Available free online at www.medjchem.com

Mediterranean Journal of Chemistry **2015**, 4(1), 1-8

Synthesis and antitubercular evaluation of 7-chloro-4-alkoxyquinoline derivatives

Marcelle de Lima Ferreira Bispo[1], Laura Nogueira de Faria Cardoso[1], Maria C. S. Lourenço[2], Flávio Augusto Ferreira Martins Bezerra[2], Rodrigo Pedro Pinto Soares[3], Caryne Margotto Bertollo[3], Marcus Vinícius Nora de Souza[1]*

[1]Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Far Manguinhos, 21041-250, Rio de Janeiro, RJ, Brazil.

[2]Fundação Oswaldo Cruz, Instituto de Pesquisas Clínicas Evandro Chagas, Departamento de Bacteriologia, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, Brazil

[3]Fundação Oswaldo Cruz, Centro de Pesquisas René Rachou, Laboratório de Biomarcadores de Diagnóstico e Monitoração, Av. Augusto de Lima, 1715
Barro Preto, 30190-002, Belo Horizonte, MG, Brazil

Abstract: A series of eight 7-chloro-4-alkoxyquinoline derivatives (**2a-h**) have been synthesized and their *in vitro* (antimycobacterial) activity against *Mycobacterium tuberculosis* was evaluated. Furthermore, all the active compounds were selected for evaluation of their cytotoxicities against the human hepatoma (HepG2) and the relative selectivity (selective index) of these compounds against *M. tuberculosis* compared to HepG2 was calculated based on MLD_{50}/MIC ratios. These biological results have been compared to a series of 7-chloro-4-aminoquinoline derivatives **1a-i**, previously identified by our research group with the aim to provide important information about the structure-activity relationship of quinoline derivatives.

Keywords: quinolone; tuberculosis; antimycobacterial activity; cytoxicity assays.

Introduction

The development of antibiotic therapies that overcome the emerging antimicrobial resistance of *Mycobacterium tuberculosis* strains is one of the major challenges to control the tuberculosis (TB). There are two types of resistance recognized by World Health Organization (WHO): multidrug-resistant tuberculosis (MDR-TB, resistant to at least isoniazid or rifampicin, with or without resistance to other first-line drugs) and extensively drug-resistant TB (XDR-TB, is MDR-TB that is also resistant to any fluoroquinolone and also to any of the three second-line injectables-amikacin, capreomycin, and kanamycin). WHO estimates that there are about 450,000 MDR-TB cases in the world in 2012 and about 10% of MDR-TB cases worldwide have XDR-TB. Besides, the presence of XDR-TB strains has been reported at least once in 92 countries worldwide by the end of 2012[1].

**Corresponding author: Vinícius Nora de Souza*
E-mail address: marcos_souza@far.fiocruz.br
DOI: http://dx.doi.org/10.13171/mjc.4.1.2015.19.02.10.18/desouza

Considering the high impact of resistant strains for the tuberculosis treatment, there is an urgent requirement of developing new drugs to treat this disease efficiently. Therefore, in previous studies conducted by our research program for the development of new anti-TB agents, we have prepared and evaluated a series of 7-chloro-4-aminoquinoline derivatives **1a-i**, among them, three compounds (**1b-d**) have been interesting antimycobacterial activities. In this previous report, the study of structure-activity relationship of this class of compounds pointed out that the presence of the chlorine at C7 position and the increase of the lipophilicity are significant features for the biological activity[2]. Considering these results, we decided to continue studying the potential of quinoline derivatives as anti-TB agents through the synthesis and antitubercular evaluation of a series of 7-chloro-4-alkoxyquinoline derivatives **2a-h**. These compounds were designed by a classic isosteric replacement (NH x O). Besides, the selection of the substituents attached at oxygen atom was based on the best biological results obtained in the previous series of 7-chloro-4-aminoquinoline derivatives **1a-i** and also in order to increase the lipophilicity and the molecular surface. Furthermore, the relevance of this report also arises from the need of ongoing researches on the structure-activity relationship of quinoline derivatives (Figure **1**)[3].

