Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues

Maria C. S. Lourenço,[a] Marcus V. N. de Souza,[b*] Alessandra C. Pinheiro,[b] Marcelle de L. Ferreira,[b] Raoni S. B. Gonçalves,[b] Thais Cristina M. Nogueira,[b] and Mônica A. Peralta[b]

[a]IPEC-Instituto de Pesquisas Clínicas Evandro Chagas, Departamento de Bacteriologia
Av. Brasil, 4365 -Manguinhos, Rio de Janeiro - RJ, Brazil
[b]FioCruz-Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Far-Manguinhos
Rua Sizenando Nabuco, 100, Manguinhos, 21041-250 - Rio de Janeiro-RJ
E-mail: marcos_souza@far.fiocruz.br

Abstract

Several nicotinic and isoniazid derivatives, most of them containing nitro groups were synthesized by our group, and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosi*s H$_{37}$Rv using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in mg/mL. The compound **11d** exhibited the best result (1.2 μg/mL) when compared with first line drugs such as isoniazid (INH) and rifampicin (RIP). Therefore this class of compounds could be a good starting point to develop new lead compounds in the treatment of multi-drug resistant tuberculosis.

Keywords: Tuberculosis, isoniazid, nicotinic acid, analogs

Introduction

The pyridine nucleus is an important heteroaromatic class of compounds with a wide range of activities and it is present in many products such as drugs, vitamins, food, flavorings, plants, dyes, rubber products, adhesives, insecticides and herbicides[1]. In this context, nicotinic acid (pyridine-3-carboxylic acid), also known as niacin and vitamin B3, is found in various plants and animals and has vital roles in such biological processes as production of energy, signal transduction, regulation of gene expression and synthesis of fatty acids, cholesterol and steroids[2,3]. Nicotinic acid derivatives and its isomers have also been investigated as an agent for the prevention or delay of the onset of type 1 diabetes mellitus[4]. They also have anti-bacterial, anti-oxidant, anti-inflammatory and anti-carcinogenic activities, and have putative activity against osteoarthritis and granuloma annulare[3]. For example, it can be mentioned the importance

of anti-tuberculosis first-line drug isoniazid[5], which is an analogue of isonicotinic acid, an isomer of nicotinic acid. Nicotinic acid derivatives are also an important start material for the preparation of other biological activity compounds. Considering that, 2-chloro-3-pyridinecarbonyl chloride is a useful intermediate for preparation of Nevirapine (Viramune, **1**), a valuable anti-AIDS drug[6]. Due to the importance of nicotinic acid derivatives and its isomers, the aim of this article is to evaluate the *in vitro* antibacterial activity against *Mycobacterium tuberculosis* of several compounds of this class synthesized by our group.
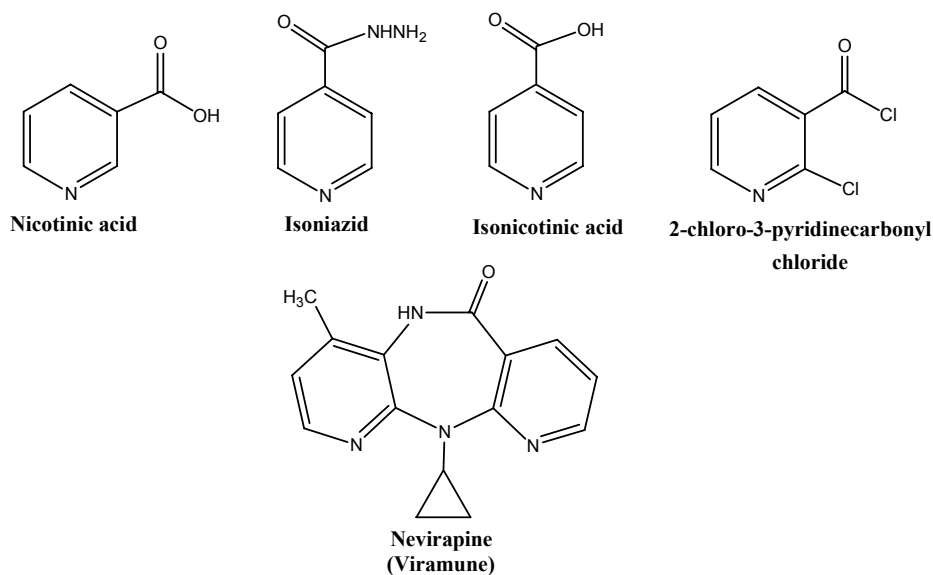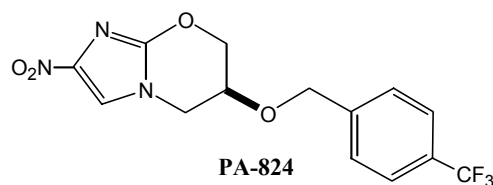

