 H, $2NCH_2$), 4.71 (s, 2 H, $ArCH_2$), 5.53 (d, $J = 7.3$ Hz, 1 H, H-5), 7.27 (d, $J = 8.1$ Hz, 2 H, ArH), 7.40 (d, $J = 8.4$ Hz, 2 H, ArH), 7.77 (d, $J = 7.0$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.4$ (CH_2), 23.5 ($2CH_3$), 25.9, 26.9 ($2CH_2$), 31.3 ($3CH_3$), 34.4 ($C(CH_3)_3$), 38.7 (C-3), 49.2, 49.3 ($N(CH_2)_2$), 53.1 ($ArCH_2$), 57.4 (C-2), 87.6 (C-5), 125.6, 127.4 (ArC), 134.3 (ArC_q), 150.4 (ArC_q), 157.4 (C-6), 163.3 (C-4) ppm; HRMS (EI^+): 338.2716 $C_{23}H_{34}N_2$ [M-HBr^+] (calcd. 338.2722).

N-[2,2-Dimethyl-1-(1-naphthylmethyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10l) Compound **7** (392 mg, 2.04 mmol) reacted for 2 days with 1-bromomethyl naphthalene (760 mg, 3.44 mmol) in $CHCl_3$ (12 mL) to 317 mg (38%) of **10l** as a yellowish precipitate. mp (acetone) 270 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 223 (4.836), 352 (4.388), 279 (3.758) nm; IR (KBr) ν_{max} 2938, 1607, 1553, 1399, 1360, 1249, 1179, 1114, 812 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.37$ (s, 6 H, $2CH_3$), 1.66 (br, s, 6 H, $(CH_2)_3$), 3.12 (s, 2 H, H-3), 3.68 – 3.73 (m, 4 H, $2NCH_2$), 5.22 (s, 2 H, $ArCH_2$), 5.51 (d, $J = 7.0$ Hz, 1 H, H-5), 7.44 (d, $J = 7.3$ Hz, 1 H, H-6), 7.49 – 7.64 (m, 4 H, ArH), 7.94 (d, $J = 7.7$ Hz, 1 H, ArH), 8.00 (dd, $J = 7.0$, 1.5 Hz, 1 H, ArH), 8.07 (d, $J = 8.0$ Hz, 1 H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.0$ ($2CH_3$), 23.3, 25.9, 26.9 ($3CH_2$), 38.5 (C-3), 49.2, 49.3 ($N(CH_2)_2$), 50.7 ($ArCH_2$), 57.6 (C-2), 87.9 (C-5), 123.1, 125.7, 125.8, 126.4, 126.9, 128.5, 128.9 (ArC), 130.4, 132.3, 133.4 (ArC_q), 156.8 (C-6), 163.4 (C-4) ppm; HRMS (EI^+): 332.2264 $C_{23}H_{28}N_2$ [M-HBr^+] (calcd. 332.2253).

N-{1-[(Benzo[d][1,3]dioxol-5-yl)methyl]-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene}piperidinium bromide (10m) Compound **7** (236 mg, 1.23 mmol) reacted overnight with 5-(bromomethyl)benzo[d][1,3]dioxole (445 mg, 2.07 mmol) in $CHCl_3$ (12 mL) to 113 mg (23%) of **10m** as yellowish solid. mp (acetone) 194 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 352 (4.415), 287 (3.614) nm; IR (KBr) ν_{max} 2935, 1607, 1560, 1489, 1455, 1405, 1387, 1359, 1275, 1233, 1183, 1104, 1032, 854, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.23$ (s, 6 H, $2CH_3$), 1.62 (br, s, 6 H, $(CH_2)_3$), 2.93 (s, 2 H, H-3), 3.68 (br, s, 4 H, $2NCH_2$), 4.64 (s, 2 H, $ArCH_2$), 5.50 (d, $J = 7.0$ Hz, 1 H, H-5), 6.02 (s, 2 H, OCH_2O), 6.84 (d, $J = 8.1$ Hz, 1 H, ArH), 6.91 – 6.93 (m, 2 H, ArH), 7.75 (d, $J = 7.0$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.3$ (CH_2), 23.4 ($2CH_3$), 25.9, 26.9 ($2CH_2$), 38.7 (C-3), 49.2 ($N(CH_2)_2$), 53.2 ($ArCH_2$), 57.4 (C-2), 87.5 (C-5), 101.3 (OCH_2O), 108.2, 108.5, 121.4 (ArC), 130.8, 147.0, 147.7 (ArC_q), 157.2 (C-6), 163.3 (C-4) ppm; HRMS (EI^+): 326.2011 $C_{20}H_{26}N_2O_2$ [M-HBr^+] (calcd. 326.1994).

N-[2,2-Dimethyl-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium chloride (10n) Compound **7** (935 mg, 4.9 mmol) reacted overnight with 3,4,5-trimethoxybenzyl chloride (1.7 g, 7.8 mmol) in $CHCl_3$ (12 mL) to 775 mg (39%) of **10n** as yellowish precipitate. mp (acetone) 227 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 209 (4.645), 350 (4.540) nm; IR (KBr) ν_{max} 2934, 1613, 1595, 1549, 1507, 1452, 1418, 1359, 1330, 1274, 1255, 1236, 1186, 1152, 1118, 1104, 1017, 856, 613 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.25$ (s, 6 H, $2CH_3$), 1.62 (br, s, 6 H, $(CH_2)_3$), 2.97 (s, 2 H, H-3), 3.64 (s, 3 H, OCH_3), 3.68 – 3.70 (m, 4 H, $2NCH_2$), 3.76 (s, 6 H, $2OCH_3$), 4.66 (s, 2 H, $ArCH_2$), 5.51 (d, $J = 7.0$ Hz, 1 H, H-5), 6.67 (s, 2 H, ArH), 7.82 (d, $J = 7.3$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.3$ (CH_2), 23.4 ($2CH_3$), 25.9, 26.9 ($2CH_2$), 38.7 (C-3), 49.1, 49.2 ($N(CH_2)_2$), 53.6 ($ArCH_2$), 56.2 ($2OCH_3$), 57.5 (C-2), 60.2 (OCH_3), 87.6 (C-5), 105.4 (ArC), 132.6, 137.2, 153.2 (ArC_q), 157.3 (C-6), 163.4 (C-4) ppm; HRMS (EI^+): 372.2428 $C_{22}H_{32}N_2O_3$ [M-HCl^+] (calcd. 372.2413).

