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Synthesis and antimycobacterial activity of (3,4-diaryl-3*H*-thiazol-2-ylidene)-hydrazide derivatives☆

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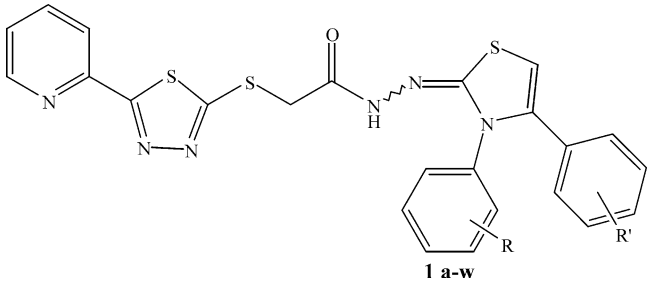
Abstract

[5-(Pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid (3,4-diaryl-3*H*-thiazol-2-ylidene)-hydrazide derivatives were synthesized and tested for their in vitro antimycobacterial activity. Some compounds showed an interesting activity against a strain of *Mycobacterium tuberculosis* $H_{37}Rv$ and three clinical isolates of *M. tuberculosis*.

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

1. Introduction

In our search for new antimycobacterial agents we already synthesized a series of [5-pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid arylidene-hydrazide derivatives [1], some of which exhibited a moderate in vitro antimycobacterial activity against a strain of *Mycobacterium tuberculosis* $H_{37}Rv$ sensitive to isoniazid and rifampicin and a strain of *Mycobacterium avium* resistant to ciprofloxacin and rifampicin. None of the synthesized compounds showed activity against the tested strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. With the aim to obtain more active and selective antimycobacterial compounds we sinthesized a series of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid (3,4-diaryl-3*H*-thiazol-2-ylidene)-hydrazide derivatives **1a**–**w** (Scheme 1) in which the arylidene moiety of the previously described compounds was replaced by the 3,4-diaryl-3*H*-thiazol-2-ylidene one.



1 a-w

Actually, 3,4-disubstituted 3*H*-thiazol-2-ylidene-hydrazide derivatives have been described for their antibacterial and antifungal properties [2,3]. Moreover, the thioacetyl hydrazone moiety, linked to the 2 position of the 1,3,4-thiadiazole derivatives **1a**–**w**, was present in other compounds characterized by antibacterial [4], antimicotic [4,5] and antimycobacterial [1] activity and hydrazido–hydrazone derivatives have been described for their antimycobacterial properties [6].

☆ A preliminary account of this work was presented at Italian–Hungarian–Polish Joint Meeting on Medicinal Chemistry Giardini Naxos, Ramada Hotel, 28 September–1 October 1999.

