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

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Abstract

Some 4-phenyl-1,8-naphthyridine derivatives with a piperazino group in the 2- and/or 7-position have been synthesized and evaluated for their tuberculostatic activity. The compounds **1**, **6**, **10**, **17b**,**c** and **19i** showed a marked activity against *Mycobacterium tuberculosis* $H_{37}Rv$. For this series of compounds, submitted to biological screening, no structure–activity relationship can be deduced.

Keywords: Tuberculostatic agents; 1,8-Naphthyridines; Piperazino derivatives

1. Introduction

Tuberculosis can be considered today as the major opportunistic emerging infection [1], because of its increasing during the last years.

Moreover, it has been reported that only a few compounds with a bicyclic structure have been synthesized and tested for their antituberculosis activity. In particular, the preliminary antimycobacterial screening against *Mycobacterium tuberculosis* showed that some compounds, having the quinoline and quinazoline ring system with morpholino group in the 7-position and phenyl group in the 2- or 3-position, respectively, possess tuberculostatic activity [2–6].

These considerations induced us to plan a program of synthesis and antiinfective screening of a series of 1,8-naphthyridine derivatives, structurally correlated to quinoline and quinazoline as already mentioned.

In this paper we report the synthesis and the biological results of some 4-phenyl-1,8-naphthyridine derivatives.

2. Chemistry

The carbethoxypiperazino derivative **1**, previously described [7], was converted to compound **2** by alkaline hydrolysis. The acetyl derivatives **3** and **4** were then prepared from **1** and **2**, respectively, by reaction with acetic anhydride (Scheme 1, Table 1).

When the hydroxy derivative **5** [7] was treated with phosphoryl chloride, the chloro derivative **6** was obtained. The acid hydrolysis of this compound gave a recovery of the starting compound **6** instead of the chloro derivative **7**, whereas by alkaline hydrolysis of **6** the hydroxy derivative **8** was obtained; this was also prepared by alkaline hydrolysis of **5**. Compound **6** was then treated with sodium methoxide to give, by transesterification, the methoxy derivative **9**, and treatment with a hydroalcoholic solution of sodium hydroxide gave the corresponding piperazino derivative **10** (Scheme 2, Table 1).

Attempts of dehalogenation of **6**, carried out with hydrogen at 2 atm in acetic acid in the presence of sodium acetate using 10% palladium on charcoal as catalyst at 70°C or in methanol using a Ni-Raney catalyst at room temperature, gave the corresponding tetrahydro derivative **11** (Scheme 3, Table 1).

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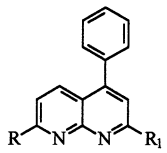
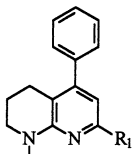
Table 1
Physical data of 1,8-naphthyridine and 1,2,3,4-tetrahydro-1,8-naphthyridine derivatives



