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Synthesis and Preliminary *In Vitro* Evaluation of Antimycobacterial Activity of New Pyrrolo[1,2-*a*] quinoxaline-carboxylic Acid Hydrazide Derivatives

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New pyrrolo[1,2-*a*]quinoxaline-2- or -4-carboxylic acid hydrazide derivatives were synthesized from nitroaniline or 1,2-phenylenediamine, and evaluated *in vitro* for their antimycobacterial activity as part of a TAACF TB screening program. Two compounds 7c and 13 showed an interesting activity at 6.25 μg/mL against *Mycobacterium tuberculosis* H_{37}Rv, with a 94 and 100 percentage inhibition, respectively.

Keywords: Pyrrolo[1,2-*a*]quinoxaline-carboxylic acid hydrazide derivatives; *Mycobacterium tuberculosis*; Antimycobacterial agents; Tuberculosis

INTRODUCTION

Tuberculosis (TB), an infection of *Mycobacterium tuberculosis*, still remains the leading cause of worldwide death among infectious diseases;[1,2] one-third of the population is infected with *M. tuberculosis* and the World Health Organization (WHO) estimates that within the next 20 years about 30 million people will develope tuberculosis.[3–7] The poor and the wealthy countries alike are at increased risk of infection. As of now, in most parts of the world, we are limited to a five drug combination to treat tuberculosis effectively: rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide. Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to any of these "first-line" tuberculosis drugs and because "second-line" drugs such as ethionamide, aminosalicylic acid, cycloserine, amikacin, kanamycin and capreomycin are too toxic and cannot be employed simultaneously.[8] The infuriating truth is that the pharmaceutical industry has produced nothing, in the way of new tuberculosis drugs, since rifampicin was introduced in the 1960s, even if rifapentine, a long-acting rifamycin drug, was more recently investigated by Hoechst Marion Roussel.[9] In the course of screening to discover new compounds employed in the chemotherapy of tuberculosis, we identified new pyrrolo[1,2-*a*]quinoxaline-carboxylic acid hydrazide derivatives which inhibited *in vitro M. tuberculosis* H_{37}Rv. Thus, taking into account our experience in the field of the synthesis of new bioactive heterocyclic compounds of the type pyrrolo[1,2-*a*]quinoxaline,[10–13] we have prepared pyrrolo[1,2-*a*]quinoxaline-2- or -4-carboxylic acid hydrazide derivatives bearing hydrazide or arylmethylenehydrazide groups in various positions (Figure 1). We present preliminary results concerning the synthesis and the initial *in vitro* antituberculosis activity of the first representative compounds of this chemical series.

MATERIALS AND METHODS

Chemistry

Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and reported

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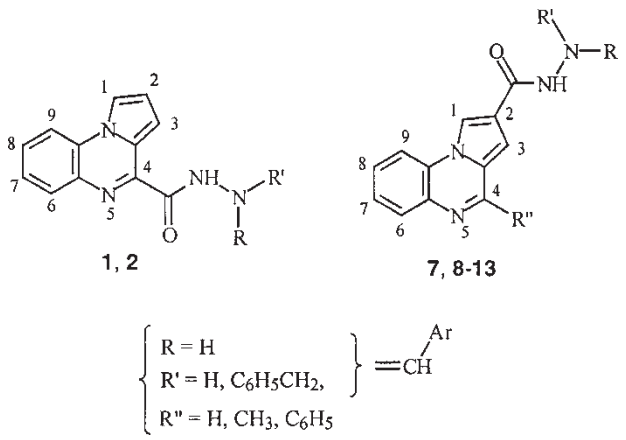


FIGURE 1 Structures of pyrrolo[1,2-*a*]quinoxaline-2- or -4-carboxylic acid hydrazide derivatives **1**, **2** and **7**, **8**–**13**.

uncorrected. Infrared (IR) spectra were determined in KBr discs on a BRUKER IFS-25 spectrometer. NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer (300 MHz). Chemical shifts refer to tetramethylsilane which was used as an internal reference. Elemental analyses were conducted by CNRS, Vernaison, France.

General Procedure for the Preparation of Pyrrolo[1,2-a]quinoxaline-4- or -2-carboxylic Acid Hydrazides 1 and 7a–c

To a solution of ethyl pyrrolo[1,2-*a*]quinoxaline-4-carboxylate **6** or ethyl pyrrolo[1,2-*a*]quinoxaline-2-carboxylates **14a**–**c** (0.004 mol) in 30 mL of ethanol, was added an excess of hydrazine hydrate (98%). The mixture was refluxed for 7 h. The precipitate formed was filtered, washed with ethanol then with diethyl ether and dried.

