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Synthesis and antituberculosis activity of some new 2-quinoxalinecarbonitriles

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Abstract

Tuberculosis, an ancient disease undergoing recent control by public hygiene and drug therapy, has experienced a recrudescence throughout the world. New and effective therapies are rapidly needed to combat infections caused by these strains. Some new 2-quinoxalinecarbonitriles have been synthesized and tested as antituberculosis agents and interesting results have been obtained from the first screening.

Keywords: Antituberculosis activity; 2-Quinoxalinecarbonitriles; Tuberculosis

1. Introduction

Mycobacterium tuberculosis infects over one-third of the world's population and causes almost three million deaths every year [1–3]. The World Health Organization suggests that at least 5.6 million persons worldwide, mostly in developing countries, carry dual infection, and $>$ 1.4 million cases of HIV-related tuberculosis will occur by the year 2000 [4]. Ethambutol is one of the primary drugs used in combination with isoniazid, rifampin, streptomycin and pyrazinamide to treat tuberculosis. But *M. tuberculosis* is often resistant to multiple drugs, therefore antituberculosis agents with bactericidal mechanisms different from those of available first-line drugs, are urgently needed [5].

Some quinoxalines and quinoxalines 1,4-di-*N*-oxides were synthesized and evaluated as antibacterial agents [6–8]. Other quinoxaline 1,4-di-*N*-oxides were similar to vitamin K, and their use for the treatment of the tuberculosis was suspected in the past [9]. The possibility that these type of compounds could be useful for treatment of tuberculosis was proposed. With this idea, some new quinoxaline 1,4-di-*N*-oxides were prepared in our laboratory and screened at the GWL Hansen's Disease Center within the TAACF (Tuberculosis Antimicrobial Acquisition Coordinating Facility). The primary screening has been very interesting and some quinoxaline 1,4-di-*N*-oxide derivatives with very different substituents in the 2, 3, 6 and 7 positions have shown antituberculosis activity [10,11].

We described substituted quinoxaline 1,4-di-*N*-oxides. This type of structure seemed fundamental for the intended activity. Now, we propose to establish the importance of quinoxaline1,4-di-*N*-oxides derivatives versus quinoxaline derivatives.

2. Chemistry

2-Quinoxalinecarbonitriles derivatives were prepared using the synthetic methods outlined in Scheme 1. The starting products 5(6)-substituted benzofuroxane (**1a–1c**), and 3-amino-6(7)-substituted-2-quinoxalinecarbonitrile 1,4-di-*N*-oxides (**2a–2c**), were obtained by previously described methods [12]. Compounds **2a**, **2b** or **2c** were reacted with sodium ditionite in methanol to give 3-amino-6(7)-substituted-2-quinoxalinecarbonitriles (**3a–3c**); **3a** had been described previously [13] and **3b, 3c** were prepared from a similar synthesis, but the purification process was different to that described. We obtained isomer mixtures in both cases although in different proportions. The reaction of **3a**, **3b** or **3c** with a fresh solution of sodium nitrite gave 3-chloro-6(7)-substituted-2-quinoxalinecarbonitriles (**4a–4c**); **4a** had been described previously [14] and we prepared **4b, 4c** from a similar synthesis, but the purification of these

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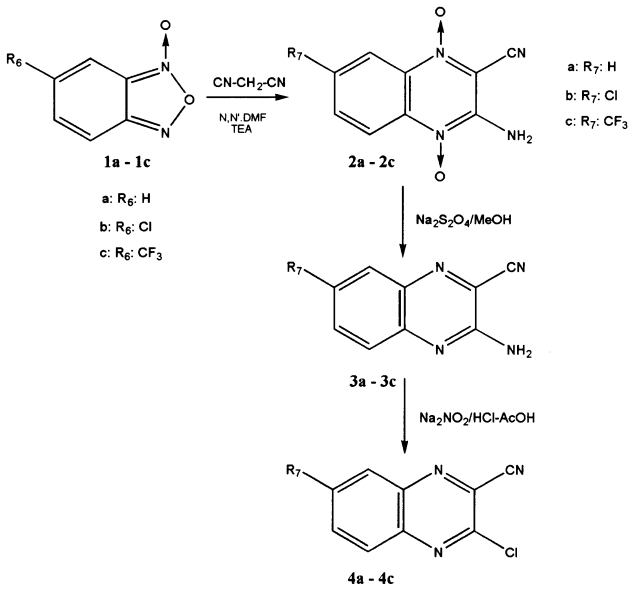


Scheme 1.