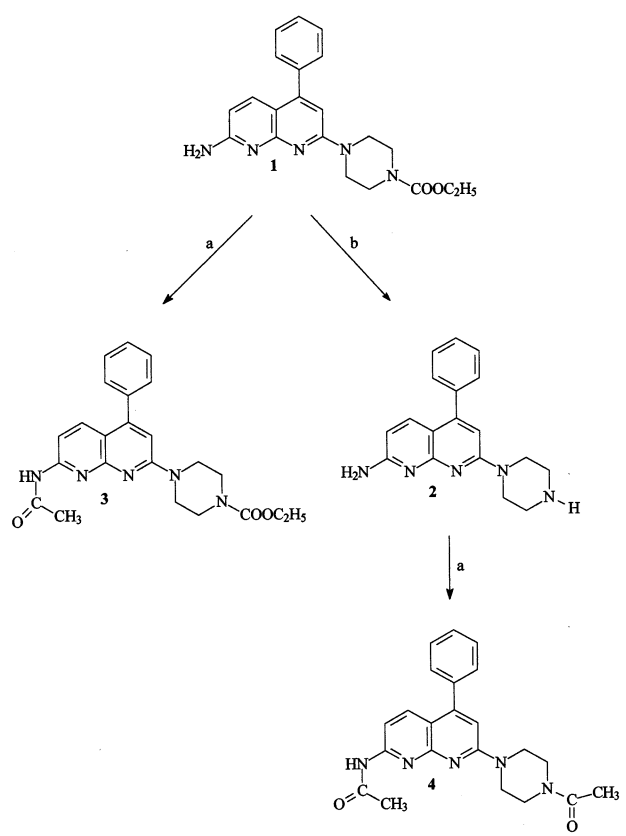
2-4,6,8-10,12,14,16a,17,18



11,13

Comp.	R[a]	R_1[a]	Yield (%)	Crystallization solvent[b]	M.p. (°C)	Empirical formula
2	NH_2	Pipz	98	Toluene	249–251	$C_{18}H_{19}N_5$
3	$NHCOCH_3$	Cep	93	Toluene	127–129	$C_{23}H_{25}N_5O_3$
4	$NHCOCH_3$	Apipz	98	Ethanol–water	146–149	$C_{22}H_{23}N_5O_2 \cdot H_2O$
6	Cl	Cep	93	Petroleum ether	159–161	$C_{21}H_{21}N_4O_2Cl$
8	OH	Pipz	97	Benzene	207–210	$C_{18}H_{18}N_4O$
9	OCH_3	Cmp	87	Benzene/petroleum ether	163–166	$C_{21}H_{22}N_4O_3$
10	OCH_3	Pipz	94	Petroleum ether	85–88	$C_{19}H_{20}N_4O$
11	–	Cep	85	2-Propanol	142–144	$C_{21}H_{26}N_4O_2$
12	H	Cep	68	Petroleum ether	125–128	$C_{21}H_{22}N_4O_2$
13	–	Pipz	84	Petroleum ether	125–127	$C_{18}H_{22}N_4$
14	H	Pipz	90	Cyclohexane	112–113	$C_{18}H_{18}N_4$
16a	Cl	OCH_3	93	Ethanol	152–153	$C_{15}H_{11}N_2OCl$
17a	Cep	OH	93	Toluene	216–218	$C_{21}H_{22}N_4O_3$
17b	Cep	Cep	78	Cyclohexane	173–175	$C_{28}H_{34}N_6O_4$
17c	Cep	OCH_3	94	Benzene	154–157	$C_{22}H_{24}N_4O_3$
18a	Pipz	OH	93	Toluene	183–185	$C_{18}H_{18}N_4O$
18b	Pipz	Pipz	98	Benzene	115–117	$C_{22}H_{26}N_6 \cdot H_2O$
18c	Pipz	OCH_3	96	Petroleum ether	71–74	$C_{19}H_{20}N_4O$

[a] Pipz, piperazinyl; Cep, 4-carbethoxypiperazinyl; Apipz, 4-acetylpiperazinyl; Cmp, 4-carbomethoxypiperazinyl.
[b] Petroleum ether 100–140°C was used.



Scheme 1. (a) Acetic anhydride; (b) 10% aqueous NaOH, ethanol.

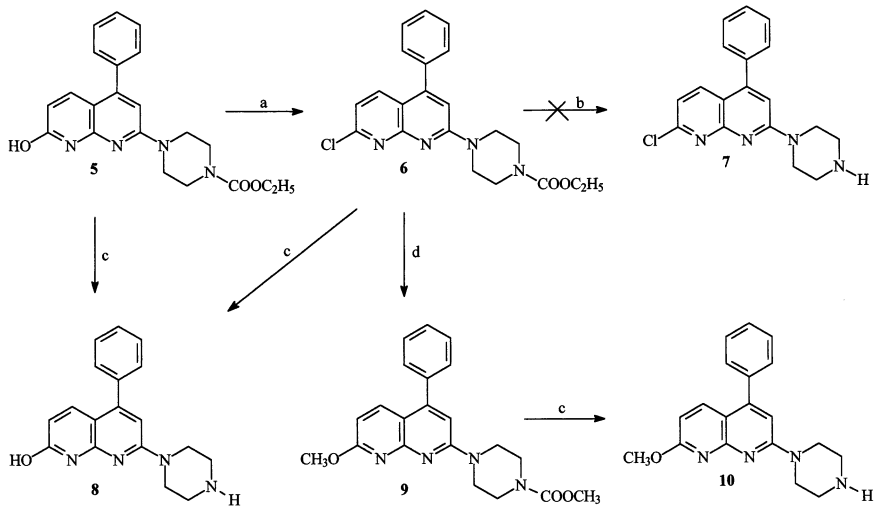
The structure of this compound was confirmed by ^1H NMR spectroscopy; in fact, the ^1H NMR spectrum shows three multiplets at δ 2.61, 2.01 and 3.47 due to H_5, H_6 and H_7, respectively, and a singlet at δ 5.72 due to H_3 (Table 2).

