Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 859–866
IL FARMACO
www.elsevier.com/locate/farmac

Synthesis of 3- or 4-phenyl-1,8-naphthyridine derivatives and evaluation of antimycobacterial and antimicrobial activity

Muwaffag Badawneh [a], Laura Bellini [b], Tiziana Cavallini [b], Jalal Al jamal [a], Clementina Manera [b,*], Giuseppe Saccomanni [b], Pier Luigi Ferrarini [b]

[a] *Philadelphia University, P.O. Box 1101, Sweileh, Jordan*
[b] *Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno 6, I-56126 Pisa, Italy*

Received 23 December 2002; accepted 22 March 2003

Abstract

A series of 3- or 4-phenyl-1,8-naphthyridine derivatives variously substituted in the positions 2, 6 and 7 were synthesized and evaluated for in vitro evaluation for their antimycobacterial activity as part of a TAACF TB screening program under the direction of the US National Institute of Health, NIAID division. Several compounds showed an interesting activity when tested at a concentration of 6.25 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ and in particular compounds **2a**, **4a**,**d**, **8a**,**d** and **8i**, exhibit a % inhibition from 91 to 99. Among these, compounds **2a**, **8a** and **8d** appeared to have a good activity with minimum inhibitory concentrations (MICs) of 6.25 μg/ml. On the basis of the biological results, the most effective substituent in position 2 or 7 seems to be the piperidinyl group. The introduction of a morpholinyl group either in position 2 or 7 of the heterocycle ring caused a decrease in activity. The 1,8-naphthyridine derivatives were also tested in vitro for their antimicrobial activity against *Staphylococcus aureus* as Gram-positive bacteria and *Escherichia coli* as Gram-negative bacteria.

Keywords: Tuberculostatic agents; Antimycobacterial agents; Antimicrobial agents; 1,8-Naphthyridines; Piperidinyl; Morpholinyl; Ethylpiperazinyl; Phenylpiperazinyl

1. Introduction

Contrary to general expectations, the incidence of mycobacterial disease has significantly increased worldwide since 1990 and in spite of the therapeutic protocols used so far, tuberculosis continues to represent one of the major threats to public health in the world [1–4]. Furthermore, other mycobacteria, especially the *Mycobacterium avium* complex, have emerged as important pathogens due mainly to the AIDS epidemic [5]. The prevalence of HIV infection, and the emergence of drug-resistant and multi-drug-resistant strains of *Mycobacterium tuberculosis* are contributing to the worsening impact of the disease [6]. The recent emergence of drug-resistant *M. tuberculosis* has also become a serious concern [7]. There is therefore a pressing need to develop novel chemotherapeutic agents to hinder the emergence of resistance and, ideally, to shorten the duration of therapy of this disease [8,9].

In a previous paper [10], we reported the preparation and the antimycobacterial activity of some 4-phenyl-1,8-naphthyridine derivatives variously substituted in positions 2 and 7 tested in vitro at a concentration of 12.5 μg/ml against *M. Tuberculosis* $H_{37}Rv$. Some of these compounds showed an activity with an inhibition > 50%. More recently [11], we described the synthesis and antimycobacterial activity of some 1,8-naphthyridine derivatives variously substituted in positions 2, 3, 4 and 7. Several compounds, when tested in vitro at a concentration of 6.25 μg/ml against *M. Tuberculosis* $H_{37}Rv$ showed an interesting activity with % inhibition in the range 38–96%. These studies showed that the most effective substituents in position 2 or 7 of the 1,8-naphthyridine nucleus seem to be the piperidinyl or *N*-carbethoxypiperazinyl groups.

* Corresponding author.
E-mail address: manera@farm.unipi.it (C. Manera).

0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(03)00144-7

Now we report the antituberculosis activity of a new series of 3- or 4-phenyl-1,8-naphthyridine derivatives variously substituted in positions 2, 6 and 7.

Furthermore, it was recently reported that some 1,8-naphthyridine derivatives show an interesting bactericidal activity [12]. For this reason the 1,8-naphthyridine derivatives reported in this paper were also tested in vitro for their antimicrobial activity against *Staphylococcus aureus* as Gram-positive bacteria and *Escherichia coli* as Gram-negative bacteria.

2. Chemistry

When the 2,7-dichloro-4-phenyl-1,8-naphthyridine (**1**) [13] was treated with piperidine, morpholine, ethylpiperazine and phenylpiperazine in a sealed tube at 140 °C, the 2,7-dicycloalkylamino derivatives **2** were obtained (Scheme 1).

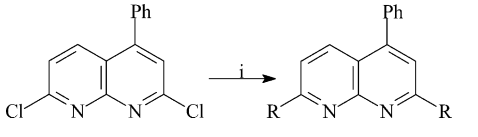
Under the same conditions the reaction of the 7-amino-2-chloro-4-phenyl-1,8-naphthyridine (**3**) [14] with the suitable cycloalkylamine afforded the 2-cycloalkylamino derivatives **4** (Scheme 2). Diazotization of 7-amino-2-cycloalkylamino-4-phenyl-1,8-naphthyridines **4**, carried out at −12 °C in sulfuric acid, gave in a good yield the corresponding 7-hydroxy derivatives **5**, which were converted, by reaction with phosphoryl chloride under reflux, into the corresponding 7-chloro-2-cycloalkylamino-4-phenyl-1,8-naphthyridines **6**. These last compounds **6** were then treated with sodium methoxide to give the corresponding 7-methoxy derivatives **7** (Scheme 2).