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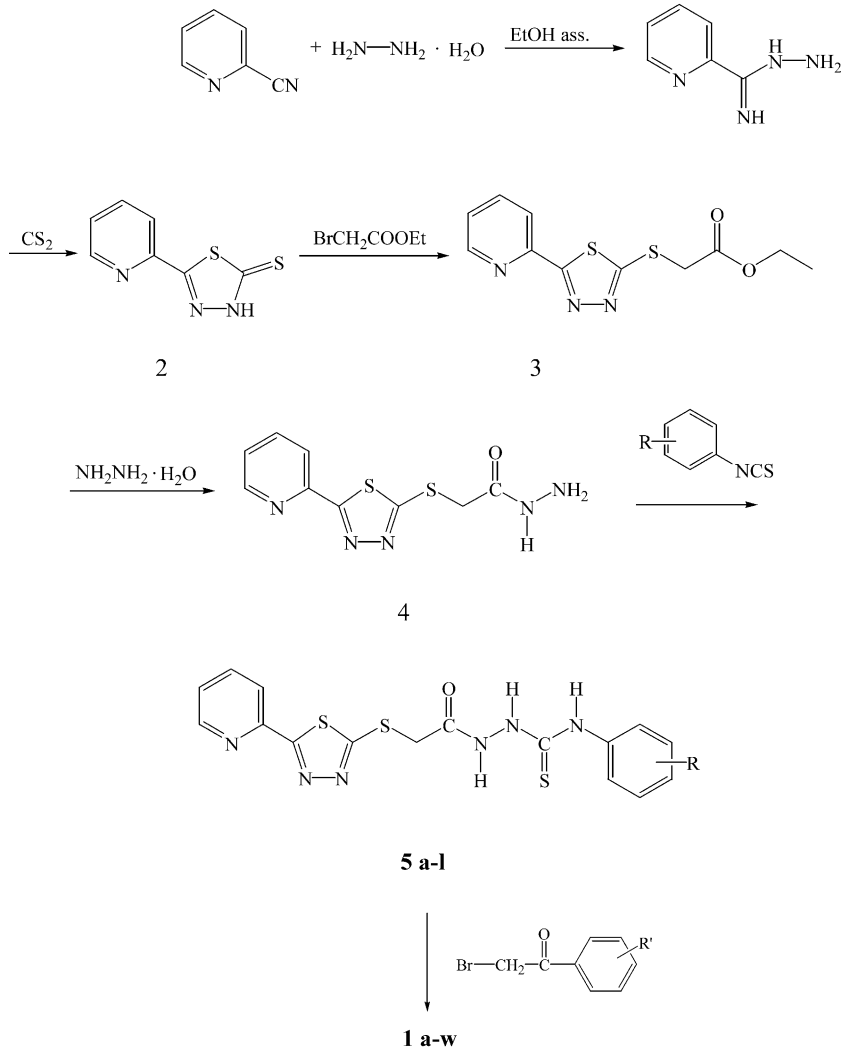
The new synthesized compounds have been tested for their in vitro antimycobacterial activity toward a strain of *M. tuberculosis* $H_{37}Rv$ and three clinical isolates of *M. tuberculosis*.

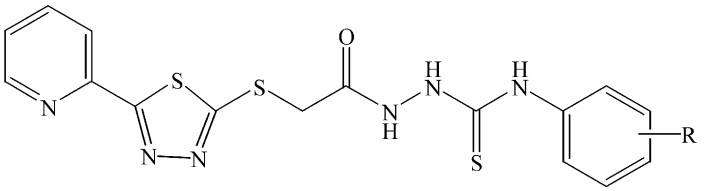
2. Chemistry

The synthesis of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid (3,4-diaryl-3*H*-thiazol-2-ylidene)-hydrazide derivatives **1a**–**w** (Table 1) was carried out (Scheme 1) by treating pyridine-2-carboxamidrazone with carbon disulfide to obtain 5-(pyridin-2-yl)-3*H*-1,3,4-thiadiazole-2-thione 2 [7]. Compound **2** was made to react with ethyl bromoacetate to afford the [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid ethylester (**3**) [1] from which the [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio] acetic acid hydrazide (**4**) [1] was obtained by treatment with hydrazine hydrate. The hydrazide **4** was treated with substituted arylisothiocianates to obtain the thiosemicarbazide derivatives **5a**–**l** (Table 2) which were cyclized with variously substituted 2-bromoacetophenones to yield the corresponding derivatives **1a**–**w**, isolated as free bases or in the form of hydrobromides. IR spectra of thiosemicarbazide derivatives **5a**–**l** exhibited characteristic broad stretching bands in the 3250–3115 and 3350–3257 cm^{-1} regions. The C=O bands were observed in the 1702–1659 cm^{-1} range. Their ^1H NMR spectra exhibited N^4–H, N^2–H and N^1–H in the 9.27–9.89, 9.72–10.16 and 10.42–10.61 ppm regions, respectively [8]. The methylene S–CH_2 protons resonated as a singlet in the 4.05–4.25 ppm region. The IR spectra of thiazoline derivatives **1a**–**w** exhibited NH and C=O bands in the 3471–3279 and 1690–1625 cm^{-1} regions, respectively, attributed to the CO–NH–N= function. ^1H NMR spectra displayed a single NH resonance at 10.32–12.39 ppm. The absence of the thiosemicarbazide moiety N^2–H and N^4–H