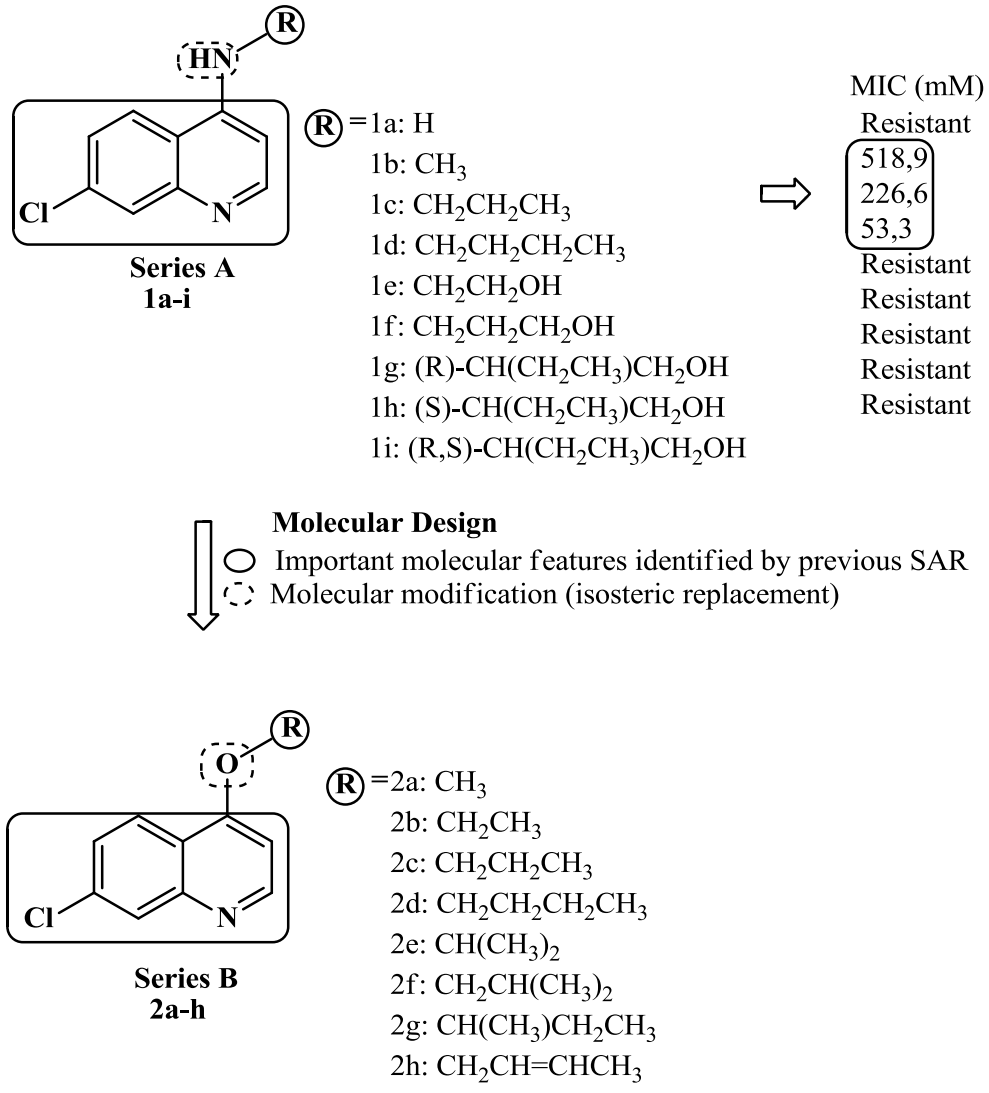


Figure 1. Molecular design of 7-chloro-4-alkoxyquinoline derivatives (**2a-h**).

Experimental Section

General Procedures

Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. Mass spectra (ESI assay in solution of ammonium chloride) were recorded on Micromass ZQ Waters mass spectrometer. LTQ Orbitrap XL ETD (mass spectrometry facility RPT02H PDTIS/Carlos Chagas Institute-Fiocruz Parana). NMR spectra were recorded on a Bruker Avance 400 operating at 400 MHz (^1H) and 100.0 MHz (^{13}C) and Bruker Avance 500 spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C), in deuterated dimethylsulfoxide. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and *J*-coupling in Hertz (Hz). Proton and carbon spectra were typically obtained at room temperature. For TLC plates coated with silica gel were run in chloroform/methanol mixture and spots were developed in ultraviolet.

