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Antimycobacterial and H_1-antihistaminic activity of 2-substituted piperidine derivatives

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ABSTRACT

2-Substituted derivatives of the antihistaminic agents *Bamipine*, *Diphenylpyraline* and of their 1-phenyl analogues were tested for their antimycobacterial and H_1-antagonistic activities. They are strong H_1-receptor antagonists and also inhibit the growth of mycobacterials with a maximum MIC of 6.25 μg/mL against *Mycobacterium tuberculosis* $H_{37}Rv$. H_1-receptor antagonistic potency was slightly decreased by substitution in ring position 2 and distinctly diminished by *N*-aryl substitution. The antimycobacterial potency of *Diphenylpyraline* was in general increased by substitution in ring position 2, whereas only a few *Bamipine* derivatives showed markedly improved activity. A correlation between the two activities was not detected for those compounds.

1. Introduction

With more than 1.6 million deaths and 8.8 million new cases being reported per year tuberculosis is the leading infectious cause of death today.[1] Due to the spreading of multi-drug-resistant and extensively drug-resistant strains, new antimycobacterial drugs with alternative mechanism of action are urgently needed for the therapy of tuberculosis.[2] A number of compounds with H_1-receptor antagonistic activity were far more active against *Mycobacterium tuberculosis* than against other bacterials.[3–5] Since histamine is a growth-promotor for mycobacterials and the growth inhibition was blocked by the addition of histamine, the antimycobacterial activity of antihistaminics has been argued to be linked to their histamine-receptor antagonistic potency.[4,5] We modified the structures of two of those antihistaminic compounds, *Diphenylpyraline* (**1**) and *Bamipine* (**2**), in ring positions 1 and 2 (Fig. 1).

A great number of derivatives of *Diphenylpyraline* and *Bamipine* derivatives with different substitution on the ring nitrogen or at the aromatic ring system have been reported, whereas substitution of the piperidine ring was restricted to a few methyl analogues. Recently, we reported the synthesis of 2-alkyl and 2-aryl substituted derivatives of **1** and **2** and their 1-phenyl analogues which have been prepared via new pathways.[6–8] The *Diphenylpyraline* derivatives were tested against *Mycobacterium tuberculosis* $H_{37}Rv$. Compounds with alkyl substituents in ring position 2 showed higher activity.[7]

In the present paper, the synthesis of some new analogues of *Bamipine* will be described, which were prepared to complete the series for the purpose of comparison. The antimycobacterial activities and the cytotoxic properties of all 2-substituted derivatives of *Bamipine* (**2**) were examined and compared to those of compounds **1** and **2** in order to elucidate structure–activity relationships. In addition, the H_1-antihistaminic activity of selected compounds was tested and investigated for a possible correlation between those activities.

2. Results

2.1. Chemistry

Considering that enhanced lipophilicity was argued to increase the antimycobacterial activity of antihistamines,[4] we prepared

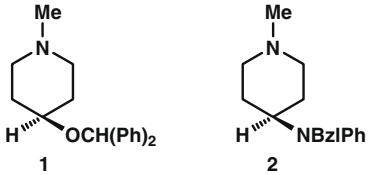

Figure 1. Structures of *Diphenylpyraline* (**1**) and *Bamipine* (**2**).

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2-alkyl and 2-aryl derivatives of **1** and **2** as well as the corresponding 1-phenyl analogues. In contrast to the formerly reported syntheses of *Diphenylpyraline* or *Bamipine* derivatives our method is based on the conversion of easily available 4-aminodihydropyridine-2(1*H*)-thiones to 2-substituted piperidines. The syntheses of the *Diphenylpyraline* derivatives **3–7** and of the *Bamipine* derivatives **8–10** have already been reported.[6–8]

The new 2,2-dimethyl analogues **11–13** of *Bamipine* were obtained by means of slightly modified procedures. The corresponding *N*-arylbenzylamines[9] were fused with the 4-hydroxypyridine-2(1*H*)-thione **14**,[10] giving the 4-aminodihydropyridine-2(1*H*)-thiones **15**, **16** in good yields. Their *S*-methyl derivatives **17**, **18** were further methylated yielding the 1-methylpyridinium salts **19**, **20**. Compounds **19**, **20** were converted to the 4-anilino piperidines **21**, **22** via hydrogenation with Raney nickel W-2[11] at 45 psi. Final N-alkylation with benzyl chloride gave the *Bamipine* derivatives **11**, **12** (Scheme 1).

The corresponding 1-phenyl analogue **13** was synthesized from the 1-substituted 4-anilinodihydropyridine-2(1*H*)-thione **23**,[12] which was converted to its isomeric methoiodides *cis*-**24** and *trans*-**24**. Those were hydrogenated giving the 4-anilino-1-phenylpiperidine **25**, which was alkylated with benzyl chloride affording the 1-phenyl substituted analogue **13** of compound **11**.

The discrimination between the isomeric compounds *cis*-**24** and *trans*-**24** was accomplished via NMR experiments. In the ^{1}H NMR spectrum the resonance of the 5-H of *cis*-**24** was shifted to lower frequencies due to shielding of the phenyl ring. The assignment was confirmed by an NOE from the 5-H of *cis*-**24** to aromatic ortho protons.

The structures of all new piperidine derivatives were established by NMR spectroscopy as outlined in Ref. 8 All of the new piperidine derivatives gave NOEs between the axial protons in ring positions 3 and 5 as well as between 4 and 6. The preference for the chair conformation of the piperidine rings was confirmed by the characteristic coupling constants of the corresponding ring protons in the ^{1}H NMR spectra. The distinction between the ^{1}H- and ^{13}C-resonances of axial and equatorial methyl groups in ring position 2 of the piperidine derivatives succeeded by means of NOE experiments. Through-space interactions from the axial protons in ring positions 4 or 6 to protons of the axial 2-methyl groups were observed.