Pyrrolo[1,2-*a*]quinoxaline-4-carboxylic acid hydrazide (**1**)

As yellow crystals (71%), m.p. 205°C (ethanol). IR (KBr), cm^{-1}:3300 and 3270 (NH and NH_2), 1675 (CO); ^1H NMR (d_6-DMSO), δ, ppm; J, Hz:4.68 (br s, 2H, NH_2), 6.99 (dd, J = 3.70 and 2.90, 1H, H-2), 7.49 (dd, J = 3.70 and 1.30, 1H, H-3), 7.51 (t, J = 7.60, 1H, H-8), 7.62 (t, J = 7.60, 1H, H-7), 7.92 (d, J = 7.60, 1H, H-6), 8.31 (d, J = 7.60, 1H, H-9), 8.54 (dd, J = 2.90 and 1.30, 1H, H-1), 9.99 (br s, 1H, NH). Anal. Calcd for $C_{12}H_{10}N_4O$:C, 63.71; H, 4.45; N, 24.77. Found: C, 63.85; H, 4.38; N, 24.71%.

Pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide (**7a**)

As beige crystals (81%), m.p. > 300°C. IR (KBr), cm^{-1}:3290 and 3210 (NH and NH_2), 1650 (CO); ^1H NMR (d_6-DMSO), δ, ppm; J, Hz:4.49F (s, 2H, NH_2), 7.37 (d, J = 1.30, 1H, H-3), 7.55 (t, J = 7.85, 1H, H-8), 7.59 (t, J = 7.85, 1H, H-7), 7.88 (d, J = 7.85, 1H, H-6), 8.30 (d, J = 7.85, 1H, H-9), 8.87 (d, J = 1.30, 1H, H-1), 8.90 (s, 1H, H-4), 9.70 (s, 1H, NH). Anal. Calcd for $C_{12}H_{10}N_4O$:C, 63.71; H, 4.45; N, 24.77. Found: C, 63.78; H, 4.40; N, 24.92%.

4-Methylpyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide (**7b**)

As white crystals (65%), m.p. > 300°C. IR (KBr), cm^{-1}:3300 and 3280 (NH and NH_2), 1620 (CO); ^1H NMR (d_6-DMSO), δ, ppm; J, Hz:2.63 (s, 3H, CH_3), 4.48 (br s, 2H, NH_2), 7.42 (d, J = 1.35, 1H, H-3), 7.53 (t, J = 7.90, 1H, H-8), 7.58 (t, J = 7.90, 1H, H-7), 7.82 (d, J = 7.90, 1H, H-6), 8.23 (d, J = 7.90, 1H, H-9), 8.83 (d, J = 1.35, 1H, H-1), 9.63 (br s, 1H, NH). Anal. Calcd for $C_{13}H_{12}N_4O$:C, 64.98; H, 5.03; N, 23.32. Found: C, 65.08; H, 4.93; N, 23.41%.

4-Phenylpyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide (**7c**)

As white crystals (76%), m.p. > 300°C. IR (KBr), cm^{-1}:3310 and 3285 (NH and NH_2), 1655 (CO); ^1H NMR (d_6-DMSO), δ, ppm; J, Hz:4.47 (br s, 2H, NH_2), 7.51 (d, J = 1.40, 1H, H-3), 7.53 (m, 1H, H-8), 7.61 (m, 4H, H-7, H-3′, H-4′ and H-5′), 7.93 (d, J = 8.00, 1H, H-6), 8.00 (m, 2H, H-2′ and H-6′), 8.35 (d, J = 8.00, 1H, H-9), 8.95 (d, J = 1.40, 1H, H-1), 9.69 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{14}N_4O$:C, 71.51; H, 4.67; N, 18.53. Found: C, 71.65; H, 4.59; N, 18.77%.

General Procedure for the Preparation of (N′-benzylidene)pyrrolo[1,2-a]quinoxaline-4- or -2-carboxylic Acid Hydrazides 2 and 8–12

To a suspension of pyrrolo[1,2-*a*]quinoxaline-4-carboxylic acid hydrazide **1** or pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide **7a** (0.002 mol) in 20 mL of ethanol containing two drops of a 37% aqueous hydrochloric acid solution, was added the corresponding aromatic aldehyde derivative (0.0021 mol). The mixture was refluxed for 5 h. The precipitate formed was filtered, washed with ethanol then with diethyl ether and dried.