When the chloro derivative **6** was allowed to react with hydrogen in methanol at room temperature and pressure using 10% palladium on charcoal as catalyst, compound **12** was obtained (Scheme 3, Table 1). The structure of this compound was again confirmed by ^1H NMR spectroscopy, with the spectrum of **12** showing three multiplets at δ 8.03, 7.30 and 9.00 due to H_5, H_6 and H_7, respectively (Table 2).

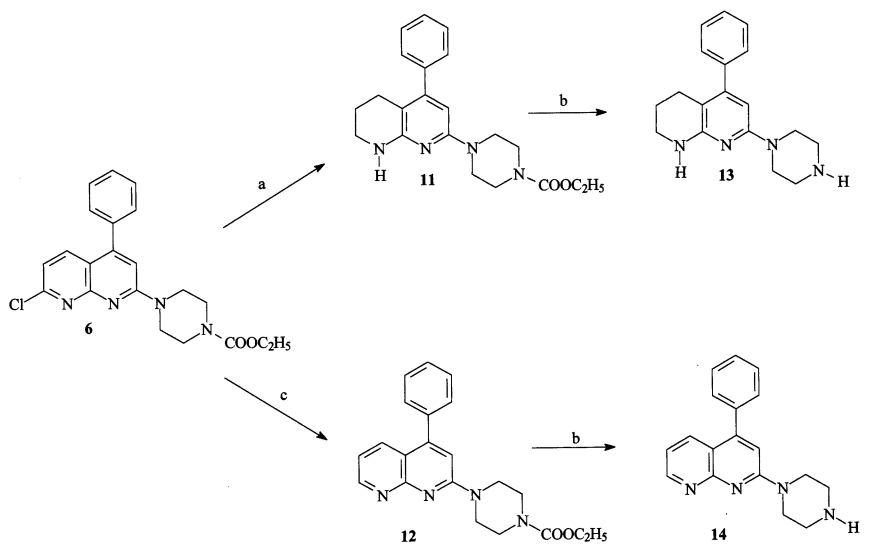
Compounds **11** and **12**, when treated with a hydroalcoholic solution of sodium hydroxide, gave the corresponding piperazino derivatives **13** and **14**, respectively (Scheme 3, Table 1)

The hydroxy derivative **15** [7] treated with phosphoryl chloride at 60°C gave the chloro derivative **16a** (Scheme 4, Table 1).

When the chloro derivatives **16a** and **16b** [8] were allowed to react with *N*-carbethoxypiperazine in sealed tube at 140°C, the hydroxy derivative **17a** and the dicarbethoxy derivative **17b** were obtained, respectively (Scheme 5, Table 1). Compound **17c** was prepared by reaction of **16a** with *N*-carbethoxypiperazine in toluene at 80°C (Scheme 5, Table 1). Alkaline hydrolysis of



Scheme 2. (a) $POCl_3$; (b) 20% HCl; (c) 10% aqueous NaOH, ethanol; (d) sodium methoxide.



Scheme 3. (a) H_2, 10% Pd/C, CH_3COONa, CH_3COOH or Ni-Raney, methanol; (b) 10% aqueous NaOH, ethanol; (c) H_2, 10% Pd/C, methanol.

carbethoxypiperazino derivatives **17** gave the corresponding compound **18** (Scheme 5, Table 1).

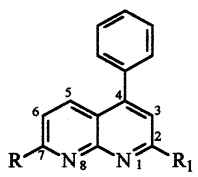
All the synthesized compounds were characterized by elemental analysis and ^1H NMR spectroscopy. The ^1H NMR spectra of the 1,8-naphthyridine derivatives **2–4**, **6**, **8–10**, **16a**, **17a–c** and **18a–c** prepared show two doublets between δ 6.35–7.38 and 7.52–8.10 due to H_6 and H_5, respectively, and a singlet ranging from δ 6.48 to 6.95 due to H_3 (Table 2).