2.2. Antimycobacterial and antihistaminic activity, cytotoxicity

The antimycobacterial activity and the cytotoxicity of *Bamipine* (**2**) and its derivatives **8–13** have been determined and are compared to those of the corresponding *Diphenylpyraline* analogues **1, 3–7** in Table 1, which in addition contains the H_1-antihistaminic activity of selected compounds.

3. Discussion

The antimycobacterial activities of *Diphenylpyraline* (**1**) and *Bamipine* (**2**) were only slightly changed by dimethylation in position 2 (compounds **4**, **11** and **12**). However, an additional equatorial isopropyl substituent increased the activity remarkably, as was observed for compounds *cis*-**3** and *cis*-**8**. In the 1-phenyl series the influence of the substitution was not the same in both compared series. The *Diphenylpyraline* derivatives *cis*-**5** and *cis*-**6** (36–39% inhibition at 6.25 μg/mL) showed improved potency and *trans*-**6** (75% inhibition at 6.25 μg/mL) had significantly increased activity. Their corresponding analogues in the *Bamipine* series *cis*-**9**, *cis*-**10** and *trans*-**10** were less or slightly more active than their parent compound **2**. However, compounds *trans*-**9** (52% inhibition at 6.25 μg/mL) and **13** (91% inhibition at 6.25 μg/mL) were the most active 1-phenyl derivatives of **2**, whereas their *Diphenylpyraline* analogues *trans*-**5** and **7** were among the least active compounds. For the most active compound **13** a minimum inhibitory concentration (MIC) of 6.25 μg/mL against *M. tuberculosis H$_{37}$Rv* was determined (Table 1).

With the exception of *cis*-**8** all of the tested compounds were less cytotoxic than *Bamipine* (**2**). Fortunately the cytotoxicity of the most active compound **13** is comparable to that of *Diphenylpyraline* (**1**) which is still used in the therapy of rhinitis. However, compared to the antimycobacterial drugs in use (*INH, Rifampicin*) the cytotoxicity of the prepared compounds needs further improvement.

The H_1-receptor antagonist potencies of compounds **1**, **2**, **4**, **11** and **13** were examined. *Diphenylpyraline* (**1**) was slightly more potent than *Bamipine* (**2**). Their 2,2-dimethyl derivatives **4** and **11** were still potent H_1-receptor antagonists, but the dimethylation in position 2 led to loss of activity of about 0.6 log units retaining the *Diphenylpyraline* analogue **4** as the more effective compound. Both substances showed competitive antagonism at concentrations between 0.3×10^{-7} mol/L and 1×10^{-6} mol/L shifting the dosage–response curve parallel to the right. At concentrations between 0.3 and 1×10^{-5} mol/L the dosage–response curves were depressed indicating non-competitive antagonism. Only slight activity ($-\log K_B = 5$) was detectable for the 1-phenyl compound **13** at 0.3×10^{-4} mol/L (Table 1). For compounds **1**, **2**, **4**, **11** and **13** molecular modeling studies were undertaken in order to investigate the reasons for the decreased H_1-receptor antagonist potency of the prepared 2-substituted and especially the 1-phenyl analogues of compounds **1** and **2**. Neutral molecules as well as protonated and diprotonated dications were considered. The conformer populations were not affected by dimethylation at C-2. The loss of activity of about 0.6 log units (**1** vs **4**, **2** vs **11**) can be attributed to steric hindrance and to the changes in the molecular electrostatic and lipophilic potential. Thus, the drop in H_1-receptor antagonist potency in **4** and **11** can be attributed to a "shielding" effect of the 2,2-dimethyl moiety of the positive charge at the protonated N-1 center. The very low H_1-antihistaminic activity (loss of >3 log units) of the 1-phenyl compound **13** can be explained by a combination of increased "shielding" of N-1 and diminished basicity (For computational details see Supplementary material).

In both series a 1-phenyl derivative was the most active antimycobacterial agent. Since those compounds possess negligible H_1-antihistaminic activity the presumed linkage between H_1-antihistaminic and antimycobacterial activity has not been confirmed.

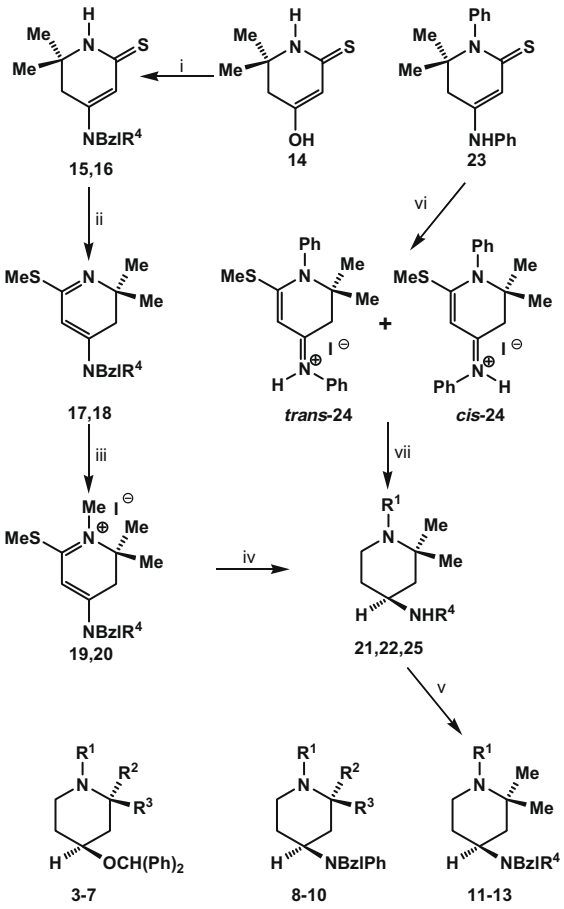
4. Conclusion

The H_1-receptor antagonistic activities of *Diphenylpyraline* and *Bamipine* were slightly decreased by substitution in ring position 2 and nearly lost by replacement of the 1-methyl by a 1-phenyl group. In contrast the antimycobacterial activity was nearly always increased by this derivatization. The supposed correlation of antimycobacterial and H_1-receptor antagonistic activity of piperidine derivatives was not observed for the tested analogues of *Diphenylpyraline* and *Bamipine*. Moreover, the compound with the highest activity against *Mycobacterium tuberculosis H$_{37}$Rv* (MIC: 6.25 μg/mL) exhibits neglectable H_1-antihistaminic activity indicating an alternative mechanism of action.