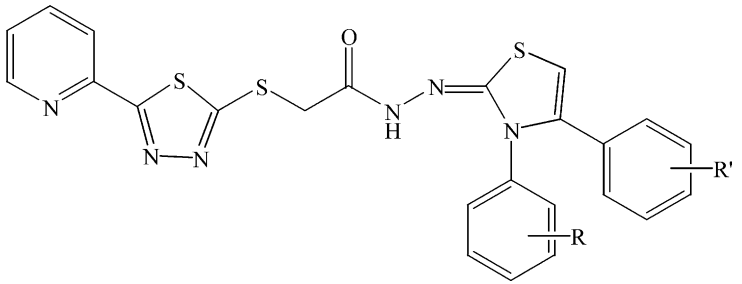
Scheme 1.

Table 1
Spectral data of compounds **5a**–**l**



Comp.	R	Yield (%)	M.p. (°C)	IR (nujol, cm^{-1})	^{1}H-NMR ($CDCl_3$) (δ)	Mass m/z [M^+]	Formula (C,H,N)
5a	H	79	192–4	3270, 3158, 1683	4.23 (s, 2H, S–CH_2), 7.01–8.80 (m, 9H, arom. and pyr.), 9.56 (1H, NH, disappearing on deuteration, N^4H), 9.81 (1H, NH, disappearing on deuteration, N^2H), 10.42 (1H, NH, disappearing on deuteration, N^1H)	402	$C_{16}H_{14}N_6OS_3$
5b	2–Cl	61	196–8	3305, 3250, 1671	4.22 (s, 2H, S–CH_2), 7.12–8.72 (m, 8H, arom. and pyr.), 9.44 (1H, NH, disappearing on deuteration, N^4H), 10.00 (1H, NH, disappearing on deuteration, N^2H), 10.61 (1H, NH, disappearing on deuteration, N^1H)	436, 438	$C_{16}H_{13}N_6S_3OCl$
5c	3-Cl	81	181–3	3257, 3150, 1682	4.22 (s, 2H, S–CH_2), 7.06–8.84 (m, 8H, arom. and pyr.), 9.61 (1H, NH, disappearing on deuteration, N^4H), 9.96 (1H, NH, disappearing on deuteration, N^2H), 10.49 (1H, NH, disappearing on deuteration, N^1H)	436, 438	$C_{16}H_{13}N_6S_3OCl$
5d	4-Cl	79	197–9	3297, 3140, 1698	4.22 (s, 2H, S–CH_2), 7.26–8.75 (m, 8H, arom. and pyr.), 9.60 (1H, NH, disappearing on deuteration, N^4H), 9.90 (1H, NH, disappearing on deuteration, N^2H), 10.49 (1H, NH, disappearing on deuteration, N^1H)	436, 438	$C_{16}H_{13}N_6S_3OCl$
5e	2-Br	83	203–5	3330, 3230, 1702	4.19 (s, 2H, S–CH_2), 7.05–8.70 (m, 8H, arom. and pyr.), 9.41 (1H, NH, disappearing on deuteration, N^4H), 9.91 (1H, NH, disappearing on deuteration, N^2H), 10.56 (1H, NH, disappearing on deuteration, N^1H)	480, 482	$C_{16}H_{13}N_6OS_3Br$
5f	3-Br	71	176–8	3300, 3160, 1680	4.22 (s, 2H, S–CH_2), 7.16–8.74 (m, 8H, arom. and pyr.), 9.60 (1H, NH, disappearing on deuteration, N^4H), 9.96 (1H, NH, disappearing on deuteration, N^2H), 10.46 (1H, NH, disappearing on deuteration, N^1H)	480, 482	$C_{16}H_{13}N_6OS_3Br$