Figure 1

Results and Discussion

1. Nitro nicotinic derivatives

The synthesis of nitro compounds described in the schemes (**1-4**) was based on the reaction between 2-chloro-3-pyridinecarbonylchloride and nitro anilines or nitro phenylhydrazines, leading to the compounds **2a-c**,[7] **4a-g**,[8] **6a-c**,[9] **8a-d**,[10] and **9a-d**[10] or the coupling of nitro acid chlorides or aldehydes with isoniazid, leading to the products **11a-d**[11]. Nowadays, important attention has been payed for nitro compounds as anti-TB, especially due to the discovery of the compound nitroimidazole PA-824 (**Figure 2**)[5], which has shown important TB activity against MDR-TB strains, promising activity in latent state TB and potent sterilization effects compared with those of rifampicin and isoniazid. Another advantage of PA-824 is that no significant inhibition of cytochrome P450 isozymes has been observed suggesting that it could be used with HIV medications, being useful in the TB/HIV treatment. Due to its promising activity against TB, the nitroimidazole PA-824 is under clinical phase studies being developed by TB Alliance.
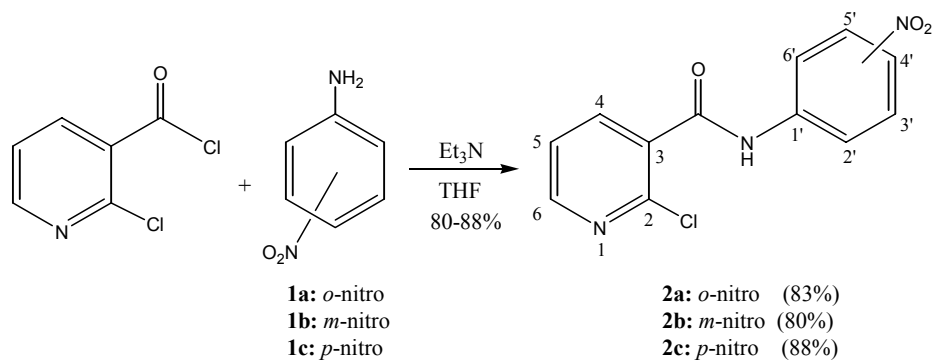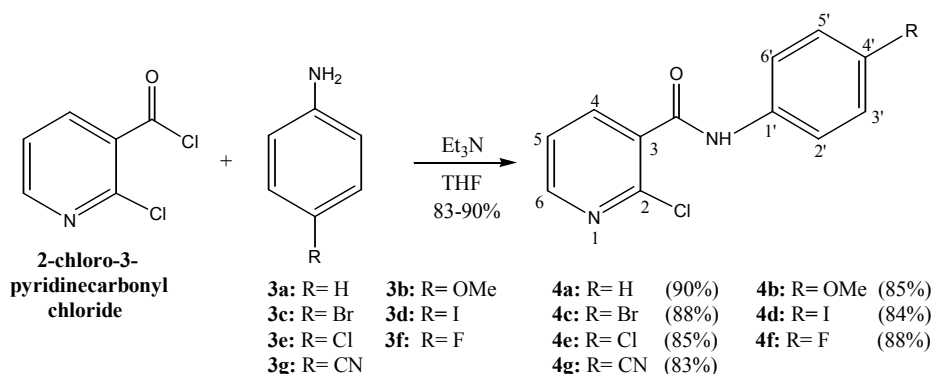
PA-824