General procedures of synthesis of 7-chloro-4-alkoxyquinoline

Metallic sodium (0.172g, 7.48 mmol) was slowly added in the appropriate alcohol (15.0 mL, 468-202 mmol). After the complete dissolution of sodium, 4,7 dichloroquinoline (0.3g, 1.51 mmol) was added in the solution and the mixture was stirred for 8-72h at the boiling point of the corresponding alcohol. Besides, the excess of solvent was concentrated under reduced pressure and the residue was extracted with water (15 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. After that, some desired products were obtained pure (**1-3** and **5**) or the crude products were purified by different process according to the substituent attached to oxygen (see bellow; **4** and **6-9**) to afford the pure derivatives **2a-i** as pale yellow or white solids in 60-95% yields.

7-chloro-4-methoxyquinoline (2a)

Yield: 95%. m.p. 137-138°C[4]. ^1H NMR (400 MHz; DMSO-d6): δ: 8.76 (1H, d, *J* = 4.1 Hz, H-2); 8.15 (1H, d, *J* = 7.1 Hz, H-5); 8.00 (1H, d, *J* = 1.5 Hz, H-8); 7.59 (1H, dd, *J* = 7.2 and 1.6 Hz, H-6); 7.08 (1H, d, *J* = 4.2 Hz, H-3); 4.06 (3H, s, H-2').
^{13}C NMR (100 MHz; DMSO-d6): δ 161.6 (C-4); 153.1 (C-2); 149.1 (C-4a); 134.4 (C-7); 127.3 (C-8); 126.3 (C-5); 123.7 (C-6); 119.3 (C-8a); 101.6 (C-3); 56.3 (C-2').
MS/ESI: [M+H]: 193.0. IR ν_{max} (cm^{-1}; KBr pellets): 1124 (C-O stretching vibration).

7-chloro-4-ethoxyquinoline (2b)

Yield: 82%. m.p. 98-99°C[5]. ^1H NMR (400 MHz; DMSO-d6): δ: 8.75 (1H, d, *J* = 5.2 Hz, H-2); 8.16 (1H, d, *J* = 8.9 Hz, H-5); 7.99 (1H, d, *J* = 2.0 Hz, H-8); 7.59 (1H, dd, *J* = 8.9 and 2.1 Hz, H-6); 7.05 (1H, d, *J* = 5.3 Hz, H-3); 4.32 (2H, q, *J* = 7.0 Hz, H-2'); 1.48 (3H, t, *J* = 7.0 Hz, H-3'). ^{13}C NMR (100 MHz; DMSO-d6): δ 160.7 (C-4); 153.1 (C-2); 149.2 (C-4a); 134.3 (C-7); 127.3 (C-8); 126.2 (C-5); 123.8 (C-6); 119.4 (C-8a); 102.1 (C-3); 64.4 (C-2'); 14.2 (C-3'). MS/ESI: [M+H]: 208.2. IR ν_{max} (cm^{-1}; KBr pellets): 1123 (C-O stretching vibration).

7-chloro-4-propoxyquinoline (2c)

Yield: 75%. m.p. 70-71°C. ^1H NMR (500 MHz; DMSO-d6): δ:8.75 (1H, d, *J* = 5.2 Hz, H-2); 8.16 (1H, d, *J* = 8.9 Hz, H-5); 7.99 (1H, d, *J* = 1.8 Hz, H-8); 7.59 (1H, dd, *J* = 8.9 and 1.9 Hz, H-6); 7.05 (1H, d, *J* = 5.2 Hz, H-3); 4.21 (2H, t, *J* = 6.4 Hz, H-2'); 1.89 (2H, sx, *J* = 7.1 and 6.8 Hz, H-3') 1.07 (3H, t, *J* = 7.4 Hz, H-4'). ^{13}C NMR (125 MHz, DMSO-d6) δ:

160.9 (C-4); 153.1 (C-2); 149.2 (C-4a); 134.4 (C-7); 127.3 (C-8); 126.2 (C-5); 123.8 (C-6); 119.4 (C-8a); 102.1 (C-3); 70.0 (C-2'); 21.8 (C-3'); 10.4 (C-4'). MS/ESI: [M+H]: 222.0. IR ν_{max} (cm^{-1}; KBr pellets): 1117 (C-O stretching vibration).

