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Synthesis and antimycobacterial activity of 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridines

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Abstract—4-(5-Substituted-1,3,4-oxadiazol-2-yl)pyridine derivatives **1**–**12** were synthesized and evaluated for their in vitro antimycobacterial activity. Some compounds showed an interesting activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and five clinical isolates (drug-sensitive and -resistant strains). Compound **4** [4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine] was 10 times more active than isoniazid, 20 times more active than streptomycin, and 28 times more potent than ethambutol against drug-resistant strain CIBIN 112. Compound **5** [4-(5-heptadecyl-1,3,4-oxadiazol-2-yl)pyridine] showed the same behavior as compound **4**. Both of the above structures bear a high lipophilic chain bonded to the 5-position of the oxadiazole moiety. This fact implies that there exists a contribution of lipophilicity, which could facilitate the entrance of these molecules through lipid-enriched bacterial cell membrane.

1. Introduction

Tuberculosis (TB) is one of the most common infectious diseases known by the mankind. About 32% of the world's population is infected by *Mycobacterium tuberculosis*, the main causal agent of TB. Every year, approximately 8 million of the infected people develop active TB, and 2 million die.[1] The World Health Organization estimates that about 30 million people will be infected by *M. tuberculosis* within the next 20 years.[2] The incidence of TB infection has steadily risen in the last decade. The reemergence of TB infection has been further complicated by an increase in the prevalence of drug-resistant TB cases. Current control efforts are severely hampered due to *M. tuberculosis* being a leading opportunistic infection in patients with acquired immune deficiency syndrome and the spreading of multidrug-resistant strains (MDR-MTB). Problems in the chemotherapy of tuberculosis arise when patients develop bacterial resistance to the first-line drugs: isoniazid (INH), rifampicin (RIF), ethambutol (ETH), streptomycin (STR), and pyrazinamide (PYR).[3]

The ever-increasing drug resistance, toxicity, and side effects of currently used antituberculosis drugs, and the absence of their bactericidal activity highlight the need for new, safer, and more effective antimycobacterial compounds. Since no effective vaccine is available, the major strategy to combat the spreading of TB is the chemotherapy.[4]

New chemical entities with novel mechanisms of action will most likely possess activity against MDR-MTB. There are two sources of these new chemical entities. The first one is the extraordinary diversity provided by natural product extraction, biological evaluation, and structural elucidation. The second one comes from synthetic compounds made through drug design. Some natural and synthetic scaffolds have been tested as antimycobacterial drugs,[5–9] and the research for novel vaccines is in progress.[10] Three critical reviews have been published recently, and they may give an outlook on the latest research developments on antimycobacterial substances, either of synthetic or natural products.[11–13]

Keywords: *Mycobacterium tuberculosis*; 1,3,4-Oxadiazoles; Multidrug-resistant strain.
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However, these alone will not provide the breakthrough that is needed. The key to improving therapy is to develop new agents with potent sterilizing activity that will lead to a shortening of the duration of chemotherapy.[13]

One of the most effective first-line anti-TB drugs is INH. Many analogues featuring the structure of INH have been synthesized and tested as antimycobacterials. In a critical review published recently, the existence of more than 3000 compounds based on the INH core was reported, about 66% of them being hydrazones.[11]

It has been reported that conversion of INH to oxadiazoles produces the corresponding 5-substituted 3*H*-1,3,4-oxadiazol-2-thione and 3*H*-1,3,4,-oxadiazol-2-one and their 3-alkyl or aralkyl derivatives, characterized by their high activity against *M. tuberculosis* strain $H_{37}Rv$.[14,15] 1,3,4-Oxadiazoles conform to an important class of heterocyclic compounds with a wide range of biological activities such as antiviral,[16] tyrosinase inhibitors,[17] antimicrobial,[18,19] cathepsin K inhibitors,[20] fungicidal,[19,21] and antineoplastic properties.[22] Accordingly, their synthesis and transformations have been a focus of interest for a long time.

Here, we report the synthesis of some 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridine derivatives and their in vitro activity on two first-line drug-sensitive and three drug-resistant *M. tuberculosis* clinical isolates and the H_{37} Rv strain.