5. Experimental

5.1. Instrumentation and chemicals

Melting points were obtained on a melting point apparatus Dr. Tottoli (Büchi 510) or on a digital melting point apparatus Electrothermal IA 9200 and are uncorrected. IR spectra: infrared spectrometer system 2000 FT (Perkin-Elmer). NMR spectra: Varian



Compound	R^1	R^2	R^3	R^4
cis-**3**, *cis*-**8**	Me	H	iPr	---
4	Me	Me	Me	---
cis-**5**, *cis*-**9**	Ph	H	iPr	---
trans-**5**, *trans*-**9**	Ph	iPr	H	---
cis-**6**, *cis*-**10**	Ph	H	Ph	---
trans-**6**, *trans*-**10**	Ph	Ph	H	---
7	Ph	Me	Me	---
11, 21	Me	---	---	Ph
12, 22	Me	---	---	Ar
13, 25	Ph	---	---	Ph
15, 17, 19	---	---	---	Ph
16, 18, 20	---	---	---	Ar

Ar=4-methoxyphenyl; Bzl=benzyl

[a]Reagents and reaction conditions: (i) NHBzlPh or NHBzlAr, 120 °C, 16h; (ii) (1) CH_3I, $CHCl_3$, room temperature (rt), 16 h, (2) NaOH, rt, 1 h; (iii) CH_3I, $CHCl_3$, rt, 16 h; (iv) Raney nickel W-2, EtOH, rt; (v) BzlCl, $NaNH_2$, PhMe, 110 °C, 6 h; (vi) CH_3I, $CHCl_3$, rt, 16 h; (vii) Raney nickel W-2, ethanol, 30-45 psi (H_2), rt, 16 h.

Scheme 1.

Table 1
Antimycobacterial activity (Alamar Blue Assay), cytotoxicity and H1-receptor antagonist activity

Compound	% Inhibition 6.25 μg/mL[a]	MIC μg/mL[a]	Cytotoxicity (LC50) μg/mL (μM)[a]	H1-receptor guinea-pig ileum (−logKB)[b]
Diphenylpyraline (**1**)	5	>6.25	107.59	8.79
cis-**3**	68	>6.25	98.06	n.t.
4	18	>6.25	104.67	8.19
cis-**5**	36	>6.25	69.38	n.t.
trans-**5**	7	>6.25	97.06	n.t.
cis-**6**	39	>6.25	77.82	n.t.
trans-**6**	75	>6.25	111.67	n.t.
7	3	>6.25	65.08	n.t.
Bamipine (**2**)	29	>6.25	64.08	8.55
cis-**8**	66	>6.25	35.93	n.t.
cis-**9**	7	>6.25	126.91	n.t.
trans-**9**	52	>6.25	>28	n.t.
cis-**10**	37	>6.25	>28	n.t.
trans-**10**	22	>6.25	>28	n.t.
11	31	>6.25	69.46	7.93
12	26	>6.25	81.96	n.t.
13	91	6.25	107.84	5.0
Isoniazid (*INH*)	—	0.025–0.2	>200	n.t.
Rifampicin	—	0.06–0.5	>200	n.t.

[a] Data for compounds **1**, **3–7** were taken from Ref. 7.
[b] n.t., not tested.

Gemini 200, Varian Inova 400 (298 K) 5 mm tubes, TMS as internal standard. ^{1}H- and ^{13}C-resonances were assigned using ^{1}H,^{1}H- and ^{1}H,^{13}C-correlation spectra. Microanalyses: EA 1108 CHNS-O apparatus (Carlo Erba) at the Microanalytical Laboratory at the Institute of Physical Chemistry, University of Vienna; Hydrogenations were performed in a Parr hydrogenation apparatus shaker type 3911. Chromatography: TLC: plates (Merck) silica gel 60 F_{254}.

5.2. Syntheses

5.2.1. Synthesis of dihydropyridine derivatives

5.2.1.1. General procedure for the synthesis of 4-(*N*-Benzylarylamino)-6,6-dimethyl-5,6-dihydropyridine-2(1*H*)-thiones (15, 16). Compound **14**[10] was fused with the corresponding *N*-arylbenzylamine for 16 h at 120 °C in an argon atmosphere. Then the mixture was cooled to room temperature and triturated with EtOH (15 mL). The precipitate was filtered with suction and dried. Then it was stirred for 1 h at 60 °C in $CHCl_3$ (100 mL). The suspension was cooled to room temperature and filtered. The filtrate was concentrated and recrystallized from EtOH.