3. Results and discussion

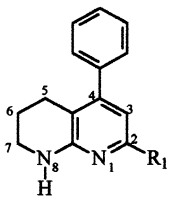
Some of these new compounds, **1–6**, **8–10**, **12**, **14**, **16b** and **17–19** (Table 3) previously prepared, were tested in vitro at a concentration of 12.5 μg/ml against *M. tubercolosis* $H_{37}Rv$ by a new method recently described by Collins and Franzblau [9]. The results of the biological evaluation indicate that only compounds **1**, **6**, **10**, **17b**,**c** and **19i** showed a marked activity against *M. tubercolosis* $H_{37}Rv$, whereas the other compounds tested were found to be slowly active or inactive at the assayed concentration (Table 4).

For this series of compounds, on the basis of the biological results obtained, the activity does not appear to be related directly with the substituents present on the different positions of the 1,8-naphthyridine nucleus and, consequently, no structure–activity relationship can be deduced.

Table 2
^{1}H NMR of 1,8-naphthyridine and tetrahydro-1,8-naphthyridine derivatives[a]



2-4,6,8-10,12,14,16a,17,18



11,13 [a]

Comp.	H_3(s)	H_5	H_6	H_7	C_6H_5(m)	Pipz[b](m)	Others
2	6.63	7.83(d)	6.53(d)	–	7.56	3.00, 3.83	NH 2.00; NH_2 4.96
3	6.90	8.10(d)	6.80(d)	–	7.56	3.40, 3.75	C_2H_5 1.33(t), 4.30(q); $COCH_3$ 2.23(s); NH 10.25
4	6.93	8.10(d)	6.91(d)	–	7.56	3.40, 4.00	$COCH_3$ 2.16(s); NH 8.70
6	6.95	7.96(d)	7.10(d)	–	7.60	3.63, 3.87	C_2H_5 1.33(t), 4.05(q)
8	6.48	7.60(d)	6.35(d)	–	7.56	3.56, 2.74	NH 2.40
9	6.76	7.83(d)	6.63(d)	–	7.55	3.50, 3.75	OCH_3 3.95(s); CH_3 3.75(s)
10	6.76	7.90(d)	6.63(d)	–	7.63	2.88, 3.69	OCH_3 4.20(s)
11	5.72	2.41(m)	1.64(m)	3.50(m)	7.30	3.32	C_2H_5 1.33(t), 4.03(q); NH 6.00
12	7.00	8.03(d)	7.30(dd)	8.90(d)	7.56	3.65, 4.00	C_2H_5 1.33(t), 4.23(q)
13	5.80	2.50(m)	1.85(m)	3.50(m)	7.28	3.00, 3.35	NH 3.35
14	6.93	7.96(d)	7.20(dd)	8.83(d)	7.45	3.86, 3.00	NH 2.20
16a	7.01	8.10(d)	7.38(d)	–	7.56	–	OCH_3 4.20(s)
17a	6.30	7.55(d)	6.42(d)	–	7.45	3.65	C_2H_5 1.33(t), 4.16(q)
17b	6.80	7.60(d)	6.92(d)	–	7.56	3.00, 3.80	C_2H_5 1.33(t), 4.16(q)
17c	6.71	7.93(d)	6.95(d)	–	7.53	3.50, 3.60	C_2H_5 1.33(t), 4.16(q); OCH_3 4.26(s)
18a	6.30	7.52(d)	6.80(d)	–	7.41	3.00, 3.65	NH 2.34
18b	6.67	7.73(d)	7.30(d)	–	7.53	3.00, 3.83	NH 2.20
18c	6.66	7.85(d)	7.33(d)	–	7.50	2.80, 3.60	NH 2.30; OCH_3 4.16(s)

[a] The numeration of the structure of the tetrahydro-1,8-naphthyridine derivatives reported in this table, is used only for their ^{1}H NMR data on the analogy of that of the 1,8-naphthyridine derivatives.
[b] Pipz, piperazinyl.