5g	4-Br	82	190–2	3320, 3233, 1699	4.23 (s, 2H, S–CH_2), 7.29–8.75 (m, 8H, arom. and pyr.), 9.59 (1H, NH, disappearing on deuteration, N^4H), 9.87 (1H, NH, disappearing on deuteration, N^2H), 10.46 (1H, NH, disappearing on deuteration, N^1H)	480, 482	$C_{16}H_{13}N_6OS_3Br$
5h	2-CH_3	89	203–5	3278, 3165, 1682	2.09 (s, 3H, CH_3), 4.23 (s, 2H, S–CH_2), 6.94–8.82 (m, 8H, arom. and pyr.), 9.35 (1H, NH, disappearing on deuteration, N^4H), 9.72 (1H, NH, disappearing on deuteration, N^2H), 10.51 (1H, NH, disappearing on deuteration, N^1H)	416	$C_{17}H_{16}N_6OS_3$
5i	3-CH_3	59	177–9	3280, 3130, 1680	2.17 (s, 3H, CH_3), 4.21 (s, 2H, S–CH_2), 7.15–8.92 (m, 8H, arom. and pyr.), 9.27 (1H, NH, disappearing on deuteration, N^4H), 9.78 (1H, NH, disappearing on deuteration, N^2H), 10.45 (1H, NH, disappearing on deuteration, N^1H)	416	$C_{17}H_{16}N_6OS_3$
5j	4-CH_3	68	179–80	3350, 3250, 1660	2.23 (s, 3H, CH_3), 4.22 (s, 2H, S–CH_2), 7.00-8.79 (m, 8H, arom. and pyr.), 9.47 (1H, NH, disappearing on deuteration, N^4H), 9.72 (1H, NH, disappearing on deuteration, N^2H), 10.44 (1H, NH, disappearing on deuteration, N^1H)	416	$C_{17}H_{16}N_6OS_3$
5k	3-NO_2	79	196–98	3290, 3130, 1697	4.25 (s, 2H, S–CH_2), 7.37–8.75 (m, 8H, arom. and pyr.), 9.89 (1H, NH, disappearing on deuteration, N^4H), 10.16 (1H, NH, disappearing on deuteration, N^2H), 10.57 (1H, NH, disappearing on deuteration, N^1H)	446	$C_{16}H_{13}N_7O_2S_3$
5l	4-NO_2	77	214–6	3312, 3115, 1659	4.04 (s, 2H, S–CH_2), 7.46–8.75 (m, 8H, arom. and pyr.), 9.47 (1H, NH, disappearing on deuteration, N^4H), 9.87 (1H, NH, disappearing on deuteration, N^2H), 10.57 (1H, NH, disappearing on deuteration, N^1H)	446	$C_{16}H_{13}N_7O_2S_3$