N′-(2-hydroxybenzylidene)-pyrrolo[1,2-*a*]-quinoxaline-4-carboxylic acid hydrazide (**2**)

As yellow crystals (43%), m.p. 238°C (methanol). IR (KBr), cm^{-1}:3210 (NH), 1665 (CO), 1625 (C=N); ^1H NMR (d_6-DMSO), δ, ppm; J, Hz:6.95 (m, 2H, Ar-H′), 7.07 (dd, J = 3.80 and 2.80, 1H, H-2), 7.33 (m, 1H, Ar-H′), 7.59 (m, 3H, H-7, H-3 and Ar-H′), 7.74 (t, J = 7.95, 1H, H-8), 8.07 (d, J = 7.95, 1H, H-6), 8.39 (d, J = 7.95, 1H, H-9), 8.63 (dd, J = 2.80 and 1.35, 1H, H-1), 8.89 (s, 1H, N=CH), 11.28 (br s, 1H, OH), 12.49 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{14}N_4O_2$:C, 69.08; H, 4.27; N, 16.96. Found: C, 68.94; H, 4.39; N, 16.82%.

N′-Benzylidene-pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide (**8**)

As beige crystals (74%), m.p. 269°C IR (KBr), cm^{-1}:3200 (NH), 1660 (CO), 1625 (C=N); ^1H NMR

(d_6-DMSO), δ, ppm; J, Hz:7.47 (m, 4H, H-3, H-3′, H-4′ and H-5′), 7.73 (m, 4H, H-7, H-8, H-2′ and H-6′), 8.01 (d, J = 7.80, 1H, H-6), 8.51 (d, J = 7.80, 1H, H-9), 8.57 (s, 1H, N=CH), 9.34 (d, J = 1.35, 1H, H-1), 9.40 (s, 1H, H-4), 12.18 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{14}N_4O$: C, 72.59; H, 4.49; N, 17.82. Found: C, 72.72; H, 4.63; N, 17.96%.

N′-(2-Hydroxybenzylidene)-pyrrolo[1,2-*a*]-quinoxaline-2-carboxylic acid hydrazide (**9**)

As beige crystals (90%), m.p. > 300°C. IR (KBr), cm^{-1}:3210 (NH), 1655 (CO), 1625 (C=N); ^{1}H NMR (d_6-DMSO), δ, ppm; J, Hz:6.94 (m, 2H, H-3′ and H-4′), 7.31 (t, J = 8.60, 1H, H-5′), 7.56 (d, J = 8.60, 1H, H-6′), 7.65 (t, J = 8.10, 1H, H-7), 7.79 (t, J = 8.10, 1H, H-8), 7.94 (d, J = 1.20, 1H, H-3), 8.02 (d, J = 8.10, 1H, H-6), 8.49 (d, J = 8.10, 1H, H-9), 8.77 (s, 1H, N=CH), 9.35 (d, J = 1.20, 1H, H-1), 9.41 (s, 1H, H-4), 11.22 (br s, 1H, OH), 12.45 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{14}N_4O_2$:C, 69.08; H, 4.27; N, 16.96. Found: C, 69.16; H, 4.42; N, 16.90%.

N′-(4-Hydroxybenzylidene)-pyrrolo[1,2-*a*]-quinoxaline-2-carboxylic acid hydrazide (**10**)

As white crystals (77%), m.p. > 300°C. IR (KBr), cm^{-1}:3200 (NH), 1660 (CO), 1630 (C=N); ^{1}H NMR (d_6-DMSO), δ, ppm; J, Hz:6.85 (d, J = 8.20, 2H, H-2′ and H-6′), 7.59 (m, 3H, H-7, H-3′ and H-5′), 7.68 (d, J = 1.30, 1H, H-3), 7.70 (t, J = 8.00, 1H, H-8), 7.95 (d, J = 8.00, 1H, H-6), 8.39 (s, 1H, N=CH), 8.44 (d, J = 8.00, 1H, H-9), 9.14 (d, J = 1.30, 1H, H-1), 9.17 (s, 1H, H-4), 9.98 (br s, 1H, OH), 11.75 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{14}N_4O_2$:C, 69.08; H, 4.27; N, 16.96. Found: C, 69.02; H, 4.33; N, 17.08%.

N′-(Thien-2-ylmethylidene)-pyrrolo[1,2-*a*]-quinoxaline-2-carboxylic acid hydrazide (**11**)

As yellow crystals (72%), m.p. 279°C. IR (KBr), cm^{-1}:3210 (NH), 1660 (CO), 1630 (C=N); ^{1}H NMR (d_6-DMSO), δ, ppm; J, Hz:7.16 (m, 1H, H-4′), 7.50 (m, 1H, H-3′), 7.65 (t, J = 7.85, 1H, H-7), 7.70 (d, J = 1.30, 1H, H-3), 7.79 (t, J = 7.85, 1H, H-8), 8.04 (m, 2H, H-6 and H-5′), 8.53 (d, J = 7.85, 1H, H-9), 8.83 (s, 1H, N=CH), 9.43 (d, J = 1.30, 1H, H-1), 9.50 (s, 1H, H-4), 12.29 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{12}N_4OS$:C, 63.73; H, 3.77; N, 17.49. Found: C, 63.95; H, 3.86; N, 17.54%.

