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Synthesis and antimycobacterial activity of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives

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Abstract

[5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives were synthesized and tested for their in vitro antimycobacterial activity. Some compounds showed a feable activity against a strain of *Mycobacterium tuberculosis* and a strain of *Mycobacterium avium*.

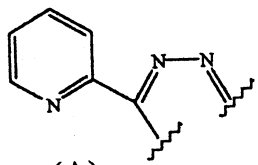
Keywords: [5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives; Antimycobacterial activity

1. Introduction

The increase of tuberculosis due to emergence of multidrug-resistant strains (MDR) of *Mycobacterium tuberculosis*, together with the increased incidence of severe disseminated infections produced by mycobacteria other than tuberculosis (MOTT) in immunocompromised patients, have promted the search for new antimycobacterial drugs.

Continuing our search for new antimycobacterial agents, we synthesized a series of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives (**4**–**37**) (Table 1).

The synthesized compounds are characterized by the presence of the sequence (**A**) which partially resembles to pyridine-2-carboxamidrazone moiety, whose importance with respect to the antimycobacterial activity of a number of derivatives has been previously described [1–6].



(A)

Moreover, the thioacetyl hydrazone moiety, linked to the 2 position of the 1,3,4-thiadiazole derivatives (**4**–**37**), was present in other compounds characterized by antibacterial and antifungal activity [7,8] and hydrazido-hydrazone derivatives have been described for antimycobacterial properties [9].

The new synthesized compounds have been tested for their in vitro antimycobacterial activity towards a strain of *Mycobacterium tuberculosis* $H_{37}Rv$ and a strain of *Mycobacterium avium*.

The activity of these compounds against a strain of *Staphylococcus aureus*, a strain of *Escherichia coli* and two strains of *Candida albicans* has been also evaluated.

2. Chemistry

The synthesis of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives (**4**–**37**)

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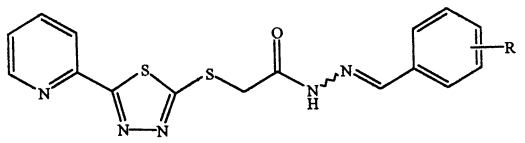
Table 1



4-37

Comp.	R	Yield (%)	M.p. (°C)	Formula (C, H, N)
4	H	80	219	$C_{16}H_{13}N_5OS_2$
5	2-Cl	81	206	$C_{16}H_{12}N_5O\ {}_2Cl$
6	3-Cl	93	223	$C_{16}H_{12}N_5OS_2Cl$
7	4-Cl	68	190	$C_{16}H_{12}N_5OS_2Cl$
8	2-Br	89	207	$C_{16}H_{12}N_5OS_2Br$
9	3-Br	95	225	$C_{16}H_{12}N_5OS_2Br$
10	4-Br	93	197	$C_{16}H_{12}N_5OS_2Br$
11	2-F	74	206	$C_{16}H_{12}N_5OS_2F$
12	3-F	34	186	$C_{16}H_{12}N_5OS_2F$
13	4-F	86	181	$C_{16}H_{12}N_5O\ S_2F$
14	2,3-Cl_2	84	205	$C_{16}H_{11}N_5OS_2Cl_2$
15	2,4-Cl_2	86	202	$C_{16}H_{11}N_5OS_2Cl_2$
16	2,6-Cl_2	69	222	$C_{16}H_{11}N_5OS_2Cl_2$
17	3,4-Cl_2	95	235	$C_{16}H_{11}N_5OS_2Cl_2$
18	2-CH_3	97	203	$C_{17}H_{15}N_5OS_2$
19	3-CH_3	94	188	$C_{17}H_{15}N_5OS_2$
20	4-CH_3	54	177	$C_{17}H_{15}N_5OS_2$
21	2,4-$(CH_3)_2$	83	185	$C_{18}H_{17}N_5OS_2$
22	2,5-$(CH_3)_2$	96	212	$C_{18}H_{17}N_5OS_2$
23	2-OCH_3	95	176	$C_{17}H_{15}N_5O_2S_2$
24	3-OCH_3	97	200	$C_{17}H_{15}N_5O_2S_2$
25	4-OCH_3	96	196	$C_{17}H_{15}N_5O_2S_2$
26	2,3-$(OCH_3)_2$	92	208	$C_{18}H_{17}N_5\ O_3S_2$
27	2,4-$(OCH_3)_2$	96	170	$C_{18}H_{17}N_5O_3S_2$
28	2,5-$(OCH_3)_2$	85	192	$C_{18}H_{17}N_5O_3S_2$
29	3,4-$(OCH_3)_2$	92	218	$C_{18}H_{17}N_5O_3S_2$
30	3,5-$(OCH_3)_2$	84	223	$C_{18}H_{17}N_5O_3S_2$
31	3,4,5-$(OCH_3)_3$	87	210	$C_{19}H_{19}N_5O_4S_2$
32	2-NO_2	80	171	$C_{16}H_{12}N_6O_3S_2$
33	3-NO_2	98	260	$C_{16}H_{12}N_6O_3S_2$
34	4-NO_2	92	244	$C_{16}H_{12}N_6O_3S_2$
35	2,4-$(NO_2)_2$	67	240	$C_{16}H_{11}N_7O_5S_2$
36	2,6-$(NO_2)_2$	32	206	$C_{16}H_{11}N_7O_5S_2$
37	4-Cl,3-NO_2	67	252	$C_{16}H_{11}N_6O_3S_2Cl$