4. Experimental

4.1. Chemistry

All compounds were routinely checked for their structure by IR and ^{1}H NMR spectroscopy. Melting points were determined using a Köfler hot-stage apparatus and were uncorrected. The ^{1}H NMR spectra were determined in DMSO-d_6 or $CDCl_3$ with TMS as the internal standard on a Fourier transform spectrometer Varian Model CFT 20. Mass spectra were obtained using a Hewlett–Packard 5988A spectrometer operating at 70 eV. Analytical TLC was carried out on Merck 0.2 mm precoated silica-gel glass plates (60 F-254) and the location of spots was detected by illumination with a UV lamp. Flash chromatography was carried out on silica gel (60 size 0.04–0.063 mm) at low pressure. Elemental analyses of all synthesized compounds for C, H and N were within $\pm 0.4\%$ of the theoretical values and were performed by our analytical laboratory.

4.1.1. 7-(4-Carbethoxypiperazin-1-yl)-5-phenyl-1,2,3,4-tetrahydro-1,8-naphthyridine ***11***

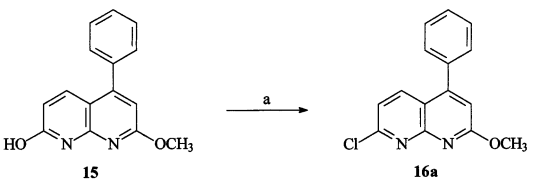
1. A solution of 1 mmol of **6** and 2 mmol of anhydrous sodium acetate in 30 ml of glacial acetic acid was hydrogenated in presence of 0.2 g of 10% palladium on charcoal at 2 atm of pressure and at 60°C for 16 h. After cooling, the catalyst was filtered and the solution was evaporated to dryness in vacuo. The resulting residue was treated with water, made alkaline with 10% aqueous sodium hydroxide and extracted twice with chloroform. The combined organic extracts were dried (magnesium sulfate) and evaporated in vacuo to obtain the title compound **11**, which was purified by crystallization (Tables 1 and 2).
2. A solution of 1 mmol of **6** in 50 ml of methanol was hydrogenated in presence of 2 g of Ni-Raney at room temperature and at atmospheric pressure for 12 h. Compound **11** was then obtained by an analogous procedure to that reported above.

4.1.2. 2-(4-Carbethoxypiperazin-1-yl)-4-phenyl-1,8-naphthyridine ***12***

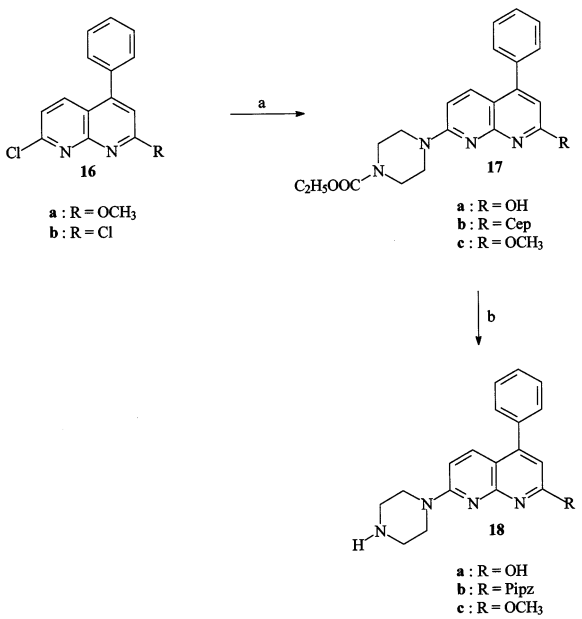
A solution of 1 mmol of **6** in 50 ml of methanol was hydrogenated in the presence of 0.2 g of 10% palladium on charcoal at room temperature and atmospheric pressure for 12 h. The catalyst was filtered and the solvent was evaporated to dryness in vacuo. The residue obtained was treated with water, made alkaline with 10% aqueous sodium hydroxide and then extracted twice with chloroform. The combined organic extracts, dried (magnesium sulfate) and evaporated to dryness in vacuo, gave an oil, which was purified by flash chromatography eluting with a mixture of ethyl acetate/petroleum ether (40–70°C)/diethylamine (9:3:0.5) and then crystallized to give **12** (Tables 1 and 2).