N′-[1-(3,4-dichlorophenyl)ethylidene]-pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide (**12**)

As pale-yellow crystals (69%), m.p. > 300°C. IR (KBr), cm^{-1}:3200 (NH), 1655 (CO), 1630 (C=N); ^{1}H NMR (d_6-DMSO), δ, ppm; J, Hz:2.59 (s, 3H, CH_3), 7.54 (m, 2H, H-7 and H-5′), 7.58 (t, J = 7.85, 1H, H-8), 7.71 (m, 2H, H-3 and H-6), 7.93 (d, J = 8.00, 1H, H-6′), 8.08 (s, 1H, H-2′), 8.37 (m, 1H, H-9), 8.97 (d, J = 1.30, 1H, H-1), 9.08 (s, 1H, H-4), 11.78 (br s, 1H, NH). Anal. Calcd for $C_{20}H_{14}Cl_2N_4O$:C, 60.47; H, 3.55; N, 14.10. Found: C, 60.65; H, 3.68; N, 14.37%.

N′-Benzyl-pyrrolo[1,2-a]quinoxaline-2-carboxylic Acid Hydrazide (13)

To a suspension of (*N′*-benzylidene)pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide **8** (0.001 mol) in methanol (25 mL) was added, portion-wise at room temperature, sodium borohydride (0.002 mol). The reaction mixture was then heated under reflux for 3 h and then evaporated to dryness under reduced pressure. After cooling the residue was triturated in water, filtered, washed with ethanol and then with hot methylene chloride to give beige crystals (56%) **13**. m.p. 295°C. IR (KBr), cm^{-1}:3330 and 3200 (NH), 1630 (CO); ^{1}H NMR (d_6-DMSO), δ, ppm; J, Hz : 4.30 (s, 2H, CH_2), 6.22 (br s, 1H, NH), 6.52 (d, J = 1.35, 1H, H-3), 6.80 (m, 2H, H-7 and H-8), 6.96 (d, J = 7.90, 1H, H-6), 7.43 (m, 3H, H-3, H-3′, H-4′ and H-5′), 7.52 (d, J = 7.90, 1H, H-9), 7.71 (m, 2H, H-2′ and H-6′), 8.11 (d, J = 1.35, 1H, H-1), 8.38 (s, 1H, H-4), 11.36 (br s, 1H, NH). Anal. Calcd for $C_{19}H_{16}N_4O$:C, 72.13; H, 5.10; N, 17.71. Found: C, 72.39; H, 5.02; N, 17.78%.

Primary Antimycobacterial Activity

All 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).[14] The reference compound used in this primary assay was Rifampicin (MIC = 0.25 μg/mL). Compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations against *M. tuberculosis* H_{37}Rv to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

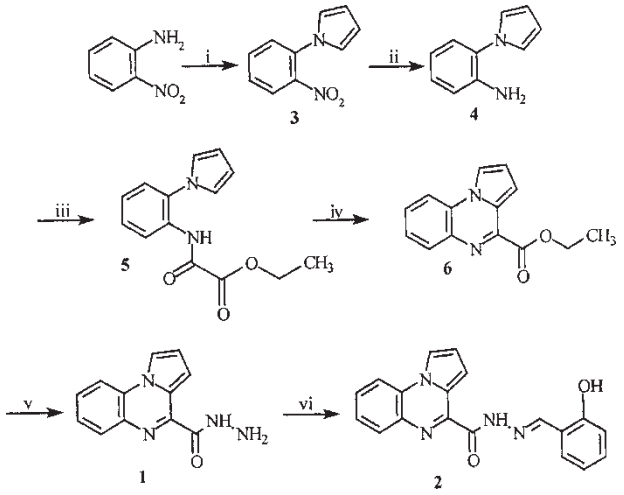
Cytotoxicity Assay

Concurrent with the determination of MICs, compounds are tested for cytotoxicity (IC_{50}) in VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC for *M. tuberculosis* H_{37}Rv. After 72 h exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay.