4-butoxy-7-chloroquinoline (2d)

Yield: 62%. m.p. 56-57°C. ^{1}H NMR (400 MHz; DMSO-d6): δ: 8.75 (1H, d, *J* = 4.2 Hz, H-2); 8.16 (1H, d, *J* = 7.1 Hz, H-5); 7.98 (1H, d, *J* = 1.5 Hz, H-8); 7.59 (1H, dd, *J* = 7.1 and 1.6 Hz, H-6); 7.06 (1H, d, *J* = 4.2 Hz, H-3); 4.26 (2H, t, *J* = 5.1 Hz, H-2'); 1.85 (2H, qi, *J* = 5.6 Hz H-3'); 1.54 (2H, sx, *J* = 6.0 Hz, H-4'); 0.98 (3H, t, *J* = 5.9 Hz, H-5'). ^{13}C NMR (100 MHz, DMSO-d6) δ: 160.8 (C-4); 153.1 (C-2); 149.2 (C-4a); 134.3 (C-7); 127.2 (C-8); 126.2 (C-5); 123.7 (C-6); 119.4 (C-8a); 102.0 (C-3); 68.2 (C-2'); 30.3 (C-3'); 18.8 (C-4'); 13.6 (C-5'). HRMS/ESI: [M+H]: 236.0834. IR ν_{max} (cm^{-1}; KBr pellets): 1119 (C-O stretching vibration).

7-chloro-4-isopropoxyquinoline (2e)

Purification by chromatography column (hexane / ethyl acetate (8:2).Yield: 60%. ^{1}H NMR (500 MHz; DMSO-d6): δ: 8.66 (1H, d, *J* = 5.5 Hz, H-2); 8.20 (1H, d, *J* = 8.9 Hz, H-5); 7.90 (1H, d, *J* = 2.0 Hz, H-8); 7.51 (1H, dd, *J* = 8.9 and 2.1 Hz, H-6); 7.01 (1H, d, *J* = 5.5 Hz, H-3); 4.98 (1H, sp, H-2'); 1.49 (6H, d, *J* = 6.1 Hz, H-3' and H-4'). ^{13}C NMR (125 MHz, DMSO-d6) δ: 159.7 (C-4); 153.0 (C-2); 149.4 (C-4a); 134.2 (C-7); 127.2 (C-8); 126.0 (C-5); 123.9 (C-6); 119.8 (C-8a); 102.6 (C-3); 70.9 (C-2'); 21.4 (C-3' and C-4'). MS/ESI: [M+H]: 222.0. IR ν_{max} (cm^{-1}; KBr pellets): 1118 (C-O stretching vibration).

7-chloro-4-isobutoxyquinoline (2f)

The crude product was purified by washing with cold hexane (3 x 10 mL).
Yield: 67%. m.p. 56-58°C. ^{1}H NMR (400 MHz; DMSO-d6): δ: 8.74 (1H, d, *J* = 5.2 Hz, H-2); 8.18 (1H, d, *J* = 9.2 Hz, H-5); 7.99 (1H, d, *J* = 2.0 Hz, H-8); 7.60 (1H, dd, *J* = 8.8 and 2.0 Hz, H-6); 7.05 (1H, d, *J* = 5.2 Hz, H-3); 4.04 (2H, d, *J* = 6.4 Hz, H-2'); 2.21-2.17 (1H, m, H-3'); 1.07 (6H, d, *J* = 6.8 Hz; H-4' and H-5'). ^{13}C NMR (100 MHz, DMSO-d6) δ: 160.8 (C-4); 153.0 (C-2); 149.1 (C-4a); 134.3 (C-7); 127.2 (C-8); 126.2 (C-5); 123.6 (C-6); 119.4 (C-8a); 102.0 (C-3); 74.3 (C-2'); 39.6 (C-1'); 27.5 (C-3'); 18.9 (C-4'). HRMS/ESI: [M+H]: 236.0833. IR ν_{max} (cm^{-1}; KBr pellets): 1120 (C-O stretching vibration).