*5.2.1.1.1. 4-(N-Benzylanilino)-6,6-dimethyl-5,6-dihydropyridine-2(1H)-thione (**15**).* Compound **14** (7.86 g, 50 mmol) gave with *N*-phenylbenzylamine (10.1 g, 55 mmol) pale yellow crystals of **15**. Mp 214 °C; yield: 10.7 g (66%). IR (KBr) 2960, 1554, 1495, 1448, 1390, 1112, 1103, 703 cm^{-1}. ^{1}H NMR (DMSO-d_6, 400 MHz) δ(ppm) 1.16 (s, 6H, $(CH_3)_2$), 2.30 (s, 2H, 5-H), 4.93 (s, 2H, NCH_2), 5.36 (s, 1H, 3-H), 7.25–7.43 (m, 10H, aromatic H), 8.64 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ(ppm) 26.84 ($(CH_3)_2$), 38.75 (C-5), 52.91 (C-6), 55.86 (NCH_2), 100.60 (C-3), 126.64, 127.00, 127.24, 127.42, 128.73, 129.70, 137.13, 143.86 (aromatic C), 151.01 (C-4), 189.26 (C-2). Anal. Calcd for $C_{20}H_{22}N_2S$: C, 74.49; H, 6.88; N, 8.69; S, 9.94. Found: C, 74.44; H, 6.76; N, 8.73; S, 9.68.

*5.2.1.1.2. 4-((N-Benzyl)-p-anisidino)-6,6-dimethyl-5,6-dihydropyridine-2(1H)-thione (**16**).* Compound **14** (7.89 g, 50 mmol) gave with *N*-(4-methoxyphenyl)benzylamine (10.1 g, 55 mmol) pale yellow crystals of **16**. Mp 208 °C; yield: 8.89 g (50%). IR (KBr) 2967, 1565, 1509, 1446, 1397, 1110, 720 cm^{-1}. ^{1}H NMR (DMSO-d_6, 200 MHz) δ(ppm) 1.16 (s, 6H, $(CH_3)_2$), 2.27 (s, 2H, 5-H), 3.73 (s, 3H, OCH_3), 4.85 (s, 2H, NCH_2), 5.31 (s, 1H, 3-H), 6.91–7.37 (m, 9H, aromatic H), 8.54 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz) δ(ppm) 26.88 ($(CH_3)_2$), 38.70 (C-5), 52.82 (C-6), 55.45 (OCH_3), 56.00 (NCH_2), 99.61 (C-3), 114.79, 126.76, 127.23, 128.71, 128.80, 136.46, 137.09, 157.97 (aromatic C), 151.77 (C-4), 189.16 (C-2). Anal. Calcd for $C_{20}H_{22}N_2S$: C, 71.56; H, 6.86; N, 7.95; S, 9.10. Found: C, 71.51; H, 6.99; N, 7.90; S, 8.95.

5.2.1.2. General procedure for the synthesis of 1-unsubstituted 2,2-dimethyl-6-methylthio-2,3-dihydropyridines (17, 18). To an ice-cooled solution of the dihydropyridinethiones **15, 16** in $CHCl_3$ (80 mL), a solution of iodomethane in $CHCl_3$ (20 mL) was added through a dropping funnel within 1 h. The reaction mixture was stirred for 16 h at room temperature, and the solvent was removed in vacuo. The residue was triturated with ethyl acetate, filtered with suction, dried with Na_2SO_4 and recrystallized from $CHCl_3$/ethyl acetate. The salts were stirred in 1 M NaOH (90 mL, 90 mmol) for 1 h. Then the suspension was extracted with ether repeatedly. The combined organic layers were washed three times with H_2O and dried with Na_2SO_4. The solvent was removed in vacuo and the residue was recrystallized.

*5.2.1.2.1. 4-(N-Benzylanilino)-2,2-dimethyl-6-methylthio-2,3-dihydropyridine (**17**).* Compound **15** (9.67 g, 30 mmol) gave with iodomethane (5.1 g, 36 mmol) pale yellow crystals of **17**. Mp 89 °C (EtOH); yield: 7.80 g (77%). IR (KBr) 2970, 1604, 1544, 1496, 1397, 1098, 764, 705 cm^{-1}. ^{1}H NMR (DMSO-d_6, 200 MHz) δ(ppm) 1.06 (s, 6H, $(CH_3)_2$), 2.10 (s, 2H, 3-H), 2.17 (s, 3H, SCH_3), 4.85 (s, 1H, 5-H), 4.86 (s, 2H, NCH_2), 7.18–7.41 (m, 10H, aromatic H). ^{13}C NMR (DMSO-d_6, 50 MHz) δ(ppm) 11.23 (SCH_3), 28.42 ($(CH_3)_2$), 38.15 (C-3), 55.53 (C-2), 55.72 (NCH_2), 91.86 (C-5), 126.10, 126.64, 126.89, 127.05, 128.60, 129.45, 137.74, 144.37 (aromatic C), 150.74 (C-4), 160.10 (C-6). Anal. Calcd for $C_{21}H_{24}N_2S$: C, 74.96; H, 7.19; N, 8.32; S, 9.53. Found: C, 75.15; H, 7.49; N, 8.35; S, 9.46.

*5.2.1.2.2. 4-(N-Benzyl-p-anisidino)-2,2-dimethyl-6-methylthio-2,3-dihydropyridine (**18**).* Compound **16** (10.58 g, 30 mmol) gave with iodomethane (5.1 g, 36 mmol) pale yellow crystals of **18**. Mp 120 °C (EtOH/H_2O); yield: 8.40 g (76%). IR (KBr) 2966, 1603, 1541, 1509, 1249, 1093, 764, 738 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ(ppm) 1.15 (s, 6H, $(CH_3)_2$), 2.06 (s, 2H, 3-H), 2.31 (s, 3H, SCH_3), 3.78 (s, 3H, OCH_3), 4.73 (s, 2H, NCH_2), 4.94 (s, 1H, 5-H), 6.82–7.32 (m, 10H, aromatic H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ(ppm) 11.98 (SCH_3), 28.25 ($(CH_3)_2$), 38.54 (C-3), 55.38 (OCH_3), 55.76 (C-2), 56.25 (NCH_2), 91.40 (C-5), 114.37, 126.77, 127.08, 128.53, 137.40, 137.54 (aromatic C), 151.47 (C-4), 157.86 (aromatic C), 161.71 (C-6). Anal. Calcd for $C_{22}H_{26}N_2OS$: C, 72.09; H, 7.15; N, 7.64; S, 8.75. Found: C, 72.08; H, 7.32; N, 7.60; S, 8.66.