2. Chemistry

The common synthetic route to 1,3,4-oxadiazoles involves cyclization of diacylhydrazines with a variety of anhydrous reagents such as thionyl chloride,[19,23] phosphorus pentoxide,[24] phosphorus oxychloride,[25] polyphosphoric acid,[26] and sulfuric acid.[27] They have also been prepared by oxidation of acylhydrazones with different oxidizing agents.[28–30] One-pot syntheses of 1,3,4-oxadiazoles from acid hydrazides and hydrazine with an acid chloride,[31] as well as from hydrazines with carboxylic acids,[32] have also been reported. The sequence followed in the present study is shown in Scheme 1. Compounds **1** and **2** were prepared using acetic and trifluoroacetic acid, respectively, and INH. A catalytic amount of sulfuric acid was added to promote the dehydration and intramolecular cyclocondensation via microwave irradiation with low yields (<35%). Compounds **3**–**7** and **9** were obtained by treatment of INH with acyl chlorides in DMF, through one-pot N-acylation and cyclodehydration.

Reaction of INH and different aldehydes in presence of sodium metabisulfite and dimethoxyethane afforded N^1-(arylmethylene)isonicotinohydrazides **13**–**16**, which were used immediately in a subsequent step without purification. Oxidation of N^1-(arylmethylene)isonicotinohydrazides **13**–**16** with potassium permanganate in a mixture of acetone and water (5:1)[33] under microwave irradiation yielded compounds **8** and **10**–**12**. Compounds **8** and **10** were obtained with low yields due to decomposition and superoxidation subproducts formed in the last step. Solid compounds were purified by recrystallization. The structure of the purified compounds was established by spectroscopic and spectrometric data.

3. Results and discussion

Following the Microplate Alamar Blue Assay (MABA),[34] compounds **1**–**12** were tested in vitro for their antimycobacterial activity against *M. tuberculosis* strains $H_{37}Rv$ (ATCC 27294), and two drug-sensitive and three drug-resistant clinical isolates. All the active compounds were further analyzed for intrinsic cytotoxicity in mammalian cells from the VERO line. The results are summarized in Table 1.

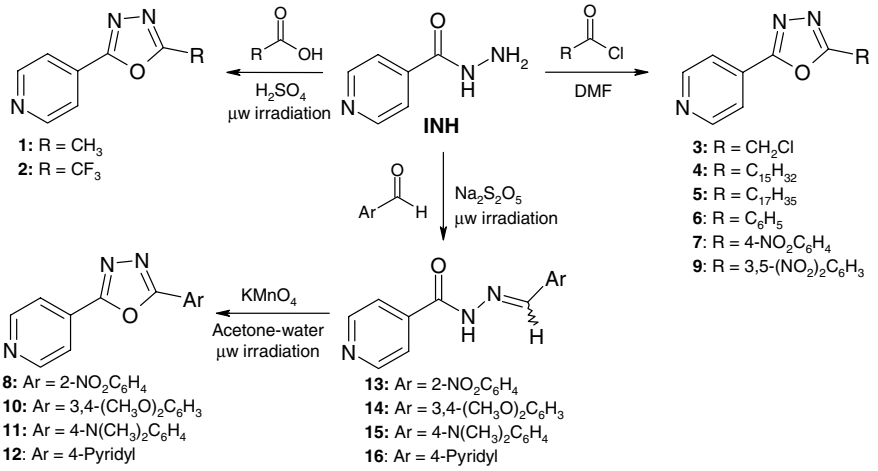
Results revealed that compounds **4** and **5** exhibited high antimycobacterial activity. Among others, these structures were found to be the most potent compounds with MIC's 0.35 and 0.65 μM, respectively, showing similar activity as INH (0.44 μM) against *M. tuberculosis* strain $H_{37}Rv$. Compounds **6**, **8**, **11** and **12** showed biological activity against this strain in the range of 3.76–8.97 μM. The remaining compounds did not show important activity against this strain.

Compounds were also tested against five clinical isolates of *M. tuberculosis* (Table 2). Compounds **4** and **5** were as active as INH and also were the most active for the series, showing nanomolar activities against CIBIN 687 strain. In particular, compound **4** was 10-fold more active than INH and as active as STR against CIBIN 650 strain. Compound **5** was six times more potent than INH against this strain. Against CIBIN 687 strain, compounds **1**, **6**, **8**, **10**–**12** showed MIC's ranging between 3.53 and 6.39 μM. Compound **12** showed moderate activity against drug-sensitive clinical isolates, with IC_{50}'s < 9 μM. This compound has been reported before and showed good activity against different strains of bacteria and fungi.[19]