Table 2
Spectral data of compounds **1a**–**w**



Comp.	R	R′	Yield (%)	M.p. (°C)	IR (nujol, cm^{-1})	^{1}H-NMR ($CDCl_3$) (δ)	Mass m/z [M^+]	Formula (C,H,N)
1a	H	H	48	192	3383,1702	4.27 (s, 2H, CH_2), 6.82 (s, 1H, CH thiazoline), 7.18–8.77 (m, 14H, arom. and pyr.), 11.92 (1H, NH, disappearing on deuteration)	502	$C_{24}H_{18}N_6OS_3$
1b	H	2-Cl	46	210	3378, 1664	4.12 (s, 2H, CH_2), 6.50 (s, 1H, CH thiazoline), 6.95–8.77 (m, 13H, arom. and pyr.), 10.41 (1H, NH, disappearing on deuteration)	536–538	$C_{24}H_{17}N_6OS_3Cl$
1c	H	3-Cl	52	226	3471,1721	4.15 (s, 2H, CH_2), 6.63 (s, 1H, CH thiazoline), 6.92–8.80 (m, 13H, arom. and pyr.), 10.41 (1H, NH, disappearing on deuteration)	536–538	$C_{24}H_{17}N_6OS_3Cl$
1d	H	4-Cl	50	235	3342, 1678	4.24 (s, 2H, CH_2), 6.52 (s, 1H, CH thiazoline), 6.74–8.84 (m, 13H, arom. and pyr.), 11.54 (1H, NH, disappearing on deuteration)	536–538	$C_{24}H_{17}N_6OS_3Cl$
1e	H	2-Br	55	290	3342,1663	4.28 (s, 2H, CH_2), 6.96 (s, 1H, CH thiazoline), 7.10–8.85 (m, 13H, arom. and pyr.), 10.39 (1H, NH, disappearing on deuteration)	580–582	$C_{24}H_{17}N_6OS_3Br$
1f	H	3-Br	57	250	3390, 1674	4.21 (s, 2H, CH_2), 6.67–8.82 (m, 14H, thiaz., arom. and pyr.), 10.85 (1H, NH, disappearing on deuteration)	580–582	$C_{24}H_{17}N_6OS_3Br$
1g	H	4-Br	50	245	3279, 1651	4.15 (s, 2H, CH_2), 6.65 (s, 1H, CH thiazoline), 6.86–8.79 (m, 13H, arom. and pyr.), 10.53 (1H, NH, disappearing on deuteration)	580–582	$C_{24}H_{17}N_6OS_3Br$
1h	H	2-CH_3	55	215	3393, 1657	2.08 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 6.33 (s, 1H, CH thiazoline), 6.95–8.78 (m, 13H, arom. and pyr.), 10.32 (1H, NH, disappearing on deuteration)	516	$C_{25}H_{20}N_6OS_3$
1i	H	3-CH_3	61	178	3389, 1677	2.11 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 6.61 (s, 1H, CH thiazoline), 6.74–8.77 (m, 13H, arom. and pyr.), 10.57 (1H, NH, disappearing on deuteration)	516	$C_{25}H_{20}N_6OS_3$
1j	H	4-CH_3	64	255	3382, 1692	2.17 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 6.50 (s, 1H, CH thiazoline), 6.91–8.75 (m, 13H, arom. and pyr.), 10.47 (1H, NH, disappearing on deuteration)	516	$C_{25}H_{20}N_6OS_3$
1k	H	3-NO_2	54	162	3421, 1718	4.19 (s, 2H, CH_2), 6.65 (s, 1H, CH thiazoline), 6.86–8.79 (m, 13H, arom. and pyr. and 1H, N^+H, disappearing on deuteration), 10.53 (1H, NH, disappearing on deuteration)	547	$C_{24}H_{17}N_7O_3S_3HBr$
1l	H	4-NO_2	45	210	3445, 1638	4.15 (s, 2H, CH_2), 6.65 (s, 1H, CH thiazoline), 6.86–8.79 (m, 13H, arom. and pyr.), 10.53 (1H, NH, disappearing on deuteration)	547	$C_{24}H_{17}N_7O_3S_3$
1m	2-Cl	H	50	252	3384, 1708	4.26 (s, 2H, CH_2), 6.79 (s, 1H, CH thiazoline), 7.20–8.68 (m, 13H, arom. and pyr.), 8.46 (1H, N^+H, disappearing on deuteration) 12.01 (1H, NH, disappearing on deuteration)	536–538	$C_{24}H_{17}N_6OS_3Cl{\cdot}HBr$
1n	3-Cl	H	53	250	3381,1698	4.32 (s, 2H, CH_2), 6.89 (s, 1H, CH thiazoline), 7.19–8.71 (m, 13H, arom. and pyr. and 1H, N^+H, disappearing on deuteration), 12.22 (1H, NH, disappearing on deuteration)	536–538	$C_{24}H_{17}N_6OS_3Cl{\cdot}HBr$
1o	4-Cl	H	49	275	3392, 1701	4.29 (s, 2H, CH_2), 6.94 (s, 1H, CH thiazoline), 7.15–8.84 (m, 13H, arom. and pyr. and 1H, N^+H, disappearing on deuteration), 12.10 (1H, NH, disappearing on deuteration)	536–538	$C_{24}H_{17}N_6OS_3Cl{\cdot}HBr$
1p	2-Br	H	56	244	3386,1706	4.30 (s, 2H, CH_2), 6.91 (s, 1H, CH thiazoline), 7.12–8.75 (m, 13H, arom. and pyr.), 11.13 (1H, N^+H, disappearing on deuteration), 12.18 (1H, NH, disappearing on deuteration)	580–582	$C_{24}H_{17}N_6OS_3Br{\cdot}HBr$