All the compounds synthesized were characterized by elemental analysis, IR and ^{1}H NMR.

3. Experimental protocols

3.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra in Nujol mulls were recorded on an ATI Mattson Genesis Series

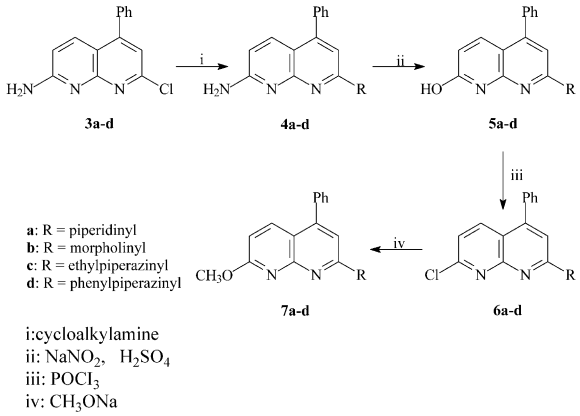


1 **2a-d**

a: R = piperidinyl
b: R = morpholinyl
c: R = ethylpiperazinyl
d: R = phenylpiperazinyl

i: cycloalkylamine

Scheme 1.



3a-d **4a-d** **5a-d**

7a-d **6a-d**

a: R = piperidinyl
b: R = morpholinyl
c: R = ethylpiperazinyl
d: R = phenylpiperazinyl

i:cycloalkylamine
ii: $NaNO_2$, H_2SO_4
iii: $POCl_3$
iv: CH_3ONa

Scheme 2.

FTIR spectrometer. ^{1}H NMR spectra were recorded with a Bruker AC-200 spectrometer in δ units from TMS as an internal standard. Analytical TLC was carried out on Merck 0.2 mm precoated silica-gel glass plates (60 F-254) and location of spots was detected by illumination with an UV lamp. Elemental analyses of all compounds synthesized for C, H and N were within ± 0.4% of the theoretical values and were performed on a Carlo Erba elemental analyzer model 1106 apparatus.

3.1.1. General procedure for the preparation of 2,7-dicycloalkylamino-4-phenyl-1,8-naphthyridine derivatives *2a–d*

A mixture of 2,7-dichloro-4-phenyl-1,8-naphthyridine (**1**) (3.64 mmol) and the suitable cycloalkylamine (16.4 mmol) was heated at 140 °C in a sealed tube for 24 h. After cooling the reaction mixture was treated with H_2O and the solid obtained was collected by filtration, washed with diethyl ether and purified by crystallization from petroleum ether 100–140 °C to give the title compounds **2a**–**d**.

*3.1.1.1. 2,7-Di(piperidin-1-yl)-4-phenyl-1,8-naphthyridine (**2a**).* 1.22 g, yield 90%; m.p. 125–127 °C; ^{1}H NMR (DMSO): δ 7.57 (d, 1H, H_5), 7.46 (m, 5H, Ar), 6.81 (d, 1H, H_6), 6.70 (s, 1H, H_3), 3.67 (m, 8H, piperidinyl), 1.56 (m, 12H, piperazinyl). *Anal*. $C_{24}H_{28}N_4$ (C, H, N).

*3.1.1.2. 2,7-Di(morpholin-1-yl)-4-phenyl-1,8-naphthyridine (**2b**).* 1.18 g, yield 86%; m.p. 155–157 °C; ^{1}H NMR (DMSO): δ 7.62 (d, 1H, H_5), 7.49 (m, 5H, Ar), 6.89 (d, 1H, H_6), 6.79 (s, 1H, H_3), 3.68 (m, 8H, morpholinyl). *Anal*. $C_{22}H_{24}N_4O_2$ (C, H, N).

*3.1.1.3. 2,7-Di(4-ethylpiperazin-1-yl)-4-phenyl-1,8-naphthyridine (**2c**).* 1.3 g, yield 82%; m.p. 100–102 °C; ^{1}H NMR (DMSO): δ 7.63 (d, 1H, H_5), 7.47 (m, 5H, Ar) 6.82 (d, 1H, H_6), 6.76 (s, 1H, H_3), 3.66 (m, 8H,

piperazinyl), 2.44 (m, 12H, piperazinyl and CH_2), 1.03 (t, 6H, CH_3). *Anal*. $C_{26}H_{34}N_6$ (C, H, N).

*3.1.1.4. 2,7-Di(4-phenylpiperazin-1-yl)-4-phenyl-1,8-naphthyridine (**2d**).* 1.5 g, yield 77%; m.p. 175–178 °C; ^{1}H NMR ($CDCl_3$): δ 7.58 (d, 1H, H_5), 7.35 (m, 5H, Ar), 7.27 (m, 5H, Ar), 6.94 (m, 5H, Ar), 6.71 (d, 1H, H_6),6.68 (s, 1H, H_3), 3.99 (m, 4H, piperazinyl), 3.35 (m, 4H, piperazinyl). *Anal*. $C_{25}H_{24}N_4O$ (C, H, N).