(Table 1) was carried out (Scheme 1) by treating pyridine-2-carboxamidrazone with carbon disulfide to obtain 5-(pyridin-2-yl)-*3H*-1,3,4-thiadiazole-2-thione **1** [10]. Compound **1** was reacted with ethyl bromoacetate to afford the [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid ethylester (**2**), from which the [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid hydrazide (**3**) was obtained by treatment with hydrazine hydrate. Condensation of the hydrazide **3** with variously substituted benzaldehydes gave the corresponding arylidene hydrazides (**4**–**37**).

The ^{1}H NMR spectra of the synthesized compounds were consistent with the presence of both *E* and *Z* geometric isomers.

The azomethine linkage together with the hydrogen bond between the hydrazide –NH– proton and sulfur atom produce, in accordance with literature findings for similar compounds [7,8,11–13], three pairs of singlets associated with –S–CH_2–, –CH=N– and –CO–NH– protons, with ppm values in the range 4.22–4.33 and 4.33–4.76, 7.97–8.45 and 8.08–8.64, 11.50–12.24 and 11.72–12.40, respectively. The resonances associated with hydrazide and azomethine protons of the isomers with the higher percentage in the mixture occur at higher fields.

From literature data, the signals of the azomethine protons of the *Z* isomers of hydrazido-hydrazone derivatives and hydrazone derivatives appear at higher fields with respect to the corresponding signals of the *E* isomers [14–18]. On the basis of these findings, we assigned the *Z* configuration to the isomers with the higher percentage in the mixtures. The percentage of each isomer was calculated using the integral values of each singlet pair.

The isomers *Z* and *E* were present in the synthesized compounds **4**–**37** with percentages ranging from 72 to 67% and 28 to 33%, respectively.