Chiron Corporation, TB Alliance

Figure 2

1.1. *N*-Nitro-2-chloronicotinamides

The *N*-nitro-2-chloronicotinamides, as well others *N*-aryl-2-chloronicotinamides   (**Schemes 1** and **2**) were synthesized and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in mg/mL. However, none of the compounds above mentioned were active. The compounds **2a-c** and **4a-g** were identified by spectral data, which are presented in the **Tables 1-3**.

1a: *o*-nitro   **2a:** *o*-nitro   (83%)
1b: *m*-nitro   **2b:** *m*-nitro (80%)
1c: *p*-nitro   **2c:** *p*-nitro   (88%)

Scheme 1

2-chloro-3-pyridinecarbonyl chloride

3a: R= H   **3b:** R= OMe   **4a:** R= H   (90%)   **4b:** R= OMe (85%)
3c: R= Br   **3d:** R= I   **4c:** R= Br   (88%)   **4d:** R= I   (84%)
3e: R= Cl   **3f:** R= F   **4e:** R= Cl   (85%)   **4f:** R= F   (88%)
3g: R= CN   **4g:** R= CN (83%)

Scheme 2

Table 1. Data of compounds **2a-c** and **4a-g**

Products	Formula	$[M]^+$	PF(^{o}C)	Yield (%)	IR (cm^{-1}) C=O	IR (cm^{-1}) NH
2a	$C_{12}H_8ClN_3O_3$	277	157-159	83	1687	3327
2b	$C_{12}H_8ClN_3O_3$	277	155-158	80	1672	3358
2c	$C_{12}H_8ClN_3O_3$	277	206-208	88	1649	3266
4a	$C_{12}H_9ClN_2O$	232	122-124	90	1662	3250
4b	$C_{13}H_{11}ClN_2O_2$	262	110-111	85	1655	3235
4c	$C_{12}H_8BrClN_2O$	312	126-127	88	1649	3266
4d	$C_{12}H_8ClIN_2O$	358	175-177	84	1645	3221
4e	$C_{12}H_8Cl_2N_2O$	267	148-149	85	1658	3280
4f	$C_{12}H_8ClFN_2O$	250	93-94	88	1661	3135
4g	$C_{13}H_8ClN_3O$	257	172-173	83	1688	3261

Table 2. ^{1}H-NMR chemical shifts of compounds **2a-c** and **4a-g**

				δ ^{1}H (ppm)			
Products	NH	H_4	H_5	H_6	$H_{2', 6'}$	$H_{3', 5'}$	$H_{4'}$
2a	11.06	7.67	7.62	8.58	-- ; 8.02	8.06, 7.49	7.78
2b	11.15	8.02	7.61	8.58	8.74, 8.00	--; 7.69	8.15
2c	11.27	8.17	7.62	8.59	7.97	8.30	--
4a	10.63	8.08	7.56	8.54	7.71	7.37	7.14
4b	10.50	8.06	7.55	8.53	7.63	6.95	--
4c	10.79	8.10	7.59	8.55	7.57	7.70	--
4d	10.75	8.10	7.56	8.55	7.55	7.72	--
4e	10.79	8.11	7.58	8.55	7.44	7.75	--
4f	10.71	8.10	7.58	8.55	7.22	7.74	--
4g	11.16	8.15	7.60	8.57	7.86	7.89	--