N-{2,2-Dimethyl-1-[2-(trifluoromethyl)benzyl]-1,2,3,4-tetrahydropyridin-4-ylidene}piperidinium bromide (10o) Compound **7** (122 mg, 0.634 mmol) reacted overnight with 2-(trifluoromethyl)benzyl bromide (256 mg, 1.07 mmol) in $CHCl_3$ (6 mL) to 166 mg (61%) of **10** as yellowish precipitate. mp (ethanol/acetone) 243 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.292) nm; IR (KBr) ν_{max} 2951, 1609, 1555, 1445, 1406, 1359, 1313, 1270, 1252, 1187, 1157, 1126, 1037, 788 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 1.23$ (s, 6 H, $2CH_3$), 1.67 (br, s, 6 H, $(CH_2)_3$), 3.10 (s, 2 H, H-3), 3.73 (br, s, 4 H, $2NCH_2$), 4.87 (s, 2 H, $ArCH_2$), 5.62 (d, $J = 7.3$ Hz, 1 H, H-5), 7.57 – 7.61 (m, 3 H, H-6, ArH), 7.76 (t, $J = 7.7$ Hz, 1 H, ArH), 7.83 (d, $J = 7.7$ Hz, 1 H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.0$ ($2CH_3$), 23.3, 26.0, 26.9 ($3CH_2$), 38.5 (C-3), 49.4, 49.5 ($N(CH_2)_2$), 50.1 ($ArCH_2$), 57.4 (C-2), 88.4 (C-5), 126.5 (q, $^2J(C,F) = 30.8$ Hz, ArC_q), 126.5 (q, $^3J(C,F) = 5.8$ Hz, ArC), 127.1 (q, $^1J(C,F) = 274$ Hz, CF_3), 128.6 (ArC), 128.9 (ArC), 133.3 (ArC), 135.6 (ArC_q), 157.9 (C-6), 163.9 (C-4) ppm; HRMS (EI^+): 350.1977 $C_{20}H_{25}F_3N_2$ [M-HBr^+] (calcd. 350.1970).

N-{2,2-Dimethyl-1-[4-(trifluoromethyl)benzyl]-1,2,3,4-tetrahydropyridin-4-ylidene}piperidinium bromide (10p) Compound **7** (500 mg, 2.60 mmol) reacted overnight with 4-(trifluoromethyl)benzyl bromide (1 g, 4.2 mmol) in $CHCl_3$ (5 mL) to 900 mg (80%) of **10p** as yellowish precipitate. mp (ethanol/acetone) 180 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 349 (4.389), 206 (4.212) nm; IR (KBr) ν_{max} 2945, 1608, 1558, 1469, 1446, 1407, 1355, 1326, 1277, 1238, 1189, 1156, 1113, 1066, 1017, 743 cm^{-1}; ^1H NMR

(DMSO-d_6, 400 MHz): $\delta = 1.20$ (s, 6 H, $2CH_3$), 1.58 – 1.72 (m, 6 H, $(CH_2)_3$), 3.00 (s, 2 H, H-3), 3.68 – 3.72 (m, 4 H, $2NCH_2$), 4.89 (s, 2 H, $ArCH_2$), 5.59 (d, $J = 7.3$ Hz, 1 H, H-5), 7.59 (d, $J = 8.1$ Hz, 2 H, ArH), 7.75 (d, $J = 8.4$ Hz, 2 H, ArH), 7.86 (d, $J = 7.3$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.4$ (CH_2, $2CH_3$), 26.0, 27.0 ($2CH_2$), 38.7 (C-3), 49.4 ($N(CH_2)_2$), 53.0 ($ArCH_2$), 57.6 (C-2), 88.0 (C-5), 125.7 (q, ^3J(C,F) = 3.8 Hz, ArC), 127.2 (q, ^1J(C,F) = 277 Hz, CF_3), 128.2 (ArC), 128.39 (q, ^2J(C,F) = 31.7 Hz, ArC_q), 142.8 (ArC_q), 158.0 (C-6), 163.6 (C-4) ppm; HRMS (EI^+): 350.1960 $C_{20}H_{25}F_3N_2$ [M-HBr^+] (calcd. 350.1970).