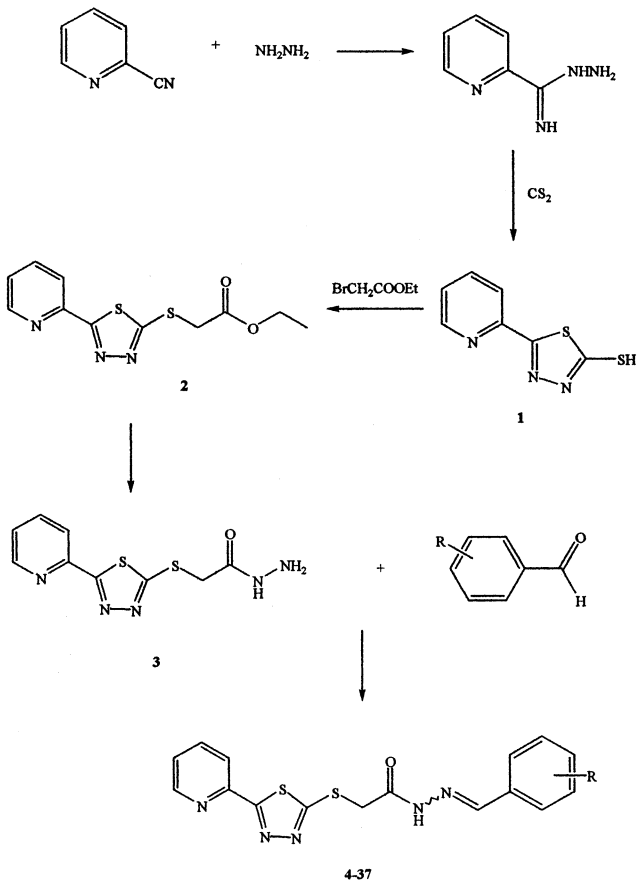
3. Experimental

3.1. Chemistry

Melting points were determined with a Büchi 510 capillary apparatus, and are uncorrected. Infrared spectra in nujol mulls were recorded on a Jasko FT 200 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian Gemini 200 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as internal standard, dimethylsulfoxide as solvent. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. EI-MS spectra (70 eV) were taken on a VG 7070 spectrometer. Elemental analyses (C, H, N) were performed on a Carlo Erba analyzer and were within $\pm$0.3 of the theoretical value.

*3.1.1. [5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid ethylester (**2**)*

To a suspension of 1.66 g (12 mmol) of K_2CO_3 and KI (catalytic amount) in 30 ml of anhydrous acetone, 2.02 g (10 mmol) of **1** dissolved in 30 ml of the same solvent were added under stirring. Ethyl bromoacetate (1.84 g, 11 mmol) was dropped slowly into the stirred mixture. After 24 h, the solvent was completely evaporated in vacuo and the solid residue was whased with distilled water, collected by filtration and recrystallized from absolute ethanol to obtain 1.6 g (62%) of **2**; m.p. 85°C.



Scheme 1.

IR (Nujol, cm^{-1}): 1730. ^1H NMR (DMSO/Me_4Si): δ 1.30 (t, 3H, CH_3), 4.15–4.35 (m, 4H, –S–CH_2– and CH_2–CH_3), 7.30–8.75 (m, 4H, pyr.). MS: m/z 281 [M^+]. *Anal.* ($C_{11}H_{11}N_3O_2S_2$): C, H, N.

*3.1.2. [5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid hydrazide (**3**)*

To a solution of 3.87 g (13.7 mmol) of **2** in 30 ml of absolute ethanol, 0.86 g (16.9 mmol) of 98% hydrazine monohydrate were added. The mixture was refluxed under stirring for 72 h. After cooling, the formed precipitate was collected by filtration and recrystallized from absolute ethanol to give 1.95 g (53%) of **3**; m.p. 174–75°C.

IR (Nujol, cm^{-1}): 3320, 3240, 3180, 1670. ^1H NMR (DMSO/Me_4Si): δ 3.80 (br.s., 2H, NH_2, disappearing on deuteration), 4.02 (s, 2H, CH_2), 7.35–8.65 (m, 4H, pyr.), 8.4 (t, 1H, NH, disappearing on deuteration). MS: m/z 267 [M^+]. *Anal.* ($C_9H_9N_5OS_2$): C, H, N.

*3.1.3. [5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid benzylidene-hydrazide (**4**)*

Benzaldehyde (0.4 g, 3.74 mmol) was dropped slowly into a stirred solution of 1 g (3.74 mmol) of **3** in 50 ml of absolute ethanol.

The mixture was left to react under reflux for 4 h. At the end of the reaction, the solution was concentrated under reduced pressure. After cooling the white solid formed was filtered off and crystallized from ethanol to obtain 1.07 g (80%) of **4**; m.p. 219°C.

IR (Nujol, cm^{-1}): 3180, 1680. ^1H NMR (DMSO/Me_4Si): δ 4.28 and 4.71 (2s, 2H, CH_2), 7.40–8.69 (m, 9H, arom. and pyr.), 8.08 and 8.18 (2s, 1H, CH=N), 11.78 and 11.85 (2s, 1H, NH, disappearing on deuteration). MS: m/z 355 [M^+]. *Anal.* ($C_{16}H_{13}N_5OS_2$): C, H, N.