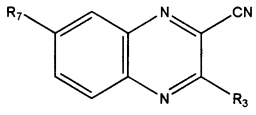
products, **4a–4c**, was different to that described; **4a** and **4c** were obtained as isomer mixtures.

All the compounds were characterized by physical constants (Table 1), elemental analysis (Table 2), IR, ^{1}H NMR and MS spectra.

3. Pharmacology

Compounds **3b**, **3c**, **4a**, **4b** and **4c** were tested for their 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 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 virulent *Mycobacterium tuberculosis*.

Table 1
Physicochemical properties of the tested compounds



Compound	R_3	R_7	Formula	M.p. (°C)	Yield (%)
3b	NH_2	Cl	$C_9H_5ClN_4$	245 (d)	75
3c	NH_2	CF_3[a]	$C_{10}H_5F_3N_4$	198–201	65
4a	Cl	H	$C_9H_4ClN_3$	156–158	42
4b	Cl	Cl	$C_{10}H_3ClF_3N_3$	140–142	46
4c	Cl	CF_3[a]	$C_9H_3Cl_2N_3$	45–47	42

[a] Isomeric mixture (R_6 or R_7).

Table 2
Elemental analysis

Compound		C	H	N
3b	Calc.	52.82	2.46	27.38
	Found	52.88	2.43	27.54
3c	Calc.	50.43	2.11	23.52
	Found	50.04	2.13	23.25
4a	Calc.	56.99	2.11	22.16
	Found	57.38	2.24	21.67
4b	Calc.	48.24	1.34	18.75
	Found	48.58	1.38	18.77
4c	Calc.	46.62	1.17	16.31
	Found	46.89	1.15	16.34

4. Results and discussion

The results of in vitro evaluation of antituberculosis activity appears in Table 3. The minimum inhibitory concentration (MIC) is > 12.5 μg/ml for **3b**, **3c**, **4b** and **4c**, and is < 12.5 μg/ml for **4a**. The last compound, which shows an inhibition greater than 90% (94), has been selected for confirmatory and advanced screening. The other compounds, considered inactive, still have significant inhibitory activity (inhibition from 23 to 68).

We can establish preliminary structure–activity relationships on the basis of the primary screening. If we compare these results with those obtained for quinoxaline 1,4-di-*N*-oxides [10,11], it seems that the N-oxide function is important for the activity: seven 3-amino or 3-amino-substituted 2-quinoxalinecarbonitrile 1,4-di-*N*-oxides with one or two chloros in position 6(7) are active [10,11], and the compound **3b**, with the same structure but not N-oxide, does not present activity. But according to these results, the presence of the N-oxide moiety in the structure is not fundamental; the compound **4a**, 3-chloro-2-quinoxalinecarbonitrile, shows an inhibition of 94% (MIC < 12.5 μg/ml). In the case of the inactive compounds, the 3-chloro substitution with the 6(7)-chloro derivative is better than the 3-amino substitution (**4b** versus **3b**, with 58 and 23% inhibition respectively), and the 3-amino substitution with the 6(7)-trifluoromethyl derivative is better than the 3-chloro

Table 3
In vitro evaluation of antituberculosis activity: rifampin (RMP) MIC 0.25 μg/ml versus *Mycobacterium tuberculosis*

Compound	MIC (μg/ml)	% Inhibition
3b	> 12.5	23
3c	> 12.5	68
4a	< 12.5	94
4b	> 12.5	58
4c	> 12.5	37

substitution (**3c** versus **4c**, with 68 and 37% inhibition respectively).

On the basis of these results, 3-chloro-2-quinoxalinecarbonitrile shows good antituberculosis activity in the primary screening. We propose to confirm the results, to advance further on in the screening, to synthesize new 2-quinoxalinecarbonitrile compounds with different substituents in positions 3, 6 and 7 in order to get the best antituberculosis activity, and to study these structures beside the corresponding N-oxides, because quinoxalines versus quinoxaline 1,4-di-*N*-oxides may be better from the point of view of biological biodisponibility.