RESULTS AND DISCUSSION

Chemistry

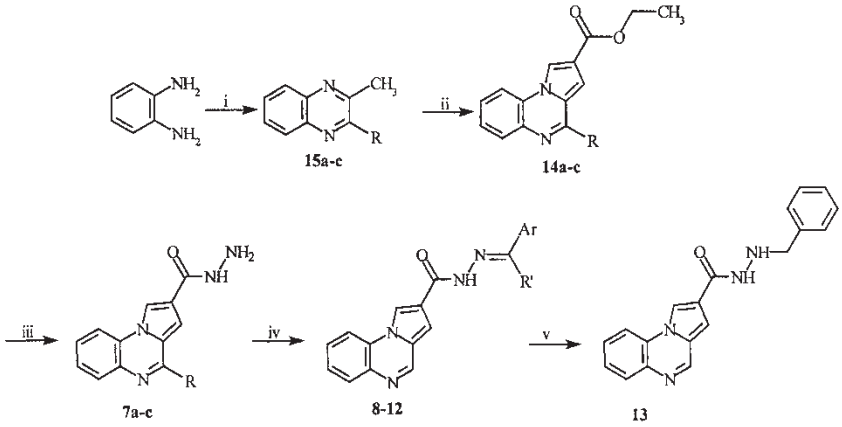
The synthesis of the new pyrrolo[1,2-*a*]quinoxaline-4-carboxylic acid hydrazide derivatives **1**–**2** has been



SCHEME 1 Synthesis of compounds **1**, **2**. Reagents: (i) 2,5-dimethoxyTHF, CH_3COOH; (ii) $NaBH_4$—$BiCl_3$, C_2H_5OH; (iii). $(COOCH_2CH_3)_2$; (iv) $POCl_3$; (V) H_2N—NH_2, H_2O, C_2H_5OH; (vi) 2-HOC_6H_5—CHO, EtOH.

accomplished in five or six steps starting from 2-nitro-aniline according to the sequence depicted in Scheme 1. The Clauson-Kaas reaction[15,16] of 2-nitroaniline with 2,5-dimethoxytetrahydrofuran (DMTHF) in acetic acid gave the pyrrolic derivative **3**, which was reduced using $BiCl_3$—$NaBH_4$ treatment to provide the required 1-(2-aminophenyl)pyrrole **4**.[17,18] The ethyl oxamidate **5** was synthesized from compound **4** by action of diethyl oxalate. The ethyl pyrrolo[1,2-*a*]quinoxaline-4-carboxylate **6** was then prepared by cyclization of the amide **5** in refluxing phosphorus oxychloride.[19] Treatment of the ester **6** in refluxing ethanol with hydrazine hydrate yielded the pyrrolo[1,2-*a*]quinoxaline-4-carboxylic acid hydrazide **1**. The new carboxyhydrazone derivative **2** was obtained by condensing compound **1** with salicylaldehyde in ethanol, using hydrochloric acid as a catalyst (Scheme 1).[20,21]

The hydrazide derivatives **7**–**13**, structural analogues of hydrazides **1**–**2** in position 2 of the pyrrolo[1,2-*a*]quinoxaline moiety, were prepared in three to five steps from 1,2-phenylenediamine *via* the ethyl pyrrolo[1,2-*a*]quinoxaline-2-caboxylates **14a**–**c** (Scheme 2). Reaction of commercially available phenylenediamine with 1-substituted propan-1,2-dione in acetic acid gave the methylquinoxalines **15a**–**c** according to the von Auwers method.[10,22] Treatment of **15** with ethyl bromopyruvate in refluxing ethanol led to ethyl pyrrolo[1,2-*a*]quinoxaline-2-carboxylates **14a**–**c**. Pyrrolo[1,2-*a*]quinoxaline-2-carboxyhydrazides **7a**–**c** were obtained, in 65–81% yield, from esters **14a**–**c** by applying



15, 14, 7	R
a	H
b	CH_3
c	C_6H_5

Compound	R'	Ar
8	H	
9	H	
10	H	
11	H	
12	CH_3	

SCHEME 2 Synthesis of compounds **7a**–**c**, **8**–**12** and **13**. Reagents: (i) R—CO—CO—CH_3, CH_3COOH; (ii) Br—CH_2—CO—$COOC_2H_5$, C_2H_5OH; (iii) H_2N—NH_2, H_2O, C_2H_5OH; (iv) ArCOR′, EtOH; (v) $NaBH_4$, MeOH.

