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Antimicrobial activity of rhodanine-3-acetic acid derivatives

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ABSTRACT

Twenty-four 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid (rhodanine-3-acetic acid)-based amides, esters and 5-arylalkylidene derivatives were synthesized, characterized and evaluated as potential antimicrobial agents against a panel of bacteria, mycobacteria and fungi. All of the derivatives were active against mycobacteria. *N*-(4-Chlorophenyl)-2-[5-(2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl] acetamide demonstrated the highest activity against *Mycobacterium tuberculosis* with minimum inhibitory concentrations (MIC) of 8–16 μM. Non-tuberculous mycobacteria were the most susceptible to 2-[5-(2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acids (MIC values ⩾32 μM). The highest antibacterial activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* exhibited 4-(trifluoromethyl)phenyl 2-(4-oxo-2-thioxothiazolidin-3-yl)acetate (MIC ⩾ 15.62 μM). Several structure-activity relationships were identified. The activity against Gram-negative and fungal pathogens was marginal.

1. Introduction

Currently, novel antimicrobial agents active especially against drug-resistant bacteria and fungi are required to overcome limitations of the treatment opportunities. Microorganisms that are resistant to most of the clinically used antibiotics are emerging at a rapid rate. The spread of methicillin-resistant *Staphylococcus aureus*, biofilm-producing bacteria, nosocomial infections caused by *Enterococci* and *Streptococci*, a wide spectrum of Gram-negative pathogens or fungal species, increasing incidence of tuberculosis, onset of HIV/AIDS epidemic may serve as examples of serious threats for public health. One additional reason for developing new antibiotics is related to their own toxicity and side effects. Unfortunately, the number of innovative classes of the antimicrobial agents being developed has decreased dramatically recently and it is lagging behind clinical requirements.[1–3]

Rhodanine (2-thioxothiazolidin-4-one) as a privileged scaffold in medicinal chemistry offers various possibilities of chemical modification. Principally, N-3 and/or "active methylene" C-5 substitution has brought a wide range of potentially bioactive compounds. Illustratively, derivatives of rhodanine-3-acetic acid (RAA; 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid) are able to inhibit various enzymes, e.g., cholinesterases,[4,5] 15-lipoxygenase,[5] aldose reductase,[6] dolicholphosphate mannose synthase,[7] deoxyxylulose 5-phosphate reductoisomerase,[8] gyrase B,[9] Mur ligases,[10] as well as growth of pathogenic protozoa,[7] fungi,[11] bacteria,[8,10,12,13] and *Mycobacterium tuberculosis*[14] in whole-cell screening assays. It is beyond doubt that rhodanines including those with C-5 substitution are a well-established scaffold for both microbial enzymes inhibition and *in vitro* antimicrobial activity.

Based on here mentioned facts and as a part of our research effort focused on synthesis and identification of novel antimicrobial agents,[15–19] we evaluated previously reported *N*-phenylamides and phenyl esters of rhodanine-3-acetic acid,[4] novel salicylaldehyde-based C-5 arylmethylidene derivatives of the RAA and their mutual conjugates against mycobacteria, Gram-positive and Gram-negative bacteria and also eight fungal species. The selected panel of twenty microbes covers a wide range of important human pathogens including strains with an acquired resistance. It is a useful screening tool for an initial identification of potential antimicrobial activity of new compounds.

2. Results and discussion

2.1. Chemistry

The synthesis of amides **1** and esters **2** from RAA using predominantly *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)-based coupling was described previously.[4]

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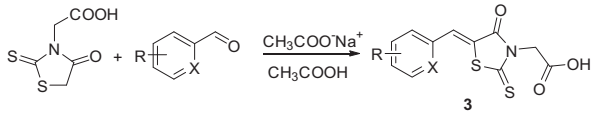


Scheme 1. Synthesis of 5-arylidenerhodanine-3-acetic acids **3** (X = CH, N; R = H, 2-OH, 3-OH, 4-OH, 2-OH-5-Br, 2-OH-5-Cl).