Table 3. ^{13}C-NMR chemical shifts of compounds **2a-c** and **4a-g**

Products	C$_2$	C$_3$	C$_4$	C$_5$	C$_6$	C=O	C$_{1'}$	C$_{2', 6'}$	C$_{3', 5'}$	C$_{4'}$
						δ (ppm)				
2a	146.5	131.8	138.3	123.1	151.0	163.6	133.9	130-126.3	126-124.9	143.2
2b	146.3	132.4	138.2	123.1	150.8	164.0	139.6	119-113.6	131-125.5	147.9
2c	146.3	132.4	138.3	123.2	150.9	164.2	142.8	119.4	125.0	144.6
4a	146.4	133.1	138.1	123.1	150.4	163.4	138.1	119.5	128.8	124.0
4b	146.5	131.8	138.1	123.1	150.3	163.1	133.3	114.0	121.1	155.8
4c	146.4	132.9	138.2	123.1	150.6	163.6	138.0	121.5	131.7	115.8
4d	146.3	132.8	138.1	123.1	150.5	163.5	138.4	121.6	137.5	87.7
4e	146.4	132.9	138.1	123.1	150.5	163.5	137.5	121.1	128.7	127.7
4f	146.4	133.0	138.1	123.1	150.5	163.4	135.0	115.4	121.3	158.4
								J=22Hz*	*J*=8Hz*	*J*=241Hz*
4g	146.2	132.5	138.2	123.2	150.8	164.1	142.7	119.50	133.4	105.8

*C-F couplings

1.2. 1-(2-Chloronicotinoyl)-2-(nitrophenyl)hydrazines

The 1-(2-chloronicotinoyl)-2-(nitrophenyl)hydrazines **6a-c** (**Scheme 3**) were synthesized and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H$_{37}$Rv using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in μg/mL. However, none of the compounds above mentioned were active.
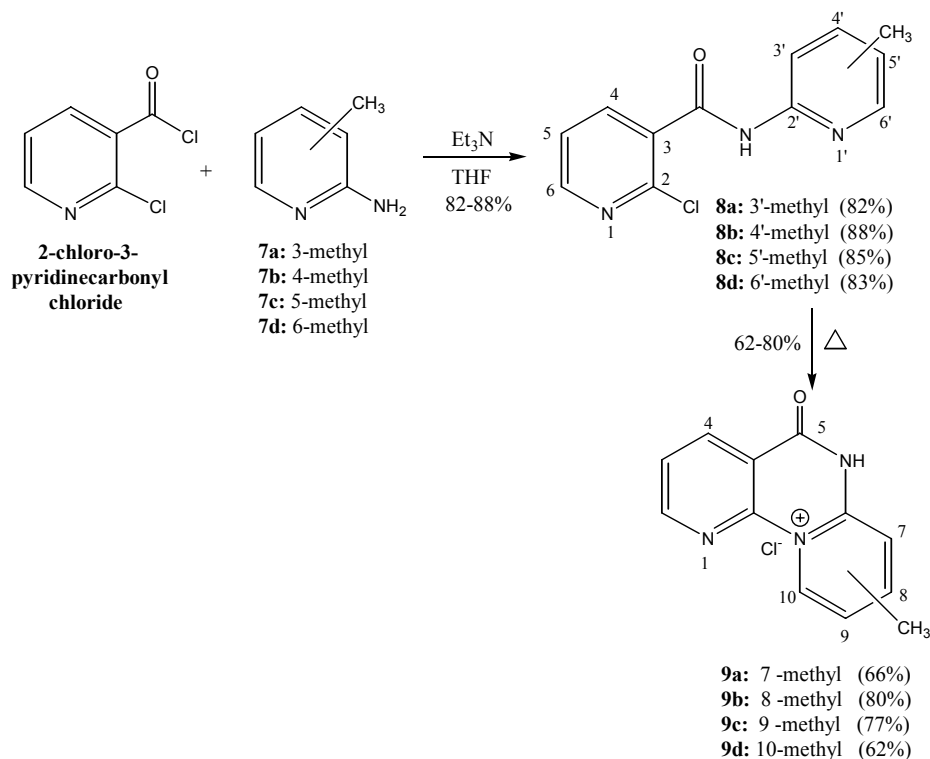

Scheme 3

2. 2-Chloro-*N*-(2-pyridinyl)nicotinamides

The 2-chloro-*N*-(2-pyridinyl)nicotinamides **8a-d** (**Scheme 4**), were synthesized and its cyclization lead 5-oxo-5,6-dihydrodipyrido[1,2-*a*:3',2'-*e*]pyrimidin-11-ium chlorides **9a-d**, which were identified by spectral data, which are presented in the **Tables 4** and **5**. The compounds **8a-d**

and **9a-d** were evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H$_{37}$Rv using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in µg/mL. However, none of the compounds above mentioned were active.