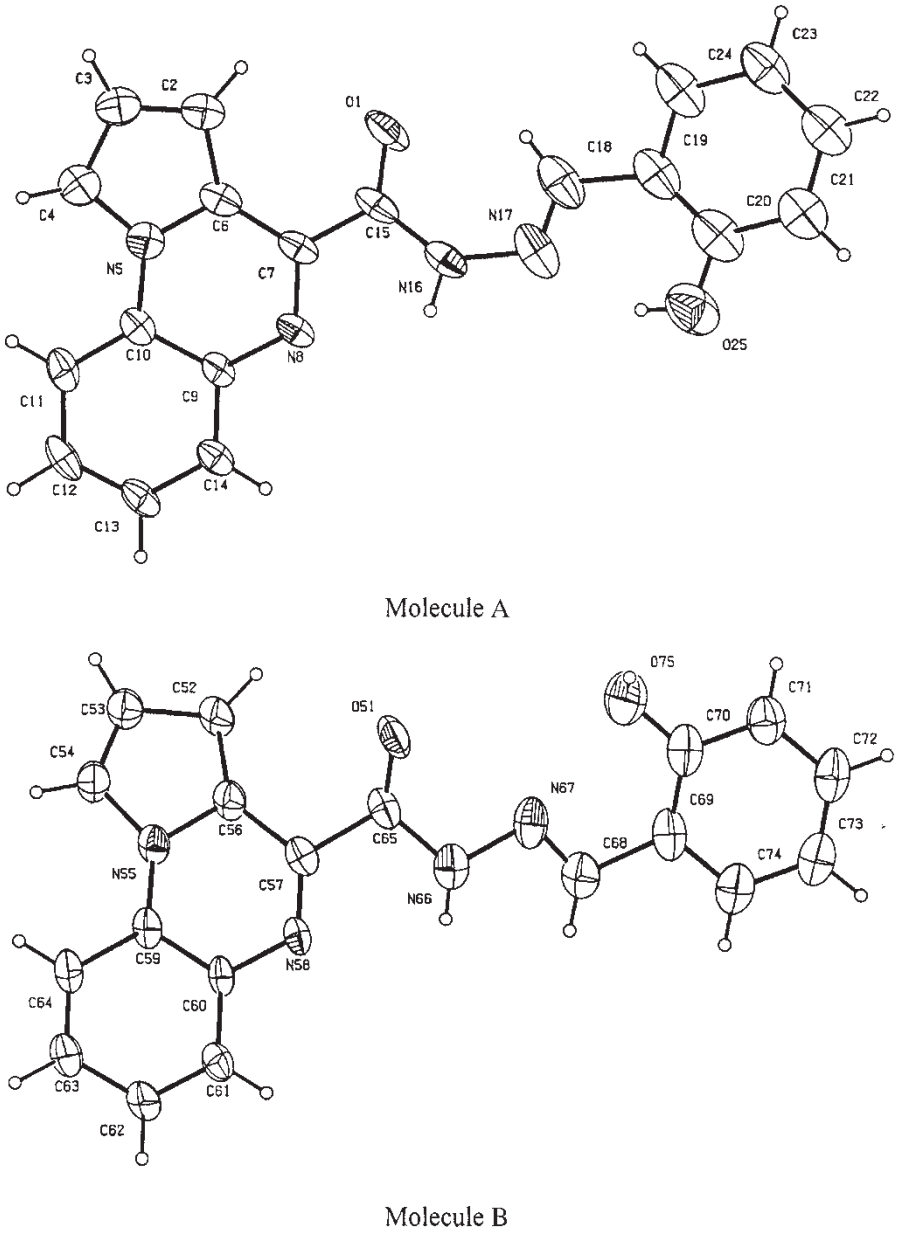


FIGURE 2 A view of **2** (molecules A and B) with our numbering scheme. Displacement ellipsoids are drawn at the 30% probability level.

the same experimental procedure as described in the synthesis of hydrazide **1**. The desired benzylidenecarboxyhydrazide compounds **8**–**12** were prepared by condensation of hydrazide derivatives **7a**–**c** with various arylaldehydes.

The hydrazone **8** was reduced into the hydrazide **13** using sodium borohydride in methanol.[23]

The 3D spatial structure of benzylidenecarboxyhydrazide **2** was established by X-ray crystallography and showed the (*E*)-isomerism of the imino double bond in the solid state as anticipated on the basis of ^{1}H NMR data (Figure 2). The analysis of the ^{1}H-NMR spectra of **2**, **8**–**11**, allowed us to detect the presence of only one imino hydrogen signal, which indicated reference to the (*E*)-diastereomer, on the basis of previously described results.[24,25] Indeed, compounds **2** and **8**–**12** presented, in their ^{1}H-NMR spectra, a singlet at δ 8.10–8.89 ppm, which indicates reference to the (*E*)-diastereomer. Two independent molecules designated as A and B were found in the asymmetric crystallographic unit of **2**.[†] These two conformers only differ from one another through a rotation of the N—N bonds bearing the *N′*-benzilidene moiety.