4-*sec*-butoxy-7-chloroquinoline (2g)

Purification by chromatography column (hexane / ethyl acetate (8:2). Yield: 74%. ^{1}H NMR (400 MHz; DMSO-d6): δ: 8.74 (1H, d, *J* = 6.6 Hz, H-2); 8.16 (1H, d, *J* = 11.1 Hz, H-5); 7.98 (1H, d, *J* = 2.6 Hz, H-8); 7.57 (1H, dd, *J* = 11.1 and 2.6 Hz, H-6); 7.07 (1H, d, *J* = 6.7 Hz, H-3); 4.77 (1H, q, J = 7.4 Hz, H-2'); 1.86-1.73 (2H, m, H-3'); 1.38 (3H, d, *J* = 7.6 Hz, H-5'); 0.98 (3H, t, *J* = 9.2 Hz, H-4'). ^{13}C NMR (100 MHz, DMSO-d6) δ: 160.0 (C-4); 153.0 (C-2); 149.4 (C-4a); 134.3 (C-7); 127.2 (C-8); 126.0 (C-5); 123.9 (C-6); 119.9 (C-8a); 118.2 (C-3); 102.6 (C-3); 75.5 (C-2'); 28.3 (C-5'); 18.5 (C-3'); 9.3 (C-4'). HRMS/ESI: [M+H]: 236.0834. IR ν_{max} (cm^{-1}; KBr pellets): 1116 (C-O stretching vibration).

7-chloro-4-[(1*E*)-prop-1-en-1-yloxy]quinoline (2h)

The crude product was purified by recrystallization using hexane as the solvent.
Yield: 65%. m.p. 68-70°C. ^{1}H NMR (500 MHz; DMSO-d6): δ 8.75 (1H, d, *J* = 5.3 Hz, H-2); 8.16 (1H, d, *J* = 8.9 Hz, H-5); 7.99 (1H, d, *J* = 2.0 Hz, H-8); 7.57 (1H, dd, *J* = 11.0 and 2.1 Hz, H-6); 7.05 (1H, d, *J* = 5.3 Hz, H-3); 5.86-5.78 (1H, m, H-3'); 4.78 (2H, d, *J* = 6.0 Hz, H-2'); 1.76 (3H, dd, *J* = 6.0 and 5.0 Hz, H-5'); 1.66 (1H, d, *J* = 5.3 Hz, H-4'). ^{13}C NMR (125 MHz, DMSO-d6) δ:160.5 (C-4); 153.0 (C-2); 149.2 (C-4a); 134.3 (C-7); 131.0 (C-2');

127.2 (C-8); 126.2 (C-5); 123.8 (C-6); 119.4 (C-8a); 102.4 (C-3'); 17.6 (C-4'). HRMS/ESI: [M-H]: 233.0672. IR ν_{max} (cm^{-1}; KBr pellets): 1115 (C-O stretching vibration).

General procedures for biological tests

Antimycobacterial Activity

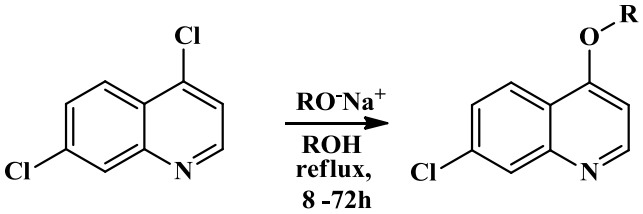
Briefly, 200 μL of sterile deionized water was added in all outer-perimeter wells of sterile 96 well plates (falcon, 3072: Becton Dickinson, Lincoln Park, NJ) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100μL of the Middlebrook 7H9 broth containing the mycobacterial cells (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **(2a-i)** was made directly on the plate. The final drug concentrations tests were 0.01-100 μg/mL. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25 μL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake, Ohio) reagent and 10% tween 80 was added in plate and incubated for 24h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The MIC (minimal inhibition concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink. Ethambutol (EMB) was used by pattern in the tests.

Cell Viability Assay

Cytotoxicity was determined using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and the hepatoma cell lineage Hep G2 A16. Cells were kept in RPMI medium supplemented with 10% FBS and confluent monolayers were trypsinized, washed in RPMI and applied in 96-well microtiter plates (4 X 10^4 cells/well). Compounds and amphotericin B in the same conditions described above were incubated with the cells (37°C, 5% CO_2, 24h). Colorimetric reaction was developed after incubation with MTT (37°C, 4 h) followed by addition of acidified isopropanol as previously described[5]. The reaction was read spectrophotometrically with a 570 nm filter and a background of 630 nm. Incubations were tested in triplicate in two independent experiments.

Results and Discussion

The synthesis of 7-chloro-4-alkoxyquinoline derivatives (2a-h) was performed through reactions between 4,7-dichloroquinoline and a solution of the alkoxyde in the corresponding alcohol as a solvent, under the appropriated reflux temperature, as indicated in Scheme **1** and Table **1**.