Interestingly, compound **7**, substituted with a 4-nitro phenyl moiety, did not show significant activity against any *M. tuberculosis* strains (MIC = 29.85 μM), whereas its regioisomeric compound **8** (2-nitrophenyl-substituted) showed MICs ranging from 3.76 to 7.46 μM. The presence of additional nitro group in compound **9** (3,5-dinitrophenyl-substituted) resulted in a 2-fold less potency against CIBIN 687 strain, but a 2-fold improvement in activity against CIBIN 112 strain compared to compound **8**. The bioactivity of these compounds could be related to their reduced amino forms, which were also reported previously with antibacterial and antifungal activity.[19]

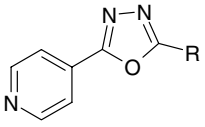
All compounds were more active than INH, RIF, and STR against MDR-MTB CIBIN 234 strain. Compounds **6**, **8**, **11**, and **12** showed anti-TB activity against $H_{37}Rv$ in the range of 3.76–8.93 μM. The remaining compounds showed an activity similar to that of INH against INH-resistant strains and higher than that of INH in the sensitive clinical isolates.

Anti-TB activity of compounds **6**, **8**, **10**, and **12** was in the range of 7.06–8.97 μM against CIBIN 650 strain.



Scheme 1. Synthetic pathway of 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridine derivatives (**1**–**12**).

Table 1. Physicochemical data and in vitro antimycobacterial activity of **1**–**12** against *M. tuberculosis* $H_{37}Rv$ and two drug-sensitive and three drug-resistant clinical isolates



Compound	R	MW	Mp (°C)	*C*log *P*	$H_{37}Rv$	*M. tuberculosis* clinical isolates MIC (μM)					IC_{50} VERO cells (μM)
						CIBIN 687	CIBIN 650	CIBIN 675	CIBIN 234	CIBIN 112	
1	$-CH_3$	161	239.9–241.2	0.70 ± 0.6	49.69	6.21	49.69	49.69	49.69	49.69	nd
2	$-CF_3$	215	257.2–258.5	1.71 ± 0.98	37.21	37.21	37.21	37.21	37.21	37.21	nd
3	$-CH_2Cl$	195	nd	1.22 ± 0.62	41.03	41.03	41.03	41.03	41.03	41.03	64.6
4	$-C_{15}H_{31}$	357	121.1–122.2	8.14 ± 0.60	0.35	0.70	0.09	11.19	22.38	2.80	95.5
5	$-C_{17}H_{35}$	385	112.2–113.0	9.20 ± 0.60	0.65	0.65	0.16	10.37	20.75	2.59	86.7
6	$-C_6H_5$	223	152.2–155.1[36]	2.89 ± 0.62	8.97	4.48	8.97	35.87	35.87	35.87	67.8
7	$4\text{-}NO_2C_6H_4$	268	203.5–205.4[37]	2.85 ± 0.62	29.85	29.85	29.85	29.85	29.85	29.85	nd
8	$2\text{-}NO_2C_6H_4$	268	66.9–68.7	2.38 ± 0.62	7.46	3.73	7.46	29.85	29.85	29.85	32.4
9	$3,5\text{-}(NO_2)_2C_6H_3$	313	225.7–226.8	2.65 ± 0.63	25.54	6.39	25.54	25.54	25.54	12.77	77.1
10	$3,4,5\text{-}(CH_3)_3OC_6H_3$	283	217.6–219.2	3.13 ± 0.63	14.12	3.53	7.06	28.25	28.25	14.12	250.2
11	$4\text{-}N(CH_3)_2C_6H_3$	266	204.9–206.8	3.30 ± 0.63	3.76	3.76	30.04	30.04	30.04	30.04	50.4
12	4-Pyridyl	224	235.2–236.3	1.63 ± 0.62	8.93	4.46	8.93	35.71	35.71	35.71	92.41
Isoniazid	—	137	—	−0.89 ± 0.24	0.44	0.91	0.91	29.19	58.38	29.19	39.8
Streptomycin	—	581	—	−3.20 ± 1.04	0.86	nd	0.10	6.87	55.02	55.02	>100
Rifampicin	—	822	—	0.49 ± 0.74	0.07	nd	nd	0.94	121.51	3.79	>100
Ethambutol	—	204	—	−0.05 ± 0.44	9.80	nd	nd	nd	nd	78.31	>100

MIC, minimal inhibitory concentration; nd, not determined.

Table 2. Profile of susceptibility of clinical isolated of *M. tuberculosis*

Clinical isolate	Drug-resistance profile
CIBIN 687	Sensitive[a]
CIBIN 650	Sensitive[a]
CIBIN 675	STR, INH,
CIBIN 234	STR, INH, RIF, PYR
CIBIN 112	STR, INH, ETH

[a] Of all first-line antituberculosis drugs.