*3.1.2. General procedure for the preparation of 7-amino-2-cycloalkylamino-4-phenyl-1,8-naphthyridine derivatives **4a–d***

A mixture of 7-amino-2-chloro-4-phenyl-1,8-naphthyridine (**3**) (3.9 mmol) and the suitable cycloalkylamine (11.7 mmol) was heated at 140 °C in a sealed tube for 24 h. After cooling, the reaction mixture was treated with H_2O, and the precipitate formed was collected by filtration and purified by crystallization to give compounds **4a–c**. To obtain **4d** the reaction mixture was treated with H_2O and the solution was extracted with $CHCl_3$; the combined extracts were dried ($MgSO_4$) and evaporated to dryness under a vacuum to obtain a solid, which was purified by crystallization.

*3.1.2.1. 7-Amino-4-phenyl-2-(piperidin-1-yl)-1,8-naphthyridine (**4a**).* 0.78 g, yield 65%; m.p. 232–236 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 7.66 (d, 1H, H_5), 7.46 (m, 5H, Ar), 6.65 (s, 1H, H_3), 6.36 (d, 1H, H_6), 4.83 (brs, 2H, NH_2), 3.80 (m. 4H, piperidinyl), 1.68 (m, 6H, piperidinyl). *Anal*. $C_{19}H_{20}N_4$ (C, H, N).

*3.1.2.2. 7-Amino-2-(morpholin-1-yl)-4-phenyl-1,8-naphthyridine (**4b**).* 0.50 g, yield 49%; m.p. 153–156 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 7.53 (d, 1H, H_5), 7.46 (m, 5H, Ar), 6.63 (s, 1H, H_3), 6.43 (d, 1H, H_6), 4.94 (brs, 2H, NH_2), 3.81 (m, 8H, morpholinyl). *Anal*. $C_{18}H_{18}N_4O$ (C, H, N).

*3.1.2.3. 7-Amino-4-phenyl-2-(4-ethylpiperazin-1-yl)-1,8-naphthyridine (**4c**).* 0.55 g, yield 42%; m.p. 198–200 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 7.69 (d, 1H, H_5), 7.43 (m, 5H, Ar), 6.65 (s, 1H, H_3), 6.39 (d, 1H, H_6), 4.82 (brs, 2H, NH_2), 3.85 (m, 4H, piperazinyl), 2.49 (m, 6H, CH_2 and piperazinyl), 1.14 (t, 3H, CH_3). *Anal*. $C_{20}H_{23}N_5$ (C, H, N).

*3.1.2.4. 7-Amino-4-phenyl-2-(4-phenylpiperazin-1-yl)-1,8-naphthyridine (**4d**).* 0.50 g, yield 34%; m.p. 262–265 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 7.71 (d, 1H, H_5), 7.45 (m, 4H, Ar), 7.31 (m, 3H, Ar), 7.01 (m, 3H, Ar), 6.70 (s, 1H, H_3), 6.42 (d, 1H, H_6), 4.85 (brs, 2H, NH_2), 4.00 (m, 4H, piperazinyl), 3.32 (m, 4H, piperazinyl). *Anal*. $C_{24}H_{23}N_5$ (C, H, N).

*3.1.3. General procedure for the preparation of 2-cycloalkylamino-7-hydroxy-4-phenyl-1,8-naphthyridine derivatives **5a–d***

Sodium nitrite (1.8 mmol) was added portion wise to an ice-cooled solution (−12 °C) of the appropriate 7-amino-2-cycloalkylamino-4-phenyl-1,8-naphthyridine derivative **4a–d** (1.50 mmol) in concentrated sulfuric acid (5 ml). The reaction mixture was stirred at room temperature for 2 h and then treated with crushed ice and then with concentrated NH_4OH until the pH was 8. The solid was collected by filtration, washed with H_2O and purified by flash chromatography eluting with ethyl acetate for **5a** and **5b**, ethyl acetate/diethylamine 12:1 for **5c** and ethyl acetate/diethylamine 10:1 for **5d**.

*3.1.3.1. 7-Hydroxy-4-phenyl-2-(piperidin-1-yl)-1,8-naphthyridine (**5a**).* 0.38 g yield 37%; m.p. 242–244 °C (toluene). ^{1}H NMR (DMSO): δ 11.55 (brs, 1H, OH), 7.46 (m, 6H, Ar and H_5), 6.59 (s, 1H, H_3), 6.09 (d, 1H, H_6), 3.69 (s, 4H, piperidinyl), 1.56 (s, 6H, piperidinyl). *Anal*. $C_{19}H_{19}N_3O$ (C, H, N).

*3.1.3.2. 7-Hydroxy-2-(morpholin-1-yl)-4-phenyl-1,8-naphthyridine (**5b**).* 0.42 g, yield 32%; m.p. 254–256 °C (toluene). ^{1}H NMR (DMSO): δ 11.58 (brs, 1H, OH), 7.47 (m, 6H, Ar and H_5), 6.62 (s, 1H, H_3), 6.14 (d, 1H, H_6), 3.67 (s, 8H, morpholinyl). *Anal*. $C_{18}H_{17}N_3O_2$ (C, H, N).

