Centro de Investigación en Farmacobiología Aplicada[1], Universidad de Navarra, España, and Facultad de Farmacia y Bioquímica[2], Universidad Nacional Mayor de San Marcos, Lima, Perú

Synthesis and antituberculosis activity of new 2-quinoxalinecarbonitrile 1,4-di-*N*-oxides

M. A. Ortega[1], Y. Sainz[1], M. E. Montoya[2], A. López De Ceráin[1] and A. Monge[1]

With the advent of multidrug-resistant *Mycobacterium tuberculosis* strains, new and effective therapies are rapidly needed to combat infections caused by these strains. Some new 2-quinoxalinecarbonitrile 1,4-di-*N*-oxides have been synthesized and tested as antituberculosis agents and interesting results have been obtained from the first screening.

1. Introduction

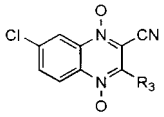
Tuberculosis, an ancient disease undergoing recent control by public hygiene and drug therapy, has experienced a recrudescence in all the world. Factors leading to the increased incidence of tuberculosis include the high incidence of tuberculosis among the AIDS population and the emergence of drug-resistant strains of mycobacteria [1]. The World Health Organization suggests that at least 5.6 million persons worldwide, mostly in developing countries, carry dual infection, and additional >1.4 million cases of HIV-related tuberculosis will occur by the year 2000 [2]. New and effective therapies are rapidly needed to combat infections caused by this mycobacteria [3].
Some quinoxaline 1,4-di-*N*-oxides were synthesized and evaluated as antibacterial agents [4, 5]. Other quinoxaline 1,4-di-*N*-oxides were similar to vitamin K, and their use for the treatment of the tuberculosis was intuited in the past [6. We have prepared new quinoxaline 1,4-di-*N*-oxides as new anticancer compounds, selectives in hypoxia conditions: we demonstrated that several quinoxaline 1,4-di-*N*-oxides were more potent and selective than tirapazamine when assayed on V79 cells [7–11]. In a previous paper we have demonstrated that some quinoxaline 1,4-di-*N*-oxides could be useful for the treatment of tuberculosis [12. The activity of these compounds can be modulated for a great variety of substituents in positions 2, 3, 6 and 7. Now we report on new 7-chloro-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides that present an interesting antituberculosis activity.

2. Investigations, results and discussion

2.1. Chemistry

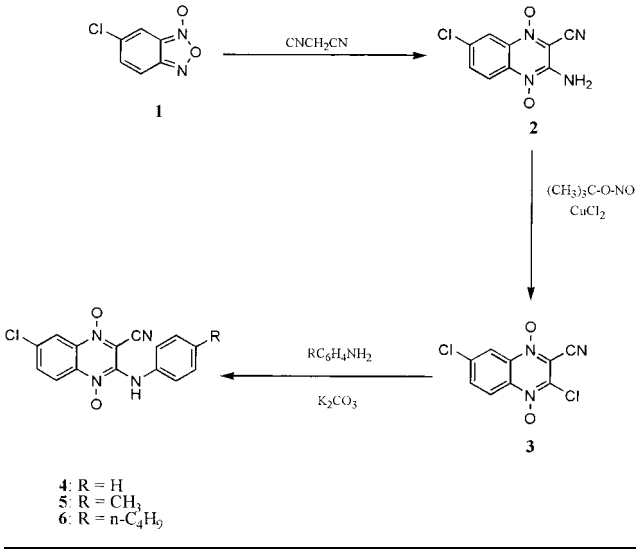
2-quinoxalinecarbonitrile 1,4-di-*N*-oxides were prepared according to the procedures shown in the Scheme. From 5(6)-chloro benzofuroxane (**1**), we obtained 3-amino-6(7)-chloro-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides **2**, as described previously [9]. The amino group of **2** was replaced by chlorine using tert-butyl nitrite in dry acetonitrile and in the presence of copper(II) chloride as chlorine donor [10]. In this reaction a isomers mixture was obtained and the isomers were separated by flash chromatography, being the major the 7-chloro isomer, 3,7-dichloro-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide, (**3**), in 98% (we identified these isomers by ^{1}H NMR). The reaction of **3** with an aromatic amine gave the corresponding products, 7-chloro-3-phenylamino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide, (**4**, (with aniline), 7-chloro-3-[(4-methyl)phenylamino]-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide, (**5**, with *p*-toluidine) and 7-chloro-3-[4-(*n*-butyl)-phenylamino]-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide, (**6**, with 4-*n*-butylaniline).
All the compounds were characterized by physical constants, elemental analysis, IR, ^{1}H NMR and MS spectra. We include physical data in Table 1.