It is interesting to note that these 5-arylsubstituted compounds possessed moderate activity, whereas 5-low alkyl and haloalkyl ($-CH_3$, $-CF_3$, and $-CH_2Cl$) substituted derivatives did not show significant activity. It was reported that 5-low alkyl homologues (methyl, ethyl, and propyl-1,3,4-oxadiazole-2-yl)pyridines showed a low tuberculostatic in vitro effect.[35] Apparently, it is necessary to increase the steric hindrance at position 5 of oxadiazole moiety to improve the biological activity of these derivatives. It also implies that lipophilicity plays an important role in the bioactivity of these 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridines.

Compound **4** showed 10 and 20 times more potency than INH and STR, respectively, against the INH-resistant *M. tuberculosis* CIBIN 112 strain. This compound was 27 times more active than ETH. Compound **5** showed the same behavior as **4** against this strain. These compounds bear a highly lipophilic chain bonded to the 5-position of oxadiazole moiety. When we compared the

values of *C*log *P* of INH (−0.89 ± 0.24) and synthesized compounds **4** and **5** (8.14 ± 0.60 and 9.20 ± 0.60, respectively), we realized that there exists a contribution of lipophilicity, which could facilitate the entrance of these molecules through lipid-enriched bacterial membrane, which is formed by long chain fatty acids. Moreover, β-ketoacyl-acyl carrier protein synthase III (FabH) catalyzes a two-step reaction that initiates the pathway of fatty acid biosynthesis in plants and bacteria. FabH catalyzes extension of lauroyl, myristoyl, and palmitoyl groups from which cell wall mycolic acids of the bacterium are formed.[38]

Another raised hypothesis explores the possibility that compounds **1**–**3**, **6**–**8**, and **11** and **12** could be acting as INH prodrugs.[12] None of them showed more potency than INH against the three drug-resistant *M. tuberculosis* clinical isolates. Although oxadiazole nucleus is very stable to acid hydrolysis, it has been reported that it may be chemically hydrolyzed with an strong base and heating,[39,40] leading thus to the generation of acyl-INH, which are very likely to be completely hydrolyzed to INH. According to Scior and Garces-Eisele[12] the pharmacological role of INH derivatives (isonicotinoyl hydrazones, hydrazides, and amides) must be considered as bio-reversible prodrugs of INH or isonicotinic acid. Worse activities showed by these kinds of structures can be explained by the compounds with a structural gain of stability against prodrug hydrolysis. The best activity could be related to decomposition products formed in situ, inferring membrane toxicity. Such membrane toxicity can only be of true help in an in vitro study, since they would be undesired for the host cells of any patient, too.

On the other hand, compounds **4** and **5** could not be considered as INH prodrugs, because INH derivatives cannot be expected to overcome INH-resistance, as the molecular action mechanism is identical.

All active compounds were examined for cytotoxicity (IC_{50}) in a mammalian VERO cell line. All of them showed moderate toxicity levels, with IC_{50}'s > 33 μM, and selective indexes (IC_{50}/MIC) ranging from 2- to 6-fold (Table 1).

We also evaluated the toxicity of most promissory compounds (**4** and **5**) on peripheral blood mononuclear human cells (PBMC; Table 3). Compounds **4** and **5** showed IC_{50} similar to INH on adherent cells, and both compounds showed IC_{50} 6- and 2-fold more toxic, respectively, on non-adherent cells. These results are important because PBMC are the mainly human cells implicated on the immune response versus mycobacterial infection.[41–43]

Table 3. Toxicity of compounds **4** and **5**

PBMC	IC_{50} (μg/mL)		
	4	**5**	Isoniazid
Adherent cells	125.21	312.18	>100[a]
Non-adherent cells	15.84	45.41	87.08

PBMC, peripheral blood mononuclear cells.
[a] Isoniazid treated adherent cells (100 μg/mL) survive more than 95%.

4. Conclusion

A series of 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridine derivatives were synthesized and tested against *M. tuberculosis* drug-sensitive and drug-resistant strains. The present results highlight the importance of lipophilicity of these compounds to present good antimycobacterial activity. The high bioactivity of compounds **4** and **5** makes them suitable hits for additional in vitro and in vivo evaluations, in order to develop new antimycobacterial drugs or prodrugs with potential use in the tuberculosis treatment. Further studies in this area are in progress in our laboratory.