*3.1.3.3. 7-Hydroxy-4-phenyl-2-(4-ethylpiperazin-1-yl)-1,8-naphthyridine (**5c**).* 0.54 g, yield 53%; m.p. 157–160 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 9.08 (brs, 1H, OH), 7.58 (d, 1H, H_5), 7.40 (m, 5H, Ar), 6.46 (s, 1H, H_3), 6.31 (d, 1H, H_6), 3.75 (m, 4H, piperazinyl), 2.53 (m, 6H, piperazinyl and CH_2), 1.14 (t, 3H, CH_3). *Anal*. $C_{20}H_{22}N_4O$ (C, H, N).

*3.1.3.4. 7-Hydroxy-4-phenyl-2-(4-phenylpiperazin-1-yl)-1,8-naphthyridine (**5d**).* 0.30 g, yield 33%; m.p. 253–256 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 9.08 (brs, 1H, OH), 7.60 (d, 1H, H_5), 7.43 (m, 6H, Ar), 6.96 (m, 4H, Ar), 6.51 (s, 1H, H_3), 6.33 (d, 1H, H_6), 3.88 (m, 4H, piperazinyl), 3.32 (m, 6H, piperazinyl). *Anal*. $C_{24}H_{22}N_4O$ (C, H, N).

*3.1.4. General procedure for the preparation of 7-chloro-2-cycloalkylamino-4-phenyl-1,8-naphthyridine derivatives **6a–d***

A mixture of 0.98 mmol of the appropriate 2-cycloalkylamino-7-hydroxy-4-phenyl-1,8-naphthyridine derivative **5a–d** and 3 ml of phosphoryl chloride was heated at 130 °C for 3 h. The cooled mixture was poured into crushed ice and treated with concentrated NH_4OH until the pH was 8. The resulting precipitate was collected by filtration, washed with water and purified by crystallization to give **6a–6d**.

*3.1.4.1. 7-Chloro-4-phenyl-2-(piperidin-1-yl)-1,8-naphthyridine (**6a**).* 0.28 g, yield 87%; m.p. 143–146 °C (petroleum ether 100–140 °C). ^{1}H NMR ($CDCl_3$): δ 7.82 (d, 1H, H_5), 7.45 (m, 5H, Ar), 7.01 (d, 1H, H_6), 6.92 (s, 1H, H_3), 3.87 (m, 4H, piperidinyl), 1.71 (m, 6H, piperidinyl). *Anal*. $C_{19}H_{18}N_3Cl$ (C, H, N).

*3.1.4.2. 7-Chloro-2-(morpholin-1-yl)-4-phenyl-1,8-naphthyridine (**6b**).* 0.27 g yield 84%; m.p. 91–94 °C (petroleum ether 40–60 °C). ^{1}H NMR ($CDCl_3$): δ 7.89 (d, 1H, H_5), 7.45 (m, 5H, Ar), 7.10 (d, 1H, H_6), 6.91 (s, 1H, H_3), 3.86 (m, 8H, morpholinyl). *Anal*. $C_{18}H_{16}ClN_3O$ (C, H, N).

*3.1.4.3. 7-Chloro-4-phenyl-2-(4-ethylpiperazin-1-yl)-1,8-naphthyridine (**6c**).* 0.31 g yield 89%; m.p. 167–170 °C (petroleum ether 100–140 °C). ^{1}H NMR ($CDCl_3$): δ 7.87 (d, 1H, H_5), 7.48 (m, 5H, Ar), 7.08 (d, 1H, H_6), 6.92 (s, 1H, H_3), 3.94 (m, 4H, piperazinyl), 2.53 (m, 6H, piperazinyl and CH_2). *Anal*. $C_{20}H_{21}ClN_4$ (C, H, N).

*3.1.4.4. 7-Chloro-4-phenyl-2-(4-phenylpiperazin-1-yl)-1,8-naphthyridine (**6d**).* 0.36 g yield 93%; m.p. 90–93 °C (petroleum ether 100–140 °C). ^{1}H NMR ($CDCl_3$): δ 7.89 (d, 1H, H_5), 7.42 (m, 7H, Ar and H_6), 7.00 (m, 5H, Ar and H_3), 4.08 (m, 4H, piperazinyl), 3.47 (m, 4H, piperazinyl). *Anal*. $C_{24}H_{21}ClN_4$ (C, H, N).

*3.1.5. General procedure for the preparation of 2-cycloalkylamino-7-methoxy-4-phenyl-1,8-naphthyridine derivatives **7a**–**d***

A solution of 15 mmol of freshly prepared sodium methoxide and 1.5 mmol of the appropriate 7-chloro-2-cycloalkylamino-4-phenyl-1,8-naphthyridine derivative **6a**–**d** in 30 ml of absolute methanol was refluxed for 5.5 h. After cooling, water was added and the pH of the mixture was adjusted to 8 with 10% HCl. The solid obtained was collected by filtration and crystallized from petroleum ether 100–140 °C to give **7a**–**d**.

*3.1.5.1. 7-Methoxy-4-phenyl-2-(piperidin-1-yl)-1,8-naphthyridine (**7a**).* 0.33 g yield 69%; m.p. 80–82 °C. ^{1}H NMR ($CDCl_3$): δ 7.78 (d, 1H, H_5), 7.47 (m, 5H, Ar), 6.76 (s, 1H, H_3), 6.57 (d, 1H, H_6), 4.11 (m, 3H, OCH_3), 3.81 (m, 4H, piperidinyl), 1.70 (m, 6H, piperidinyl). *Anal*. $C_{20}H_{21}N_3O$ (C, H, N).

