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Synthesis of new tetrahydropyridinylidene ammonium salts and their antiprotozoal potency

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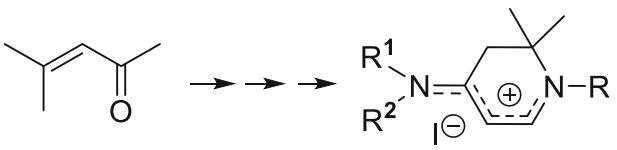
Abstract Several new tetrahydropyridinylidene ammonium salts were prepared by selective reduction. They were characterized using UV–Vis spectroscopy, FT-IR spectroscopy, and HRMS. Their structure was established by NMR spectroscopy and a single X-ray structure analysis. One compound shows a distinct antiplasmodial potency ($IC_{50} = 0.34$ μM) against the multiresistant K_1-strain of *Plasmodium falciparum* and low cytotoxicity ($IC_{50} = 199.8$ μM) against L-6 cells (rat skeletal myoblasts).

Graphical abstract



Keywords Reductions · X-ray structure determination · Heterocycles · Drug research

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Introduction

According to the latest estimates, malaria mortality rates were reduced by about 42 % globally and by 49 % in the WHO African Region between 2000 and 2012. But parasite resistance to artemisinin—the core compound in the world's most effective antimalarial medicines—and mosquito resistance to insecticides remain major concerns. If not addressed with appropriate urgency, they could threaten the remarkable progress made since 2000 [1]. So the development of new and effective antimalarial drugs is of urgent need.

Human African trypanosomiasis, which is fatal if left untreated, affects rural populations in sub-Saharan Africa. Although the present number of cases (50,000–70,000) seems negligible on a worldwide scale, the characteristics and focal distribution of the disease can have a great socioeconomic effect on affected villages. Diagnosis and treatment are unsatisfactory and needs more research and development [2]. Only five drugs are in use at the present, which are not effective against all stages and strains of trypanosomes. Melarsoprol, an organic arsenical, is the only drug used to treat late stage *Trypanosoma brucei rhodesiense* infection. Unfortunately, this drug induces an extremely severe post-treatment reactive encephalopathy (PTRE) in up to 10 % of treated patients, half of whom die from this complication [3]. Therefore, new trypanocidal compounds with less side effects are of great demand.

Several 4-aminopyridines showed antimalarial activity [4–6]. We, therefore, prepared a series of new pyridinylidene salts with differing 4-amino substitution and investigated them for their activities against *Plasmodium falciparum* and *T. b. rhodesiense* as well as for their cytotoxicity.

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Results and discussion

Chemistry

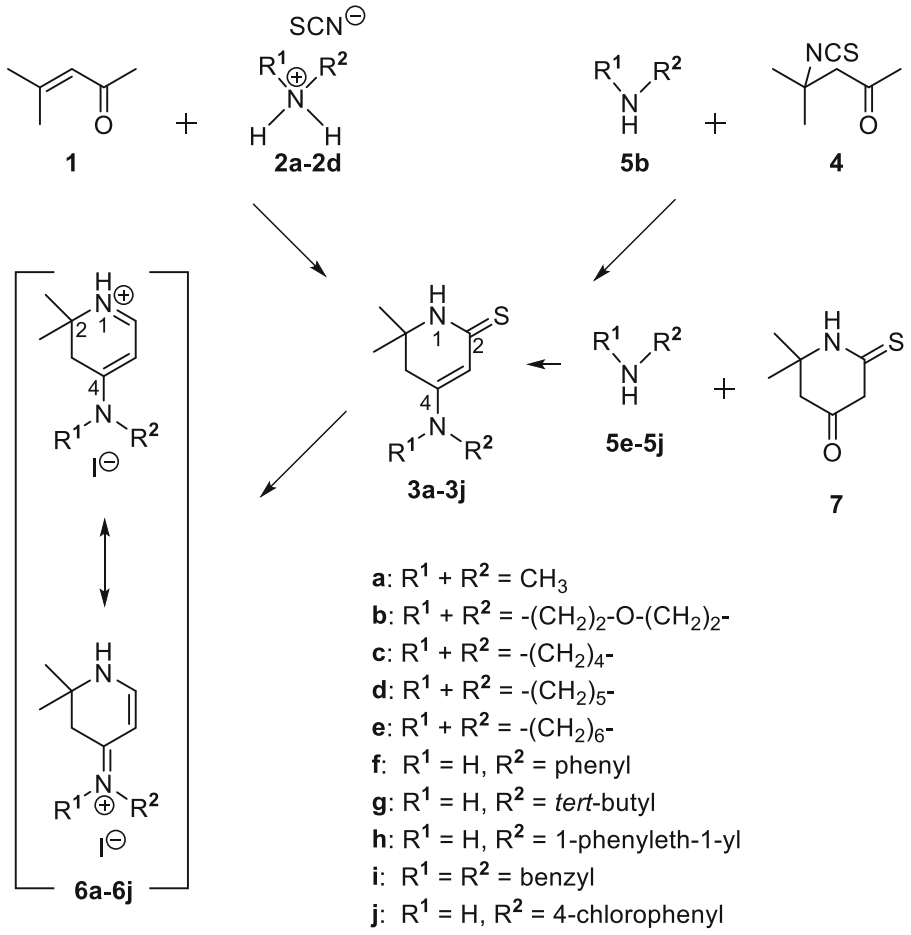
Our synthetic pathway started from mesityl oxide (**1**) and thiocyanates of secondary amines **2a**–**2d** reacting to tetrahydropyridinethiones **3a**–**3d** [7]. Alternatively, a reaction of 4-isothiocyanato-4-methylpentan-2-one (**4**) with secondary amine **5b** yielded **3b** [8]. Thiones **3a**–**3d** were transformed to the tetrahydropyridinylidene salts **6a**–**6d** by a S-methylation/reduction procedure using deactivated Raney nickel for the selective reduction process. To vary the 4-amino substituents of **6**, it was in some cases easier to prepare thiones **3e**–**3j** via reaction of compound **7** and the appropriate amine **5e**–**5j** [9] followed by above-mentioned steps to yield **6e**–**6j** (Scheme 1).

The preservation of the double bonds in compounds **6** was verified by ^{1}H NMR measurements. Instead of a singlet at 5.2 ppm for the olefinic proton in compounds **3**, two doublets with a coupling constant of approximately 6 Hz appear at 5.2 and 7.6 ppm for the olefinic protons at positions 5 and 6 in compounds **6**. In ^{13}C spectra, the signal of a quaternary carbon for the thione group at 188 ppm is exchanged with a signal for an olefinic carbon at 155 ppm. The crystal structure analysis of **6a** confirmed the compound as 4-(dimethylamino)-2,2-dimethyl-2,3-dihydropyridinium iodide (Fig. 1) and is the first structure determination of a protonated 4-amino-2,3-dihydropyridine. All atoms lie on general positions. By the protonation of N1 a planarization of N4 (sum of the three bond angles: 359.44° at N4, 358.7° at N1) is observed according to the two following mesomeric structures which are supported by the determined bond lengths (N1–C2 1.473(2) Å, N1–C6 1.317(2) Å, C4–N4 1.3229(19) Å, C2–C3 1.532(2) Å, C4–C3 1.506(2) Å, C4–C5 1.402(2) Å, C5–C6 1.377(2) Å). In the packing (Fig. 2), the ion-pairs [N1–H1···I1 172(2)°, N1–H1···I1 3.5421(15) Å] are arranged with the iodides in tubes parallel to the a axis.