5. Experimental

Melting points were determined on a EZ-Melt MPA120 automated melting point apparatus from Stanford Research Systems and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated silica gel 60 F_{254} plates (E. Merck). ^{1}H NMR and ^{13}C NMR spectra were measured with a Varian EM-390 (300 and 75.5 MHz) spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane (Me_4Si, $\delta = 0$) in $CDCl_3$; *J* values are given in Hz. The following abbreviations are used: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; br s, broad signal. MS were recorded on a JEOL JMS-SX102A spectrometer by electron impact (EI). Reactions under microwave irradiation were performed in a domestic microwave oven, Samsung MW1446WC, 1000 W. The *C*log *P* values were obtained using ACD/labs software v.4.5.

5.1. Synthesis of 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridines

5.1.1. General method of synthesis of derivatives 1 and 2. Isoniazid (0.0036 mol), 1.6 equiv of CH_3COOH or CF_3COOH, and 1 drop of concentrated H_2SO_4 were mixed and introduced in an open Erlenmeyer flask. The mixture was irradiated in a household microwave oven for 120 s. TLC was used to monitor the reaction. After irradiation, the cooled mixture was neutralized with saturated $NaHCO_3$ solution, and the crude compound was extracted with AcOEt. The solvent was removed under vacuum, and the resulting solid was isolated by filtration through a fritted 60 mL glass funnel packed with Al_2O_3, basic type, and then crystallized.

5.1.1.1. 4-(5-Methyl-1,3,4-oxadiazol-2-yl)pyridine (1). Recrystallized from methanol. Yield 0.176 g (30%) of white solid. Mp 239.9–241.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (s, 3H, CH_3), 8.00–8.02 (m, 2H, H-3, H-5), 9.01–9.03 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.50 (*C*H_3), 117.94 (C-3, C-5), 132.03 (C-4), 151.20 (C-2, C-6), 152.21 (C-2′), 164.70 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 161 (M^+, 100), 146 (78), HRMS: Calcd for $C_8H_7N_3O$: 161.0589. Found: 161.0595.

5.1.1.2. 4-[5-(Trifluoromethyl)-1,3,4-oxadiazol-2-yl]pyridine (2). Recrystallized from ethanol. Yield 0.247 g (32%) of pale yellow solid. Mp 257.2–258.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27–8.29 (m, 2H, H-3, H-5), 9.02–9.04 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 118.08 (q, CF_3, J = 285.2 Hz), 119.18 (C-3, C-5), 131.97 (C-4), 138.30 (q, C-5′, J = 45.2 Hz) 152.72 (C-2, C-6), 163.37 (C-2′) ppm; MS: *m*/*z* (% rel. int.) 215 (M^+, 100), 196 (20); HRMS: Calcd for $C_8H_4F_3N_3O$: 215.0306. Found: 215.0312.

5.1.2. General method of synthesis of derivatives 3–7 and 9. A mixture of INH (0.0036 mol) and 1.1 equiv of appropriate acyl chloride in 10 mL of DMF was heated to reflux for 3–4.5 h. TLC was used to monitor the reaction. After cooling, the mixture was neutralized with saturated $NaHCO_3$ solution and the precipitate formed was filtered by suction. The crude product was purified by recrystallization from adequate solvent.

5.1.2.1. 4-[5-(Chloromethyl)-1,3,4-oxadiazol-2-yl]pyridine (3). Recrystallized from methanol. Yield 0.582 g (83%) of yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.51 (s, 2H, CH_2), 8.22 (dd, 2H, H-3, H-5), 8.94 (dd, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 34.11 (*C*H_2), 118.41 (C-3, C-5), 133.60 (C-4), 146.29 (C-5′), 151.14 (C-2, C-6), 160.56 (C-2′) ppm; MS: *m*/*z* (% rel. int.) 313 (M^+, 98), 248 (100); HRMS: Calcd for $C_8H_6ClN_3O$: 195.0199. Found: 195.0210.

5.1.2.2. 4-(5-Pentadecyl-1,3,4-oxadiazol-2-yl)pyridine (4). Recrystallized from methanol. Yield 1.19 g (93%) of white solid. Mp 121.1–122.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, 3H, CH_3), 1.24–1.36 (m, 24H, H-2′, H-3′, H-4′, H-5′ H-6′), 1.52–1.60 (m, 2H, CH_2), 2.37–2.41 (m, 2H, CH_2), 8.00–8.02 (m, 2H, H-3, H-5), 8.93–8.96 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 14.22 (CH_3), 22.68 (C-14″), 29.39, 29.36, 29.56, 29.48, 29.78, 32.22, 117.90 (C-3, C-5), 131.73 (C-4), 151.17 (C-2, C-6), 161.03 (C-2′), 170.18 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 357 (M^+, 100), 328 (10), 217 (30), 174 (80), 161 (80); HRMS: Calcd for $C_{22}H_{35}N_3O$: 357.2780. Found: 357.2792.