*3.1.5.2. 7-Methoxy-2-(morpholin-1-yl)-4-phenyl-1,8-naphthyridine (**7b**).* 0.35 g yield 72%; m.p. 85–87 °C. ^{1}H NMR ($CDCl_3$): δ 7.83 (d, 1H, H_5), 7.48 (m, 5H, Ar), 6.74 (s, 1H, H_3), 6.38 (d, 1H, H_6), 4.14 (m, 3H, OCH_3), 3.83 (m, 8H, morpholinyl). *Anal*. $C_{19}H_{19}N_3O_2$ (C, H, N).

*3.1.5.3. 7-Methoxy-4-phenyl-2-(4-ethylpiperazin-1-yl)-1,8-naphthyridine (**7c**).* 0.39 g yield 74%; m.p. 139–141 °C. ^{1}H NMR ($CDCl_3$): δ 7.80 (d, 1H, H_5), 7.47 (m, 5H, Ar), 6.76 (s, 1H, H_3), 6.60 (d, 1H, H_6), 4.14 (m, 3H, OCH_3), 3.88 (m, 4H, piperazinyl), 2.59 (m, 6H, piperazinyl and CH_2), 1.15 (t, 3H, CH_3). *Anal*. $C_{21}H_{24}N_4O$ (C, H, N).

*3.1.5.4. 7-Methoxy-4-phenyl-2-(4-phenylpiperazin-1-yl)-1,8-naphthyridine (**7d**).* 0.45 g yield 76%; m.p. 85–88 °C. ^{1}H NMR ($CDCl_3$): δ 7.83 (d, 1H, H_5), 7.31 (m, 6H, Ar), 6.87 (m, 5H, Ar and H_3), 6.63 (d, 1H, H_6), 4.16 (m, 3H, OCH_3), 4.02 (m, 4H, piperazinyl), 3.35 (m, 4H, piperazinyl). *Anal*. $C_{25}H_{24}N_4O$ (C, H, N).

3.2. Biological assay

3.2.1. Antimycobacterial activity

All of the compounds were evaluated for in vitro antituberculosis activity against *M. tuberculosis* as part of a TAACF TB screening program under the direction of the US National Institute of Health, NIAID division.

Primary screening was conducted at a single concentration, 6.25 μg/ml against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the microplate Alamar Blue Assay (MABA) [15]. The standard compound used in this primary assay was rifampicin (MIC = 0.25 μg/ml).

Compounds effecting $<90\%$ inhibition in the primary screening (MIC $>$ 6.25 μg/ml) were not generally evaluated further. The active compounds were re-tested by serial dilution beginning at 6.25 μg/ml against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) in BABTEC 460. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% compared with controls.

3.2.2. Cytotoxicity assay

The compounds were screened by serial dilution to assess toxicity on a VERO cell line, generally beginning at 10 × the MIC, if permitted by the sample solubility in culture media. The selectivity index (SI) is defined as the ratio of the IC_{50} measured in VERO cells to the MIC described above.

3.2.3. Antimicrobial activity

The 1,8-naphthyridine derivatives were evaluated in vitro for their antimicrobial activity against *S. aureus* as Gram-positive bacteria and *E. coli* as Gram-negative bacteria, using the cup diffusion technique [16].

The compounds were dissolved in DMSO at a concentration of 1 mg/ml. Sterile nutrient agar (oxoid) was incubated with the organisms tested. Each 100 ml of the medium received 1 ml of 24 h broth culture and 3 drops of the test compounds were placed separately in cups (8 mm diameter) cut in the agar. The plates were incubated at 37 °C for 24 h. DMSO as a blank showed

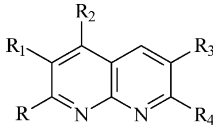
no inhibition zone. A solution of 0.1% of penicillin G or streptomycin sulfate in DMSO was used as the standard for Gram-positive and Gram-negative bacteria, respectively. The resulting inhibition zone diameters (I.Z.) were measured in mm. For compounds, which exhibited reasonable inhibition zones (≥ 20 mm), the MIC was determined. The organisms tested were grown in suitable broth media for 24 h at 37 °C. The compounds selected were dissolved in DMSO (400 μg/ml) and twofold serial dilutions were prepared using the appropriate broth. The tubes were then inoculated with 100 μl of 24 h test organism culture and incubated at 37 °C for 48 h.

4. Results and discussion

4.1. Antimycobacterial activity

The new compounds **2a**–**d**, **4a**–**d**, **5a**–**d**, **6a**–**d** and **7a**–**d** and compounds previously reported **8a**–**n** [17], **8o**,**p** [18], and **8q** [19] were tested in vitro at a concentration

Table 1
Antimycobacterial in vitro activity of the tested compounds expressed as % inhibition of *M. tuberculosis* $H_{37}Rv$ at a concentration of 6.25 μg/ml