Antiprotozoal activity

All new tetrahydropyridinylidene salts **6a**–**6j** were tested against *Plasmodium falciparum* (NF54 strain) and

Scheme 1



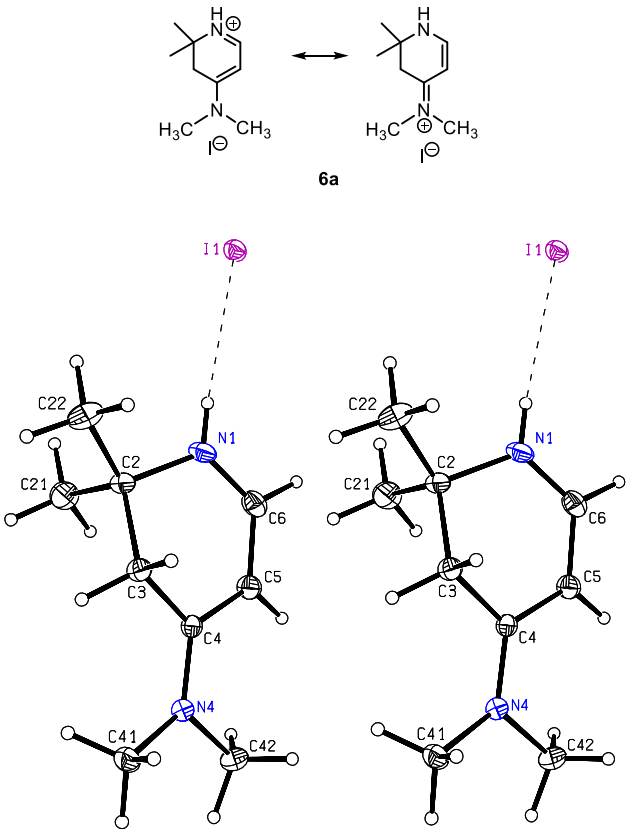


Fig. 1 Stereoscopic ORTEP [10] plot of the asymmetric unit of **6a** showing the atomic numbering scheme. The probability ellipsoids are drawn at the 50 % probability level. The H atoms are drawn with arbitrary radii. The hydrogen bond is indicated by a *dashed line*

Trypanosoma brucei rhodesiense as well as for their cytotoxicity which was determined using L-6 cells. For comparative reasons, some of the thiones **3a**–**3d** were tested too. The results are presented in Table 1.

The antiprotozoal potency of thiones **3a**–**3d** is low, in fact they are not active at all. Concerning the antiprotozoal properties of the tetrahydropyridinylidene salts **6a**–**6j**, the promising antitrypanosomal potency of compounds **6i** ($IC_{50} = 3.52$ μM) and **6j** ($IC_{50} = 5.40$ μM) is worth mentioning. Furthermore, the selective reduction to tetrahydropyridinylidene salts **6a**–**6d** causes a distinct increase of the antiplasmodial activity in case of substitution **a**, **c**, and **d**. The other compounds **6d**–**6j** show comparable potencies. The most active compound of this series is *N*-(2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)pyrrolidiniumiodide (**6d**, $IC_{50} = 2.53$ μM) showing a good selectivity ($SI = IC_{50tox.}/IC_{50p.f.} = 76$). It was tested against the multiresistant strain *Plasmodium falciparum* K_1 (resistant to chloroquine and pyrimethamine) showing an even higer activity ($IC_{50} = 1.73$ μM). This was the reason for a further derivatization of compound **6c**. To insert an aromatic residue attached to the ring nitrogen, we started from thiopyrane thione **8** which was S-methylated to **9** giving after aminolysis with aniline compound **10**. A subsequent Dimroth reaction leads to thione **11** which can be converted by our S-methylation/reduction process to the desired product **12** (Scheme 2).

This compound **12** was also tested for its antiprotozoal activities. The result was a distinct increase of the antiplasmodial activity into the submicromolar area ($IC_{50} = 0.36$ μM), so the attachment of the phenyl ring causes a 7-fold increase in activity (Table 1). Fortunately, the toxicity remains nearly the same compared with **6c** (**6c**: $IC_{50} = 192.4$ μM; **12**: $IC_{50} = 199.8$ μM), therefore, compound **12** shows high selectivity ($SI = 555$). The activity against the multiresistant strain *Plasmodium falciparum* K_1 was even better, the IC_{50} was 0.34 μM.

Conclusion

The investigation of a series of tetrahydropyridinylidene salts, which were produced by a selective S-methylation/reduction process from their corresponding thiones revealed, that the amino substitution in position 4 of such compounds influences the antiprotozoal activity distinctly. The 4-pyrrolidino-substituted compound showed highest antiplasmodial activity which was 7-fold increased by an attachment of a phenyl residue to the pyridine nitrogen. From these results, further investigations seem to be worthwhile.

Experimental

Melting points were obtained on a digital melting point apparatus Electrothermal IA 9200. IR spectra: infrared spectrometer system 2000 FT (Perkin Elmer). UV/VIS: Lambda 17 UV/VIS-spectrometer (Perkin Elmer) carried out in CH_3OH solutions. NMR spectra: Varian Inova 400 (300 K) 5 mm tubes, spectra were acquired in DMSO-d_6. Chemical shifts were recorded in parts per million (ppm), for ^1H spectra the solvent signal at 2.49 ppm was used as standard and for ^{13}C spectra the central peak of the DMSO peak was used as the internal reference (39.7). Abbreviations: aromatic H, ArH; aromatic C, ArC, quaternary aromatic C, ArC_q. Signal multiplicities are abbreviated as follows: br, broad; d, doublet; m, multiplet; q, quartet; s, singlet. Coupling constants (*J*) are reported in Hertz (Hz). ^1H and ^{13}C resonances were assigned using $^1H,^1H$- and $^1H,^{13}C$-correlation spectra. ^1H and ^{13}C resonances are numbered as given in the formulae. The signals with an asterisk are interchangeable. HR-MS: GCT-Premier, Waters (EI, 70 eV), Micromass tofspec 3E spectrometer (MALDI). Crystal structure analysis was performed on a STOE four circle diffractometer. Materials: column chromatography (CC): silica gel (Merck, silica gel 60