Scheme 1. Preparation of 7-chloro-4-alkoxyquinoline derivatives (**2a-h**).

Table 1. Yields, reaction times and melting points of 7-chloro-4-alkoxyquinoline derivatives (**2a-h**).

Substances	R	Yield (%)	Time (h)	m.p. (°C)
2a	Methyl	95	4	137-138[4]
2b	Ethyl	82	8	98-99[5]
2c	Propyl	75	10	70-71
2d	Butyl	62	24	56-57
2e	i-Propyl	60	15	Oil[4]
2f	i-Butyl	67	30	56-58
2g	s-Butyl	74	72	Oil
2h	(1*E*)-prop-1-en-1-yl	65	12	68-70

In general, these results showed that both reaction times and yields could be related to the size of the alkyl group attached to oxygen atom. As larger is the alkyl chain, more slowly is the reaction and poorer is the yield. Furthermore, all the compounds were identified by the spectral data. In general, IR spectra of 7-chloro-4-alkoxyquinoline derivatives (**2a-h**) showed the C-O stretching vibrations at 1115-1124 cm^{-1}. The nuclear magnetic resonance spectra (^{1}H NMR) showed the six aromatic protons at 8.76-8.66, 8.20-8.15, 8.00-7.90, 7.51-7.60 and 7.08-7.01 ppm relative to protons of quinoline nucleus (H-2, H-5, H-8, H-6 and H-3, respectively) and the corresponding aliphatic protons (4.98-0.98 ppm). The ^{13}C NMR spectrum showed the C-O (C-4) signals at 161.6-159.7 and C=N (C-2) signals at 153.1-153.0 ppm.

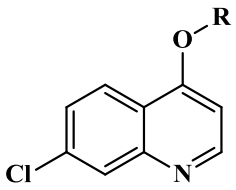
Biological evaluation

The antimycobacterial activities of the derivatives **2a-h** were assessed against *M. tuberculosis* ATCC 27294[7] using the microplate Alamar Blue assay (MABA)[8] (Table **2**). This nontoxic methodology uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods[9,10].

Furthermore, all the active compounds were selected for evaluation of their cytotoxicities against the human hepatoma (HepG2). The cellular viability in the presence and absence of the test compounds was determined using the Mosman's MTT (3-(4.5-demethylthylthiazol-2-yl)-2.5-dimethyltetrazolium bromide; Merck) microcultured tetrazolium assay[11]. The results are expressed as the minimum lethal dose that killed 50% of the cells (MLD_{50}) in µM are listed in Table **2**. The relative selectivity (selective index) of these compounds against *M. tuberculosis* compared to HepG2 was calculated based on MLD_{50}/MIC ratios (Table **2**).

In general, it was observed that the biological activity of this series also could be related to the increase of the size of the alkyl group attached to oxygen atom, which can be see when the compounds **2a-e** are compared. Besides, the ramification of alkyl chain also could be important since the presence of branched alkyl groups (i-Butyl, **2g**, s-Butyl, **2h**) improve the biological activity when compared to a linear alkyl group (Butyl, **2e**). Another important observation is that the presence of an unsaturated substituent improved the antimycobacterial activity, being the derivative **2i** the most active of this series of compounds.

Table 2. Antimycobacterial activity (MIC), cytotoxicity (MLD_{50}) and selective index of 7-chloro-4-alkoxyquinoline derivatives **(2a-h)**.

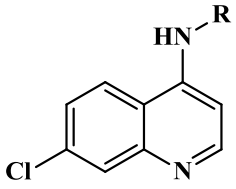


Substances	R	MIC (µM)	MLD_{50} (µM)	Selective Índex (SI)
2a	Methyl	516.5	25826.4	50.0
2b	Ethyl	Resistant	----	----
2c	Propyl	225.5	992.3	4.4
2d	Butyl	212.1	1400.1	6.6
2e	i-Propyl	225.5	315.7	1.4
2f	i-Butyl	53.0	424.3	8.0
2g	s-Butyl	106.1	721.3	6.8
2h	(1*E*)-prop-1-en-1-yl	53.5	1240.9	23.2
EMB		15.3	25380.9	1659.5

In regard to cytotoxicity evaluation, it was observed that these compounds were very cytotoxic when compared to ethambutol (EMB). The best SI value was observed for **2a** followed by **2h**. EMB, our drug control, had the best SI value as expected. Compounds exhibiting SI lower than 20 (**2c-h**) were considered toxic[12,13].