4.1.3. General procedure for the preparation of 7-chloro-4-phenyl-1,8-naphthyridine derivatives ***6*** *and* ***16a***

A solution of 5 mmol of **5** and **15** [7] in 20 ml of phosphoryl chloride was allowed to react at 80 and 60°C, respectively. The cooled solution was poured into crushed ice and the mixture was alkalinized with concentrated ammonium hydroxide. The resulting precipitate was collected, washed with water and crystallized to obtain **6** or **16a** (Tables 1 and 2).

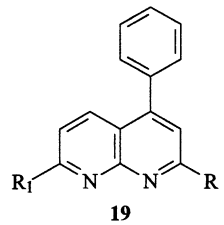


Scheme 4. (a) $POCl_3$.



Scheme 5. (a) *N*-Carbethoxypiperazine; (b) 10% aqueous NaOH, ethanol.

Table 3
1,8-Naphthyridine derivatives previously reported



Comp.	R	R_1	Ref.
a	$N(CH_2CH_2OH)_2$	NH_2	[10]
b	OH	$NHCOCH_3$	[8]
c	$N(CH_2CH_2OH)_2$	OH	[10]
d	$N(CH_2CH_2OH)_2$	$N(CH_2CH_2OH)_2$	[11]
e	OH	NH_2	[8]
f	SH	NH_2	[12]
g	NH_2	OH	[13]
h	Cl	$NHCOCH_3$	[13]
I	NH_2	OCH_3	[7]

4.1.4. 2-(4-Carbomethoxypiperazin-1-yl)-7-methoxy-4-phenyl-1,8-naphthyridine ***9***

To a solution of 50 mmol of sodium in 50 ml of absolute methanol, 5 mmol of **6** were added and the mixture was refluxed for 5 h and then the solvent was removed by evaporation in vacuo. The crude residue was treated with water and then 10% hydrochloric acid was added until pH 8. The solid obtained was collected, washed with water and crystallized to give **9** (Tables 1 and 2).

4.1.5. Preparation of 7-(4-carbethoxypiperazin-1-yl)-2-hydroxy- and of 2,7-di(4-carbethoxypiperazin-1-yl)-4-phenyl-1,8-naphthyridines ***17a,b***

A mixture of 5 mmol of **16a**,**b** and 15 mmol of *N*-carbethoxypiperazine was heated in sealed tube at 140°C for 20 h. The crude residue was treated with water and the solid obtained, collected by filtration and washed with water, was purified by crystallization to obtain **17a**,**b** (Tables 1 and 2).

4.1.6. 7-(4-Carbethoxypiperazin-1-yl)-2-methoxy-4-phenyl-1,8-naphthyridine ***17c***

A solution of 5 mmol of **16a** and 5.5 mmol of *N*-carbethoxypiperazine in 30 ml of toluene was kept at 80°C for 24 h. The solvent was evaporated to dryness in vacuo and the residue was treated with water, filtered and purified by crystallization to give **17c** (Tables 1 and 2).

Table 4
Antimycobacterial in vitro activity of the tested compounds expressed as % inhibition of *M. tuberculosis* $H_{37}Rv$ at a concentration of 12.5 μg/ml

Comp.	% Inhibition	Comp.	% Inhibition	Comp.	Ref.	% Inhibition
1	70	**12**	0	**19a**	[10]	15
2	0	**14**	0	**19b**	[8]	2
3	0	**16b**	4	**19c**	[10]	1
4	0	**17a**	0	**19d**	[11]	0
5	12	**17b**	75	**19e**	[8]	0
6	71	**17c**	77	**19f**	[12]	0
8	7	**18a**	0	**19g**	[13]	0
9	0	**18b**	10	**19h**	[13]	0
10	67	**18c**	34	**19i**	[7]	54
RMP[a]	98					

[a] MIC 0.25 μg/ml.