N-[1-(2-Cyanobenzyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium bromide (10q) Compound **7** (680 mg, 3.54 mmol) reacted with 2-(bromomethyl)benzonitrile (708 mg, 3.6 mmol) in $CHCl_3$ (12 mL) to 1.18 g (86%) of **10q** as yellowish precipitate. mp (ethanol/acetone) 183 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 349 (4.451), 204 (4.307) nm; IR (KBr) ν_{max} 2952, 1604, 1549, 1470, 1436, 1401, 1389, 1373, 1358, 1344, 1280, 1251, 1238, 1181, 1116, 779 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.24$ (s, 6 H, $2CH_3$), 1.66 (br, s, 6 H, $(CH_2)_3$), 3.07 (s, 2 H, H-3), 3.73 (br, s, 4 H, $2NCH_2$), 4.96 (s, 2 H, $ArCH_2$), 5.62 (d, $J = 7.3$ Hz, 1 H, H-5), 7.53 – 7.89 (m, 4 H, ArH), 7.91 (d, $J = 7.0$ Hz, 1 H, H-6) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta =$ 23.1 ($2CH_3$), 23.3, 26.1, 27.0 (($CH_2)_3$), 38.6 (C-3), 49.5, 49.6 ($N(CH_2)_2$), 51.8 ($ArCH_2$), 57.6 (C-2), 88.4 (C-5), 110.4 (ArC_q), 117.4 (CN), 128.8, 128.9, 133.8, 134.0 (ArC), 140.8 (ArC_q), 158.0 (C-6), 163.9 (C-4) ppm; HRMS (EI^+): 292.1830 $C_{19}H_{22}N_3$ [M-HBr-CH_3^+] (calcd. 292.1814).

N-{2,2-Dimethyl-1-[(pyridin-2-yl)methyl]-1,2,3,4-tetrahydropyridin-4-ylidene}piperidinium chloride (10r) Compound **7** (308 mg, 1.60 mmol) reacted with 2-(chloromethyl)pyridine (263 mg, 2.06 mmol) in $CHCl_3$ (12 mL) to 148 mg (29%) of **10r** as yellowish precipitate. mp (ethanol/acetone) 213 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 349 (4.408), 205 (3.929) nm; IR (KBr) ν_{max} 2921, 1613, 1585, 1570, 1550, 1468, 1417, 1400, 1374, 1357, 1266, 1249, 1239, 1186, 1108, 1008, 772 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.16$ (s, 6 H, $2CH_3$), 1.58 – 1.70 (m, 6 H, $(CH_2)_3$), 2.94 (s, 2 H, 3-H), 3.67 – 3.70 (m, 4 H, $2NCH_2$), 4.86 (s, 2 H, $ArCH_2$), 5.57 (d, $J = 7.3$ Hz, 1 H, 5-H), 7.34 (dd, $J = 7.3$, 4.8 Hz, 1 H, ArH), 7.50 (d, $J = 7.7$ Hz, 1 H, ArH), 7.83 (t, $J = 7.7$ Hz, 1 H, ArH), 7.88 (d, $J = 7.3$ Hz, 1 H, 6-H), 8.56 (d, $J = 4.8$ Hz, 1 H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta =$ 23.4 (CH_2, $2CH_3$), 25.9, 26.9 ($2CH_2$), 38.6 (C-3), 49.2, 49.3 ($N(CH_2)_2$), 55.1 ($ArCH_2$), 57.2 (C-2), 87.6 (C-5), 122.4, 123.3, 137.5, 149.6 (ArC), 156.6 (ArC_q), 158.4 (C-6), 163.3 (C-4) ppm; HRMS (EI^+): 283.2050 $C_{18}H_{25}N_3$ [M-HCl^+] (calcd. 283.2048).

N-{2,2-Dimethyl-1-[(quinolin-2-yl)methyl]-1,2,3,4-tetrahydropyridin-4-ylidene}piperidinium chloride (10s) Compound **7** (584 mg, 3.04 mmol) reacted during 4 days with 2-(chloromethyl)quinoline (900 mg, 5.1 mmol) in $CHCl_3$ (10 mL) to 349 mg (31%) of **10s** as yellowish precipitate. mp (ethanol/acetone) 163 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 207 (4.554), 227 (4.540), 350 (4.480) nm; IR (KBr) ν_{max} 2941, 2857, 1605, 1548, 1505, 1468, 1440, 1410, 1363, 1345, 1278, 1257, 1238, 1184, 1116, 956, 826, 773, 758 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.19$ (s, 6 H, $2CH_3$), 1.58 – 1.70 (m, 6 H, $(CH_2)_3$), 3.00 (s, 2 H, H-3), 3.68 – 3.72 (m, 4 H, $2NCH_2$), 5.10 (s, 2 H, $ArCH_2$), 5.62 (d, $J = 7.3$ Hz, 1 H, H-5), 7.58 – 7.64 (m, 2 H, ArH), 7.76 (t, $J = 7.5$ Hz, 1 H, ArH), 7.96 – 8.03 (m, 3 H, H-6, ArH), 8.44 (d, $J = 8.4$ Hz, 1 H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta =$ 23.4 (CH_2, $2CH_3$), 26.0, 26.9 ($2CH_2$), 38.6 (C-3), 49.2, 49.3 ($N(CH_2)_2$), 55.8 ($ArCH_2$), 57.3 (C-2), 87.8 (C-5), 120.1, 126.9, 128.2, 128.7, 130.2, 137.4 (ArC), 127.3, 147.1, 157.6 (ArC_q), 158.4 (C-6), 163.5 (C-4) ppm; HRMS (EI^+): 333.2217 $C_{22}H_{27}N_3$ [M-HCl^+] (calcd. 333.2205).