Comp.	R	R_1	R_2	R_3	R_4	% Inhibition
2a	Pip	H	Ph	H	Pip	99
2b	Morph	H	Ph	H	Morph	0
2c	Ethylpipz	H	Ph	H	Ethylpipz	81
2d	Phenylpipz	H	Ph	H	Phenylpipz	47
4a	Pip	H	Ph	H	NH_2	91
4b	Morph	H	Ph	H	NH_2	47
4c	Ethylpipz	H	Ph	H	NH_2	69
4d	Phenylpipz	H	Ph	H	NH_2	94
5a	Pip	H	Ph	H	OH	0
5b	Morph	H	Ph	H	OH	0
5c	Ethylpipz	H	Ph	H	OH	29
5d	Phenylpipz	H	Ph	H	OH	32
6a	Pip	H	Ph	H	Cl	70
6b	Morph	H	Ph	H	Cl	36
6c	Ethylpipz	H	Ph	H	Cl	68
6d	Phenylpipz	H	Ph	H	Cl	55
7a	Pip	H	Ph	H	OCH_3	85
7b	Morph	H	Ph	H	OCH_3	35
7c	Ethylpipz	H	Ph	H	OCH_3	72
7d	Phenylpipz	H	Ph	H	OCH_3	86
8a	Pip	Ph	H	H	Pip	99
8b	Pip	Ph	H	NO_2	Pip	79
8c	Pip	Ph	H	NH_2	Pip	71
8d	Pip	Ph	H	H	Cep	91
8e	Pip	Ph	H	H	Pipz	89
8f	Pip	Ph	H	H	Morph	83
8g	Pip	Ph	H	H	NH_2	59
8h	Cep	Ph	H	H	Cep	48
8i	Cep	Ph	H	H	Pip	92
8l	Cep	Ph	H	H	Morph	64
8m	Cep	Ph	H	H	Cl	67
8n	Cmp	Ph	H	H	OCH_3	43
8o	Pipz	Ph	H	H	Pipz	73
8p	Pipz	Ph	H	H	Pip	88
8q	Pipz	Ph	H	H	OCH_3	67
RMP						98 [a]

Pip, piperidinyl; Morph, morpholinyl; Ethylpipz, ethylpiperazinyl; Phenylpipz, phenylpiperazinyl; Cep, ethylcarbethoxypiperazinyl; Cmp, methylcarbethoxypiperazinyl. Pipz, piperazinyl.

[a] At a concentration of 0.25 μg/ml.

of 6.25 μg/ml against *M. tuberculosis* $H_{37}Rv$ by the method described by Collins and Franzblau [15]. The results of the biological evaluation, expressed as % inhibition of the growth of mycobacterium, are summarized in Table 1 and for the sake of comparison the % inhibition of Rifampicin (RMP) is also included. All the compounds tested proved to be less active, against *M. tuberculosis* $H_{37}Rv$, than Rifampicin. In particular, compounds **2a**, **4a**,**d**, **8a**,**d** and **8i** showed an appreciable activity with inhibition from 91 to 99%. Compounds **2c**, **4c**, **6a**,**c**,**d**, **7a**,**c**,**d**, **8b**,**c**, **8e**–**g**,**l**,**m**,**o**,**p** and **8q**, showed a moderate activity, with inhibition from 50 to 90%, whereas compounds **2b**,**d**, **4b**, **5a**–**d**, **6b**, **7b**, **8h** and **8n**, with inhibition $< 50\%$, were found to possess little or no activity at the concentration assayed (Table 1).

Compounds **2a**, **4a**,**d**, **8a**,**d** and **8i**, which demonstrated an inhibition $> 90\%$ in the primary screening, were retested at a lower concentration against *M. tuberculosis* $H_{37}Rv$ to determine the actual MIC with the same method used in the primary screening. The results of the MIC evaluation (Table 2) indicate that only compounds **2a**, **8a** and **8d** showed an MIC of 6.25 μg/ml, whereas compounds **4a**,**d** and **8i** showed an MIC > 6.25 μg/ml.

Concurrent with the determination of MICs, compounds **2a**, **8a** and **8d** were tested for cytotoxicity (IC_{50}) on a VERO cell line. The cytotoxicity data indicate that compounds **2a** and **8a** exhibit a high degree of toxicity and a low selectivity index level (SI) (Table 2). In the case of compound **8d**, the cytotoxicity was not determined because of its insolubility in tissue culture media (Table 2).

The biological results of this series of 1,8-naphthyridine derivatives, which present a wide number of structural variables, linked to several molecular regions, allow us to confirm [10,11] or clarify the influence of the substituents in positions 2, 3, 4 and 7 of the 1,8-naphthyridine nucleus.

On the basis of the biological results, the most effective substituent in positions 2 and/or 7 seems to be the piperidinyl group. This result is in agreement with previous findings [11].

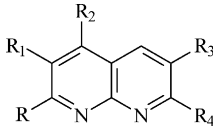
The ethylcarbethoxypiperazinyl group and the new ethylpiperazinyl and phenylpiperazinyl groups seem to be effective substituents in positions 2 and/or 7 of the 1,8-naphthyridine nucleus for the antimycobacterial activity. Moreover, also the amino, chloro or methoxy groups in position 7 of the heterocyclic ring seem to be effective for the antimycobacterial activity. In fact, the 7-amino derivatives **4a**,**c**,**d**, the 7-chloro derivatives **6a**,**c**,**d** and the 7-methoxy derivatives **7a**,**c**,**d** showed a moderate activity with inhibition from 55 to 91%. The analogous derivatives **4b**, **6b** and **7b** showed a very poor activity: most probably, the lack of activity is due to the presence of the morpholinyl group in position 2 of the 1,8-naphthyridine ring.