In an analogous way the compounds **5**–**37** have been prepared. Yields and melting points of compounds **4**–**37** are reported in Table 1 and their spectral data are recorded in Table 2.

3.2. Microbiology

The determination of antimycobacterial activity was performed by viable count employing the agar dilution method [19]. Midlebrook and Cohn 7H10 agar, supplemented with Midlebrook OADC enrichment, was used to prepare quadrant plates with serial dimethylsulfoxide twofold dilutions of the different compound tested.

We employed two strains of *Mycobacterium spp.*: *Mycobacterium tuberculosis* reference strain $H_{37}Rv$ and *Mycobacterium avium*, strain 485, from our bacterial collection, emulsified in diluting fluid containg 0.2% fatty acid free albumin and 0.02% polysorbate 80, pH 6.9.

Control plates were included with known antimycobacterial drugs; all plates were incubated at 35°C in 5% CO_2 for 3–4 weeks.

The MIC was defined as the lowest compound dilution associated with at least a 99% reduction in the number of the visible colonies. The results were indicated in Tables 3 and 4.

The other microbial strains tested were *Escherichia coli* strain ML35, *Staphylococcus aureus* ATCC25923 and *Candida albicans* strains C873 and C644, a recent clinical isolate. The *E. coli* strain was grown overnight in Mueller Hinton broth, and the fungal strain was grown overnight in Sabouraud dextrose broth; the test inocula were prepared diluting the overnight suspension to a density of 10^4 microorganisms/ml. The MIC determinations were performed by the agar dilution method; Mueller Hinton agar (Oxoid) and Sabouraud Dextrose agar (Oxoid) were used for bacterial and fungal strains, respectively, to prepare quadrant plates with serial dimethylsulfoxide twofold dilutions of the different chemical tested. A 20 μl sample of each 10^4/ml microbial suspension was inoculated onto each chemical containing quadrant. Control plates consisted of Mueller Hinton agar or Sabouraud dextrose agar alone, culture medium with dimethylsulfoxide and culture medium