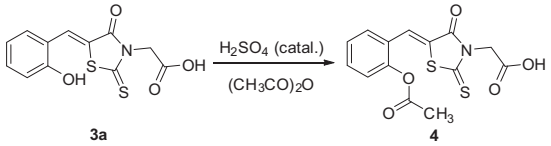
C-5 substituted rhodanine-3-acetic acids were synthesized by heating of parent RAA together with 1.1 equivalents of appropriate hydroxybenzaldehydes (Scheme 1, X = CH) and pyridine-2-carbaldehyde (X = N, R is missing) in glacial acetic acid in the presence of sodium acetate. This procedure takes 5 h proving satisfactory yields of 78–97%. We selected salicylaldehyde as an initial compound (derivative **3a**), followed by their positional isomers 3-/4-hydroxybenzaldehyde (**3b** and **3c**) and 5-halogenated salicylaldehydes (chlorine and bromine derivatives **3d** and **3e**, respectively). This halogenation of salicylic ring has been reported to be beneficial for enhancing of antimicrobial activity.[15,16] Supported by a previous report of Dolezel et al.,[11] we also performed condensation of rhodanine-3-acetic acid with pyridine-2-carbaldehyde leading to conjugate **3f**.

To discover the possible importance of 2-hydroxybenzylidene free phenolic group or to reveal potential advantage of its esterification,[20] we treated **3a** with acetic anhydride (yield of 78%; Scheme 2).

Based on results of antimycobacterial activity evaluation, we decided to combine both active fragments, *N*-(4-chlorophenyl)-2-(4-oxo-2-thioxothiazolidin-3-yl)acetamide **1a** and 2-[5-(2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acids **3a**, **3d** and **3e**, in one molecular entity. A conjugate with the pyridine derivative **3f** was synthesized too. From two possible two-step synthetic pathways, we opted for the reaction of amide **1d** with a mild excess of aromatic aldehydes under identical conditions (acetic acid, sodium acetate, reflux; Scheme 3) used for the synthesis of the compounds **3**. Yields of this step were within the range of 77–85%. The reaction in a reverse order, i.e., synthesis of **3** followed by conversion of free carboxyl to *N*-(4-chlorophenyl)amide, could be complicated by a side reaction of free hydroxyl group.

Compounds were characterized by melting points, IR and NMR spectra; their purity was checked by thin-layer chromatography, melting point and elemental analysis.

5-Salicylidene or pyridin-2-ylmethylene derivatives **3**–**5** can form two geometric isomers (*E* and *Z*) due to the presence of exocyclic double bond. In NMR spectra of all of the derivatives **3**–**5**, only single isomer was observed uniformly. According to the literature[11] and references therein, this type of synthesis leads to the thermodynamically more stable *Z*-isomers. The identity of *Z*-isomers was also confirmed by the comparison with previously reported NMR data of known or structurally close similar compounds.[6,10,21]



Scheme 2. Esterification of **3a**.

2.2. Pharmacology

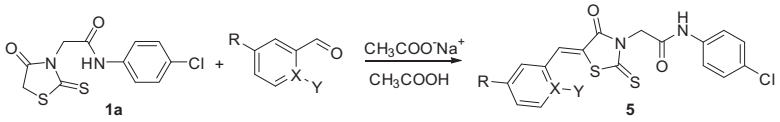
2.2.1. Antimycobacterial activity

Initially, we evaluated previously reported amides **1** and esters **2** of rhodanine-3-acetic acid,[4] parent RAA and newly synthesized 5-arylmethylidenerhodanine-3-acetic acids **3** as potential antimycobacterial agents. A drug-susceptible strain of *Mycobacterium tuberculosis* (*Mtb.*) 331/88 ($H_{37}Rv$) and three strains of atypical (non-tuberculous) mycobacteria (*M. avium*, *M. kansasii* including one clinical isolate – 6509/96 strain) were involved (Table 1). With one exception (**5b**), there were no solubility problems in the testing medium up to the concentration of 1 mM.

Among the RAA *N*-phenylamides **1** and phenyl esters **2**, only 4-chloroderivative **1b** exhibited a moderate activity against *Mtb.* with MIC values of 32–62.5 μM and 125–250 μM for *M. kansasii* 6509/96. The removal or the replacement of chlorine by other substituent decreases the antimycobacterial activity of **1b** especially against *Mtb.* The switch of the *N*-(4-chlorophenyl)-2-(4-oxo-2-thioxothiazolidin-3-yl)acetamide **1b** to the corresponding ester **2b** modulates the action in the same way. In general, the esters **2** are not superior to the amides **1**. *M. avium* displayed the lowest susceptibility to all the derivatives **1** and **2** (MIC ⩾ 1000 μM). Unsubstituted RAA is virtually inactive with a uniform MIC value of 1000 μM.