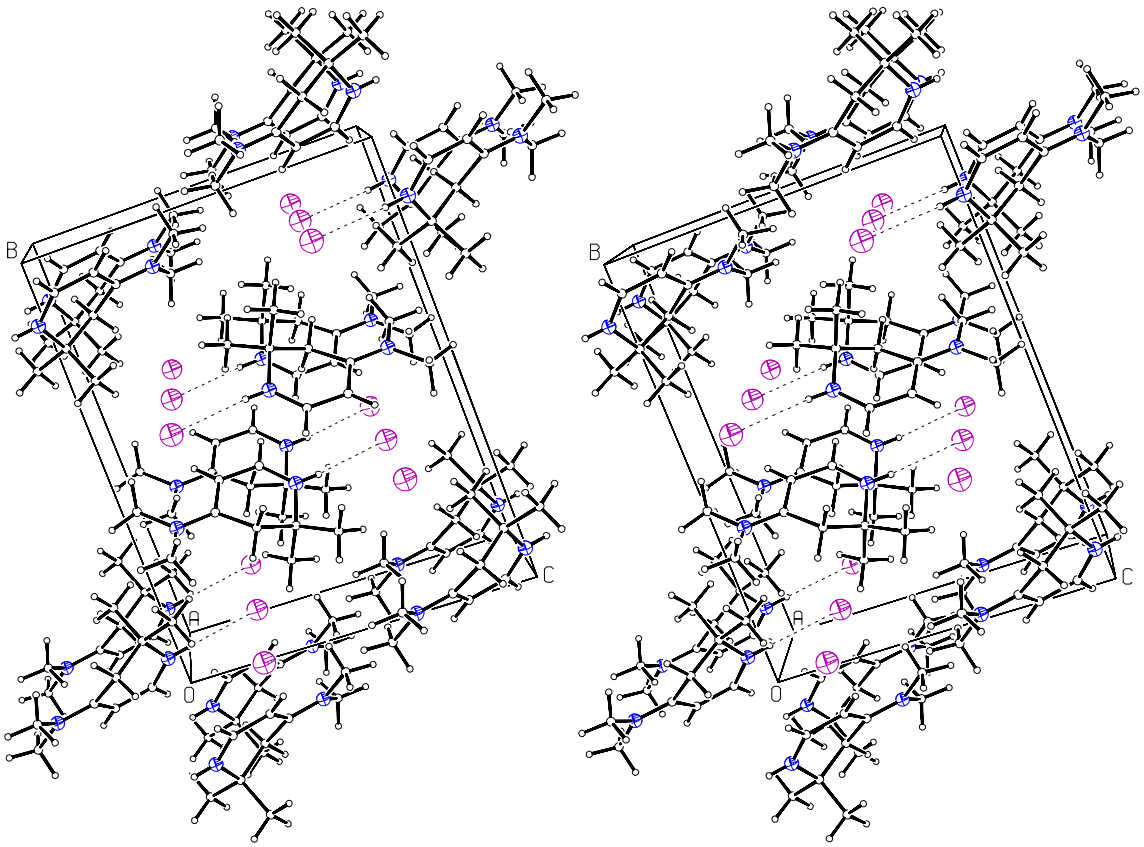


Fig. 2 Stereoscopic ORTEP [10] plot of the packing of **6a**. The atoms are drawn with arbitrary radii, the hydrogen bonds are indicated by *dashed lines*

(0.063–0.200 mm); thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F_{254} 0.2 mm, 200 × 200 mm); the substances were detected in UV light at 254 nm.

General procedure for the preparation of 4-(alkylamino)- and 4-(dialkylamino)-5,6-dihydro-6,6-dimethylpyridin-2(1H)-thiones 3a–3d

A mixture of the thiocyanates of the secondary amines, mesityloxide, and bromobenzene was refluxed over a water separator, filled with molecular sieves (0.4 nm) as long as the boiling point of pure bromobenzene was reached. This process took usually 4 h. The product precipitated from the solution and was further purified.

*4-(Dimethylamino)-5,6-dihydro-6,6-dimethyl-2(1H)-pyridinthione (**3a**)*

A mixture of 52 g of *N*,*N*-dimethylammonium thiocyanate (0.5 mol) and 98 g of mesityloxide (1 mol) in 350 cm^3 bromobenzene reacted to a product which crystallized upon cooling. It was sucked off, washed with 2-propanol, and recrystallized from 2-propanol giving 80.6 g (88 %) of **3a** as light brownish prisms. M.p.: 225 °C, corresponds well with the reported melting point: 220 °C [11].

*5,6-Dihydro-6,6-dimethyl-4-(1-morpholinyl)pyridine-2(1H)-thione (**3b**)*

A mixture of 23.5 g of morpholinium thiocyanate (0.16 mol) and 32.1 g of mesityloxide (0.321 mol) in 125 cm^3 bromobenzene gave a product which crystallized over night at room temperature. It was sucked of, washed with 2-propanol, and recrystallized from 2-propanol/CH_2Cl_2 giving 27.1 g (74 %) of **3b** as yellowish needles. M.p.: 256 °C, corresponds well with the reported melting point: 255–258 °C [7].

*5,6-Dihydro-6,6-dimethyl-4-(1-pyrrolidinyl)pyridine-2(1H)-thione (**3c**)*

A mixture of 54.6 g of pyrrolidinium thiocyanate (0.45 mol) and 90 g of mesityloxide (0.9 mol) in 350 cm^3 bromobenzene gave a solution from which some of the solvent was removed by evaporation in vacuo. The resulting solution was left in the refrigerator and the product crystallized overnight. It was sucked of, washed with 2-propanol, and recrystallized from 2-propanol giving 19 g (20 %) of **3c** as green needles. M.p.: 250 °C, corresponds well with the reported melting point: 254 °C [12].

Table 1 Antiprotozoal activities (IC50/μM) of compounds **3**, **6**, and **12**

Entry	*P.falc.*NF54	*T.b.rhod.*	Cytotox.
3a	>271.3	266.4	310.4
3b	>220.9	329.6	292.5
3c	120.3	80.82	161.2
3d	77.10	27.86	140.0
6a	10.21	>357.0	305.9
6b	>155.2	310.4	212.0
6c	2.53	>326.6	192.4
6d	21.61	249.2	277.6
6e	7.69	97.62	>299.2
6f	69.93	19.17	193.7
6g	25.37	173.1	>324.5
6h	68.58	47.27	185.6
6i	6.99	3.52	80.77
6j	26.06	5.40	125.0
12	0.36	37.35	199.8
chl	0.006		188.5
mel		0.004	7.78
mef			11.37

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

chl chloroquine, *mel* melarsoprol, *mef* mefloquine

*5,6-Dihydro-6,6-dimethyl-4-(1-piperidinyl)pyridine-2(1H)-thione (**3d**)*

A mixture of 32.5 g of piperidinium thiocyanate (0.23 mol) and 45 g of mesityloxide (0.45 mol) in 175 cm^3 bromobenzene gave a product which crystallized overnight. It was sucked off, washed with 2-propanol, and recrystallized from 2-propanol giving 27.4 g (54 %) of **3d** as bright yellow needles. M.p.: 217 °C, corresponds well with the reported melting point: 217 °C [12].