Table 2
Spectral data of compounds **4**–**37**

Comp.	R	IR (Nujol, cm^{-1})	^1H NMR (DMSO/Me_4Si) (δ)	Mass m/z [M^+]
4	H	3180, 1680	4.28 and 4.71 (2s, 2H, CH_2), 7.40–8.69 (m, 9H, arom. and pyr.), 8.08 and 8.18 (2s, 1H, CH=N), 11.78 and 11.85 (2s, 1H, NH, disappearing on deuteration)	355
5	2-Cl	3180, 1670	4.22 and 4.75 (2s, 2H, CH_2), 7.15–8.80 (m, 8H, arom. and pyr. and 1H, CH=N), 11.4 and 12.0 (2s, br.sign., 1H, NH, disappearing on deuteration)	389, 391
6	3-Cl	3200, 1680	4.30 and 4.75 (2s, 2H, CH_2), 7.40–8.70 (m, 8H, arom. and pyr.), 8.03 and 8.18 (2s, 1H, CH=N), 11.85 and 12.01 (2s, 1H, NH, disappearing on deuteration)	389, 391
7	4-Cl	3180, 1670	4.24 and 4.66 (2s, 2H, CH_2), 7.40–8.65 (m, 8H, arom. and pyr. and 1H, CH=N), 11.79 and 11.88 (2s, 1H, NH, disappearing on deuteration)	389, 391
8	2-Br	3200, 1680	4.26 and 4.68 (2s, 2H, CH_2), 7.20–8.65 (m, 8H, arom. and pyr.), 8.35 and 8.53 (2s, 1H, CH=N), 11.93 and 12.09 (2s, 1H, NH, disappearing on deuteration)	433, 435
9	3-Br	3200, 1690	4.24 and 4.67 (2s, 2H, CH_2), 7.31–8.64 (m, 8H, arom. and pyr. and 1H, CH=N), 11.83 and 11.93 (2s, 1H, NH, disappearing on deuteration)	433, 435
10	4-Br	3160, 1680	4.29 and 4.71 (2s, 2H, CH_2), 7.55–8.70 (m, 8H, arom. and pyr.), 8.00 and 8.08 (2s, 1H, CH=N), 11.85 and 11.98 (2s, 1H, NH, disappearing on deuteration)	433, 435
11	2-F	3180, 1670	4.30 and 4.73 (2s, 2H, CH_2), 7.20–8.70 (m, 8H, arom. and pyr.), 8.27 and 8.46 (2s, 1H, CH=N), 11.90 and 12.03 (2s, 1H, NH, disappearing on deuteration)	373
12	3-F	3120, 1690	4.30 and 4.74 (2s, 2H, CH_2), 7.25–8.70 (m, 8H, arom. and pyr.), 8.00 and 8.18 (2s, 1H, CH=N), 11.50 and 12.00 (2s, 1H, NH, disappearing on deuteration)	373
13	4-F	3160, 1680	4.30 and 4.72 (2s, 2H, CH_2), 7.20–8.70 (m, 8H, arom. and pyr.), 7.99 and 8.18 (2s, 1H, CH=N), 11.80 and 11.89 (2s, 1H, NH, disappearing on deuteration)	373
14	2,3-$(Cl)_2$	3200, 1670	4.30 and 4.73 (2s, 2H, CH_2), 7.40–8.70 (m, 7H, arom. and pyr.), 8.45 and 8.64 (2s, 1H, CH=N), 12.04 and 12.20 (2s, 1H, NH, disappearing on deuteration)	423, 425, 427
15	2,4-$(Cl)_2$	3190, 1675	4.29 and 4.70 (2s, 2H, CH_2), 7.38–8.72 (m, 7H, arom. and pyr. and 1H, CH=N), 11.80 and 12.12 (2s, 1H, NH, disappearing on deuteration)	423, 425, 427
16	2,6-$(Cl)_2$	3200, 1670	4.32 and 4.65 (2s, 2H, CH_2), 7.40–8.70 (m, 7H, arom. and pyr.), 8.30 and 8.42 (2s, 1H, CH=N), 12.05 and 12.18 (2s, 1H, NH, disappearing on deuteration)	423, 425, 427
17	3,4-$(Cl)_2$	3190, 1685	4.30 and 4.73 (2s, 2H, CH_2), 7.55–8.80 (m, 7H, arom. and pyr. and 1H, CH=N), 11.98 and 12.10 (2s, 1H, NH, disappearing on deuteration)	423, 425, 427
18	2-CH_3	3180, 1670	2.10 (s, 3H, CH_3), 4.30 and 4.73 (2s, 2H, CH_2), 7.29–8.72 (m, 8H, arom. and pyr.), 8.34 and 8.51 (2s, 1H, CH=N), 11.74 and 11.88 (2s, 1H, NH, disappearing on deuteration)	369
19	3-CH_3	3180, 1680	2.35 (s, 3H, CH_3), 4.29 and4.71 (2s, 2H, CH_2), 7.20–8.70 (m, 8H, arom. and pyr. and 1H, CH=N), 11.76 and 11.86 (2s, 1H, NH, disappearing on deuteration)	369
20	4-CH_3	3200, 1670	2.30 (s, 3H, CH_3), 4.29 and 4.70 (2s, 2H, CH_2), 7.20–8.70 (m, 8H, arom. and pyr.), 8.02 and 8.18 (2s, 1H, CH=N), 11.67 and 11.82 (2s, 1H, NH, disappearing on deuteration)	369
21	2,4-$(CH_3)_2$	3180, 1670	2.30 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.25 and 4.70 (2s, 2H, CH_2), 7.05–8.70 (m, 7H, arom. and pyr.), 8.19 and 8.44 (2s, 1H, CH=N), 11.65 and 11.79 (2s, 1H, NH, disappearing on deuteration)	383
22	2,5-$(CH_3)_2$	3180, 1680	2.25 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.30 and 4.70 (2s, 2H, CH_2), 7.15–8.70 (m, 7H, arom. and pyr. and 1H, CH=N), 11.70 and 11.85 (2s, 1H, NH, disappearing on deuteration)	383
23	2-OCH_3	3180, 1670	3.85 (s, 3H, OCH_3), 4.28 and 4.71 (2s, 2H, CH_2), 7.0–8.88 (m, 8H, arom. and pyr.), 8.40 and 8.58 (2s, 1H, CH=N), 11.75 and 11.84 (2s, 1H, NH, disappearing on deuteration)	385
24	3-OCH_3	3200, 1680	3.80 (s, 3H, OCH_3), 4.29 and 4.72 (2s, 2H, CH_2), 6.99–8.70 (m, 8H, arom. and pyr. and 1H, CH=N), 11.81 and 11.88 (2s, 1H, NH, disappearing on deuteration)	385
25	4-OCH_3	3210, 1680	3.80 (s, 3H, OCH_3), 4.26 and 4.69 (2s, 2H, CH_2), 7.0–8.70 (m, 8H, arom. and pyr.), 8.03 and 8.22 (2s, 1H, CH=N), 11.73 and 11.76 (2s, 1H, NH, disappearing on deuteration)	385