Table 2 (*Continued*)

Comp.	R	R′	Yield (%)	M.p. (°C)	IR (nujol, cm^{-1})	^{1}H-NMR ($CDCl_3$) (δ)	Mass *m/z* [M^+]	Formula (C,H,N)
1q	3-Br	H	50	242	3383, 1698	4.29 (s, 2H, CH_2), 6.90 (s, 1H, CH thiazoline), 7.20–8.74 (m, 13H, arom. and pyr.), 9.02 (1H, N^+H, disappearing on deuteration), 12.04 (1H, NH, disappearing on deuteration)	580–582	$C_{24}H_{17}N_6OS_3Br{\cdot}HBr$
1r	4-Br	H	59	277	3384, 1702	4.25 (s, 2H, CH_2), 6.81 (s, 1H, CH thiazoline), 7.02–8.82 (m, 13H, arom. and pyr. and 1H, N^+H, disappearing on deuteration), 11.90 (1H, NH, disappearing on deuteration)	580–582	$C_{24}H_{17}N_6OS_3Br{\cdot}HBr$
1s	2-CH_3	H	55	255	3380, 1710	2.00 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.29 (s, 1H, CH thiazoline), 6.76–8.74 (m, 13H, arom. and pyr. and 1H, N^+H, disappearing on deuteration), 11.24 (1H, NH, disappearing on deuteration)	516	$C_{25}H_{20}N_6OS_3HBr$
1t	3-CH_3	H	58	250	3382, 1697	2.31 (s, 3H, CH_3), 4.35 (s, 2H, CH_2), 7.11 (s, 1H, CH thiazoline), 7.16–8.75 (m, 13H, arom. and pyr. and 1H, N^+H, disappearing on deuteration), 12.39 (1H, NH, disappearing on deuteration)	516	$C_{25}H_{20}N_6OS_3HBr$
1u	4-CH_3	H	61	280	3384, 1698	2.31 (s, 3H, CH_3), 4.28 (s, 2H, CH_2), 6.98 (s, 1H, CH thiazoline), 7.10–8.77 (m, 13H, arom. and pyr. and 1H, N^+H, disappearing on deuteration), 12.15 (1H, NH, disappearing on deuteration)	516	$C_{25}H_{20}N_6OS_3HBr$
1v	3-NO_2	H	64	240	3373, 1695	4.30 (s, 2H, CH_2), 6.83 (s, 1H, CH thiazoline), 7.18–8.68 (m, 13H, arom. and pyr.), 9.47 (1H, N^+H, disappearing on deuteration),10.95 (1H, NH, disappearing on deuteration)	547	$C_{24}H_{17}N_7O_3S_3HBr$
1w	4-NO_2	H	51	220	3390, 1703	4.20 (s, 2H, CH_2), 6.59 (s, 1H, CH thiazoline), 6.73 (1H, N^+H, disappearing on deuteration), 7.04–8.70 (m, 13H, arom. and pyr.), 11.50 (1H, NH, disappearing on deuteration)	547	$C_{24}H_{17}N_7O_3S_3HBr$

signals, the =CH– resonance at 6.29–7.11 ppm and the –S–CH_2– resonance at 4.11–4.35 ppm confirmed the cyclization to the thiazoline derivatives **1a**–**w**. The thiazoline derivatives **1k** and **1m**–**w**, isolated as hydrobromides, presented an additional NH^+ signal.