A comparison of the activity of morpholinyl-substituted derivatives versus the activity of the other 1,8-naphthyridine derivatives clearly indicates that the introduction of a morpholinyl group either in position 2 or 7 of the heterocycle ring causes a decrease in activity as previously reported [11].

Furthermore, the biological results showed that the introduction of nitro or amino group in position 6 of the 2,7-dipiperidinyl-3-phenyl-1,8-naphthyridine (**8a**), compounds **8b** and **8c**, respectively, resulted in a decrease of the antimycobacterial activity.

Moreover, the biological results of this series of 1,8-naphthyridine derivatives confirmed that, when a hydroxy group was introduced into position 7 of the 1,8-naphthyridine nucleus, inactive compounds were obtained, as also reported in a previous paper [10,11].

Table 2
Antimycobacterial in vitro activity and cytotoxicity effect of compounds **2a**, **4a**,**d**, **8a**,**d**,**i**



Comp.	R	R_1	R_2	R_3	R_4	MIC (μg/ml)	IC_{50} (μg/ml)	SI
2a	Pip	H	Ph	H	Pip	6.25	2.49	0.40
4a	Pip	H	Ph	H	NH_2	> 6.25	nd [a]	nd [a]
4d	Phenylpipz	H	Ph	H	NH_2	> 6.25	nd [a]	nd [a]
8a	Pip	Ph	H	H	Pip	6.25	2.74	0.44
8d	Pip	Ph	H	H	Cep	6.25	[b]	[b]
8i	Cep	Ph	H	H	Pip	> 6.25	nd [a]	nd [a]
RMP						0.25		

Pip, piperidinyl; Phenylpipz, phenylpiperazinyl; Cep, ethylcarbethoxypiperazinyl.
[a] nd: not determined.
[b] Insoluble in tissue culture media.

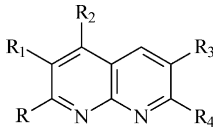
Finally, the phenyl group in position 3 or 4 of the heterocyclic ring seems to play an important role for the antimycobacterial activity of 1,8-naphthyridine derivatives, confirming that in these positions a large lipophilic group is indispensable, as shown in a previous paper for the benzylic group in position 3 [11].

4.2. Antimicrobial activity

The new compounds were evaluated in vitro for their antibacterial activity against *S. aureus* as Gram-positive bacteria and *E. coli* as Gram-negative bacteria (Table 3). Furthermore, the MIC values in μg/ml using twofold serial dilution method were calculated for compounds which exhibited reasonable inhibition zones (≥ 20 mm).

The results of the biological evaluation indicate that all the compounds tested were less active than the reference standards. Compound **8b** showed an appreciable activity against *S. aureus* with an MIC value of 25, compounds **5a**, **6a** and **7d** possessed a moderate activity with an MIC value of 100 and compounds **4b**, **5b**, **8f** and **8i**, with MIC values from 140 to 200, showed a poor

Table 3
Inhibition zone diameters (I.Z.), values in mm and MIC values in μg/ml



Comp.	R	R_1	R_2	R_3	R_4	*S. aureus*		*E. coli*	
						I.Z.	MIC	I.Z.	MIC
2a	Pip	H	Ph	H	Pip	14		28	25
2b	Morph	H	Ph	H	Morph	12		–	
2c	Ethylpipz	H	Ph	H	Ethylpipz	12		12	
2d	Phenylpipz	H	Ph	H	Phenylpipz	12		18	
4a	Pip	H	Ph	H	NH_2	14		20	100
4b	Morph	H	Ph	H	NH_2	20	200	33	25
4c	Ethylpipz	H	Ph	H	NH_2	16		36	12.5
4d	Phenylpipz	H	Ph	H	NH_2	16		32	50
5a	Pip	H	Ph	H	OH	24	100	–	
5b	Morph	H	Ph	H	OH	20	200	26	200
5c	Ethylpipz	H	Ph	H	OH	12		–	
5d	Phenylpipz	H	Ph	H	OH	14		14	
6a	Pip	H	Ph	H	Cl	26	100	–	
6b	Morph	H	Ph	H	Cl	18		12	
6c	Ethylpipz	H	Ph	H	Cl	–		16	
6d	Phenylpipz	H	Ph	H	Cl	22		14	
7a	Pip	H	Ph	H	OCH_3	–		20	200
7b	Morph	H	Ph	H	OCH_3	–		24	100
7c	Ethylpipz	H	Ph	H	OCH_3	–		–	
7d	Phenylpipz	H	Ph	H	OCH_3	28	100	–	
8a	Pip	Ph	H	H	Pip	16		20	100
8b	Pip	Ph	H	NO_2	Pip	30	25	–	
8c	Pip	Ph	H	NH_2	Pip	–		26	50
8d	Pip	Ph	H	H	Cep	15		18	
8e	Pip	Ph	H	H	Pipz	18		14	
8f	Pip	Ph	H	H	Morph	22	160	26	130
8g	Pip	Ph	H	H	NH_2	18		–	
8h	Cep	Ph	H	H	Cep	17		18	
8i	Cep	Ph	H	H	Pip	23	140	25	120
8l	Cep	Ph	H	H	Morph	13		17	
8m	Cep	Ph	H	H	Cl	–		24	45
8n	Cmp	Ph	H	H	OCH_3	16		12	
8o	Pipz	Ph	H	H	Pipz	14		12	
8p	Pipz	Ph	H	H	Pip	–		18	
	Pipz	Ph	H	H	OCH_3	12		16	
Penicillin G						42	4		
Streptomycin								38	6

Pip, piperidinyl; Cep, ethylcarbethoxypiperazinyl; Ethylpipz, ethylpiperazinyl; Phenylpipz, phenylpiperazinyl; Morph, morpholinyl; Pipz, piperazinyl; Cmp, methylcarbethoxypiperazinyl.

activity. The other 1,8-naphthyridine derivatives were found to be inactive.