Scheme 4
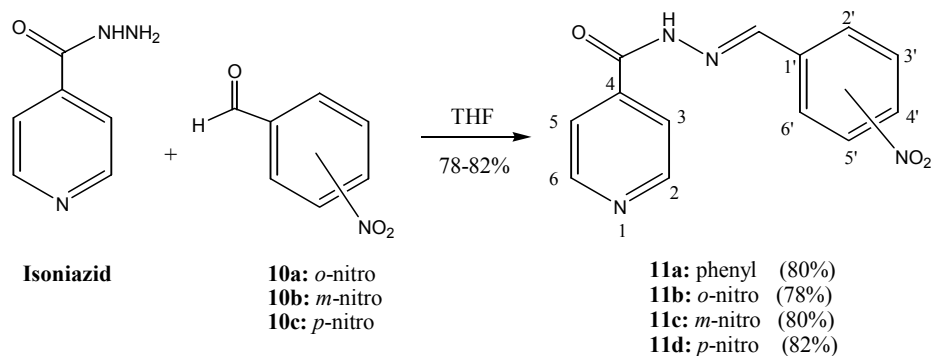
Table 4. ^{1}H-NMR chemical shifts of compounds **8a-d**

Products	δ ^{1}H (ppm)							
	NH	H$_4$	H$_5$	H$_6$	H$_{3'}$	H$_{4'}$	H$_{5'}$	H$_{6'}$
8a	--	8.16	7.48	8.47	--	7.82	7.32	8.24
8b	11.25	8.19	7.48	8.45	7.60	--	7.20	8.23
8c	11.06	8.04	7.52	8.50	8.08	7.68	--	8.20
8d	11.21	8.03	7.52	8.50	8.00	7.74	7.05	--

Table 5. ^{1}H-NMR chemical shifts of compounds **9a-d**

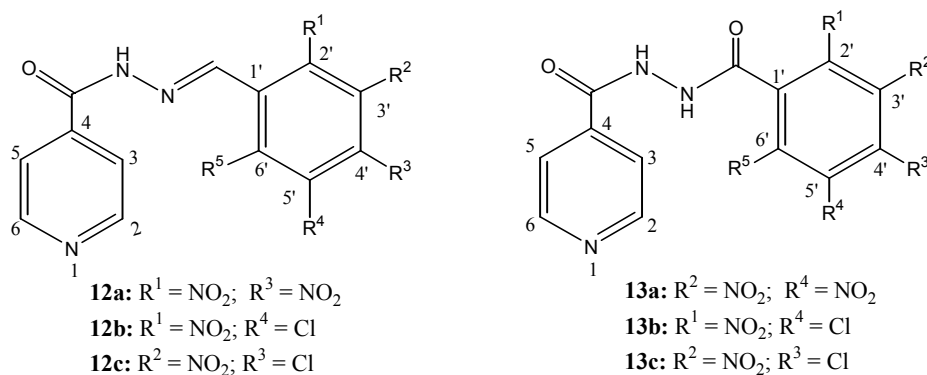| Products	δ ^{1}H (ppm)						
	H$_2$	H$_3$	H$_4$	H$_7$	H$_8$	H$_9$	H$_{10}$
9a	8.98	7.84	8.63	--	7.79	7.05	9.32
9b	9.15	8.03	8.80	7.71	--	7.64	9.80
9c	9.18	8.06	8.82	8.07	8.46	--	9.78
9d	9.03	7.95	8.72	7.68	8.20	7.41	--