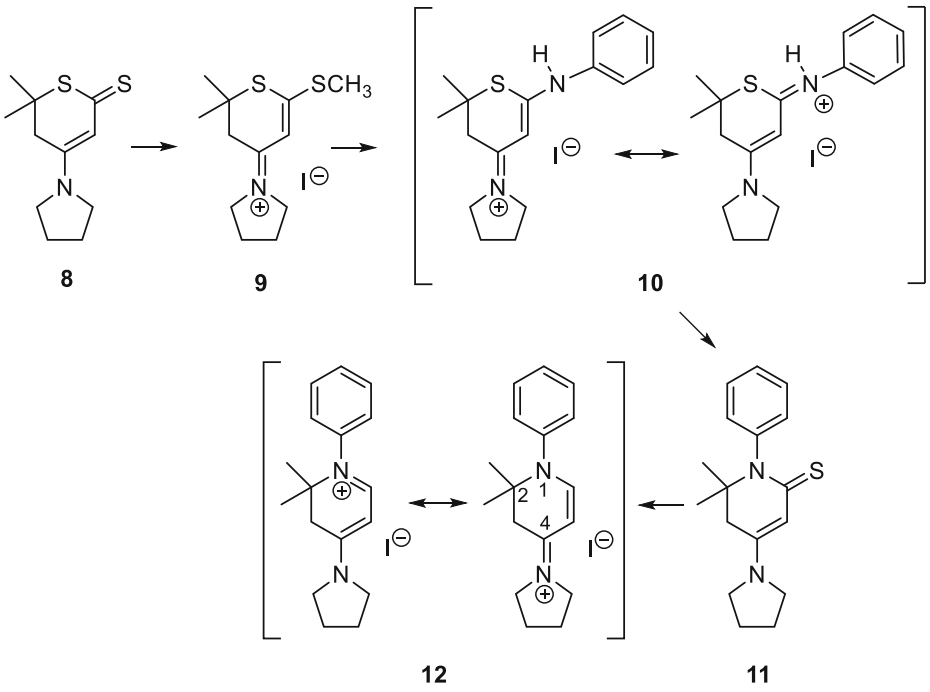
General procedure for the preparation of 4-(alkylamino)- and 4-(dialkylamino)-5,6-dihydro-6,6-dimethylpyridine-2(1H)-thiones 3e, 3g–3j

Compound **7** was dissolved in benzene, glacial acetic acid was added, and a solution of the primary or secondary amine in benzene was added too. The mixture was refluxed for 4 h on a water separator and cooled to room temperature. The product crystallized from the reaction mixture, was sucked off, and recrystallized from ethanol or 2-propanol.

4-(Azepan-1-yl)-6,6-dimethyl-5,6-dihydropyridine-2(1H)-thione (**3e**, $C_{13}H_{22}N_2S$)

Reaction of 3.15 g of **7** (20 mmol) dissolved in 50 cm^3 with 1.98 g azepane (20 mmol) dissolved in 16 cm^3

Scheme 2



benzene in the presence of 1.2 cm^3 glacial acetic acid yielded 2.04 g (43 %) of **3e**. For analytical purposes, a further crystallization was done from ethanol after treatment with charcoal. R_f ($CHCl_3$:benzene:EtOH = 4:4:1) = 0.44; m.p.: 233 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.18 (s, 6H, 2 CH_3), 1.47 (br, s, 4H, H-3′), 1.63 (br, s, 4H, H-2′), 2.40 (s, 2H, H-5), 3.38 (br, s, 4H, H-1′), 5.19 (s, 1H, H-3), 8.15 (s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.52 (C-2′), 26.11 (C-3′), 26.94 (2 CH_3), 36.90 (C-5), 49.66 (C-1′), 52.30 (C-6), 95.64 (C-3), 152.61 (C-4), 188.40 (C-2) ppm; IR (KBr): $\bar{\nu}$ = 3182, 2968, 2931, 1560, 1542, 1507, 1485, 1449, 1412, 1376, 1363, 1345, 1164, 1105, 1028, 772 cm^{-1}; UV (CH_3OH): λ (log ε) = 346 (4.495), 206 (4.066) nm; HRMS (EI^+): calcd. $C_{13}H_{22}N_2S$ [M^+] 238.1504, found 238.1516.

*6,6-Dimethyl-4-(phenylamino)-5,6-dihydropyridine-2(1H)-thione (**3f**)*

This compound was prepared according a reported procedure [8]. Its melting point m.p.: 230 °C (Ref. [8] 234 °C) corresponds well with the reported one.

4-(tert-Butylamino)-6,6-dimethyl-5,6-dihydropyridine-2(1H)-thione (**3g**, $C_{11}H_{20}N_2S$)

Reaction of 4.71 g of **7** (30 mmol) dissolved in 100 cm^3 benzene with 2.19 g *tert*-butylamine (30 mmol) dissolved in 20 cm^3 benzene in the presence of 1.7 cm^3 glacial acetic acid yielded 960 mg (15 %) of **3g** after recrystallization from ethanol. R_f ($CHCl_3$:benzene:EtOH = 4:4:1) = 0.30; m.p.: 296 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.14 (s, 6H, 2 CH_3), 1.28 (s, 9H, $C(CH_3)_3$), 2.18 (s, 2H, H-5), 5.27 (s, 1H, H-3), 6.44 (s, 1H, NH), 8.20 (s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 26.80 (2 CH_3), 28.69 ($C(CH_3)_3$), 40.86 (C-5), 51.49, 52.19 (C-6, $C(CH_3)_3$), 96.31 (C-3), 150.07 (C-4), 188.71 (C-2) ppm; IR (KBr): $\bar{\nu}$ = 3262, 3174, 2969, 1578, 1536, 1510, 1462, 1406, 1366, 1209, 1142, 1105, 1003, 806, 723 cm^{-1}; UV (CH_3OH): λ (log ε) = 331 (4.351), 210 (3.859) nm; HRMS (EI^+): calcd. $C_{11}H_{20}N_2S$ [M^+] 212.1347, found 212.1349.

6,6-Dimethyl-4-(1-phenylethylamino)-5,6-dihydropyridine-2(1H)-thione (**3h**, $C_{15}H_{20}N_2S$)

Reaction of 3.15 g of **7** (20 mmol) dissolved in 60 cm^3 benzene with 2.42 g of 1-phenylethylamine (20 mmol) dissolved in 15 cm^3 benzene in the presence of 1.2 cm^3 glacial acetic acid yielded 4.51 g (87 %) of **3h**. For analytical purposes, a further crystallization was done after treatment with charcoal from 2-propanol. R_f ($CHCl_3$:benzene:EtOH = 4:4:1) = 0.32; m.p.: 208 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.12 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.37 (d, J = 7.0 Hz, 3H, $CHCH_3$), 2.29 (s, 2H, H-5), 4.49 (t, J = 6.6 Hz, 1H, $CHCH_3$), 4.91 (s, 1H, H-3), 7.20–7.35 (m, 6H, NH, ArH), 8.19 (s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 23.96 ($CHCH_3$), 26.71, 27.10 (2 CH_3), 39.89 (C-5), 51.90 ($CHCH_3$), 52.37 (C-6), 95.47 (C-3), 125.70, 127.04, 128.70 (ArC), 144.43 (ArC_q), 150.89 (C-4), 189.21 (C-2) ppm; IR (KBr): $\bar{\nu}$ = 3202, 3041, 2973, 1580, 1543, 1508, 1447, 1415, 1181, 1101, 967, 700 cm^{-1}; UV (CH_3OH): λ (log ε) = 331 (4.456), 209 (4.166) nm; HRMS (EI^+): calcd. $C_{15}H_{20}N_2S$ [M^+] 260.1347, found 260.1340.