As regards activity against *E. coli*, compounds **2a**, **4b**–**d**, **8c** and **8m** showed an appreciable activity with MIC value from 12.5 to 50. The compounds **4a**, **5b**, **7a**,**b**, **8a**,**f** and **8i** showed a moderate activity with MIC values from 100 to 200. The other 1,8-naphthyridine derivatives were found to be inactive.

On the basis of these results, the 1,8-naphthyridine derivatives seem to be more active against *E. coli* than against *S. aureus*. At this time the biological results do not permit us to deduce a structure–activity relationship.

Acknowledgements

The in vitro evaluation of the antituberculosis activity was carried out in the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) at the National Institute of Allergy and Infectious Disease, Southern Research Institute, GWL Hansen's Disease Center and Colorado State University, USA; we thank J.A. Maddry, Ph.D. for his collaboration.

References

[1] C. Dye, Global burden of tuberculosis, J. Am. Med. Assoc. 282 (1999) 667–686.
[2] A.M. Rohui, Tuberculosis: a tough adversary, C&EN 17 (1999) 52–69.
[3] M.C. Raviglione, D.E. Snider, A. Kochi, Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic, J. Am. Med. Assoc. 273 (1995) 220–226.
[4] N.E. Billo, Global aspects of tuberculosis, in: P.R.J. Gangadharam, P.A. Jenkins (Eds.), Mycobacteria II Chemotherapy, Chapman and Hall, New York, 1998, pp. 1–14.
[5] G.J. Churchyard, A.D. Grant, HIV infection, tuberculosis and non-tuberculous mycobacteria, South African Med. J. 91 (2000) 472–476.
[6] B. Petrini, S. Hoffner, Drug-resistant and multidrug-resistant tubercle bacilli, Int. J. Antimicrob. Agents 13 (1999) 93–97.
[7] J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, Goodman and Gilman's The Pharmacological Basis of Therapeutics, ninth ed., McGraw-Hill, New York, 1996, p. 1155.
[8] S. Jyoti, Taking toll of TB, Trends Microbiol. 9 (2001) 255.
[9] C.E.I. Barry, New horizons in the treatment of tuberculosis, Biochem. Pharmacol. 54 (1997) 1165–1172.
[10] P.L. Ferrarini, C. Manera, C. Mori, M. Badawneh, G. Saccomanni, Synthesis and evaluation of antimycobacterial activity of 4-phenyl-1,8-naphthyridine derivatives, Farmaco 53 (1998) 741–746.
[11] M. Badawneh, C. Manera, C. Mori, G. Saccomanni, P.L. Ferrarini, Synthesis of variously substituted 1,8-naphthyridine derivatives and evaluation of their antimycobacterial activity, Farmaco 57 (2002) 631–639.
[12] F. Al-Omran, R.M. Mohareb, A.A. El-Khair, Synthesis and biological effects of new derivatives of benzotriazole as antimicrobial and antifungal agents, J. Heterocycl. Chem. 39 (2002) 877–882.
[13] A. Mangini, M. Colonna, Contributo alla conoscenza delle naftiridine, Gazz. Chim. Ital. 72 (1942) 183–197.
[14] S. Carboni, A. Da Settimo, P.L. Ferrarini, I. Tonetti, Ricerca nel campo delle antiridine, Gazz. Chim. Ital. 97 (1967) 1262–1273.
[15] L.A. Collins, S.G. Franzblau, Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium, Antimicrob. Agents Chemother. 42 (1997) 1004–1009.
[16] S.R. Jain, A. Kar, The antibacterial activity of some essential oils and their combinations, Planta Med. 20 (1971) 118–122.
[17] P.L. Ferrarini, M. Badawneh, F. Franconi, C. Manera, C. Mori, M. Miceli, G. Saccomanni, Synthesis and antiplatelet activity of some 2,7-di(*N*-cycloamino)-3-phenyl-1,8-naphthyridine derivatives, Farmaco 56 (2001) 311–318.
[18] P.L. Ferrarini, C. Mori, M. Badawneh, F. Franconi, C. Manera, M. Miceli, G. Saccomanni, Synthesis and antiplatelet activity of some 3-phenyl-1,8-naphthyridine derivatives, Farmaco 55 (2001) 603–610.
[19] P.L. Ferrarini, C. Mori, M. Badawneh, C. Manera, A. Martinelli, M. Miceli, F. Romagnoli, G. Saccomanni, Unusual nitration of substituted 7-amino-1,8-napthyridine in the synthesis of compounds with antiplatelet activity, J. Heterocyclic Chem. 34 (1997) 1501–1510.