3. Nitroisoniazid derivatives

The *N*'-[(*E*)-(nitrophenyl)methylidene]isonicotinohydrazides **11b-d** (**Scheme 5**), were synthesized and identified by spectral data, which are presented in the **Table 6**. These compounds were evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H$_{37}$Rv using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in µg/mL (**Table 7**). Depending on the position of the nitro group in the aromatic ring, different MIC values were obtained when compared with phenyl group, demonstrating the importance of the nitro group for the biological activity in this class of compounds. In *ortho* or *meta* position, MIC values were higher (5.0 µg/mL) when compared with the phenyl system (3.2 µg/mL). However, when the nitro group was placed in the *para* position it increases by three folds the biological activity, with MIC values of 1.2 µg/mL. It can be observed some modifications in the anti-tubercular activity when *N*'-[(*E*)-(nitrophenyl)methylidene]isonicotinohydrazides **11b-d**[12] were compared with the *N*'-[(*E*)-(disubstituted-phenyl)methylidene]isonicotino-hydrazides **12a-c**[13] and *N*'-(disubstitutedbenzoyl)isoniazid **13a-c**[14] derivatives containing nitro groups, which were previously evaluate by our group (**Scheme 6**). For example, the *N*'-(disubstitutedbenzoyl)isoniazid **13a-c** were more activity when compared with isonicotino-hydrazides **11b-d** and **12a-c**. The dinitro compound **12a** and the nitrochloro compound **12b** did not show increased anti-tubercular activity when compared with the mononitroisonicotino-hydrazide **11d**. However, the compound **12c**, which has respectively, the nitro group and chloro atom at 2 and 3 positions in the aromatic ring presented increased anti-tubercular activity, when compared with the mononitroisonicotino-hydrazide **11b** indicating that chloro atom at 3 position is important for the biological activity.

Scheme 5

Table 6. ^{1}H-NMR chemical shifts of compounds **11a-d**

					$\delta\ {}^{1}H$ (ppm)				
Products	NH	N=CH	$H_{2,6}$	$H_{3,5}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{6'}$
11a	12.10	8.47	8.79	7.83	7.76	7.47-7.57	7.47-7.57	7.47-7.57	7.76
11b	12.42	8.89	8.80	7.85-7.82	--	8.13-8.10	7.71 7.85-7.82	7.71 7.85-7.82	8.13-8.10
11c	12.34	8.58	8.81	7.84	8.58	--	8.29 8.19	7.78	8.29 8.19
11d	12.37	8.57	8.81	7.84	8.02	8.32	--	8.32	8.02

12a: R^1 = NO$_2$; R^3 = NO$_2$
12b: R^1 = NO$_2$; R^4 = Cl
12c: R^2 = NO$_2$; R^3 = Cl

13a: R^2 = NO$_2$; R^4 = NO$_2$
13b: R^1 = NO$_2$; R^4 = Cl
13c: R^2 = NO$_2$; R^3 = Cl

Scheme 6

Table 7. Antimycobacterial activities and log P measurements of derivatives **11a-d**, **12a-c** and **13a-c**

Products	MIC (µg/mL)	log P [a]
11a	3.12	1.810
11b	5.00	1.720
11c	5.00	1.744
11d	1.20	1.768
12a	5.00	1.655
12b	5.00	2.374
12c	1.20	3.664
13a	2.00	0.480
13b	0.50	1.199
13c	0.50	1.199
RIP	1.0	2.788
INH	0.2	-0.969

[a] Calculated by http://www.molinspiration.com/

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 (CG/MS) were recorded on a Agilent Tecnologies 6890 / 5972A mass spectrometer. NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.00 MHz (^{1}H) and 125.0 MHz (^{13}C), in deuterated dimethylsulfoxide. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane. 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. For column chromatographic purification under gravity, column grade silica gel 60 (0.063-0.200 mm mesh size) was employed.

Biological test. The antimycobacterial activities of compounds **2a-c**; **4a-g**; **6a-c**; **8a-d**; **9a-d**; and **11a-d** were assessed against *M. tuberculosis* ATTC 27294[15] using the micro plate Alamar Blue assay (MABA)[16]. This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods.[17,18] Briefly, 200 µL of sterile deionized water was added to 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 100 µL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **2a-c**; **4a-g**; **6a-c**; **8a-d**;

9a-d; and **11a-d** were made directly on the plate. The final drug concentrations tested were 0.01 to 20.0 μL/mL. Plates were covered and sealed with parafilm and incubated at 37°C 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 to the plate and incubated for 24 h. 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.