Table 1: Physical data of 2-quinoxalinecarbonitrile 1,4-di-*N*-oxides



Compd.	R_3	Formula	M.p. (°C)	Yield (%)
4	NHC_6H_5	$C_{15}H_9ClN_4O_2$	221–223	43
5	$NHC_6H_4CH_3$	$C_{16}H_{11}ClN_4O_2$	231–232	45
6	$NHC_6H_4(CH_2)_3CH_3$	$C_{19}H_{17}ClN_4O_2$	207–208	79

Scheme



2.2. Antituberculosis activity

In vitro evaluation of antituberculosis activity was carried out at the GWL Hansen's Disease Center (Colorado State University) within the TAACF (Tuberculosis Antimicrobial Acquisition Coordinating Facility) screening program for the discovery of novel drugs for the treatment of tuberculosis. 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

Table 2: Results of the biological screening

Compound	MIC (μg/ml)	% Inhibition
4	<12.5	99
5	<12.5	99
6	<12.5	99
Rifampin (RMP)	0.25	97

virulent *Mycobacterium tuberculosis*. The MIC (the lowest concentration inhibiting 99% of the inoculum) is <12.5 μg/ml for all the compounds and show an inhibition of 99% (Table 2). These results confirm the high interest of the new described structures. These compounds have been selected for confirmatory and advanced screening, in order to determine the actual minimum inhibitory concentration (MIC). On the basis of the obtained results, it seems that 7-chloro-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides with different aromatic amines in position 3 could be a new type of compounds in the treatment of tuberculosis.

We propose to confirm these results, increasing the presented compounds, modulate the substituents in positions 3, 6 and 7 in order to get the best antituberculosis activity.

3. Experimental

M.p.'s were determined on a Mettler FP82+FP80 apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba Elemental Analyzer Mod. 1106 and agreed with calculated values within ±0.4% of the calculated values except otherwise stated. IR spectra were recorded on a Perkin-Elmer 681 apparatus (ν max in cm^{-1}), using potassium bromide tablets. ^1H NMR spectra were determined in DMSO-d_6 solutions and TMS as an internal reference with a Brucker AC-200E Spectrometer. Chemicals shifts are given in ppm (δ-scale). Merck silica gel 60 (70–230 mesh) was used for CC. TLC (Merck silica gel 60 F_{254} analytical plates) was used to monitor reactions. The plates were scanned under UV light at 254 and 366 nm. Organic solutions were dried over anh. Na_2SO_4. Compounds **1**, **2** and **3** (see Scheme) were previously reported [9–10].

3.1. 7-Chloro-3-phenylamino-2-quinoxalinecarbonitrile 1,4-di-N-oxide (**4**)

To a solution of 3,7-dichloro-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide (**3**) [10] (0.12 g, 0.40 mmol) in dry chloroform (75 ml), aniline (20 drops) and potassium carbonate (0.11 g, 0.80 mmol) were added. The mixture was stirred isolated from light at room temperature for fifteen days. The reaction mixture was filtered and washed with ethyl acetate. The solvent was removed under reduced pressure. The impure product was purified by flash chromatography (SP: silica gel; eluting with toluene/ethyl acetate. The solvent was removed under reduced pressure and recrystallized from ethyl acetate/methanol (1:1).

4: Yield 43%. M.p. 221–223 °C. IR (cm^{-1}): 3399 (NH), 3072 (CH aromatic), 2231 (CN), 1559 (aromatic), 1356 (NO). ^1H NMR (δ ppm): 7.27 to 7.39 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.76 (s, 1 H, H_6), 8.30 (d, 1H, H_8, J = 4.1 Hz), 8.35 (d, 1H, H_5, J = 8.8 Hz), 10.24 (s b, 1H, NH). MS: m/z (%) = 312 (17) [$M^{+\cdot}$], 295 (100).

3.2. 7-Chloro-3-[(4-methyl)phenylamino]-2-quinoxalinecarbonitrile 1,4-di-N-oxide (5) and 7-chloro-3-[4-(n-butyl)phenyl)amino]-2-quinoxalinecarbonitrile 1,4-di-N-oxide (**6**)

Compounds **5** and **6** were prepared similar to **4**, using *p*-toluidine or 4-*n*-butylaniline instead of aniline.

5: Yield 45%. M.p. 231–232 °C. IR (cm^{-1}): 3406 (NH), 3086 (CH aromatic), 2936 (CH aliphatic), 1562 (aromatic), 1364 (NO). ^1H NMR (δ ppm): 2.31 (s, 3 H, CH_3), 7.17–7.28 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$), 8.00 (d, 1 H, H_6, J = 9.1 Hz), 8.32 (d, 1H, H_8, J = 1.3 Hz), 8.37 (d, 1 H, H_5, J = 9.3 Hz), 10.11 (s b, 1 H, NH). MS: m/z (%) = 326 (25) [$M^{+\cdot}$], 309 (100).