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 Jasco 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, deuterochloroform 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. 4-Phenyl-1-[[(5-pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetyl]thiosemicarbazide (**5a**)

A solution of 1.95 g (7.29 mmol) of [5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetic acid hydrazide **4** and 0.99 g (7.29 mmol) of phenylisothiocyanate was refluxed for 3 h. The solution was concentrated at reduced pressure and the solid formed was filtered off and crystallized from absolute ethanol to obtain 2.31 g (78.7%) of **5a**; m.p. 192–194 °C.

IR (Nujol, cm^{-1}): 3270, 3158, 1683. ^{1}H NMR ($CDCl_3$–TMS): δ 4.23 (s, 2H, S–CH_2), 7.01–8.80 (m, 9H, arom. and pyr.), 9.56 (1H, NH, disappearing on deuteration, N^4H), 9.81 (1H, NH, disappearing on deuteration, N^2H), 10.42 (1H, NH, disappearing on deuteration, N^1H) MS: *m/z* 402 [M^+].

In an analogous way compounds **5b**–**l** have been prepared. Yields, melting points and spectral data are recorded in Table 1.

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

A mixture of 1.5 g (3.73 mmol) of 4-phenyl-1-[[(5-(pyridin-2-yl)-1,3,4-thiadiazol-2-ylthio]acetyl]thiosemicarbazide **5a** and 0.74 (3.73 mmol) of 2-bromoacetophenone in 80 ml of absolute ethanol was refluxed for 4 h. The obtained solution was evaporated under reduced pressure and the residue was treated with saturated $NaHCO_3$ solution. The obtained precipitate was filtered, washed with cold water, dried and recrystallized from ethanol to obtain 0.88g (47.3%) of **1a**; m.p. 192 °C.

IR (Nujol, cm^{-1}): 3383, 1702. ^1H NMR ($CDCl_3$–TMS): δ 4.27 (s, 2H, CH_2), 6.82 (s, 1H, CH thiazole), 7.18–8.77 (m, 14H, arom. and pyr.), 11.92 (1H, NH, disappearing on deuteration) MS: m/z 502 [M^+].

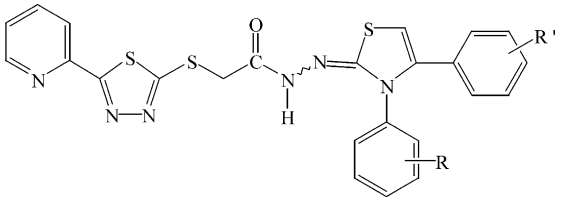
Analogously, the compounds **1a**–**j** and **1l** were prepared. With the same procedure compounds **1k** and **1m**–**w** were obtained as HBr salts. Yields, melting points and spectral data of compounds **1a**–**w** are reported in Table 2.

3.2. Microbiology

3.2.1. Antimycobacterial activity

The antimycobacterial activity has been evaluated on *M. tuberculosis* reference strain H37Rv, on two sensitive *M. tuberculosis* clinical strains and on one multiresistant *M. tuberculosis* clinical isolate, all strains belonging to our collection. The inhibiting activity of the new molecules was tested by means of a standard agar dilution technique [9] with viable counts performed in quadrant petri plates containing Middlebrook and Cohn 7H11 agar, supplemented with Middlebrook OADC enrichment. Serial dimethylsulfoxide twofold dilutions of the different chemicals tested were placed in each quadrant; control plates were included with known antitubercular drugs and no drug. A 20-μl sample of each reference strain or clinical strain suspension, containing 10^4/ml mycobacteria in sterile saline with the addition of 0.02% polysorbate 80, was inoculated onto each chemical containing quadrant. All plates were incubated at 37 °C in 5% CO_2 for 3–4 weeks. Minimal inhibiting concentration (MIC) of each compound was defined as the lowest chemical dilution associated with at least a 99% reduction in the number of visible colonies.