Conclusions

In conclusion, none of the nicotinic derivatives tested were active against the *M. tuberculosis*. In the case of the isoniazid derivatives containing nitro groups evaluated, the best result was the compound **11d** (1.2 μg/mL) when compared with first line drugs as isoniazid (INH) and rifampin (RIP). It suggests that this class of compounds may be selectively targeted to *M. tuberculosis* growth, also considering that they were not cytotoxic to host cells at the same concentration and could be a good starting point to find new lead compounds.

References

1. Elguero, J. In *Comprehensive Heterocyclic Chemistry II*; Katritzky, A. R.; Rees, C. W.; Scriven, E. F. V., Eds.; Pergamon Press: Oxford, UK, 1996; Vol.*3*, p 70.
2. *Merck Index*, 11th Edition, 6435**.**
3. http://www.mayoclinic.com/health/niacin/NS_patient-niacin
4. http://www.aafp.org/afp/981015ap/mayfield.html
5. De Souza, M. V. N. *Recent Patents on Anti-Infective Drug Discovery* **2006**, *1,* 33.
6. De Clercq, E. *Biochim. Biophys. Acta – Mol.Basis Disease* **2002**, *258*, 1587.
7. De Souza, M. V. N. Vasconcelos, T. R. A.; Wardell, S. M. S. V.; Wardell. J. L.; Low, J. N.; Glidewell, C. *Acta Cryst.* **2005**, *C61*, 204.
8. Cuffini, S.; Glidewell, C.; Low, J. N.; De Oliveira A. G.; De Souza, M. V. N.; Vasconcelos, T. R. A. Wardell, S. M. S. V. Wardell, J. L. *Acta Cryst.* **2006**, *B62*, 651.
9. Wardell, S. M. S. V.; De Souza, M. V. N.; Wardell, J. L.; Low, J. N.; Glidewell, C. *Acta Cryst.* **2007**; *B63*; 101.
10. Fernandes, E. L.; Magalhães, A.; Paes, K. C.; De Souza, M. V. N.; Vasconcelos, T. R. A.; Wardell, J. L.; Wardell, S. M. S. V. *J. Chem. Res.* **2006**, 93.
11. Wardell, S. M. S. V.; De Souza, M. V. N.; Wardell, J. L.; Low, J. N.; Glidewell, C. *Acta Cryst.* **2005**, *C61*, 683.
12. Wardell, S. M. S. V.; De Souza, M. V. N.; Wardell, J. L.; Low, J. N.; Glidewell, C. *Acta Cryst.* **2007**, *B*, In press (Forthcoming articles).

13. Souza, M. V. N.; Vasconcelos, T. A.; Mello, S. C. P.; Wardell, S. M. S. V.; Peralta, M. A.; Ferreira, B.; Henrique, M. G. M. O.; Neves Junior, I.; Lourenço, M. C. S. *Letters in Drug Design & Discovery*, **2005**, *2*, 451.
14. Junior, N. I.; Lourenço, M. C. S.; De Miranda, G. B. P.; Vasconcelos, T. R. A.; Pais, K. C.; Júnior, J. P. A.; Wardell, S. M. S. V.; Wardell, J. L.; De Souza, M. V. N. *Letters in Drug Design & Discovery*, **2006**, *3*, 424.
15. Canetti, J., Rist, E.; Grosset, R. *Pneumology* **1963**, *27*, 217.
16. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362.
17. Vanitha, J. D.; Paramasivan, C. N. *Mycobacteriology* **2004**, *49*, 179.
18. Reis, R. S.; Neves Jr., I.; Lourenço, S. L. S.; Fonseca, L. S.; Lourenço, M. C. S. *J. Clin. Microbiol.* **2004**, *42*, 2247.