[†]Supplementary X-ray crystallographic data of compound **2** (CCDC-225301): Cambridge Crystallographic Data Centre, University Chemical Lab, 12 Union Road, Cambridge, CB2 1EZ, U.K.; E-mail: deposit@ccdc.cam.ac.uk

Pharmacology

The *in vitro* evaluation of antituberculosis activity was carried out at the GW Long Hansen's Disease Center within the Tuberculosis Antimicrobial Acquisition Coordinating Facility (TAACF) screening program for the discovery of novel drugs for treatment of tuberculosis. Under the direction of the US National Institute of Allergy and Infectious Diseases (NIAID), Southern Research Institute coordinates the overall program. The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*.

All the derivatives resulting from the described reactional sequences were preliminary evaluated *in vitro* at a single 6.25 μg/mL concentration against *M. tuberculosis* H_{37}Rv by the method described by Collins and Franzblau.[14] The results of the biological evaluation, expressed as a percentage inhibition of the growth of mycobacterium, are summarized in Table I, and for the sake of comparison, the % inhibition for rifampicin, used as reference, is also included (MIC = 0.25 μg/mL). All tested compounds proved to be less active than rifampicin against *M. tuberculosis* H_{37}Rv. However, compounds **7c** and **13** showed an appreciable activity with inhibitions of 94 and 100%, respectively. Compounds **2**, **9** and **11**, showed a moderate activity, with percentage inhibition from 52–88, whereas compounds **1**, **7a–b**, **8**, **10** and **12** were found to possess no activity at the assayed concentration (Table I).

Compounds **7c** and **13**, which demonstrated an inhibition >90% in the primary screening, were selected to further testing at a lower concentration against *M. tuberculosis* H_{37}Rv to determine the actual MIC, with the same method used in the primary screening (Table II). Concurrent with the determination of MICs, compound **7c** was tested for cytotoxicity (IC_{50}) on a VERO cell line. The cytotoxicity data indicated that **7c** exhibited a small degree of toxicity and consequently a low selectivity index level (SI), defined as the ratio of the measured IC_{50} in VERO cells to the MIC (Table II).

TABLE I Antimycobacterial *in vitro* activity of the tested compounds expressed as % inhibition of *Mycobacterium tuberculosis* H_{37}Rv at a concentration of 6.25 μg/mL

Compound	% Inhibition
1	0
2	88
7a	0
7b	0
7c	94
8	0
9	52
10	0
11	57
12	0
13	100
rifampicin	98[a]

[a] At a concentration of 0.25 μg/mL.

TABLE II Antimycobacterial *in vitro* activity and cytotoxicity of compounds **7c** and **13**.

Compound	MIC (μg/mL)	IC_{50} (μg/mL)	SI
7c	6.25	>10	>1.6
13	6.25	nd[a]	nd[a]
Rifampicin	0.25	>100	>400

[a] nd: not determined.

The preliminary biological results for this new series of pyrrolo[1,2-*a*]quinoxaline derivatives, allowed us to clarify the influence of the substituents in positions 2 and/or 4 of the pyrrolo[1,2-*a*]quinoxaline nucleus. Indeed, with regard to the pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazides **7a–c** variously substituted in position 4, the 4-phenyl derivative **7c** was active at 6.25 μg/mL, whereas the unsubstituted compound **7a** and its 4-methyl analogue **7b** were inactive. However, the introduction of the carboxylic acid hydrazide function at position 4 of the pyrrolo[1,2-*a*]quinoxaline moiety in compound **1** was not found beneficial. Moreover, activity was significantly affected by the introduction of a *N′*-benzylidene function on the hydrazide moiety of **7a**, leading to compounds **8–12**. The 2-hydroxybenzylidene and thien-2-ylmethylidene groups in compounds **9** and **11** seem to be effective substituents for the antimycobacterial activity (i.e., 52 and 57% inhibition, respectively). Moreover, the same 2-hydroxybenzylidene group introduced on the hydrazide function of **1** to give **2** is beneficial, leading to a moderate activity; the percentage inhibition was 88% for **2**. A comparison of the activities of compounds **8** *versus* **13** clearly indicated that the reduction of the *N′*-benzylidene function caused a high increase in the antimycobacterial activity (i.e., 0% *versus* 100%).

In conclusion, the phenyl group in position 4 at the pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid hydrazide moiety, and the reduction of the *N′*-benzylidene function of the hydrazone group seem important for the antimycobacterial activity of pyrrolo[1,2,-*a*]quinoxaline-carboxylic acid hydrazides. Based on these preliminary structure-activity results, it could be possible to further identify new pyrrolo[1,2-*a*]quinoxaline-carboxylic acid hydrazide derivatives developed as antimycobacterial agents.