N-[2,2-Dimethyl-1-(4-nitrobenzyl)-1,2,3,4-tetrahydropyridin-4-ylidene]piperidinium chloride (10t) Compound **7** (935 mg, 4.9 mmol) reacted within 14 days with 4-nitrobenzyl chloride (1.41 g, 8.2 mmol) in $CHCl_3$ (10 mL) in the presence of 1 g potassium carbonate to 678 mg (38%) of **10t** as yellowish precipitate. mp (acetone) 218 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 350 (4.715), 206 (4.367), 262 (4.173) nm; IR (KBr) ν_{max} 2941, 1605, 1560, 1512, 1468, 1446, 1402, 1343, 1272, 1246, 1183, 1106, 1014, 794, 713 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.23$ (s, 6 H, $2CH_3$), 1.69 (br, s, 6 H, $(CH_2)_3$), 3.04 (s, 2 H, H-3), 3.73–3.76 (m, 4 H, $2NCH_2$), 5.01 (s, 2 H, $ArCH_2$), 5.64 (d, $J = 7.0$ Hz, 1 H, H-5), 7.69 (d, $J = 8.8$ Hz, 2 H, ArH), 7.96 (d, $J = 7.0$ Hz, 1 H, H-6), 8.26 (d, $J = 8.8$ Hz, 2 H, ArH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 23.3$ ($2CH_3$, CH_2), 26.0, 26.9 ($2CH_2$), 38.6 (C-3), 49.4 ($N(CH_2)_2$), 52.8 ($ArCH_2$), 57.6 (C-2), 88.1 (C-5), 123.9, 128.5 (ArC), 146.0, 147.1 (ArC_q), 158.1 (C-6), 163.7 (C-4) ppm; HRMS (EI^+): 327.1962 $C_{19}H_{25}N_3O_2$ [M-HCl^+] (calcd. 327.1947).

2,2-Dimethyl-4-(pyrrolidin-1-yl)piperidine (11)

A solution of **4** (17.8 g. 85 mmol) in $CHCl_3$ (50 mL) was treated with a solution of methyl iodide (14.27 g, 100.5 mmol) in $CHCl_3$ (50 mL) and stirred overnight at room temperature. Part of the solvent was evaporated and ethyl acetate was added till the first turbidity appeared. Upon stirring, the methylthio compound precipitated and was sucked off and dried giving 25 g (83%) of a yellow solid.

Powdered nickel/aluminum alloy (50 %Al, 50 %Ni) (75 g, containing 640 mmol Ni) was given into a big beaker, water was added, and solid NaOH (150 g, 3.75 mol) was

added cautiously in portions, so that the reaction was kept under control. After the main reaction ceased, the beaker was put into a water bath at 70 °C for 30 min. After that, the liquid was decanted and the solid nickel was washed 20 times with water until the solution reacted neutral. Then it was washed twice with ethanol. It was rinsed to a solution of the yellow methylthio compound (25 g, 71 mmol) in ethanol (400 mL) and stirred for 45 min at room temperature. The catalyst was removed by suction and washed with ethanol. The filtrate and washings were combined and evaporated in vacuo. The residue was dissolved in chloroform and filtered and the solvent evaporated in vacuo. From this residue a small amount of **11** can be distilled off at 4 mbar and 100 °C giving 560 mg (4%) of a liquid. The residue, a dark yellow powder was further purified by crystallization from chloroform/ethyl acetate giving 14.4 g (66%) of **6** (Seebacher et al. 2015).

11: Mp (diHCl): 289 °C; IR (base, neat) ν_{max} 2954, 2933, 2873, 2780, 1460, 1449, 1376, 1361, 1351, 1266, 1219, 1203, 1132, 1111, 1088, 974, 911, 876, 746, 707 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.12$ (s, 3 H, CH_3), 1.13 (s, 3 H, CH_3), 1.16–1.27 (m, 2 H, H_{ax}-3, H_{ax}-5), 1.74 – 1.83 (m, 5 H, H_{eq}-3, $2CH_2$), 1.87 – 1.92 (m, 1 H, H_{eq}-5), 2.28 (dddd, $J = 15.4$, 7.5, 4.0, 3.7 Hz, 1 H, H-4), 2.54–2.57 (m, 4 H, $2NCH_2$), 2.84 (ddd, $J = 13.2$, 12.5, 2.8 Hz, 1 H, H_{ax}-6), 2.93 (ddd, $J = 13.6$, 5.0, 2.6 Hz, 1 H, H_{eq}-6) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 23.0$ ($2CH_2$), 24.2 (CH_3), 32.8 (C-5), 33.2 (CH_3), 40.5 (C-6), 44.2 (C-3), 50.1 (C-2), 51.0 ($2NCH_2$), 58.7 (C-4) ppm; HRMS (EI): 182.1780 $C_{11}H_{22}N_2$ [M^+] (calcd. 182.1783).

1-(4-Chlorobenzyl)-2,2-dimethyl-4-(pyrrolidin-1-yl)piperidine (12b) Compound **11** (324 mg, 1.78 mmol) was dissolved in $CHCl_3$ (5 mL) and 4-chlorobenzyl bromide (438 mg, 2.13 mmol) was added. The mixture was stirred overnight and $CHCl_3$ was added and the solvent evaporated in vacuo. The residue was dissolved in 2 N HCl and the solution extracted four times with ether. The organic phases were discarded and 2 N NaOH was added to the aqueous phase till it reacted alkaline. Then it was extracted three times with $CHCl_3$ and once with CH_2Cl_2. The organic phases were combined and washed with brine and dried over Na_2SO_4. After filtration, the solvents were evaporated in vacuo and the residue subjected to column chromatography (silica gel; CH_2Cl_2:$CH_3OH = 8:2$). The fractions containing the product were combined and the solvents evaporated in vacuo. Yield: 40 mg (7%) of **12b** in form of a colorless resin. The hydrochloride was prepared by repeated treatment of a solution of **12b** in CH_2Cl_2 with an ethereal solution of HCl and subsequent evaporation. The residue was recrystallized from ethanol/ethyl acetate. mp (decomp.) = 286 °C; UV (CH_3OH) λ_{max} ($\log_\varepsilon$) 220 (3.874) nm; IR (base, neat) ν_{max} 2928, 2433, 1636, 1559, 1490, 1444, 1096, 1018, 847, 806 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta =$ 1.05 (s, 3 H, CH_3), 1.28 (s, 3 H, CH_3), 1.79 (qd, $J = 12.1$, 4.6 Hz, 1 H, H_{ax}-5), 1.87 – 1.93 (m, 3 H, H_{ax}-3, H_{eq}-3, H_{eq}-5), 2.05 (br, s, 4 H, $2CH_2$), 2.26 (ddd, $J = 12.6$, 12.5, 2.4 Hz, 1 H, H_{ax}-6), 2.61 (ddd, $J = 12.1$, 4.4, 2.9 Hz, 1 H, H_{eq}-6), 2.92–3.02 (m, 1 H, H-4), 2.96 (d, $J = 14.1$ Hz, 1 H, $ArCH_2$), 3.12 (br, s, 4 H, $2NCH_2$), 3.98 (d, $J = 14.1$ Hz, 1 H, $ArCH_2$), 7.26 (br, s, 4 H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 16.0$ (CH_3), 23.4 ($2CH_2$), 29.1 (C-5), 30.7 (CH_3), 42.5 (C-3), 44.9 (C-6), 50.6 ($2NCH_2$), 52.4 ($ArCH_2$), 54.1 (C-2), 59.8 (C-4), 128.3, 129.5 (ArC), 132.3, 138.8 (ArC_q) ppm; HRMS (EI): 306.1886 $C_{18}H_{27}ClN_2$ [M^+] (calcd. 306.1863).