5. Experimental

5.1. Chemistry

IR spectra were recorded on a Perkin-Elmer 681 infrared spectrophotometer, using KBr pellets. ^{1}H NMR spectra (reference TMS int) were taken on a Bruker AC-200E (200 MHz). The mass spectra were recorded on a Hewlett-Packard 5988-A instrument at 70 eV. Column chromatography was performed on a Merck silica gel 200 ASTM and analytical TLC on Panreac Silica gel 0.2 mm aluminium sheets. The plates were scanned under ultraviolet light at 254 and 366 nm. Melting points were determined on a Mettler FP82 + FP80 apparatus and are uncorrected. Elemental analysis were obtained on an Elemental Analyzer from vacuum-dried samples (over P_2O_5 at 1–2 mm Hg, 24 h at 60–80°C) and were within 0.4 % of the theoretical values.

Compounds **1a–1c**, **2a–2c**, **3a** and **4a** have been reported previously [12–14].

*5.1.1. General procedure for the preparation of 3-amino-2-quinoxalinecarbonitrile derivatives (**3b, 3c**)*

A solution of 6(7)-substituted-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide (5 mmol) in methanol (20 ml) was stirred at 50°C. A fresh solution of sodium ditionite (20 mmol) was added. The reaction mixture was allowed to cool, and the resulting crystals were collected and washed with water.

*5.1.1.1. 3-Amino-6(7)-chloro-2-quinoxalinecarbonitrile (**3b**)*

The mixture reaction was stirred for 1 h. The product was purified by recrystallization from methanol/dimethylformamide to afford **3b** (75% yield) as yellow solid, m.p. 245°C (d). An isomeric mixture was obtained, in which the major isomer was the 7-chloro isomer. IR (KBr, cm^{-1}) ν 1560 (C=N), 1656 (NH_2), 2232 (C≡N), 3316 (NH); ^{1}H NMR^{200} [$(CD_3)_2SO$, ppm] δ 7.44–7.60 (m, 3H, H_6 (7-chloro) + H_5(7-chloro) + H_7(6-chloro)), 7.56 (s, 2H, NH_2), 7.73 (d, 1H, H_5 (6-chloro), $J_{5-6} = 8.9$ Hz), 7,85 (d, 1H_8 (6-chloro), $J_{7-8} = 8.8$ Hz), 7.92 (s,1H, H_8, (7-chloro)); MS: *m/z* (%) 204 ($M^{+\cdot}$, 100), 177 (32), 125 (10), 110 (5), 75 (9); *Anal.* $C_9H_5ClN_4$, (C, H, N).

*5.1.1.2. 3-Amino-6(7)-trifluoromethyl-2-quinoxalinecarbonitrile (**3c**)*

The mixture reaction was stirred for 3 h. The product was purified by recrystallization from water/dimethylformamide to afford **3c** (65% yield) as yellow solid, m.p. 198–201°C. An isomeric mixture, 6 and 7-trifluoromethyl, was obtained (50:50). IR (KBr, cm^{-1}) ν 1172 (C–F), 1560 (C=N), 1664 (NH_2), 2236 (C≡N), 3331 (NH); ^{1}H NMR^{200} [$(CD_3)_2SO$, ppm] δ 7.67–7.76 (m, 2H, H_6 (7-trifluomethyl) + H_7 (6-trifluoromethyl)), 7.86 (s, 2H, NH_2 (7-trifluoromethyl)), 7.87 (s, 1H, H_5 (6-trifluoromethyl)), 7.96 (dd, H, H_5 (7-trifluoromethyl), $J_{5-7} = 1.8$ Hz, $J_{7-8} = 8.9$ Hz), 8.06 (d, 1H, H_8 (6-trifluoromethyl), $J_{7-8} = 8.7$ Hz), 8.22 (s,1H, H_8 (7-trifluoromethyl)); MS: *m/z* (%) 238 ($M^{+\cdot}$, 100), 211 (36), 159 (16), 132 (7), 117 (4), 75 (39); *Anal.* $C_{10}H_5F_3N_4$ (C, H, N).