Table 2 (*Continued*)

Comp.	R	IR (Nujol, cm^{-1})	^1H NMR (DMSO/Me_4Si) (δ)	Mass m/z [M^+]
26	2,3-$(OCH_3)_2$	3160, 1665	3.78 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.27 and 4.70 (2s, 2H, CH_2), 7.12–8.71 (m, 7H, arom. and pyr.), 8.34 and 8.50 (2s, 1H, CH=N), 11.76 and 11.92 (2s, 1H, NH, disappearing on deuteration)	415
27	2,4-$(OCH_3)_2$	3220, 1670	3.80 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.23 and 4.68 (2s, 2H, CH_2), 6.6–8.70 (m, 7H, arom. and pyr.), 8.29 and 8.47 (2s, 1H, CH=N), 11.60 and 11.72 (2s, 1H, NH, disappearing on deuteration)	415
28	2,5-$(OCH_3)_2$	3180, 1670	3.70 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.27 and 4.70 (2s, 2H, CH_2), 7.0–8.70 (m, 7H, arom. and pyr.), 8.34 and 8.49 (2s, 1H, CH=N), 11.75 and 11.91 (2s, 1H, NH, disappearing on deuteration)	415
29	3,4-$(OCH_3)_2$	3200, 1670	3.80 and 3.82 (2s, 6H, $2OCH_3$), 4.27 and 4.70 (2s, 2H, CH_2), 7.0–8.70 (m, 7H, arom. and pyr.), 7.97 and 8.2 (2s, 1H, CH=N), 11.69 and 11.75 (2s, 1H, NH, disappearing on deuteration)	415
30	3,5-$(OCH_3)_2$	3200, 1670	3.80 (s, 6H, 2 OCH_3), 4.29 and 4.71 (2s, 2H, CH_2), 6.8–8.70 (m, 7H, arom. and pyr.), 7.97 and 8.15 (2s, 1H, CH=N), 11.80 and 11.90 (2s, 1H, NH, disappearing on deuteration)	415
31	3,4,5-$(OCH_3)_3$	3180, 1670	3.70 (s, 3H, OCH_3), 3.85 (s, 6H, 2 OCH_3), 4.29 and 4.70 (2s, 2H, CH_2), 7.05–8.80 (m, 6H, arom. and pyr. and 1H, CH=N), 11.70 and 11.80 (2s, 1H, NH, disappearing on deuteration)	445
32	2-NO_2	3200, 1680	4.32 and 4.72 (2s, 2H, CH_2), 7.50–8.70 (m, 8H, arom. and pyr. and 1H, CH=N), 12.06 and 12.22 (2s, 1H, NH, disappearing on deuteration)	400
33	3-NO_2	3180, 1680	4.33 and 4.76 (2s, 2H, CH_2), 7.60–8.70 (m, 8H, arom. and pyr. and 1H, CH=N), 12.02 and 12.13 (2s, 1H, NH, disappearing on deuteration)	400
34	4-NO_2	3220, 1670	4.33 and 4.76 (2s, 2H, CH_2), 7.50–8.70 (m, 8H, arom. and pyr. and 1H, CH=N), 12.08 and 12.18 (2s, 1H, NH, disappearing on deuteration)	400
35	2,4-$(NO_2)_2$	3220, 1670	4.30 and 4.7 (2s, 2H, CH_2), 7.40–8.80 (m, 7H, arom. and pyr. and 1H, CH=N), 12.24 and 12.40 (2s, 1H, NH, disappearing on deuteration)	445
36	2,6-$(NO_2)_2$	3180, 1670	4.25 and 4.33 (2s, 2H, CH_2), 7.50–8.60 (m, 7H, arom. and pyr. and 1H, CH=N), 12.18 and 12.20 (2s, 1H, NH, disappearing on deuteration)	445
37	3-Cl,4-NO_2	3220, 1680	4.29 and 4.71 (2s, 2H, CH_2), 7.55–8.65 (m, 7H, arom. and pyr. and 1H, CH=N), 12.0 and 12.10 (2s, 1H, NH, disappearing on deuteration)	434, 436