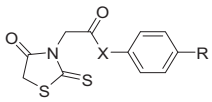
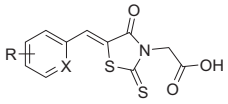
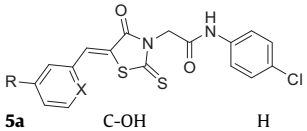
Salicylaldehyde-based derivatives **3** provide more efficient *in vitro* growth inhibition of mycobacteria (MIC within the range of 32–250 μM). Obviously, the employment of isomeric 3-hydroxybenzaldehyde (condensate **3b**) and 4-hydroxybenzaldehyde (**3c**) or pyridine-2-carbaldehyde (**3f**) led to the negligible active molecules, thus indicating the importance of phenolic group at the position 2. The acetylation of **3a** (compound **4**) and introduction of chlorine (**3d**) or bromine (**3e**) into the salicylic moiety did not influence the antimycobacterial action sharply; their activities are mostly comparable each other. This means that none of these modifications is preferred over unsubstituted salicylaldehyde (2-hydroxybenzaldehyde). The susceptibility rate of all mycobacterial strains were similar, only 5-bromosalicylidene-RAA is somewhat less active against clinically isolated *M. kansasii* 6509/96.

Keeping in mind activity data of the amide **1b** and salicylaldehyde condensates (**3a**, **3d**, **3e**), we decided to combine both these antimycobacterial pharmacophores in one molecule to determine if this "amide-condensates" conjugation will result also in a more potent suppression of mycobacteria. This initial assumption was confirmed in the case of *Mtb.* Conjugate **5a** derived from unsubstituted salicylaldehyde exhibited the highest potency in this study with MIC values of 8–16 μM, followed by 5-chloro derivative **5b** (16–32 μM). The activity against atypical mycobacteria was retained in the case of the salicylaldehyde as a starting material (**3a** vs. **5a**), but slightly diminished for 5-halogeno-salicylaldehydes (**3d** vs. **5b** and **3e** vs. **5c**). MIC values of pyridine-2-carboxaldehyde conjugate **5d** were higher than 250 μM. The evaluation at higher concentrations was prevented due to solubility problems. These data are a proof of the importance of the salicylidene structural



Scheme 3. Synthesis of 2-(5-arylmethylidene-4-oxo-2-thioxothiazolidin-3-yl)-*N*-(4-chlorophenyl)acetamides **5** (X = N, C; if X = N then Y is missing, if X = C then Y = OH; R = H, Cl, Br).

Table 1
Overview and antimycobacterial activity of rhodanine derivatives **1**–**5**.

Code	X	R	MIC [μM]									
			Mtb. 331/88		*M. avium* 330/88		*M. kansasii* 235/80			*M. kansasii* 6509/96		
			14 d	21 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
RAA	- (free acid)		1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
												
1a	NH	H	1000	1000	1000	>1000	1000	1000	1000	1000	1000	1000
1b	NH	Cl	**32**	**62.5**	1000	1000	250	500	500	**125**	**250**	**250**
1c	NH	CF_3	500	1000	>1000	>1000	1000	1000	1000	1000	1000	1000
1d	NH	CH_3	500	500	1000	>1000	250	500	500	500	500	500
1e	NH	OCH_3	500	1000	1000	>1000	500	500	1000	500	500	1000
1f	NH	NO_2	500	500	1000	1000	500	500	1000	500	500	1000
2a	O	H	1000	1000	1000	1000	500	1000	1000	500	1000	1000
2b	O	Cl	250	500	1000	1000	250	500	1000	**250**	500	500
2c	O	CF_3	500	1000	1000	1000	1000	1000	1000	1000	1000	1000
2d	O	CH_3	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
2e	O	OCH_3	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
2f	O	NO_2	500	500	1000	1000	500	500	1000	500	500	500
												