4-(Dibenzylamino)-6,6-dimethyl-5,6-dihydropyridine-2(1H)-thione (**3i**, $C_{21}H_{24}N_2S$)

Reaction of 6.29 g of **7** (40 mmol) dissolved in 120 cm^3 benzene with 7.89 g dibenzylamine (40 mmol) dissolved in 30 cm^3 benzene in the presence of 2.4 cm^3 glacial acetic acid yielded 2.24 g (17 %) of **3i**. For analytical purposes, a further crystallization from ethanol was done after treatment with charcoal. R_f ($CHCl_3$:benzene:EtOH = 4:4:1) = 0.57; m.p.: 175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.15 (s, 6H, 2 CH_3), 2.49 (s, 2H, H-5), 4.59 (s, 4H, $ArCH_2$), 5.25 (s, 1H, H-3), 7.19-7.38 (m, 10H, ArH), 8.41 (br, s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 26.99 (2 CH_3), 37.33 (C-5), 52.57 (C-6), 53.12 ($ArCH_2$), 97.92 (C-3), 126.46, 127.33, 128.87 (ArC), 137.36 (ArC_q), 152.66 (C-4), 188.90 (C-2) ppm; IR (KBr): $\bar{\nu}$ = 3170, 1555, 1509, 1454, 1411, 1373, 1342, 1178, 1110, 953, 740, 693 cm^{-1}; UV (CH_3OH): λ (log ε) = 340 (4.484), 207 (4.375) nm; HRMS (EI^+): calcd. $C_{21}H_{24}N_2S$ [M^+] 336.1660, found 336.1653.

*6,6-Dimethyl-4-(4-chlorophenylamino)-5,6-dihydropyridine-2(1H)-thione (**3j**)*

This compound was prepared according a reported procedure [8]. Its melting point m.p.: 245 °C (Ref. [8] 245–247 °C) corresponds well with the reported one.

General procedure for the preparation of (2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)ammonium iodides 6a–6j and 12

A mixture of chloroform and methyl iodide was added to the dihydropyridinethiones **3a**–**3j** or **11** and stirred over night at room temperature. The solution was concentrated, cooled with an ice bath, and the methylthio compounds precipitated upon the addition of ethyl acetate.

Powdered nickel/aluminum alloy was given into a big beaker, water was added and solid NaOH was added cautiously. 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 with water until the solution reacted neutral. After that it was washed twice with ethanol and given to a solution of the methylthio compound in ethanol. Then it was stirred for 30–45 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, filtered, and the solvent evaporated in vacuo giving the reduction products as green resins, which were dissolved in chloroform. Ethyl acetate was added. The product precipitated, was filtered with suction and dissolved in chloroform. The solution was treated with charcoal, filtered, and ethyl acetate was added. The precipitate was filtered with suction and dried at 100 °C under reduced pressure.

N,N-Dimethyl-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-iminium iodide (**6a**, $C_9H_{17}IN_2$)

A solution of 33.9 g **3a** (0.19 mol) in 90 cm^3 of chloroform reacted with 31.2 g methyl iodide (0.22 mol) dissolved in 90 cm^3 of chloroform to a precipitate which was treated in a total volume of 500 cm^3 ethanol with Raney nickel prepared from 150 g nickel/aluminum alloy and 300 g of caustic soda giving 31.7 g (74 %) **6a** as yellow needles. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.09; m.p.: 148 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.26 (s, 6H, 2 CH_3), 2.85 (s, 2H, H-3), 3.17, 3.24 (2 s, 6H, $N(CH_3)_2$), 5.20 (d, J = 6.6 Hz, 1H, H-5), 7.61 (d, J = 6.6 Hz, 1H, H-6), 9.35 (br, s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.66 (2 CH_3), 37.45 (C-3), 40.66, 41.18 ($N(CH_3)_2$), 51.40 (C-2), 86.35 (C-5), 154.05 (C-6), 165.61 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 3156, 3126, 3044, 2955, 1612, 1556, 1420, 1410, 1354, 1278, 1250, 1130, 785 cm^{-1}; UV (CH_3OH): λ (log ε) = 340 (4.246), 220 (4.175) nm; HRMS (MALDI): calcd. $C_9H_{17}N_2$ [MH^+] 153.1392, found 153.1375.

Crystal structure analysis of 6a

All the measurements were performed on a Bruker APEX-II CCD diffractometer using graphite-monochromatized Mo K_α radiation at 100 K on a single crystal (0.24 × 0.24 × 0.15 mm): $C_9H_{17}IN_2$, M_r = 280.15, monoclinic, space group P 2_1/c, a = 6.5347(4) Å, b = 15.0191(8) Å, c = 12.1232(8) Å, β = 103.884(2)°, V = 1155.07(12) Å^3, Z = 4, d_{calc} = 1.611 g cm^{-3}, μ = 2.730 mm^{-1}. A total of 8007 reflections were collected (Θ_{max} = 30.0°), from which 3342 were unique (R_{int} = 0.0205), with 2957 having $I > 2\sigma(I)$. The structure was solved by direct methods (SHELXS-97) [13] and refined by full-matrix least-squares techniques against F^2 (SHELXL-97) [13]. The non-hydrogen atoms were refined with anisotropic displacement parameters without any constraints. The H atom bonded to N1 was refined without any positional constraints with an individual isotropic displacement parameter. The H atoms of the CH_2 group were refined with a common isotropic displacement parameter and idealized geometry with approximately tetrahedral angles and C–H distances of 0.99 Å. The H atoms H5 and H6 were put at the external bisector of the C–C–C angle at a C–H distance of 0.95 Å and one common isotropic displacement parameter was refined for these H atoms. 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 rotation around the X–C bond, and C–H distances of 0.98 Å. For 123 parameters final R indices of $R1$ = 0.0181 and wR^2 = 0.0469 (GOF = 1.084) were obtained. The largest peak in a difference Fourier map was 0.528 e Å^{-3}. The final atomic parameters, as well as bond lengths and angles are deposited at the Cambridge Crystallographic Data Centre (CCDC 1047057).