Although the biological results were not very significant, they are useful to identify the most important points for the antimycobacterial activity of the 7-chloro-quinoline derivatives. For example, when the series of 7-chloro-4-alkoxyquinoline derivatives **(Series A, 2a-i)** is compared to the 7-chloro-4-aminoquinoline derivatives **(Series B, 1c-d,** Table **3**), it is important to be mentioned that the effect of the increase of the size of the alkyl chain on the biological activity is more relevant in the Series B. However, the compounds of Series B are, in general, less cytotoxic and more selective, which can indicate that the classic isosteric replacement (NH x O) produced derivatives with the same potency, but with a better cytotoxic proflie.

Table 3. Comparison between the antimycobacterial activity (MIC), cytotoxicity (MLD_{50}) and selective index of some derivatives of Series A **(1b-d)** and Series B **(2b-d)**.



Series A	R	MIC (µM)	MLD_{50} (µM)	Selective Índex (SI)	Series B	MIC (µM)	MLD_{50} (µM)	Selective Index (SI)
1b	Ethyl	518.9	726.5	1,4	**2b**	----	----	----
1c	Propyl	226.6	362.5	1,6	**2c**	225.5	992.3	4.4
1d	Butyl	53.3	724.3	13,6	**2d**	212.1	1400.1	6.6

Conclusion

In summary, eight 7-chloro-4-alkoxyquinoline derivatives (**Series B, 2a-h**) were synthesized in good yields (60-92%). Among them, four are new compounds (**2d, 2f-h**). The compounds of Series **B** were compared to 7-chloro-4-aminoquinoline derivatives (Series **A)** previously evaluated against TB by our research group and, in general, the classic isosteric replacement (NH x O) produced derivatives with the same potency, but with a better cytotoxic profile. Therefore, this study is important information about the structure-activity of 7-chloroquinoline analogs and could provide a better direction in the management of antimycobacterial activity of this class of compounds.

Acknowledgments

The authors would like to thank to CNPq, FAPERJ and the Program for Technological Development in Tools for Health-PDTIS-FIOCRUZ for use of its facilities.

References

1- WHO Global TB report 2011: http://www.who.int/tb/publications/global_report/en/ (Accessed: February, 10, 2015).
2- A. L. P. Candéa, M. L. Ferreira, K. C. Pais, L. N. F. Cardoso, C. R. Kaiser, M. G. M. O. Henriques, M. C. S. Lourenço, F. A. F. M. Bezerra, M. V. N. de Souza. Bioor. Med. Chem. Lett., **2009**, 19, 6272-6274.
3- S. S. Ravichandran; S. A. Dhanaraj; H. Rajak; S. Kumar. W.J.P.P.S., **2014**, 3, 1072-1082.
4- N. D. Heindel, S. A. Fine. J. Heterocycl. Chem., **1969**, 6, 961.
5- L. R. Gomes, J. N. Low, J. L. Wardell, L. N. F. Cardoso, M. V. N. De Souza, Acta Crystallogr., Sect. C: Cryst. Struct. Commun., **2013**, 69, 191-194.
6- F. Denizot, R. Lang. J. Immunol. Meth., **1986**, 89, 271-277.
7- J. Canetti, E. Rist, R. Grosset, Revue Tuberc. Pneumol., **1963**, *27*, 217-272.
8- S. G. Franzblau, R. S. Witzig, J. C. McLaughlin, P. Torres, G. Madico, A. Hernandez, M. T. M. B. Degnan, V. K. Cook, R. M. Quenzer, R. H. Ferguson, J. Clin. Microbiol., **1998**, 36, 362-366.
9- J. D. Vanitha, C. N. Paramasivan. Diagn. Microbiol. Infect. Dis., **2004**, 49, 179-182.
10- R. S. Reis, I. Neves Jr., S. L. S. Lourenço, L. S. Fonseca, M. C. S. Lourenço, J. Clin. Microbiol., **2004**, 42, 2247-2248.
11- T. Mossmann. J. Immunol. Meth., **1983**, 65, 55-63.
12- J. R. Loset R. Brun, T. Wenzler, M. Kaiser, V. Yardley, **2009**, DNDi and Pan-Asian Screening Network 1-74.
13- S. Nwaka, A. Hudson. Nature Rev. Drug Discov., **2006**, 5, 941-955.