5.1.2.3. 4-(5-Heptadecyl-1,3,4-oxadiazol-2-yl)pyridine (5). Recrystallized from EtOH. Yield 0.845 g (61%) of white flakes. Mp 112.2–113.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (m, 3H, CH_3), 1.21–1.35 (m, 28H, H-2′, H-3′, H-4′, H-5′ H-6′), 1.53–1.60 (m, 2H, CH_2), 2.37–2.41 (m, 2H, CH_2), 8.01 (dd, 2H, H-3, H-5), 8.94 (dd, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 14.24 (CH_3), 22.58 (CH_2), 29.22, 29.36, 29.39, 29.54, 29.56, 29.48, 29.78, 32.22, 117.94 (C-3, C-5), 131.70 (C-4), 151.20 (C-2, C-6), 163.03 (C-2′), 170.48 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 385 (M^+, 100), 356 (10), 174 (80), 161 (70); HRMS: Calcd for $C_{24}H_{39}N_3O$: 385.3093. Found: 385.3099.

5.1.2.4. 4-(5-Phenyl-1,3,4-oxadiazol-2-yl)pyridine (6). Recrystallized from ethyl acetate. Yield 0.273 g (34%) of pale yellow solid. Mp 152.2–155.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28–7.49 (m, 3H, H-3′, H-4′, H-5′), 8.09 (dd, 2H, H-3, H-5), 8.29–8.34 (m, 2H, H-2′, H-6′), 8.93 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 118.41 (C-3, C-5), 127.54 (C-3″, C-5″), 127.79 (C-2″, C-6″), 127.69 (C-1″), 132.60 (C-4), 133.53 (C-4″), 150.96 (C-2, C-6), 159.37 (C-2′), 166.61 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 223 (M^+, 100); HRMS: Calcd for $C_{13}H_9N_3O$: 223.0746. Found: 223.0750.

5.1.2.5. 4-[5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl]pyridine (7). Recrystallized from MeOH–ethyl acetate. Yield 0.261 g (27%) of pale yellow solid. Mp 203.5–205.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08–8.10 (m, 2H, H-3, H-5), 8.38–8.44 (m, 4H, H-2, H-3, H-5, H-6), 8.90–8.92 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 118.45 (C-3, C-5), 126.41 (C-2″, C-6″), 126.64 (C-3″, C-5″), 132.40 (C-4), 133.70 (C-1″), 150.83 (C-2, C-6), 151.21 (C-4″), 159.37 (C-2′), 165.85 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 268 (M^+, 100); HRMS: Calcd for $C_{13}H_8N_4O_3$: 268.0596. Found: 268.0584.

5.1.2.6. 4-[5-(2,4-Dinitrophenyl)-1,3,4-oxadiazol-2-yl]-pyridine (9). Recrystallized from methanol. Yield 0.225 g (20%) of brown solid. Mp 225.7–226.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (dd, 2H, H-3, H-5), 8.91 (dd, 2H, H-2, H-6), 9.00 (t, 1H, H-4″, J = 2.2 Hz), 9.57 (d,2H, H-2″, H-6″ J = 2.0, J = 2.2 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 118.41 (C-3, C-5), 119.37 (C-4″), 128.50 (C-2″, C-6″), 128.97 (C-3″), 132.60 (C-4), 150.96 (C-2, C-6), 151.76 (C-3″, C-5″), 159.30 (C-2′), 168.19 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 313 (M^+, 100); HRMS: Calcd for $C_{13}H_7N_5O_5$: 313.0447. Found: 313.0455.

5.1.3. General method of synthesis of derivatives 8 and 10–12. A mixture of INH (0.0036 mol) and adequate aldehyde (0.0039 mmol) was dissolved in dimethoxyethane (10 mL). Then, 1 equiv of sodium metabisulfite was added and the mixture placed in a open Erlenmeyer flask. The mixture was then subjected to microwave irradiation at 1000 W for 60 s. After complete conversion as indicated by TLC, the reaction mixture was cooled and the precipitated solids were filtered off to yield N^1-(arylmethylene)isonicotinohydrazides **13**–**16**, which were used immediately in a subsequent step without purification. Oxidation of N^1-(arylmethylene)isonicotinohydrazides.[29] A mixture of **13**–**16** and potassium permanganate (3 equiv) was dissolved in a mixture of acetone/water (10:2), and then transferred to a open Erlenmeyer flask. The mixture was then subjected to irradiation at 1000 W. After complete conversion as indicated by TLC, the solvent was removed in vacuo and the aqueous layer was extracted with ethyl acetate (3× 15 mL), washed with water (3× 20 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the precipitated solids were recrystallized from an appropriate solvent.