3a	C	2-OH	**32**	**32**	**125**	**125**	**32**	**62.5**	**62.5**	**32**	**62.5**	**62.5**
3b	CH	3-OH	1000	1000	>1000	>1000	1000	1000	1000	500	1000	1000
3c	CH	4-OH	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
3d	C	2-OH-5-Cl	**32**	**62.5**	**62.5**	**62.5**	**32**	**62.5**	**62.5**	**32**	**62.5**	125
3e	C	2-OH-5-Br	**32**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	125	250
3f	N	–	250	500	1000	1000	500	1000	1000	500	500	1000
4	C	2-AcO	**32**	**32**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**	**62.5**
												
5a	C-OH	H	**8**	**16**	**62.5**	**125**	**32**	**62.5**	125	**62.5**	125	125
5b	C-OH	Cl	**16**	**32**	250	250	125	125	250	125	250	250
5c	C-OH	Br	**32**	62.5	500	500	500	>500	>500	125	250	500
5d	N	H	250[a]	250[a]	250[a]	250[a]	250[a]	250[a]	250[a]	250[a]	250[a]	250[a]
INH			0.5	1	>250	>250	>250	>250	>250	4	8	16

Mtb: *Mycobacterium tuberculosis*. INH: isoniazid.
The best values for each strain and each group of the derivatives are provided in bold.
[a] At presented concentration the growth of strain was observed, at duplex concentration, there was present precipitate and/or turbidity, therefore it was not possible to determine exact MIC value.

fragment for the antimycobacterial activity, preferably without any halogen substitution at the position 5.

When compared to antimycobacterial agent isoniazid (INH), none of the rhodanine derivatives **1**–**5** exceeded its *in vitro* potency against *Mtb.* $H_{37}Rv$ and *M. kansasii* 6509/96. The situation is different in the case of INH-resistant non-tuberculous strains (*M. avium* 330/88, *M. kansasii* 235/80). Six derivatives (**3a**, **3d**, **3e**, **4**, **5a**, and **5b**) showed lower MIC values ($\geqslant$32 μM) than this first-line drug. The growth inhibition of the atypical mycobacteria was independent of the presence of INH-resistance or susceptibility.

2.2.2. In vitro antibacterial activity

Antibacterial activity was evaluated by the microdilution broth method against eight strains: *Staphylococcus aureus* (SA), methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* (SE) and *Enterococcus* sp. (EF) were representatives of Gram-positive bacteria, while Gram-negative strains involved in our study were *Escherichia coli* (EC), *Klebsiella pneumoniae*, ESBL-positive *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. An established drug bacitracin (BAC) used for treatment of topical bacterial infections was used as a reference drug for the comparison.

Generally, the Gram-positive strains were more susceptible than the Gram-negative species (see Table 2). None of the rhodanines **1**–**5** was able to block the growth of *P. aeruginosa* and both strains of *K. pneumoniae*, while *E. coli* was inhibited by only three salicylidene derivatives (**3a**, **3d**, **3e**) with MIC of 500 μM. MIC values for *Staphylococci* and *Enterococcus* were similar each other within the range of 15.62–500 μM. There was not any difference in the activity against a methicillin-susceptible *S. aureus* and a MRSA strain, thus indicating no cross-resistance with beta-lactams. However, several rhodanine-based molecules were inactive (parent RAA, amides **1c**-**1e**, esters **2b** and **2d**, condensate **3f** and conjugate **5a**). The highest antibacterial activity exhibited 4-(trifluoromethyl)phenyl ester **2c** (MIC = 15.62 and 62.5 μM after 24 and 48 h of incubation, respectively), followed by 5-salicylidene derivative **3a** and its acetylated analogue **4**.

4-Nitrophenyl amide **1f** showed slightly improved activity when compared to other amides **1**. The reaction of RAA with hydroxybenzaldehydes improves antibacterial activity uniformly. The presence of a hydroxyl group at the position 2 of the benzylidene moiety is an optimal choice (**3a** and **4**); its positional isomers (3-hydroxybenzaldehyde and 4-hydroxybenzaldehyde derivatives

Table 2
Antibacterial activity of rhodanines **1**–**5**.