6: Yield 79%. M.p: 207–208 °C. IR (cm^{-1}): 3086 (CH aromatic), 2929 (CH aliphatic), 2227 (CN), 1593 (aromatic), 1361 (NO). ^1H NMR (δ ppm): 0.90 (t, 3 H, CH_3, J = 6.9 Hz), 1.29–1.36 (m, 2 H, CH_2CH_3), 1.53 to 1.60 (m, 2 H, $CH_2CH_2CH_2$), 2.60 (t, 2 H, $PhCH_2$, J = 7.0 Hz), 7.23–7.25 (m, 4 H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$), 8.00 (d, 1 H, H_6, J = 9.1 Hz), 8.32 (s, 1 H, H_8), 8.38 (d, 1 H, H_5, J = 9.1 Hz), 10.12 (s b, 1 H, NH). MS: m/z (%) = 368 (22) [$M^{+\cdot}$], 309 (100).

3.3. Antituberculosis activity

In vitro evaluation of tuberculosis activity was carried out at the GWL Hansen's Disease Center. Primary screening was conducted at 12.5 μg/mL against the virulent H37Rv strain of *Mycobacterium tuberculosis*. The standard compound was rifampin (RMP), MIC: 0.25 μg/mL, 97% Inhibition. *Mycobacterium tuberculosis* H37Rv was grown in BACTEC 12B medium containing radiolabeled substrate [13]. Labeled CO_2 produced was detected and quantitated by the automatic radiometric system BACTEC 460.

Acknowledgments: The *in vitro* evaluation of the antituberculosis activity has been carried out in the TAACF (Tuberculosis Antimicrobial Acquisition & Coordinating Facility) at the National Institute of Allergy and Infectious Diseases, Southern Research Institute, GWL Hansen's Disease Center and Colorado State University, EEUU; we thank J. A. Maddry, Ph. D. for his collaboration. We also thank the program X.2.CYTED for its collaboration in the report.

The authors are grateful to Gobierno de Navarra for a predoctoral grant to M. A. Ortega and, also to I.C.I. (Instituto de Cooperación Iberoamericana) for a predoctoral grant to M. E. Montoya.

References

1 Miller, W. T.: Radiol. Clin. North. Am. **32**, 649 (1994)
2 Laal, S.; Samanich, K. M.; Sonnenberg, M. G.; Belisle, J. T.; O'Leary, J.; Simberkoff, M. S.; Zolla-Pazner, S.: J. Infect. Dis. **176**, 133 (1997)
3 Hackbarth, C. J.; Unsal, Y.; Chambers, H. F.: Antimicrob. Agents Chemother. **41**, 1182 (1997)
4 Cheeseman, G. W. H.; Cookson, R. F.; in: Weissberger, A.; Taylor, E. C. (eds): Heterocyclic Compounds, Condensed Pyrazines, Vol. 35, p. 28, Wiley, J. and sons 1979
5 Haddadin, M. J.; Issidorides, C. H.: Heterocycles **35**, 1503 (1993)
6 Iland, C. N.: Nature **161**, 1010 (1948)
7 Monge, A.; Palop, J. A.; Piñol, A.; Martínez-Crespo, F. J.; Narro, S.; González, M.; Sainz, Y.; López de Ceráin, A.: J. Heterocycl. Chem. **31**, 1135 (1994)
8 Monge, A.; Palop, J. A.; González, M.; Martínez-Crespo, F. J.; López de Ceráin, A.; Sainz, Y.; Narro, S.: J. Heterocycl. Chem. **32**, 1213 (1995)
9 Monge, A.; Palop, J. A.; López de Ceráin, A.; Senador, V.; Martínez-Crespo, F. J.; Sainz, Y.; Narro, S.; García, E.; de Miguel, C.; González, M.; Hamilton, E.; Barker, A. J.; Clarke, E. D.; Greenhow, D. T.: J. Med. Chem. **38**, 1786 (1995)
10 Monge, A.; Martínez-Crespo F. J.; López de Ceráin, A.; Palop, J. A.; Narro, S.; Senador, V.; Marín, A.; Sainz, Y.; González, M.; Hamilton, E.; Barker, A. J.: J. Med. Chem. **38**, 4488 (1995)
11 Martínez-Crespo, F. J.; Palop, J. A.; Sainz, Y.; Narro, S.; Senador, V.; González, M.; López de Ceráin, A.; Monge, A.: J. Heterocycl. Chem. **33**, 1671 (1996)
12 Sainz, Y.; Montoya, M. E.; Martínez-Crespo, F. J; Ortega, M. A.; López de Ceráin, A.; Monge A.: Arzneim.-Forsch/Drug Res. (in print)
13 Middlebrook, G., Regginardo, Z., Tigerit, W. D.; Am. Rev. Respir. Dis. **115**, 1066 (1977)

Received March 9, 1998
Accepted May 15, 1998

Prof. Dr. A. Monge
Centro de Investigación
en Farmacobiología Aplicada
Universidad de Navarra
Pamplona, E-31080
Spain