5.1.3.1. 4-[5-(2-Nitrophenyl)-1,3,4-oxadiazol-2-yl]pyridine (8). Recrystallized from methanol. Yield 0.289 g (30%) of pale yellow solid. Mp 66.9–68.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52–7.56 (m, 1H, H-4″), 7.71–7.81 (m, 1H, H-5″), 8.00–8.03 (m, 1H, H-6″), 8.08–8.11 (m, 2H, H-3, H-5), 8.23–8.25 (m, 1H, H-3″), 8.90–8.92 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$)

δ 118.41 (C-3, C-5), 124.70 (C-1″) 126.41 (C-6″), 127.39 (C-3″), 129.98 (C-4), 131.57 (C-4″), 133.83 (C-5″), 145.39 (C-2″), 150.96 (C-2, C-6), 159.37 (C-2′), 160.68 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 268 (M^+, 100); HRMS: Calcd for $C_{13}H_8N_4O_3$: 268.0596. Found: 268.0604.

5.1.3.2. 4-[5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl]pyridine (10). Recrystallized from methanol. Yield 0.141 g (13%) of white solid. Mp 217.6–219.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 (s, 3H, 4-CH_3O) 3.84 (d, 6H, 3-CH_3O, 5-CH_3O), 7.66 (d, 2H, H-2″, H-6″), 8.08–8.11 (M, 2H, H-3, H-5), 8.90–8.92 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 58.28 (3, 5-$(CH_3)_2$), 60.39 (4-CH_3O), 104.60 (C-2″, C-6″), 118.41 (C-3, C-5), 121.33 (C-1″), 132.60 (C-4), 140.89 (C-4″), 151.86 (C-2, C-6), 155.23 (C-3″, C-5″), 158.37 (C-2′), 161.88 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 313 (M^+, 100); HRMS: Calcd for $C_{16}H_{15}N_3O_4$: 313.1063. Found: 313.1070.

5.1.3.3. 4-[5-(4-*N*,*N*-dimethylaminophenyl)-1,3,4-oxadiazol-2-yl]pyridine (11). Recrystallized from ethanol. Yield 0.766 g (80%) of yellow crystals. Mp 204.9–206.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.20 (s, 6H, $(CH_3)_2N$) 7.09–7.11 (m, 2H, H-3″, H-5″), 7.85–7.89 (m, 2H, H-2″ ,H-6″), 8.08–8.10 (m, 2H, H-3′, H-5′), 8.92–8.94 (m, 2H, H-2, H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 40.44 ($(CH_3)_2N$), 114.30 (C-3″, C-5″), 118.41 (C-3, C-5), 123.64 (C-1″), 126.23 (C-2″, C-6″), 132.60 (C-4), 150.96 (C-2, C-6), 153.10 (C-4″), 159.34 (C-2′), 166.65 (C-5′) ppm; MS: *m*/*z* (% rel. int.) 266 (M^+, 100); HRMS: Calcd for $C_{15}H_{14}N_4O$: 266.1168. Found: 266.1176.

5.1.3.4. 4-(5-Pyridyl-1,3,4-oxadiazol-2-yl)pyridine (12). Recrystallized from ethanol. Yield 0.677 g (84%) of white crystals. Mp 235.2–236.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (m, 4H, H-3, H-5, H-3″, H-5″), 8.91 (m, 4H, H-2″, H-6″, H-2 ,H-6) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 118.41 (C-3, C-5, C-3″, C-5″), 132.60 (c-4), 150.96 (C-2, C-6, C-2″, C-6″), 159.37 (C-2′, C-5′) ppm; MS: *m*/*z* (% rel. int.) 224 (M^+, 100); HRMS: Calcd for $C_{12}H_8N_4O$: 224.2183. Found: 224.2196.