Code	MIC [μM]									
	SA		MRSA		SE		EF		EC	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
1a	500	500	>500	>500	500	500	500	500	>500	>500
1b	>500	>500	>500	>500	500	>500	500	>500	>500	>500
1f	500	500	500	500	250	250	250	250	>500	>500
2a	>125	>125	>125	>125	>125	>125	125	>125	>125	>125
2c	**15.62**	**62.5**	**15.62**	**62.5**	**15.62**	62.5	**15.62**	**62.5**	>500	>500
2e	>500	>500	>500	>500	500	>500	500	>500	>500	>500
2f	250	500	500	500	500	500	500	500	>500	>500
3a	**62.5**	**62.5**	**62.5**	**62.5**	125	125	125	125	**500**	>500
3b	>500	>500	500	>500	>500	>500	500	>500	>500	>500
3c	500	>500	500	>500	250	500	>500	>500	>500	>500
3d	250	250	125	125	62.5	62.5	125	500	**500**	>500
3e	250	250	125	125	62.5	62.5	500	500	**500**	**500**
4	**62.5**	**62.5**	**62.5**	**62.5**	**31.25**	**31.25**	**62.5**	125	>500	>500
5b	250	>250	125	>250	250	>250	>250	>250	>250	>250
5c	500	500	500	500	500	500	500	500	>500	>500
5d	>125	>125	>125	>125	>125	>125	125	>125	>125	>125
BAC	15.62	31.25	15.62	31.25	15.62	31.25	31.25	31.25	>500	>500

SA: *Staphylococcus aureus* CCM 4516/08; MRSA: methicillin-resistant *Staphylococcus aureus* H 5996/08; SE: *Staphylococcus epidermidis* H 6966/08; EF: *Enterococcus* sp. J 14365/08. *Escherichia coli* CCM 4517. BAC: bacitracin.
The best values for each strain are provided in bold.

3b and **3c**, respectively) share a lower activity. The acetylation of **3a** to form **4** decreased MIC values for *S. epidermidis* (125 μM vs. 31.25 μM), otherwise the activities are comparable. On the other hand, halogenation of **3a** to more lipophilic chlorine and bromine derivatives **3d** and **3e** led to an unexpected reduction of the activity towards methicillin-susceptible *S. aureus* and *Enterococcus* sp.; MIC values for MRSA and *S. epidermidis* are analogous. The conjugates **5a-5d** joining RAA, *N*-(4-chlorophenyl) and C-5 arylalkylidene structural motifs showed only a limited (**5b**-**5d**) or no antibacterial properties (**5a**). This modification mostly retained or slightly improved the activity of amide **1b**, but it abolished the activity of **3a** completely.

Based on MIC values, the ester **2c** was comparable (±one dilution) to bacitracin against all Gram-positive strains and 2-[5-(2-acetoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid **4**against *S. epidermidis*.

Interestingly, resembling catechol-rhodanine-3-acetic acid conjugates were reported as inhibitors of deoxyxylulose 5-phosphate reductoisomerase, an enzyme involved in 2-*C*-methyl-D-erythritol 4-phosphate pathway. However, this enzyme inhibition with low micromolar IC_{50} values did not result in the growth inhibition of *E. coli* for five derivatives from total six.[8]

2.2.3. In vitro antifungal activity

The antifungal action of the rhodanines **1**–**5** were screened *in vitro* against eight species: *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *Trichosporon asahii*, *Aspergillus fumigatus*, *Absidia corymbifera* and *Trichophyton mentagrophytes* (Table 3). Fluconazole was used as a reference drug.

Two fungal strains, namely *Candida krusei* E28 and *Absidia corymbifera* showed a total resistance to all rhodanines involved. Unfortunately, parent RAA, five amides (**1b**-**f**), three esters (**2a**, **2d**, **2e**), four C5-substituted derivatives (**3b**, **3d**-**f**) and all of the conjugates **5a**-**d** avoided any antifungal activity. Surprisingly, our results did not confirm previously reported an excellent antifungal activity of the compound **3f** against yeasts (MIC values $\geqslant$0.98 μM).[11] The activity against *Candidae*, *Trichosporon asahii* and *Aspergillus fumigatus* was only sporadic at the concentration of 500 μM. Only *Trichophyton mentagrophytes* showed a comparatively higher susceptibility. The growth of this mould strain was inhibited by five rhodanines (**2b**-**2f**, **3a**, **4**) with MIC values of 125–500 μM. In general, the esters **2** and derivatives of salicylaldehyde were superior to others. However, the rhodanines **1**–**5** failed in the searching for potential antimycotic drugs active at low micromolar concentrations.