N-(2,2-Dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)-morpholinium iodide (**6b**, $C_{11}H_{19}IN_2O$)

A solution of 5.8 g **3b** (25 mmol) in 10 cm^3 of chloroform reacted with 4.3 g methyl iodide (31 mmol) dissolved in 10 cm^3 of chloroform to precipitate which was were treated in a total volume of 150 cm^3 of ethanol with Raney nickel prepared from 19 g nickel/aluminum alloy and 38 g of caustic soda giving 2.8 g (46 %) **6b** as yellow needles. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.11; m.p.: 138 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.27 (s, 6H, 2 CH_3), 2.86 (s, 2H, H-3), 3.69 (s, br, 8H, $N(CH_2)_2$, $O(CH_2)_2$), 5.40 (d, J = 6.2 Hz, 1H, H-5), 7.69 (d, J = 6.2 Hz, 1H, H-6), 9.55 (br, s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.69 (2 CH_3), 36.84 (C-3), 48.08 ($N(CH_2)_2$), 51.56 (C-2), 65.74, 66.36 ($O(CH_2)_2$), 86.32 (C-5), 155.01 (C-6), 164.22 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 3134, 3091, 3009, 2961, 2933, 1603, 1552, 1427, 1398, 1368, 1348, 1319, 1258, 1181, 1108, 1066, 1036, 951, 741 cm^{-1}; UV (CH_3OH): λ (log ε) = 343 (4.246), 223 (3.956) nm; HRMS (MALDI): calcd. $C_{11}H_{19}N_2O$ [M-I]$^+$ 195.1497, found 195.1489; calc. $C_{22}H_{38}N_4IO_2$ [2M-I]$^+$ 517.2040, found 517.2068.

N-(2,2-Dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)pyrrolidinium iodide (**6c**, $C_{11}H_{19}IN_2$)

A solution of 26.4 g **3c** (124 mmol) in 40 cm^3 of chloroform reacted with 21.1 g methyl iodide (148 mmol) dissolved in 40 cm^3 of chloroform to a precipitate which was treated in a total volume of 300 cm^3 with Raney nickel prepared from 100 g nickel/aluminum alloy and 200 g of caustic soda giving 23 g (73 %) **6c** as yellow needles. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.17; m.p.: 192 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.27 (s, 6H, 2 CH_3), 1.91–1.97 (m, 4H, $(CH_2)_2$), 2.84 (s, 2H, H-3), 3.49 (t, J = 6.2 Hz, 2H, NCH_2), 3.66 (t, J = 6.2 Hz, 2H, NCH_2), 5.09 (d, J = 6.6 Hz, 1H, H-5), 7.58 (d, J = 6.6 Hz, 1H, H-6), 9.27 (br, s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 24.29, 24.54 ($(CH_2)_2$), 25.78 (2 CH_3), 38.70 (C-3), 49.52, 49.85 ($N(CH_2)_2$), 51.31 (C-2), 86.95 (C-5), 153.87 (C-6), 162.54 (C-4) ppm; IR (KBr):

$\bar{\nu}$ = 3149, 3119, 3027, 2944, 2867, 1606, 1555, 1532, 1428, 1411, 1334, 1287, 1268, 1183, 1136, 760, 730 cm^{-1}; UV (CH_3OH): λ (log ε) = 341 (4.303), 220 (4.188) nm; HRMS (MALDI): calcd. $C_{11}H_{19}N_2$ $[M\text{-}I]^+$ 179.1548, found 179.1535.

N-(2,2-Dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)piperidinium iodide (**6d**, $C_{12}H_{21}IN_2$)
A solution of 12.7 g **3d** (57 mmol) in 35 cm^3 of chloroform reacted with 9.6 g methyl iodide (68 mmol) dissolved in 35 cm^3 of chloroform to a precipitate which was treated in a total volume of 200 cm^3 ethanol with Raney nickel prepared from 55 g nickel/aluminum alloy and 110 g of caustic soda giving 10.9 g (62 %) **6d** as yellow needles. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.13;
m.p.: 133 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.26 (s, 6H, 2 CH_3), 1.60 (s, br, 6H, 3CH_2), 2.85 (s, 2H, H-3), 3.66 (s, br, 4H, N(CH_2)$_2$), 5.38 (d, J = 6.2 Hz, 1H, H-5), 7.60 (d, J = 5.5 Hz, 1H, H-6), 9.32 (br, s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 23.41 (CH_2), 25.57 (2 CH_3), 25.85, 26.97 (2 CH_2), 36.98 (C-3), 49.03, 49.23 (N(CH_2)$_2$), 51.45 (C-2), 86.07 (C-5), 154.29 (C-6), 163.61 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 3161, 3034, 2939, 2859, 1602, 1548, 1473, 1453, 1432, 1355, 1340, 1289, 1255, 1184, 1141, 1015, 784 cm^{-1}; UV (CH_3OH): λ (log ε) = 343 (4.314), 220 (4.172) nm; HRMS (MALDI): calcd. $C_{12}H_{21}N_2$ $[M\text{-}I]^+$ 193.1705, found 193.1680.

N-(2,2-Dimethyl-1,2,3,4-tetrahydropyridin-4-yliden)-azepanium iodide (**6e**, $C_{13}H_{23}IN_2$)
A solution of 1.12 g of **3e** (4.7 mmol) in 8 cm^3 of chloroform reacted with 0.76 g methyl iodide (5.4 mmol) dissolved in 8 cm^3 of chloroform to a precipitate which was treated in a total volume of 40 cm^3 ethanol with Raney nickel prepared from 6 g nickel/aluminum alloy and 12 g of caustic soda giving 755 mg **6e** (74 %) as light yellow crystals. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.17; m.p.: 141 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.27 (s, 6H, 2 CH_3), 1.50 (s, 4H, H-3′), 1.67–1.72 (m, 4H, H-2′), 2.88 (s, 2H, H-3), 3.68-3.73 (m, 4H, H-1′), 5.27 (d, J = 6.6 Hz, 1H, H-5), 7.60 (d, J = 6.6 Hz, 1H, H-6), 9.39 (br, s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.22 (C-2′), 25.36 (2 CH_3), 25.44, 25.66 (C-3′), 28.14 (C-2′), 36.86 (C-3), 51.12 (C-1′), 51.53 (C-2), 51.69 (C-1′), 86.03 (C-5), 154.28 (C-6), 164.94 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 3160, 3036, 2965, 2931, 2862, 1601, 1554, 1484, 1465, 1451, 1427, 1369, 1343, 1277, 1249, 1187, 1174, 785 cm^{-1}; UV (CH_3OH): λ (log ε) = 342 (4.355), 220 (4.274) nm; HRMS (EI^+): calcd. $C_{13}H_{22}N_2$ $[M\text{-}HI]^+$ 206.1783, found 206.1792.