5.2. Biological assays

5.2.1. Anti-*Mycobacterium tuberculosis* assay.[34] The following strains were used in the present study: *M. tuberculosis* H_{37}Rv (ATTC 27294), which is sensitive to all five first-line antituberculosis drugs (STR, INH, RIF, ETH, and PYR), and five clinical isolates (Table 2) from patients bearing advanced pulmonary tuberculosis: two sensitive to all first-line drugs and three having different drug-resistance profiles. These were isolated, identified, and characterized in the Mycobacteriology laboratory of the Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social (Monterrey, NL, México). All of these were cultured at 37 °C and 5% CO_2 atmosphere in Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC enrichment (Oleic acid-Albumin-Dextrose-Catalase) until the logarithmic phase of growth was reached. The inoculum for the assay was prepared by diluting a logarithmically growing culture to match the McFarland 1 turbidity standard and then further diluting this to 1:50 with Middlebrook 7H9 broth to obtain a concentration of 6×10^6 colony forming units/mL. The working suspension was prepared just before inoculation. The antibacterial activity of compounds against *M. tuberculosis* strains was tested using the modified MABA. The concentrations for compounds ranged from 8.000 to 0.016 μg/mL.

5.2.2. Toxicity on VERO cell line.[44] The VERO cells (ATCC Cat. No. CCL-81) were gently suspended in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) and their concentration adjusted at 1.5×10^5 cells/mL in medium with 10% (v/v) bovine fetal serum (FBS), and distributed in 200 μL aliquots into each of the 96 wells of a sterile flat-bottomed Microtest II microassay plate and incubated at 36.5 °C in 5% CO_2 atmosphere for 24 h. The spent medium in each well was replaced with 200 μL of fresh supplemented DMEM plus 10% FBS. Each microplate was divided in 9-well rows that were added (in triplicate) with 5 μL of each of the tested compounds (4000 μg of each compound/mL was dissolved in 100% DMSO, Sigma). From these stock solutions, a two-step serial dilution (100–0.390 μg/mL) was prepared. In addition, a control untreated cell culture, that was incubated in fresh supplemented DMEM plus 2.5% DMSO, was included in each plate (in triplicate) as a 100% viability internal control. These preparations were re-incubated for 24 h. The cells were trypsinized adding 100 μL of 0.25% trypsin–EDTA to each well. The cells from each of the above preparations were counted with a hemacytometer, and 50 μL of each of these was transferred to a new 1.5 mL capacity and added with 5 μL of 5% trypan blue dissolved in isotonic buffered saline phosphate, pH 7.4. Twenty microliters of this suspension was smeared on a slide and covered with a cover slide. The percentage of blue cells (dead) from a total of 100 was determined with the aid of a microscope. The number of dead cells in each well was corrected with respect to the mean of dead cells percentage determined in the 100% viability controls. The percentage of mortality was calculated by dividing the corrected number of dead cells by the total number of cells in each preparation × 100. The compound dose producing 50% of dead cells (IC_{50}) and 95% confidence limits were calculated applying a Probit analysis with the aid of the Statistical Package for Social Science (SPSS for Windows, Standard Version 10.0). Results regarding each compound and four antituberculosis drugs against each cell line were reported as means ± standard error (SE) of the three independent experiments.

5.2.3. Toxicity on peripheral blood mononuclear human cells. Blood was drawn from one healthy volunteer and peripheral blood mononuclear cells (PBMC) were isolated at room temperature following the method described by Boyum.[45] The PBMC were suspended and incubated in RPMI 1640 medium added with 10% FBS, cellular density was adjusted to 1×10^6 cells/mL, and 100 μL of this suspension was placed in each well of 96-well flat-bottomed sterile culture plates. On the other hand, compounds were dissolved in DMSO

100% and diluted in RPMI 1640 medium plus 10% of FBS to obtain a final concentration ranging from 20 to 100 μg/mL of each compound and a final concentration of 2.5% of DMSO. One hundred microliters from these solutions was tested by quintuplicate assay, except a row of wells with 100 μL of RPMI 1640 medium plus 10% of FBS in each well was placed as blank. Plates were incubated for 24 h and the non-adherent cells were separated from the adherent ones aspirating supernatants with a Pasteur pipette. One hundred microliters of tripsin–EDTA diluted in RPMI 1640 medium plus 10% of FBS was added in each cell well in order to recover adherent cells. Fifteen minutes later, adherent cells were obtained by pippeting in each cell well and aspired under sterile conditions. Adherent and non-adherent cells were placed in eppendorf 1.8 mL centrifuge tubes and seeded by centrifugation at 600*g* during 5 min, then 1 μL of supernatants was discarded and cells were resuspended in the remaining medium. Viability was determined as done with the VERO cells.

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