5.2.1.3. 4-Anilino-6,6-dimethyl-1-phenyl-5,6-dihydropyridine-2(1*H*)-thione (23). Compound **23** was prepared from 2,2-dimethyl-N-phenyl-6-phenylimino-3,6-dihydro-2*H*-thiopyran-4-amine by a reported procedure.[12] Mp 214 °C (mp 214 °C[12]). IR (KBr) 2982, 1574, 1529, 1492, 1394, 1147, 693 cm^{-1}; ^{1}H NMR (DMSO-d_6, 200 MHz) δ(ppm) 1.22 (s, 6H, $(CH_3)_2$), 2.73 (s, 2H, 5-H), 6.08 (s, 1H, 3-H), 7.08–7.42 (m, 10H, aromatic H), 8.82 (s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6, 50 MHz) δ(ppm) 26.42 ($(CH_3)_2$), 41.76 (C-5), 58.43 (C-6), 100.26 (C-3), 122.25, 123.99, 126.93, 128.43, 129.27, 129.86, 139.32, 142.98 (aromatic C), 146.32 (C-4), 191.53 (C-2) ppm.

5.2.1.4. General procedure for the synthesis of 1-substituted 2,2-dimethyl-6-methylthio-2,3-dihydropyridines (19, 20, *cis*-24, *trans*-24). To a solution of compounds **17, 18, 23** in $CHCl_3$ (60 mL), a solution of iodomethane in $CHCl_3$ (10 mL) was added through a dropping funnel within 1 h. The reaction mixture was stirred for 16 h at room temperature. The solvent was evaporated in vacuo and the residue was triturated with EtOH/ethyl acetate. The crystallizate was sucked off and recrystallized.

*5.2.1.4.1. 4-(N-Benzylanilino)-1,2,2-trimethyl-6-methylthio-2,3-dihydropyridiniumiodide (**19**).* Compound **17** (6.73 g, 20 mmol) gave with iodomethane (14.2 g, 100 mmol) yellow crystals of **19**. Mp 162 °C (EtOH/ethyl acetate); yield: 9.2 g (96%). IR (KBr) 2972, 1561, 1496, 1338, 1276, 1242, 1080, 697 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ(ppm) 1.28 (s, 6H, $(CH_3)_2$), 2.51 (s, 3H, SCH_3), 2.63 (s, 2H, 3-H), 3.21 (s, 3H, NCH_3), 5.27 (s, 2H, NCH_2), 5.64 (s, 1H, 5-H), 7.25–7.57 (m, 10H, aromatic H). ^{13}C NMR (DMSO-d_6, 50 MHz) δ(ppm) 15.65 (SCH_3), 23.16 ($(CH_3)_2$), 33.37 (NCH_3), 39.96 (C-3), 57.40 (NCH_2), 60.32 (C-2), 88.52 (C-5), 127.30, 127.55, 127.94, 128.88, 130.06, 135.16, 141.73 (aromatic C), 159.45 (C-4), 173.20 (C-6). Anal. Calcd for $C_{22}H_{27}IN_2S$: C, 55.23; H, 5.69; I, 26.52; N, 5.86; S, 6.70. Found: C, 55.27; H, 5.78; I, 26.72; N, 5.69; S, 6.65.

*5.2.1.4.2. 4-(N-Benzyl-p-anisidino)-1,2,2-trimethyl-6-methylthio-2,3-dihydropyridiniumiodide (**20**).* Compound **18** (10.58 g, 20 mmol) gave with iodomethane (5.1 g, 100 mmol) yellow crystals of **20**. Mp 177 °C (EtOH/ethyl acetate); yield: 9.0 g (89%). IR (KBr) 2973, 1566, 1496, 1346, 1294, 1251, 1171, 1112, 1083, 698 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ(ppm) 1.28 (s, 6H, $(CH_3)_2$), 2.51 (s, 3H, SCH_3), 2.60 (s, 2H, 3-H), 3.20 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 5.20 (s, 2H, NCH_2), 5.62 (s, 1H, 5-H), 7.00–7.39 (m, 9H, aromatic H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ(ppm) 15.66 (SCH_3), 23.06 ($(CH_3)_2$), 33.25 (NCH_3), 39.39 (C-3), 55.54 (OCH_3), 57.52 (NCH_2), 60.10 (C-2), 88.16 (C-5), 114.93, 127.48, 127.75, 128.37, 128.69, 134.25, 134.96, 158.95 (aromatic C), 159.76 (C-4), 172.92 (C-6). Anal. Calcd for $C_{23}H_{29}IN_2OS$: C, 54.33; H, 5.75; I, 24.96; N, 5.51; S, 6.31. Found: C, 53.96; H, 5.80; I, 24.94; N, 5.33; S, 6.27.