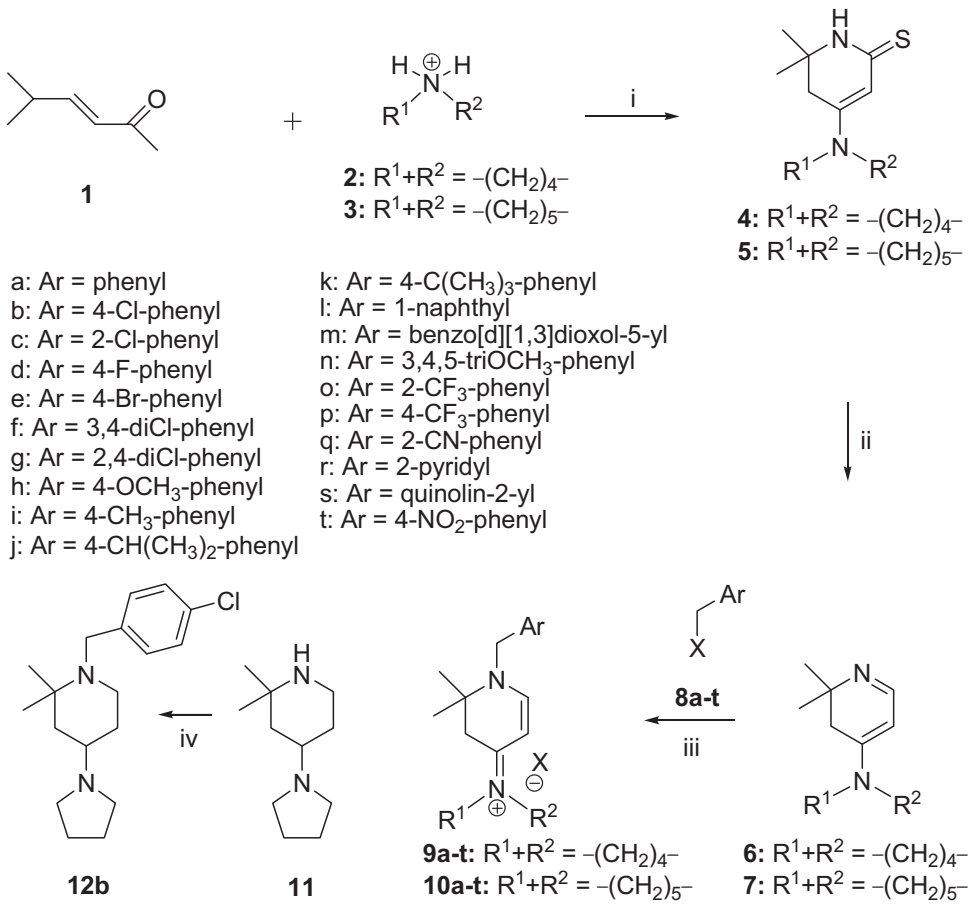
Biological activities

Antimalarial activity

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay (Desjardins et al. 1979; Matile and Pink 1990), using the drug-sensitive NF54 strain (Schipol Airport, The Netherlands, (Ponnudurai et al. 1981)) and the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubatenstitute (RPMI) 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), Albumax^R (5 g/L) and washed human red cells A^+ at 2.5% hematocrit (0.3% parasitaemia). Serial drug dilutions of 11 threefold dilution steps covering a range from 100 to 0.002 µg/mL 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 µL 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 transferred onto a glass fiber filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL 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 (Huber and Koella 1993) using Microsoft Excel. Chloroquine was used as control.

Antitrypanosomal activity and cytotoxicity against L6 cells

Minimum Essential Medium (MEM; 50 µL) supplemented according Baltz (Baltz et al. 1985) with 25 mM HEPES, 1 g/L additional glucose, 1% MEM non-essential amino acids (100 ×), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat-inactivated horse serum was added to each well of



Scheme 1 Syntheses of compounds **9a-t** (Mohsin et al. 2018a, 2018b) and **10a-t**. Reagents and conditions: (i) xylene, reflux 4−6 h, (ii) CH_3I in $CHCl_3$, r.t.; Raney nickel in EtOH, r.t., (iii) $ArCH_2X$ in $CHCl_3$, r.t. or $ArCH_2X$ in $CHCl_3$ with K_2CO_3, r.t., (iv) 4-chlorobenzyl bromide in $CHCl_3$, r.t

a 96-well microtiter plate. Serial drug dilutions of 11 threefold dilution steps covering a range from 100 to 0.002 µg/mL were prepared. Then 4×10^3 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 µL was added to each well and the plate incubated at 37 °C under a 5 % CO_2 atmosphere for 70 h. 10 µL Alamar Blue (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2–4 h (Räz et al. 1997). Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Corporation, 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 (Huber and Koella 1993) from the sigmoidal dose-inhibition curves using SoftmaxPro software (Molecular Devices Corporation, Sunnyvale, CA, USA). Melarsoprol is used as control.