with known antimicrobial drugs, like ampicillin (10 μg/disk) for bacterial strain or econazole (10 μg/disk) for *Candida albicans*. All the plates were then incubated at 37°C overnight.

Table 3
Activity of the [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives **4**–**37** against *Mycobacterium tuberculosis* $H_{37}Rv$ (MIC μg/ml) [a]

Comp.	MIC (μg/ml)	Comp.	MIC (μg/ml)
4	80	**21**	40
5	80	**22**	80
6	40	**23**	80
7	20	**24**	40
8	20	**25**	40
9	80	**26**	40
10	20	**27**	40
11	40	**28**	80
12	20	**29**	80
13	80	**30**	80
14	80	**31**	80
15	80	**32**	80
16	20	**33**	80
17	40	**34**	40
18	80	**35**	80
19	40	**36**	40
20	40	**37**	80

[a] *M. tuberculosis* strain resulted sensitive to isoniazid (5 μg/disk), rifampicin (30 μg/disk).

Table 4
Activity of the [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives **4**–**37** against *Mycobacterium avium 485* (MIC μg/ml) [a]

Comp.	MIC (μg/ml)	Comp.	MIC (μg/ml)
4	80	**21**	40
5	80	**22**	80
6	80	**23**	80
7	80	**24**	40
8	80	**25**	80
9	80	**26**	80
10	40	**27**	80
11	40	**28**	80
12	80	**29**	80
13	80	**30**	80
14	80	**31**	80
15	80	**32**	80
16	80	**33**	80
17	80	**34**	80
18	80	**35**	80
19	80	**36**	80
20	80	**37**	80

[a] *M. avium* strain resulted resistant to ciprofloxacin (5 μg/disk) and rifampicin (30 μg/disk).

4. Results and discussion

A series of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazides (**4**–**37**) (Table 1) have been synthesized with the aim to evaluating their antimycobacterial activity (Tables 3 and 4) towards a strain of *Mycobacterium tuberculosis* ($H_{37}Rv$) sensitive to isoniazid and rifampicin and a strain of *Mycobacterium avium* resistant to ciprofloxacin and rifampicin. Only compounds **7**, **8**, **10**, **12** and **16** exhibited a moderate in vitro antimycobacterial activity (MIC = 20 μg/ml) against the tested strain of *Mycobacterium tuberculosis* $H_{37}Rv$ (Table 3). These compounds are characterized by the presence of 4-chloro, 2-bromo, 4-bromo, 3-fluoro and 2,6-dichloro substituents, respectively, on the phenyl residue. The introduction of these substituents in other positions or the introduction of other substituents, as $-CH_3$, $-OCH_3$, $-NO_2$, on the phenyl residue leads to a decreased antimycobacterial activity. The obtained results are not suitable for an evaluation of structure–activity relationships, but show that the most active compounds in the series are those where lipophylic, electron-withdrawing halogen groups are linked to the benzene ring, particularly in the *ortho* or *para* position. Compounds **10**, **11**, **21** and **24** exhibited a moderate degree of activity (MIC = 40 μg/ml) towards the tested strain of *Mycobacterium avium* (Table 4). On the other hand, none of the synthesized compounds showed activity against the tested strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, at the maximal employed concentration (80 μg/ml).

It is noteworthy that among 34 tested compounds only a few showed some antimycobacterial activity. However, mycobacteria were more susceptible than bacteria and fungi to these compounds, whose moderate activity may stimulate us to individuate structural modifications with the aim to obtain more active and selective antimycobacterial compounds.

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