2,2-Dimethyl-N-phenyl-1,2,3,4-tetrahydropyridin-4-iminium iodide (**6f**, $C_{13}H_{17}IN_2$)
A solution of 0.73 g **3f** (3.1 mmol) in 7 cm^3 of chloroform reacted with 0.88 g methyl iodide (6.2 mmol) dissolved in 7 cm^3 of chloroform to a precipitate which was treated in a total volume of 40 cm^3 ethanol with Raney nickel prepared from 6 g nickel/aluminum alloy and 12 g of caustic soda giving 668 mg (67 %) **6f** as a light yellow crystals. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.11;
m.p.: 192 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.35 (s, 6H, 2 CH_3), 2.86 (s, 2H, H-3), 5.33 (d, J = 6.2 Hz, 1H, H-5), 7.38-7.56 (m, 5H, ArH), 7.78 (d, J = 6.2 Hz, 1H, H-6), 10.04 (br, s, 1H, H*-1), 10.87 (br, s, 1H, NH*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.41 (2 CH_3), 39.65 (C-3), 52.19 (C-2), 85.75 (C-5), 124.42, 127.55, 129.86 (ArC), 136.89 (ArC_q), 157.52 (C-6), 162.81 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 3159, 3101, 2999, 1600, 1559, 1538, 1509, 1492, 1464, 1449, 1372, 1318, 1286, 1265, 1214, 757 cm^{-1}; UV (CH_3OH): λ (log ε) = 219 (4.219), 350 (4.180) nm; HRMS (EI^+): calcd. $C_{13}H_{16}N_2$ $[M\text{-}HI]^+$ 200.1313, found 200.1303.

N-tert-Butyl-N-(2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-ylidene)ammonium iodide (**6g**, $C_{11}H_{21}IN_2$)
A solution of 0.73 g of **3g** (3.4 mmol) in 5 cm^3 of chloroform reacted with 0.55 g methyl iodide (3.9 mmol) dissolved in 5 cm^3 of chloroform to a precipitate which was treated in a total volume of 45 cm^3 ethanol with Raney nickel prepared from 6 g nickel/aluminum alloy and 12 g of caustic soda giving 602 mg (64 %) **6g** as light yellow crystals. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.14; m.p.: 170 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.23 (s, 6H, 2 CH_3), 1.37 (s, 9H, C(CH_3)$_3$) 2.64 (s, 2H, H-3), 5.35 (d, J = 6.2 Hz, 1H, H-5), 7.63 (d, J = 6.2 Hz, 1H, H-6), 9.20 (br, s, 2H, H-1, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.21 (2 CH_3), 28.40 (C(CH_3)$_3$), 40.57 (C-3), 51.77 (C-2), 54.30 (C(CH_3)$_3$), 86.04 (C-5), 155.08 (C-6), 163.05 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 3216, 3169, 3113, 3064, 3011, 2976, 2937, 1613, 1565, 1543, 1514, 1467, 1420, 1381, 1365, 1347, 1281, 1268, 1203, 1184, 1139, 802, 716 cm^{-1}; UV (CH_3OH): λ (log ε) = 334 (4.322), 219 (4.214) nm; HRMS (EI^+): calcd. $C_{11}H_{20}N_2$ $[M\text{-}HI]^+$ 180.1626, found 180.1633.

N-2,2-Dimethyl-N-(1-phenylethyl)-1,2,3,4-tetrahydropyridin-4-iminium iodide (**6h**, $C_{15}H_{21}IN_2$)
A solution of 1.48 g of **3h** (5.7 mmol) in 8 cm^3 of chloroform reacted with 1.04 g methyl iodide (7.3 mmol) dissolved in 8 cm^3 of chloroform to a precipitate which was treated in a total volume of 45 cm^3 ethanol with Raney nickel prepared from 6 g nickel/aluminum alloy and 12 g of caustic soda giving 710 mg (65 %) **6h** as light yellow crystals. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.17; m.p.: 167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.20 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.49 (d, J = 6.6 Hz, 3H, CHCH_3), 2.70 (s, 2H, H-3), 4.91 (q, J = 7.0 Hz, 1H, CHCH$_3$), 5.09 (d, J = 6.2 Hz, 1H, H-5), 7.29-7.39 (m, 5H, ArH), 7.62 (d, J = 6.2 Hz, 1H, H-6),

9.63 (br, s, 2H, H-1, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 22.61$ ($CHCH_3$), 25.25, 25.34 (2 CH_3), 39.70 (C-3), 51.86 (C-2), 53.12 ($CHCH_3$), 85.02 (C-5), 126.01, 127.69, 128.91 (ArC), 142.33 (ArC_q), 155.97 (C-6), 163.76 (C-4) ppm; IR (KBr): $\bar{\nu} = 3169$, 3027, 2996, 2892, 1605, 1572, 1542, 1507, 1453, 1418, 1383, 1372, 1344, 1296, 1272, 1185, 1177, 1124, 783, 734, 697 cm^{-1}; UV (CH_3OH): λ (log ε) = 336 (4.259), 218 (4.166) nm; HRMS (EI$^+$): calcd. $C_{15}H_{20}N_2$ [M-HI]$^+$ 228.1626, found 228.1643.

N,N-Dibenzyl-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-iminium iodide (**6i**, $C_{21}H_{25}IN_2$)
A solution of 1.22 g of **3i** (3.6 mmol) in 6 cm^3 of chloroform reacted with 0.67 g methyl iodide (4.7 mmol) dissolved in 6 cm^3 of chloroform to a precipitate which was treated in a total volume of 40 cm^3 ethanol with Raney nickel prepared from 6 g nickel/aluminum alloy and 12 g of caustic soda giving 243 mg (25 %) **6i** as a light yellow crystals. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.24; m.p.: 135 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.26$ (s, 6H, 2 CH_3), 3.01 (s, 2H, H-3), 4.87–4.93 (m, 4H, 2 NCH_2), 5.34 (d, $J = 6.2$ Hz, 1H, H-5), 7.25–7.40 (m, 10H, ArH), 7.71 (d, $J = 5.5$ Hz, 1H, H-6), 9.83 (br, s, 1H, H-1) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 25.36$ (2 CH_3), 37.41 (C-3), 51.92 (C-2), 54.42 (2 NCH_2), 87.57 (C-5), 126.82, 128.00, 129.06 (ArC), 134.43, 136.05 (ArC_q), 155.85 (C-6), 166.08 (C-4) ppm; IR (KBr): $\bar{\nu} = 3145$, 3102, 3024, 2946, 1547, 1496, 1451, 1413, 1346, 1325, 1285, 1255, 1178, 755, 739, 706 cm^{-1}; UV (CH_3OH): λ (log ε) = 211 (4.419), 346 (4.388) nm; HRMS (EI$^+$): calcd. $C_{21}H_{24}N_2$ [M-HI]$^+$ 304.1939, found 304.1952.