Cytotoxicity against L6 cells

Assays were performed in 96-well microtiter plates, each well containing 100 µL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L6 cells (a primary cell line derived from rat skeletal myoblasts) (Page et al. 1993; Ahmed et al. 1994). Serial drug dilutions of 11 threefold dilution steps covering a range from 100 to 0.002 µg/mL were prepared. After 70 h of incubation, the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10µL 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 Corporation, 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 (Huber and Koella 1993) from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Corporation, Sunnyvale, CA, USA). Podophyllotoxin (Sigma P4405) is used as control.

In vivo antimalarial efficacy studies, 1 day treatment and 4 days treatment

In vivo antimalarial activity was assessed basically as previously described (Peters 1987). Groups of three female Naval Medical Research Institute (NMRI) mice (20–22 g) were intravenously infected with 2×10^7 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain

Table 1 Antiprotozoal activities of **9a-t** and **10a-t**, expressed as IC_{50} values (μM)[a]

Compd.	P.falc.NF54	SI_p[b]	T.b.rhod.	SI_T[c]	Cyt.[d]
9a	0.13	94.77	61.55	0.20	12.32
9b	0.027	743.3	19.71	1.02	20.07
9c	0.12	81.08	10.46	0.93	9.73
9d	0.11	2475	39.21	6.94	>272.3
9e	0.026	445.4	14.53	0.80	11.58
9f	0.021	5348	12.44	9.03	112.3
9g	0.043	69.53	12.75	0.23	2.99
9h	0.11	1528	22.25	7.56	168.1
9i	0.10	949.2	4.24	22.39	94.92
9j	0.015	11.73	3.24	0.054	0.176
9k	0.040	0.43	0.67	0.025	0.017
9l	0.063	9.68	0.89	0.69	0.61
9m	0.046	451.1	18.69	1.11	20.75
9n	1.10	174.1	16.23	11.80	191.5
9o	0.18	38.61	9.75	0.71	6.95
9p	0.036	3481	27.22	4.60	125.3
9q	1.09	245.1	70.88	3.77	>267.15
9r	2.18	150	100.4	3.26	>327.0
9s	0.09	1392	16.63	7.53	125.3
9t	0.10	2858	79.75	3.58	>285.8
10a	0.27	516.7	1.06	131.6	139.5
10b	0.045	2576	4.42	26.22	115.9
10c	0.14	691.8	0.51	189.9	96.85
10d	0.084	364.3	1.92	15.94	30.60
10e	0.034	234.1	1.72	4.63	7.96
10f	0.031	2293	1.38	51.50	71.07
10g	0.023	1375	3.93	8.05	31.63
10h	0.080	1511	15.30	7.90	120.9
10i	0.10	534.8	1.24	43.13	53.48
10j	0.020	4.20	1.48	0.057	0.084
10k	0.038	0.34	0.12	0.11	0.013
10l	0.063	4.92	0.058	5.34	0.31
10m	0.083	67.11	8.03	0.69	5.57
10n	0.93	262.9	5.58	43.82	>244.5
10o	0.095	28.53	4.68	0.58	2.71
10p	0.030	2063	11.82	5.24	61.88
10q	0.92	279.9	15.17	16.97	>257.5
10r	1.17	267.2	4.63	67.52	>312.6
10s	0.11	767.5	1.85	45.63	84.42
10t	0.12	1471	15.53	11.37	176.5
12b	9.51	5.30	2.58	19.53	50.39
chl.[e]	0.004	–	–	–	–
mel.[f]	–	–	0.01	–	–
pod.[g]	–	–	–	–	0.012

Activities of **9a-t** were already published (Mohsin et al. 2018a, 2018b)

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

[b]Selectivity index for P. falc. (SI_p), expressed as ratio [IC_{50}(L6)/IC_{50}(P. falc.)]

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

[d]Cyt. = cytotoxicity

[e]chl. = chloroquine

[f]mel = melarsoprol

[g]pod. = podophyllotoxin

ANKA (Franke-Fayard et al. 2004). Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water and administered intraperitoneally in a volume of 10 mL/kg on day 1 (24 h post infection for 1 day treatment and 4, 24, 48, and 72 h post infection for 4 days treatment). Parasitaemia was determined on day 3 post infection (72 h post infection) by FACS analysis. Activity was calculated as the difference between the mean percent parasitaemia for the control ($n = 5$ mice) and treated groups. The survival time in days was recorded.

Table 2 In vivo activity of selected compounds from the series **9** and **10** against *P.berghei* in mice

Compd.	Application	Dose mg/kg	Activity %	MSD[a] days	Control[b]
9d	i.p.	4 × 10	31.6	4	4
9f	i.p.	4 × 10	39.9	4	4
9p	i.p.	4 × 10	54.2	7	4
9t	i.p.	4 × 50	78.4	7	4
10b	i.p.	4 × 10	22.5	4	4
10f	i.p.	4 × 10	00.0	4	4
10p	i.p.	1 × 50	00.0	1	4
chl[d]	i.p.	4 × 10	99.6	20	4

Activities of series **9** are already published (Mohsin et al. 2018a, 2018b)

[a]MSD, mean survival days

[b]Control, MSD of infected but untreated mice

[c]i.p. intraperitoneal

[d]chl, chloroquine

Results and discussion

The synthesis started from the reaction of mesityloxide **1** with ammonium thiocyanates **2** or **3** to tetrahydropyridinethiones **4** or **5** (Zigeuner and Schweiger 1976). The next step was S-methylation/reduction giving selectively compounds **6** and **7** (Seebacher et al. 2015). *N*-alkylation with benzyl halides **8a-t** yielded the target compounds **9a-t** and **10a-t** (Scheme 1). The syntheses of **9a-t** were described earlier (Mohsin et al. 2018a, 2018b). Compound **12b** is a saturated analog of **9b**. It was prepared from **11**, a by-product of the hydrogenation of **4**.