References

[1] Murray, C.J.L., Styblo, K. and Rouillon, A. (1993) "Tuberculosis", In: Jamison, D.T., Mosely, W.H., Measharn, A. and Bobadilla, J.L., eds, *Diseases Control Priorities in Developing Countries* (Oxford University Press, New York), pp 233–259.
[2] Murray, C.J.L., Styblo, K. and Rouillon, A. (1990) *Bull. Int. Union Tuberc. Lung Dis.* **65**, 6–24.

[3] Baker, R.W., Mitscher, L.A., Arain, T.M., Shawar, R. and Stover, C.K. (1996) *Ann. Rep. Med. Chem.* **31**, 161–170.
[4] Duncan, K. (1997) *Exp. Opin. Ther. Pathol.* **7**, 129–137.
[5] Niccolai, D., Tarsi, L. and Thomas, R.J. (1997) *Chem. Commun.* **24**, 2333–2342.
[6] Horsburgh, C.R. (1991) *New Engl. J. Med.* **324**, 1332–1338.
[7] Heifets, L. (1996) *Antimicrob. Agents Chemother.* **40**, 1759–1767.
[8] Mandell, G.L. and Sande, M.A. (1990) "Antimicrobial agents; drugs used in the chemotherapy of tuberculosis and leprosy", In: Gilman, A.G., Goodman, L.S., Rall, T.W., Nies, A.S. and Taylor, P., eds, *Antimicrobial Agents; Drugs Used in the Chemotherapy of Tuberculosis and Leprosy* (Pergamon Press, New York), pp 1146–1164.
[9] Mitchison, D.A. (1998) *Int.J. Tuberc. Lung Dis.* **2**, 612–615.
[10] Guillon, J., Dumoulin, H., Dallemagne, P., Reynolds, R. and Rault, S. (1998) *Pharm. Pharmacol. Commun.* **4**, 33–38.
[11] Guillon, J., Dallemagne, P., Pfeiffer, B., Renard, P., Manechez, D., Kervran, A. and Rault, S. (1998) *Eur. J. Med. Chem.* **33**, 293–308.
[12] Guillon, J., Boulouard, M., Lisowski, V., Stiebing, S., Lelong, V., Dallemagne, P. and Rault, S. (2000) *J. Pharm. Pharmacol.* **52**, 1369–1375.
[13] Prunier, H., Rault, S., Lancelot, J.C., Robba, M., Renard, P., Delagrange, P., Pfeiffer, B., Caignard, D.H., Misslin, R., Guardiola-Lemaître, B. and Hamon, M. (1997) *J. Med. Chem.* **40**, 1808–1819.
[14] Collins, L.A. and Franzblau, S.G. (1997) *Antimicrob. Agents Chemother.* **41**, 1004–1009.
[15] Clauson-Kaas, N. and Tyle, Z. (1952) *Acta Chem. Scand.* **6**, 667–670.
[16] Elming, N. and Clauson-Kaas, N. (1952) *Acta Chem. Scand.* **6**, 867–874.
[17] Borah, H.N., Prajapati, D. and Sandlu, J.S. (1994) *J. Chem. Res. Synp.* **6**, 228–229.
[18] Ren, P.D., Pan, S.F., Dong, T.W. and Wu, S.H. (1995) *Synth. Commun.* **25**, 3799–3803.
[19] Nagarajan, K., Ranga Rao, V. and Venkateswarlu, A. (1972) *Indian J. Chem.* **10**, 344–350.
[20] Maccari, R., Ottana, R., Monforte, F. and Vigorita, M.G. (2002) *Antimicrob. Agents Chemother.* **46**, 294–299.
[21] Bottari, B., Maccari, R., Monforte, F., Ottana, R., Rotondo, E. and Vigorita, M.G. (2001) *Bioorg. Med. Chem. Lett.* **11**, 301–303.
[22] von Auwers, K. (1917) *Ber.* **50**, 1177–1182.
[23] Ottana, R., Maccari, R., Vigorita, M.G. and Rotondo, E. (1998) *J. Chem. Res. (S)*, 550–551.
[24] Palla, G., Pelizzi, C., Predieri, G. and Vignali, C. (1982) *Gazz. Chim. Ital.* **112**, 339–341.
[25] Lima, P.C., Lima, L.M., da Silva, K.C.M., Léda, P.H.O., de Miranda, A.L.P., Fraga, C.A.M. and Barreiro, E.J. (2000) *Eur. J. Med. Chem.* **35**, 187–203.