Table 3
Activity of the (3,4-diaryl-3*H*-thiazol-2-ylidene) hydrazides derivatives **1a**–**w** against *M. tuberculosis* $H_{37}Rv$ and three clinical isolates of *M. tuberculosis*



Comp.	$H_{37}Rv$	180	190	331
INH	0.5	0.05	16	0.05
RIF	n.t.	0.5	> 128	0.5
1a	> 80	> 80	80	80
1b	> 80	20	> 80	> 80
1c	> 80	80	> 80	> 80
1d	40	40	40	80
1e	80	80	> 80	> 80
1f	40	40	> 80	10
1g	40	40	80	80
1h	> 80	> 80	> 80	80
1i	40	20	> 80	40
1j	40	80	40	40
1k	40	80	> 80	> 80
1l	40	> 80	40	> 80
1m	> 80	40	> 80	> 80
1n	> 80	80	> 80	> 80
1o	80	40	40	40
1p	> 80	80	> 80	> 80
1q	> 80	40	> 80	> 80
1r	> 80	40	40	> 80
1s	> 80	> 80	20	40
1t	> 80	40	> 80	> 80
1u	> 80	40	40	40
1v	> 80	80	> 80	> 80
1w	40	20	> 80	> 80

4. Results and discussion

A series of 2-[5-(pyridin-2yl)-1,3,4-thiadiazol-2-ylthio]acetic acid (3,4-diaryl-3*H*-thiazol-2-ylidene)-hydrazide derivatives **1a**–**w** (Table 1) have been synthesized with the aim to evaluating their antimycobacterial activity (Table 3) toward a strain of *M. tuberculosis* $H_{37}Rv$ and three strains of *M. tuberculosis* 180, *M. tuberculosis* 190 and *M. tuberculosis* 331, isolated from human bronchial aspirates. The clinical isolate of *M. tuberculosis* 190 was resistant to isoniazid and rifampicin. With the exception of **1w**, which explicated a weak activity (MIC = 40 μg/ml), compounds **1m**–**w**, substituted at the phenyl residue linked to the 3-position of the thiazoline cycle, were inactive against the tested strain of *M. tuberculosis* $H_{37}Rv$. However, compounds **1d**, **1f**, **1g**, **1i**–**l**, substituted at the phenyl residue linked to the 4-position of the thiazoline ring, exhibited a moderate in vitro antimycobacterial activity (MIC = 40 μg/ml) against the strain of *M. tuberculosis* $H_{37}Rv$. These compounds are characterized by the presence of a substituent in the *para* or *meta* position of the phenyl ring but their activity does not depend from electronic effects, since electron-donating and electron-withdrawing substituents produce the same level of activity. However, both the series of compounds **1a**–**l** and **1m**–**w**, substituted only at one of the phenyl residues, include compounds exhibiting a moderate in vitro antimycobacterial activity against the tested strains of clinical isolates of *M. tuberculosis*, their MIC values reaching 20 μg/ml against the strains of *M. tuberculosis* 180 (compounds **1b**, **1i** and **1w**) and *M. tuberculosis* 190 (compounds **1s**) and 10 μg/ml against the strain of *M. tuberculosis* 331 (compound **1f**). Interestingly, compounds **1d**, **1j**, **1l**, **1o**, **1r**, **1s**, **1u** exhibited a moderate antimycobacterial activity against the strain of *M. tuberculosis* 190, resistant to isoniazid and rifampicin. Only the *para*-substituted compounds **1o** and **1u** were

moderately active toward all the clinical isolates of *M. tuberculosis*.

The obtained results are not suitable for an evaluation of structure–activity relationships, but show that compounds which explicate some degree of activity against almost all of the clinical isolates of *M. tuberculosis* were those which were *para*-substituted at one of the phenyl residues. However, a few *meta* and *ortho* substituted derivatives were active against some of the tested microorganisms. Since substituents present on phenyl residue linked to the 4-position or 3-position of the thiazoline derivatives **1a**–**w** produced respectively compounds **1a**–**l** and **1m**–**w**, some of which were characterized by antimycobacterial activity, the simultaneous introduction of substituents in both phenyl rings may produce more active antimycobacterial compounds. On the basis of these considerations, the synthesis and the antimycobacterial activity evaluation of new (3,4-diaryl-3*H*-thiazol-2-ylidene)-hydrazides are now in progress.

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