*5.2.1.4.3. 2,2-Dimethyl-6-methylthio-N,1-diphenyl-2,3-dihydropyridin-4(1H)-iminiumiodide (cis-**24**, trans-**24**).* Compound **23** (6.2 g, 20 mmol) gave with iodomethane (3.4 g, 24 mmol) a mixture of *cis*-**24** and *trans*-**24**. Mp 191 °C ($CHCl_3$/ethyl acetate); yield: 8.8 g (98%). IR (KBr) 2975, 1606, 1573, 1489, 1474, 1459, 1436, 1212, 1182, 1174, 759, 702 cm^{-1}. *cis*-**24** (main component): ^1H NMR (DMSO-d_6, 400 MHz) δ(ppm) 1.32 (s, 6H, $(CH_3)_2$), 2.36 (s, 3H, SCH_3), 3.16 (s, 2H, 3-H), 5.73 (s, 1H, 5-H), 7.30–7.60 (m, 10H, aromatic H), 11.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ(ppm) 15.77 (SCH_3), 24.64 ($(CH_3)_2$), 41.12 (C-3), 61.03 (C-2), 85.93 (C-5), 124.10, 127.53, 129.38, 129.93, 130.00, 130.32, 136.61, 137.54 (aromatic C), 159.42 (C-4), 175.56 (C-6). *trans*-**24** (minor constituent): ^1H NMR (DMSO-d_6, 400 MHz) δ(ppm) 1.22 (s, 6H, $(CH_3)_2$), 2.51 (s, 3H, SCH_3), 3.16 (s, 2H, 3-H), 5.96 (s, 1H, 5-H), 7.30–7.60 (m, 10H, aromatic H), 11.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ(ppm) 15.92 (SCH_3), 24.64 ($(CH_3)_2$), 37.58 (C-3), 60.84 (C-2), 89.78 (C-5), 124.69, 127.36, 129.49, 129.75, 130.25, 135.97, 137.66 (aromatic C), 162.39 (C-4), 173.27 (C-6). Anal. Calcd for $C_{20}H_{23}IN_2S$: C, 53.34; H, 5.15; I, 28.17; N, 6.22; S, 7.12. Found: C, 53.26; H, 5.11; I, 28.05; N, 6.01; S, 7.02.

5.2.2. Piperidines

5.2.2.1. *Diphenylpyraline* analogues (3–7). Their syntheses have already been reported.[7]

5.2.2.2. General procedure for the synthesis of 1-substituted 4-anilinopiperidines 21, 22, 25. Ten grams of freshly prepared Raney nickel W-2[11] were added to a solution of compounds **19**, **20** or a mixture of *cis*-**24** and *trans*-**24** in 80 mL of EtOH. The mixture was shaken for 16 h at room temperature in hydrogen atmosphere at 45 psi in a shaker apparatus. The catalyst was sucked off through a sintered disc filter funnel and the residue rewashed with 100 mL of EtOH. The solvent was removed in vacuo and the residue dissolved in $CHCl_3$ and H_2O. The organic layers were separated and the aqueous phase was extracted once with $CHCl_3$. The combined organic layers were dried with Na_2SO_4 and the solvent evaporated. The oily base was converted to the dihydrochloride with an ethanolic solution of HCl. The solvent was removed and the residue recrystallized.

*5.2.2.2.1. (RS)-(±)-4-Anilino-1,2,2-trimethylpiperidine (**21**).* Compound **19** (2.39 g, 5 mmol) was hydrogenated with Raney nickel W-2.[11] The crude base of **21** contained *N*-phenylbenzylamine as by-product which was removed by distillation prior to the preparation of the hygroscopic dihydrochloride. Mp 220 °C (propan-2-ol/acetone); yield: 1.1 g (73%). IR (KBr) 2939, 2618, 1647, 1603, 1476, 1385, 1320, 1222, 1173, 1124, 1042, 758, 692 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ(ppm) 1.03 (s, 3H, CH_{3ax}), 1.17 (s, 3H, CH_{3eq}), 1.28 (dd, J = 12.5, 12.0 Hz, 1H, 3-H_{ax}), 1.43 (dddd, J = 12.2, 12.2, 12.2, 4.8 Hz, 1H, 5-H_{ax}), 1.89 (ddd, J = 12.5, 3.5, 2.7 Hz, 1H, 3-H_{eq}), 2.05–2.11 (m, 1H, 5-H_{eq}), 2.27 (s, 3H, NCH_3), 2.55 (ddd, J = 12.2, 12.2, 3.1 Hz, 1H, 6-H_{ax}), 2.71 (ddd, J = 12.2, 4.8, 2.7 Hz, 1H, 6-H_{eq}), 3.44–3.52 (m, 1H, 4-H_{ax}), 6.58 (d, J = 8.0 Hz, 2H, *o*-aromatic H), 6.67 (t, J = 8.0 Hz, 1H, *p*-aromatic H), 7.16 (t, J = 8.0 Hz, 2H, *m*-aromatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 14.47 (CH_{3ax}), 30.19 (CH_{3eq}), 33.61 (C-5), 37.32 (NCH_3), 46.74 (C-3), 47.25 (C-4), 49.99 (C-6), 54.13 (C-2), 113.15 (*o*-aromatic C), 117.21 (*p*-aromatic C), 129.29 (*m*-aromatic C), 147.08 (*i*-aromatic C). Anal. Calcd for $C_{14}H_{24}Cl_2N_2 \times 0.5\ H_2O$: C, 56.00; H, 8.39; Cl, 23.61; N, 9.33. Found: C, 55.80; H, 8.49; Cl, 23.25; N, 9.16.