The target compounds were tested for their antiprotozoal and cytotoxic activities using microplate assays. The in vitro activities against *T. brucei rhodesiense* and *P. falciparum NF54* as well as their cytotoxicity against L6 cells are listed in Table 1.

The compounds with the highest selectivity index for the antiplasmodial activity, **10b, 10f**, and **10p** ($SI_p = 2576$, 2293, and 1471) were further investigated in an in vivo mouse model against *P. berghei*. The results are listed in Table 2 and complemented with activities of some readily described compounds of series **9**.

Some physicochemical properties were calculated including the topological polar surface area (tPSA), molar

Table 3 Key physicochemical parameters of the synthesized compounds **9** and **10**

Compd.	MW[a]	tPSA[a] (Å^2) pH 7.4	MR[a]	Log P[a]	Log S[a]	LE [b]	LLE[b]	LELP[b]
9a	349.31	6.25	96.96	−0.06	−3.87	0.449	6.95	−0.13
9b	339.30	6.25	101.77	0.54	−4.50	0.471	7.03	1.15
9c	339.30	6.25	101.77	0.54	−4.50	0.431	6.38	1.25
9d	367.30	6.25	97.18	0.08	−4.12	0.433	6.88	0.18
9e	428.20	6.25	104.59	0.70	−4.78	0.472	6.89	1.48
9f	373.75	6.25	106.57	1.14	−5.19	0.457	6.54	2.49
9g	418.20	6.25	106.57	1.14	−5.21	0.439	6.23	2.60
9h	334.88	15.48	103.43	−0.22	−3.78	0.414	7.18	−0.53
9i	363.34	6.25	102.00	0.45	−4.36	0.436	6.55	1.03
9j	391.39	6.25	111.15	1.18	−5.26	0.447	6.64	2.64
9k	405.41	6.25	115.63	1.48	−5.38	0.405	5.92	3.65
9l	399.37	6.25	113.41	0.93	−5.63	0.395	6.27	2.36
9m	393.32	24.71	102.73	−0.44	−4.24	0.419	7.78	−1.05
9n	394.94	33.94	116.35	−0.54	−3.69	0.302	6.50	−1.79
9o	417.31	6.25	102.94	0.81	−4.80	0.370	5.93	2.19
9p	417.31	6.25	102.94	0.81	−4.80	0.408	6.63	1.99
9q	374.32	30.04	102.68	−0.21	−4.10	0.355	6.17	−0.59
9r	305.85	19.14	94.28	−1.20	−2.76	0.369	6.86	−3.25
9s	355.90	19.14	110.36	0.18	−4.45	0.386	6.87	0.47
9t	349.86	49.39	103.28	−0.12	−4.45	0.400	7.12	−0.30
10a	363.34	6.25	101.56	0.38	−4.13	0.409	6.19	0.93
10b	353.33	6.25	106.37	0.98	−4.77	0.437	6.36	2.24
10c	353.33	6.25	106.37	0.98	−4.77	0.408	5.87	2.40
10d	381.33	6.25	101.78	0.52	−4.38	0.421	6.56	1.23
10e	442.23	6.25	109.19	1.15	−5.02	0.445	6.32	2.58
10f	387.77	6.25	111.17	1.59	−5.44	0.423	5.82	3.76
10g	432.23	6.25	111.17	1.59	−5.46	0.436	6.05	3.65
10h	348.91	15.48	108.03	0.22	−4.04	0.405	6.88	0.54
10i	377.36	6.25	106.61	0.89	−4.61	0.417	6.11	2.13
10j	405.41	6.25	115.76	1.63	−5.51	0.422	6.08	3.86
10k	419.44	6.25	120.23	1.93	−5.63	0.391	5.49	4.94
10l	413.39	6.25	118.01	1.37	−5.88	0.379	5.83	3.61
10m	407.34	24.71	107.33	0.00	−4.49	0.388	7.08	0.00
10n	408.96	33.94	120.95	−0.09	−3.94	0.295	6.12	−0.30
10o	431.33	6.25	107.54	1.26	−5.04	0.370	5.76	3.41
10p	431.33	6.25	107.54	1.26	−5.04	0.396	6.26	3.18
10q	388.34	30.04	107.29	0.24	−4.35	0.345	5.80	0.70
10r	319.87	19.14	98.89	−0.76	−3.03	0.369	6.69	−2.06
10s	369.93	19.14	114.96	0.62	−4.70	0.367	6.34	1.69
10t	363.88	49.39	107.88	0.32	−4.71	0.379	6.60	0.84
12b	379.80	8.88	113.49	−3.35	−3.34	0.313	8.37	−10.71

Activities of series **9** are already published (Mohsin et al. 2018a, 2018b)

[a]Values were calculated using the ChemAxon software

[b]LE = (1.37/HAxplC$_{50}$ [kcal/mol/HA]

[c]LLE = plC$_{50}$ − cLogP

[d]LELP = cLogP/LE. HA = number of heavy atoms

plC$_{50}$ = negative logarithmic value of IC$_{50}$ values against P.falc.NF54. cLogP = calculated log *P* value

refractivity (MR), log P, logD (pH = 7.4), and log S using ChemAxon software. Moreover, the ligand efficiency (LE), the lipophilic ligand efficiency (LLE) and the lipophilicity-corrected ligand efficiency (LELP) were calculated (Hopkins et al. 2014). The results are listed in Table 3.