N-(4-Chlorophenyl)-2,2-dimethyl-1,2,3,4-tetrahydropyridin-4-iminium iodide (**6j**, $C_{13}H_{16}ClIN_2$)
A solution of 2.14 g of **3j** (8.7 mmol) in 10 cm^3 of chloroform reacted with 1.5 g methyl iodide (10.5 mmol) dissolved in 10 cm^3 of chloroform to a precipitate which was treated in a total volume of 40 cm^3 ethanol with Raney nickel prepared from 6 g nickel/aluminum alloy and 12 g of caustic soda giving 770 mg (69 %) **6j** as light yellow crystals. R_f ($CHCl_3$:benzene:MeOH:Me_2NH = 4:4:1:0.5) = 0.17; m.p.: 174 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.35$ (s, 6H, 2 CH_3), 2.85 (s, 2H, H-3), 5.35 (d, $J = 5.9$ Hz, 1H, H-5), 7.34–7.57 (m, 4H, ArH), 7.82 (d, $J = 5.9$ Hz, 1H, H-6), 10.11 (br, s, 1H, H*-1), 10.97 (br, s, 1H, NH*) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 24.39$ (2 CH_3), 38.56 (C-3), 51.26 (C-2), 84.97 (C-5), 125.25, 128.86 (ArC), 130.60, 134.63 (ArC_q), 156.90 (C-6), 161.58 (C-4) ppm; IR (KBr): $\bar{\nu} = 3154$, 3095, 2965, 1597, 1536, 1508, 1487, 1462, 1408, 1371, 1341, 1307, 1280, 1260, 1216, 1178, 1088, 1010, 744 cm^{-1}; UV (CH_3OH): λ (log ε) = 220 (4.308), 339 (4.194) nm; HRMS (EI$^+$): calcd. $C_{13}H_{15}ClN_2$ [M-HI]$^+$ 234.0924, found 234.0926.

6,6-Dimethyl-2-thioxo-4-piperidone (***7***)
This compound was prepared according a reported procedure [9]. Its melting point m.p.: 139 °C (Ref. [9] 138 °C) corresponds well with the reported one.

5,6-Dihydro-6,6-dimethyl-4-(1-pyrrolidinyl)-thiopyrane-2(1H)-thione (***8***)
This compound was prepared according a reported procedure [14]. Its melting point m.p.: 230 °C (Ref. [14] 233 °C) corresponds well with the reported one.

N-[2,3-Dihydro-2,2-dimethyl-6-(methylthio)-4H-thiopyran-4-ylidene]pyrrolidinium iodide (***9***)
This compound was prepared according a reported procedure [15]. Its melting point m.p.: 195 °C (Ref. [15] 198 °C) corresponds well with the reported one.

N-[2,3-Dihydro-2,2-dimethyl-6-(phenylimino)-4H-thiopyran-4-ylidene]pyrrolidinium iodide (***10***)
This compound was prepared according a reported procedure [16]. Its melting point m.p.: 264 °C (Ref. [16] 262 °C) corresponds well with the reported one.

5,6-Dihydro-6,6-dimethyl-1-phenyl-4-(1-pyrrolidinyl)-pyridine-2(1H)-thione (***11***)
A suspension of 8 g of **10** (19 mmol) in 140 cm^3 of 1 N NaOH was stirred for 30 min. It was extracted 3 times with chloroform, the combined organic phases were dried over sodium sulfate, filtered and the solvent evaporated in vacuo. The residue was dried by threefold codistillation with benzene and then 35 cm^3 DMF were added. The solution was refluxed overnight and then evaporated. The residue was recrystallized from ethyl acetate giving 2.76 g (51 %) of **10**. Its melting point m.p.: 235 °C (Ref. [17] 234 °C) corresponds well with the reported one.

N-[2,3-Dihydro-2,2-dimethyl-1-phenyl-4H-pyridin-4-ylidene]pyrrolidinium iodide (**12**, $C_{17}H_{23}IN_2$)
A solution of 0.8 g of **11** (2.77 mmol) in 5 cm^3 of chloroform reacted with 0.47 g methyl iodide (3.35 mmol) dissolved in 5 cm^3 of chloroform to a precipitate which was treated in a total volume of 50 cm^3 ethanol with Raney nickel prepared from 6 g nickel/aluminum alloy and 12 g of caustic soda giving 155 mg (17 %) **12** as light yellow precipitate after additional crystallization from acetone. R_f ($CHCl_3$:benzene:MeOH:Me_3N = 4:4:1:0.5) = 0.39; m.p.: 175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.30$ (s, 6H, 2 CH_3), 1.97–2.03 (m, 4H, 2 CH_2), 3.14 (s, 2H, H-3), 3.62 (t, $J = 5.9$ Hz, 2H, NCH_2), 3.78 (t, $J = 6.2$ Hz, 2H, NCH_2), 5.38 (d, $J = 7.0$ Hz, 1H, H-5), 7.35–7.47 (m, 2H, ArH), 7.49–7.52 (m, 3H, ArH), 7.61 (d, $J = 7.0$ Hz, 1H, H-6) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 24.32$

(CH_2), 24.41 (2 CH_3), 24.53 (CH_2), 40.12 (C-3), 50.24, 50.30 (2 NCH_2), 58.15 (C-2), 90.01 (C-5), 127.97, 128.98, 129.63 (ArC), 141.04 (ArC_q), 155.81 (C-6), 163.22 (C-4) ppm; IR (KBr): $\bar{\nu}$ = 2936, 1600, 1548, 1488, 1451, 1373, 1343, 1322, 1305, 1276, 1214, 1179, 1128, 974, 853, 776, 708 cm^{-1}; UV (CH_3OH): λ (log ε) = 351 (4.375), 219 (4.328) nm; HRMS (EI^+): calcd. $C_{17}H_{22}N_2$ $[M\text{-}HI]^+$ 254.1783, found 254.1784.

Antimalarial activity

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay [18, 19]), using the drug-sensitive NF54 strain (Schipol Airport, The Netherlands [20]) or the multiresistant strain K_1 and the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/cm^3 and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/dm^3), $NaHCO_3$ (2.1 g/dm^3), neomycin (100 U/cm^3), Albumax^R (5 g/dm^3) and washed human red cells A^+ at 2.5 % haematocrit (0.3 % parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/cm^3 were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4 % CO_2, 3 % O_2, 93 % N_2. After 48 h 50 mm^3 of ^{3}H-hypoxanthine (=0.5 µCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate^{TM} 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 cm^3 of scintillation fluid, and counted in a Betaplate^{TM} liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression [21] using Microsoft Excel. Chloroquine was used as control.

Antitrypanosomal activity and cytotoxicity

Minimum Essential Medium (50 mm^3) supplemented according to Baltz et al. [22] with 2-mercaptoethanol and 15 % heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 mm^3 of trypanosome suspension (*T.b.rhodesiense* STIB 900) was added to each well and the plate incubated at 37 °C under a 5 % CO_2 atmosphere for 72 h. Alamar Blue (10 mm^3) was then added to each well and incubation continued for a further 2–4 h. The plate was then read with a Millipore Cytofluor 2300 using an excitation wavelength of 530 nm and emission wavelength of 590 nm [23]. Fluorescence development was expressed as percentage of the control, and IC_{50} values determined. Melarsoprol served as standard. Cytotoxicity was assessed using the same assay and L-6 cells using mefloquine as standard.

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