*5.1.2. General procedure for the preparation of 3-chloro-2-quinoxalinecarbonitrile derivatives (**4a–4c**)*

A mixture of 6(7)-substituted-3-amino-2-quinoxalincarbonitrile (5 mmol), acetic acid (18 ml) and hydrochloric acid 35% (22 ml) was stirred at 0°C. A fresh solution of sodium nitrite (20 mmol) was added. The reaction mixture was stirred for some hours at room temperature. The resulting precipitate was collected and washed with cool water. The impure product was purified by flash chromatography (SP, silica gel; eluting with n-hexane/ethyl acetate).

*5.1.2.1. 3-Chloro-2-quinoxalinecarbonitrile (**4a**)*

The mixture reaction was stirred for 1 h. The product was purified by flash chromatography, eluting with n-hexane/ethyl acetate (75:25) to afford **4a** (42% yield) as white solid, m.p. 156–158°C. IR (KBr, cm^{-1}) ν 1556 (C=N), 2234 (C≡N), 3039 (C–H aromatic); ^{1}H NMR^{200} [$(CD_3)_2SO$, ppm] δ 8.03–8.13 (m, 3H, H_5 + H_6 + H_7), 8,24 (d, 1H, H_8, $J_{(7-8)} = 8.4$ Hz); MS: *m/z* (%) 189 ($M^{+\cdot}$, 100), 154 (33), 102 (32), 76 (19). *Anal.* $C_9H_4ClN_3$ (C, H, N).

*5.1.2.2. 3,7-Dichloro-2-quinoxalinecarbonitrile (**4b**)*

The reaction mixture was stirred for 1 h. The product was purified by flash chromatography, eluting with n-hexane/ethyl acetate (90:10) to afford **4b** (46% yield) as white solid, m.p. 140–142°C. IR (KBr, cm^{-1}) ν 1547 (C=N), 2237 (C≡N), 3041 (C–H aromatic); ^{1}H NMR^{200} [$(CD_3)_2SO$, ppm] δ 8.06–8.27 (m, 2H, H_5 + H_6), 8.43 (s, 1H, H_8); MS: *m/z* (%) 223 ($M^{+\cdot}$, 100), 136 (24), 110 (18), 75 (20); *Anal.* $C_9H_3Cl_2N_3$ (C, H, N).

*5.1.2.3. 3-Chloro-6(7)-trifluoromethyl-2-quinoxalinecarbonitrile (**4c**)*

The reaction mixture was stirred for 2 h. The product was purified by flash chromatography, eluting with n-hexane/ethyl acetate (85:15) to afford **4c** (42% yield) as white solid, m.p. 45–47°C. An isomeric mixture of 6- and 7-trifluoromethyl was obtained (50:50). IR (KBr, cm^{-1}) ν 1130 (C–F), 1560 (C=N), 2236 (C≡N), 3073 (C–H

aromatic); ^{1}H NMR^{200} [$(CD_3)_2SO$, ppm] δ 8.38–8.29 (m, 4H, H_5 (7-trifluoromethyl) + H_6 (7-trifluoromethyl) + H_7 (6-trifluoromethyl)), 8.49 (d, H_8 (6-trifluoromethyl), J_{7-8} = 8.7 Hz), 8.63 (s, 1H, H_5 (6-trifluoromethyl)), 8.74 (s, 1H, H_8 (7-trifluoromethyl)); MS: *m/z* (%) 257 ($M^{+\cdot}$, 79), 222 (26), 170 (62), 144 (28), 75 (100); *Anal.* $C_{10}H_3ClF_3N_3$ (C, H, N)

5.2. Pharmacology

Primary screening was conducted at 12.5 μg/ml against the virulent strain *Mycobacterium tuberculosis* H37Rv. *Mycobacterium tuberculosis* H37Rv was grown in BACTEC 12B medium containing radiolabelled substrate [15]. Labelled CO_2 produced was detected and quantitated by the automatic radiometric system BACTEC 460. Compounds effecting $< 90\%$ inhibition in the primary screening (MIC > 12.5 μg/ml) were not evaluated further. They were considered inactive compounds. Compounds showing $> 90\%$ inhibition in the primary screening were selected for confirmatory and advanced screening. This next phase determines the actual MIC and selectivity of the active compounds. The standard compound used in this primary assay was rifampin (RMP), MIC 0.25 μg/ml.

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