The antiplasmodial activity and the selectivity of the piperidinium compounds **10a-t** was compared with those of their formerly prepared pyrrolidinium analogs **9a-t**. The replacement of the pyrrolidinium by a piperidinium moiety changed the activity against *P. falciparum* at the maximum of factor 2. Among the more active compounds ($IC_{50} < 0.1$ μM) **10b** and **10m** ($IC_{50} = 0.045$, 0.083 μM) were about half as active as **9b** and **9m** ($IC_{50} = 0.027$, 0.046 μM). On the other hand, compounds μm**10g** and **10o** ($IC_{50} = 0.023$, 0.095 μm) were ca. twice as active as **9g** and **9o** ($IC_{50} =$ 0.043, 0.18 μM). The selectivity (IC_{50}(L6)/IC_{50}(P.falc.)) of compounds changed more significant up to a factor of ~ 20. Among the more active compounds, **10d**, **10e**, **10f**, **10l**, **10m**, and **10p** lost selectivity ($SI_P = 4.92$–2293) compared to **9d**, **9e**, **9f**, **9l**, **9m**, and **9p** ($SI_P = 9.688$–5348). However, the selectivities of **10h** and **10o** ($SI_P = 1511$, 28.53) are comparable to those of **9h** and **9o** ($SI_P = 1528$, 38.61). A significant increase of selectivity was observed for compounds **10b** and **10g** ($SI_P = 2576$, 1375) compared with **9b** and **9g** ($SI_P = 743.3$, 69.53). The most promising of the new compounds is **10g**, which showed high activity and excellent selectivity ($IC_{50} = 0.023$ μM, $SI_P = 1375$). The necessity of the conjugated double bond system in this compound series was approved by the comparison of the properties of compounds **12b** ($IC_{50} = 9.51$ μM, $SI_P = 5.30$) and **9b** (IC_{50} = 0.027 μM, $SI_P = 743.3$). Compound **12b** is aside from the aromatic ring system fully saturated. It exhibited only negligible antiplasmodial activity and selectivity.

Concerning the antitrypanosomal activity the results show clearly, that all piperidino compounds **10a-t** are more active than their pyrrolidino analogs **9a-t**. The factor is between 1.5-fold (**9h**, **10h**) and 58-fold (**9a**, **10a**). Compound **10l** showed very good activity ($IC_{50} = 0.058$ μM, SI_T = 5.34) but at the same time low selectivity. In general, compounds **10** are more selective against *T. b. rhod.* than their pyrrolidino analogoues **9** except **10m** and **10o** which show lower selectivity than **9m** and **9o**, respectively.

Compounds with the highest selectivity against *P. falciparum* NF54 **10b, 10f**, and **10p** ($SI_p = 2576$, 2293, and 1471) were further investigated in an in vivo mouse model against *P. berghei* but showed rather low in vivo activity compared with compounds **9**: no prolongation of the mean survival days (MSD) was observed and the parasitaemia was reduced only weakly (22.5%). Compound **10p** is even toxic, the mice died after one application of 50 mg/kg on day 1.

The correlation of the biological activities with the calculated physicochemical parameters have been analyzed carefully. We found out, that some relationship of the antiplasmodial activity with log *P* values can be observed and are shown in Fig. 2.

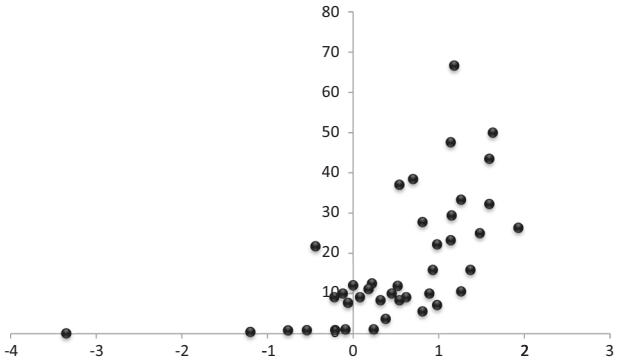


Fig. 2 Correlation between the reciprocal IC_{50} value (against *P. falc.*) and log P

In Fig. 2 it can be clearly seen, that the more-active compounds display rather higher log *P* values than the weaker ones and have, therefore, higher lipophilicity. Generally, the physicochemical properties of all the compounds meet the criteria for oral absorption of Lipinski's rule of five (MW, Log P, number of H-donors, numbers of H-acceptors) as well as the variants of Ghose (Log P, MR, MW, number of atoms, tPSA) (Ghose et al. 1999) and Veber (number of rotatable bonds, tPSA) (Veber et al. 2002). Furthermore, all compounds, except **12b** (LELP = −10.71), show values for the ligand efficiency LE (LE > ~ 0.3), for LLE (LLE > ~ 5), and LELP (−10 < LELP < 10), which are proposed acceptable values for drug candidates (Hopkins et al. 2014).

Conclusions

Summing up the results of this study, it may be said that the change of the amino residue from pyrrolidin- to a piperidin-moiety does not influence the antiplasmodial activity of 1-benzyl tetrahydropyridinylidene salts very much, whereas the antitrypanosomal activity was raised for all new compounds. The removal of the double bond system causes a complete loss of both activities so it is a pre-requisite for the antiprotozoal action of these compounds. Investigation of their physicochemical parameters revealed that they all fulfill the requirements for drug candidates.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

Ethical approval All protocols and procedures used in both in vivo studies were reviewed and approved by the local veterinary authorities of the Canton Basel-Stadt.

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