*5.2.2.2.2. (RS)-(±)-4-(p-Anisidino)-1,2,2-trimethylpiperidine (**22**).* Compound **20** (2.54 g, 5 mmol) was hydrogenated with Raney nickel W-2.[11] The crude base of **22** contained *N*-phenylbenzylamine as by-product which was removed by distillation prior to the preparation of the hygroscopic dihydrochloride. Mp 217 °C (ethyl acetate); yield: 1.1 g (68%). IR (KBr) 2928, 2624, 1647, 1598, 1476, 1384, 1321, 1220, 1171, 1114, 1041, 756, 690 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ(ppm) 0.99 (s, 3H, CH_{3ax}), 1.14 (s, 3H, CH_{3eq}), 1.22 (dd, J = 12.6, 12.0 Hz, 1H, 3-H_{ax}), 1.37 (dddd, J = 12.2, 12.2, 12.2, 4.4 Hz, 1H, 5-H_{ax}), 1.86 (ddd, J = 12.6, 3.2, 2.1 Hz, 1H, 3-H_{eq}), 2.02–2.08 (m, 1H, 5-H_{eq}), 2.24 (s, 3H, NCH_3), 2.51 (ddd, J = 12.3, 12.3, 2.7 Hz, 1H, 6-H_{ax}), 2.67 (ddd, J = 12.3, 4.4, 2.7 Hz, 1H, 6-H_{eq}), 3.22 (br, 1H, NH), 3.32–3.41 (m, 1H, 4-H_{ax}), 3.72 (s, 1H, OCH_3), 6.55 (d, J = 8.9 Hz, 2H, *o*-aromatic H), 6.75 (d, J = 8.9 Hz, 2H, *m*-aromatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ14.24 (CH_{3ax}), 30.07 (CH_{3eq}), 33.61 (C-5), 37.13 (NCH_3), 46.75 (C-3), 48.14 (C-4), 49.84 (C-6), 53.79 (C-2), 55.54 (OCH_3), 114.68 (*o*-aromatic C), 114.74 (*m*-aromatic C), 141.08 (*i*-aromatic C), 151.85 (*p*-aromatic C). Anal. Calcd for $C_{15}H_{26}Cl_2N_2O \times 0.25\ H_2O$: C, 55.30; H, 8.20; Cl, 21.76; N, 8.60. Found: C, 55.29; H, 8.20; Cl, 21.81; N, 8.50.

*5.2.2.2.3. (RS)-(±)-4-Anilino-2,2-dimethyl-1-phenylpiperidine (**25**).* A mixture of *cis*-**24** and *trans*-**24** (2.25 g, 5 mmol) was hydrogenated with Raney nickel W-2[11] giving an oily residue, which was converted to the dihydrochloride of **25**. Mp 237 °C (EtOH/ethyl acetate); yield: 1.3 g (74%). IR (KBr) 3058, 2662, 2502, 1598, 1488, 1429, 764, 703 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ(ppm) 1.08 (s, 3H, CH_{3eq}), 1.10 (s, 3H, CH_{3ax}), 1.45 (dd, J = 12.6, 11.4 Hz, 1H, 3-H_{ax}), 1.49 (dddd, J = 12.0, 12.0, 11.7, 4.4 Hz, 1H, 5-H_{ax}), 1.89 (ddd, J = 12.6, 3.6, 2.4 Hz, 1H, 3-H_{eq}), 2.16–2.22 (m, 1H, 5-H_{eq}), 2.94 (ddd, J = 12.0, 4.4, 3.0 Hz, 1H, 6-H_{eq}), 3.44 (br, 1H, NH), 3.48 (ddd, J = 12.0, 12.0, 2.7 Hz, 1H, 6-H_{ax}), 3.60–3.69 (m, 1H, 4-H_{ax}), 6.62–7.29 (m, 10H, aromatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 17.26 (CH_{3ax}), 32.21 (CH_{3eq}), 34.33 (C-5), 47.50 (C-3, C-6), 47.67 (C-4), 55.26 (C-2), 113.23, 117.24, 124.48, 127.87, 127.94, 129.33, 147.16, 149.72 (aromatic C). Anal. Calcd for $C_{19}H_{26}Cl_2N_2$: C, 64.59; H, 7.42; Cl, 20.07; N, 7.93. Found: C, 64.48; H, 7.67; Cl, 19.96; N, 7.79.

5.2.2.3. *Bamipine* analogues (8–10). Their syntheses have already been reported.[8]

5.2.2.4. General procedure for the synthesis of 2,2-dimethyl-substituted analogues of *Bamipine* (11–13). In an atmosphere of nitrogen $NaNH_2$ was added to a solution of the corresponding 4-anilinopiperidine in 30 mL of benzene. The mixture was stirred on an oil-bath at 110 °C until the evolution of NH_3 ceased. Then

benzyl chloride was added dropwise. After 6 h the mixture was cooled, diluted with 100 mL of benzene, poured into a separatory funnel and shaken twice with H_2O. The organic layer was dried over K_2CO_3 and the solvent removed in vacuo. Excess benzyl chloride was removed beyond 70 °C in high vacuum. The oily base was converted to the dihydrochlorides with an ethanolic solution of HCl. The solvent was removed and the residue recrystallized.

*5.2.2.4.1. (RS)-(±)-4-(N-Benzylanilino)-1,2,2-trimethylpiperidine (**11**).* The reaction of compound **21** (0.55 g, 2.5 mmol), $NaNH_2$ (0.12 g, 3.1 mmol) and benzyl chloride (0.40 g, 3.1 mmol) gave an oily residue, which was converted to the dihydrochloride of **11**. Mp 211 °C (2-propanol/acetone); yield: 0.70 g (73%). IR (KBr) 2927, 2375, 1630, 1605, 1493, 1477, 1435, 1392, 1379, 1221, 1168, 1119, 1042, 748, 699 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ(ppm) 1.08 (s, 3H, CH_{3ax}), 1.14 (s, 3H, CH_{3eq}), 1.57 (dd, J = 12.5, 12.5 Hz, 1H, 3-H_{ax}), 1.68 (ddd, J = 12.5, 3.6, 2.3 Hz, 1H, 3-H_{eq}), 1.73 (dddd, J = 12.2, 12.2, 12.2, 4.7 Hz, 1H, 5-H_{ax}), 1.79–1.85 (m, 1H, 5-H_{eq}), 2.24 (s, 3H, CH_3), 2.57 (ddd, J = 12.2, 12.2, 3.1 Hz, 1H, 6-H_{ax}), 2.67 (ddd, J = 12.2, 4.7, 2.7 Hz, 1H, 6-H_{eq}), 4.02–4.10 (m, 1H, 4-H_{ax}), 4.42–4.51 (2d, J = 18.0 Hz, 2H, NCH_2), 6.67–7.29 (m, 10H, aromatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 13.91 (CH_{3ax}), 30.60 (C-5), 30.75 (CH_{3eq}), 37.33 (NCH_3), 42.58 (C-3), 49.02 (NCH_2), 50.33 (C-6), 51.83 (C-4), 54.18 (C-2), 112.77, 116.51, 126.16, 126.37, 128.35, 129.19, 140.72, 149.27 (aromatic C). Anal. Calcd for $C_{21}H_{30}Cl_2N_2$: C, 66.13; H, 7.93; Cl, 18.59; N, 7.35. Found: C, 65.88; H, 7.64; Cl, 18.34; N, 7.10.