*4.1.7. General procedure for the preparation of substituted 2-, 7- and 2,7-(piperazin-1-yl)-4-phenyl-1,8-naphthyridines **2**, **8**, **10**, **13**, **14** and **18a–c***

A solution of 5 mmol of the suitable 1,8-naphthyridine derivatives **1**, **5**, **9**, **11**, **12** or **17a–c** in 20 ml of ethanol and 20 ml of 10% aqueous sodium hydroxide was refluxed for 6 h and then concentrated in vacuo to a small volume. The pH of the solution was adjusted to 8–9 with 10% hydrochloric acid and the solution was extracted twice with chloroform. The combined extracts were dried (magnesium sulfate) and evaporated to dryness in vacuo to obtain the piperazin-1-yl derivatives **2**, **8**, **10**, **13**, **14** and **18a–c**, which were purified by crystallization (Tables 1 and 2).

Compound **8** was also obtained in a similar manner by hydrolysis of **6**.

*4.1.8. General procedure for the preparation of substituted acetamido-4-phenyl-1,8-naphthyridines **3** and **4***

A mixture of 5 mmol of **1** or **2** in 20 ml of acetic anhydride was kept at 100°C for 2 h and the crude products were obtained by the following methods. To obtain **3**, the suspension was filtered and washed with H_2O. To obtain **4**, the pH of the suspension was made alkaline with concentrated ammonium hydroxide, filtered and washed with H_2O. The acetamido derivatives **3** and **4** were then purified by crystallization (Tables 1 and 2).

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References

[1] M.G. Vigorita, R. Ottanà, C. Zappalà, R. Maccari, F.C. Pizzimenti, G. Gabrielli, Halogenated isoniazid derivatives as possible antimycobacterial and anti-HIV agents-III, Farmaco 49 (1994) 775–781.
[2] T. Haruaki, S. Katsumasa, S. Hajime, I. Yoshifumi, Chem. Abstr. 119 (1993) 266305x.
[3] P.B. Trivedi, N.K. Undavia, M.A. Dave, K.N. Bhatt, N.C. Desai, Synthesis and antimicrobial activity of some heterocyclic compounds, Indian J. Chem. Sect. B 32 (1993) 497–500 (Chem. Abstr. 119 (1993) 49341b).
[4] B.R. Shah, J.J. Bhatt, H.H. Patel, N.K. Undavia, P.B. Trivedi, N.C. Desai, Synthesis of 2,3-disubstituted-3,1-quinazolin-4(4H)-ones as potential anticancer and anti-HIV agents, Indian J. Chem. Sect. B 34 (1995) 201–208 (Chem. Abstr. 123 (1995) 33013t).
[5] M. Khalifa, S. El-Basil, K.M. Youssef, Synthesis of some quinazolone derivatives with tuberculostatic activity, Zagazig J. Pharm. Sci. 4 (1995) 287–293 (Chem. Abstr. 124 (1996) 86925).
[6] P.K. Desai, P. Desai, D. Machhi, C.M. Desai, D. Patel, Quinoline derivatives as antitubercular/antibacterial agents, Indian J. Chem. Sect. B 35 (1996) 871–873 (Chem. Abstr. 125 (1996) 221530).
[7] 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-naphthyridine in the synthesis of compounds with antiplatelet activity, J. Heterocyclic Chem. 34 (1997) 1501–1510.
[8] A. Mangini, M. Colonna, Contributo alla conoscenza delle naftiridine, Gazz. Chim. Ital. 72 (1942) 183–197.
[9] 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.
[10] P.L. Ferrarini, C. Mori, G. Biagi, O. Livi, I. Tonetti, Synthesis of bis(2-chloroethyl)amino-1,8-naphthyridines for evaluation as anticancer agents, J. Heterocyclic Chem. 21 (1984) 417–419.
[11] P.L. Ferrarini, C. Mori, M. Miceli, F. Franconi, Synthesis and antiplatelet activity of some 1,8-naphthyridine derivatives, Eur. J. Med. Chem. 29 (1994) 735–741.
[12] S. Carboni, A. Da Settimo, D. Bertini, P.L. Ferrarini, O. Livi, I. Tonetti, Sintesi ed attività biologica di alcune 1,8-naftiridine, Farmaco Ed. Sci. 28 (1973) 722–732.
[13] S. Carboni, A. Da Settimo, P.L. Ferrarini, I. Tonetti, Ricerche nel campo delle antiridine, Gazz. Chim. Ital. 97 (1967) 1262–1273.