*5.2.2.4.2. (RS)-(±)-4-(N-Benzyl-p-anisidino)-1,2,2-trimethylpiperidine (**12**).* The reaction of compound **22** (0.64 g, 2.5 mmol), $NaNH_2$ (0.12 g, 3.1 mmol) and benzyl chloride (0.40 g, 3.1 mmol) gave an oily residue, which was converted to the dihydrochloride of **12**. Mp 223 °C (EtOH/ethyl acetate); yield: 0.67 g (65%). IR (KBr) 2919, 2365, 1601, 1495, 1482, 1458, 1430, 1400, 1375, 1221, 1174, 1120, 1041, 753, 696 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ(ppm) 1.08 (s, 3H, CH_{3ax}), 1.18 (s, 3H, CH_{3eq}), 1.63 (dd, J = 12.3, 12.3 Hz, 1H, 3-H_{ax}), 1.66–1.88 (m, 3H, 3-H_{eq}, 5-H), 2.28 (s, 3H, NCH_3), 2.59 (ddd, J = 12.6, 11.8, 3.7 Hz, 1H, 6-H_{ax}), 2.72–2.79 (m, 1H, 6-H_{eq}), 3.72 (s, 1H, OCH_3), 3.83–3.92 (m, 1H, 4-H_{ax}), 4.33, 4.42 (2d, J = 17.2 Hz, 2H, NCH_2), 6.67–7.27 (m, 10H, aromatic H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ(ppm) 14.44 (CH_{3ax}), 30.07 (C-5), 30.28 (CH_{3eq}), 37.15 (NCH_3), 42.31 (C-3), 49.91 (NCH_2), 50.40 (C-6), 52.93 (C-4), 55.01 (C-2), 55.64 (OCH_3), 114.60, 115.81, 126.34, 126.47, 128.27, 140.84, 143.46, 151.87 (aromatic C). Anal. Calcd for $C_{22}H_{32}Cl_2N_2O$: C, 64.23; H, 7.84; Cl, 17.23; N, 6.81. Found: C, 64.44; H, 7.62; Cl, 17.27; N, 6.68.

*5.2.2.4.3. (RS)-(±)-4-(N-Benzylanilino)-2,2-dimethyl-1-phenylpiperidine (**13**).* The reaction of compound **25** (0.7 g, 2.5 mmol), $NaNH_2$ (0.12 g, 3.1 mmol) and benzyl chloride (0.40 g, 3.1 mmol) gave an oily residue, which was converted to the dihydrochloride of **13**. Mp 207 °C (propan-2-ol); yield: 0.62 g (56%). IR (KBr) 3049, 2992, 2930, 2355, 1599, 1497, 1468, 1440, 1407, 1379, 1200, 1155, 1128, 1037, 769, 700 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ(ppm) 1.06 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 1.75 (dd, J = 12.5, 12.5 Hz, 1H, 3-H_{ax}), 1.77–1.86 (m, 1H, 3-H_{eq}, 5-H_{ax}), 1.92–1.97 (m, 1H, 5-H_{eq}), 2.92 (ddd, J = 12.0, 4.2, 3.0 Hz, 1H, 6-H_{eq}), 3.51 (ddd, J = 12.0, 12.0, 2.9 Hz, 1H, 6-H_{ax}), 4.19–4.27 (m, 1H, 4-H_{ax}), 4.48–4.59 (2d, J = 17.9 Hz, 2H, NCH_2), 7.09–7.30 (m, 15H, aromatic H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm) 16.88 (CH_3), 31.39 (C-5), 32.61 (CH_3), 43.22 (C-3), 47.99 (C-6), 49.12 (NCH_2), 52.20 (C-4), 55.58 (C-2), 112.83, 116.55, 124.52, 126.17, 126.41, 127.84, 127.95, 128.39, 129.23, 140.69, 149.25, 149.69 (aromatic C). Anal. Calcd for $C_{26}H_{32}Cl_2N_2$: C, 70.42; H, 7.27; Cl, 15.99; N, 6.32. Found: C, 70.15; H, 7.29; Cl, 15.16; N, 6.14.

5.3. Biological tests

5.3.1. Antimycobacterial activity

The primary screening of the antimycobacterial activity of all synthesized analogues of *Diphenylpyraline* and *Bamipine* was conducted *in vitro* at a concentration of 6.25 μg/mL against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[13] Isoniazid (INH) was used as standard. Compounds effecting >90% inhibition were retested at lower concentrations to determine their minimum inhibitory concentration. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

5.3.2. Cytotoxicity

The cytotoxic properties of all *Bamipine* analogues were detected indirectly via cell proliferation of a HEK-293 cell line (human embryonic kidney 293 cells) using a colorimetric assay based on the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a colored formazan product by proliferating cells.[14] INH was used as standard. The data for the *Diphenylpyraline* derivatives were taken from Ref. 7 which contains a detailed description of the test method.

5.3.3. Antihistaminic activity

The H_1-receptor antagonist potency of selected compounds was determined in whole segments of the guinea-pig ileum as previously described.[15]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.10.042.

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