Table 3
Antifungal activity of selected rhodanine derivatives.

	$MIC/IC_{80}/IC_{50}$ [μM]											
	CA		CT		CG		TA		AF		TM	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	72 h	120 h
1a	>500	>500	**500**	**500**	>500	>500	>500	>500	>500	>500	>500	>500
2b	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	**500**	**500**
2c	>500	>500	>500	>500	**500**	>500	**500**	>500	>500	>500	**125**	**250**
2f	>500	>500	>500	>500	>500	>500	>500	>500	**500**	>500	**125**	**250**
3a	**500**	**500**	>500	>500	>500	>500	>500	>500	>500	>500	**250**	**250**
3c	**500**	**500**	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
4	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	**250**	**500**
FLU	0.24	0.24	>500	>500	62.5	500	250	500	>500	>500	7.81	125

CA: *Candida albicans* ATCC 44859; CT: *Candida tropicalis*; CG: *Candida glabrata* 20/I; TA: *Trichosporon asahii* 1188; AF: *Aspergillus fumigatus* 231; TM: *Trichophyton mentagrophytes* 445. FLU: fluconazole.
The positive hits are provided in bold.

3. Material and methods

3.1. Chemistry

3.1.1. General

All of the reagents and solvents were purchased from Sigma-Aldrich (Darmstadt, Germany) or Penta Chemicals (Prague, Czech Republic), and they were used as received. The progress of the reactions and the purity of the products were monitored by thin-layer chromatography using a mixture of toluene with ethyl acetate (4:1, v/v); plates were coated with 0.2 mm Merck 60 F254 silica gel and were visualised by UV irradiation (254 nm). Melting points were determined on a Büchi Melting Point machine B-540 apparatus using open capillaries, and the reported values are uncorrected.

Elemental analysis (C, H, N) was performed on an automatic microanalyser CHNS-O CE instrument (FISONS EA 1110, Milano, Italy). Infrared spectra (ATR) were recorded on FT-IR spectrometer Nicolet 6700 FT-IR in the range of 400–4000 cm^{-1}. The NMR spectra were measured in DMSO-d_6 at ambient temperature on a Varian V NMR S500 instrument (500 MHz for ^1H and 125 MHz for ^{13}C; Varian Comp. Palo Alto, CA, USA). The chemical shifts, δ, are given in ppm, with respect to tetramethylsilane as an internal standard. The coupling constants (*J*) are reported in Hz.

3.1.2. Synthesis

3.1.2.1. Synthesis of amides and esters of rhodanine-3-acetic acid. The synthesis and characterization (m.p., NMR and IR spectra, elemental analyses) of substituted 2-(4-oxo-2-thioxothiazolidin-3-yl)-*N*-phenylacetamides **1a**-**f** and phenyl 2-(4-oxo-2-thioxothiazolidin-3-yl)acetates **2a**-**f** were published recently.[4]

*3.1.2.2. Synthesis of 2-(5-arylmethylidene-4-oxo-2-thioxothiazolidin-3-yl)acetic acids **3**.* An equivalent of 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid (rhodanine-3-acetic acid) (1 mmol) together with 1 equivalent of anhydrous sodium acetate were dissolved in glacial acetic acid (8 mL) followed by addition of 1.1 equivalents of substituted benzaldehyde/pyridine-2-carbaldehyde. The reaction mixture was refluxed for 5 h. After cooling down of the mixture, resulting crystals were filtered off, washed with a small volume of cold water and dried to give products **3**. If necessary, they were recrystallized from acetic acid.

The identity of known compounds (i.e., **3a**, **3b**, **3c**, **3d**, and **3f**) was confirmed by NMR and IR spectroscopy. All spectroscopic characteristics were in accordance with previously reported data. The purity was checked additionally by melting points measurement and elemental analysis.

3.1.2.2.1. 2-[5-(2-Hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid[5,7] ***3a***. Yellow solid; yield 81%; mp 227–230 °C decomp. Anal. Calcd. for $C_{12}H_9NO_4S_2$ (295.33): C, 48.80; H, 3.07; N, 4.74. Found: C, 48.70; H, 3.19; N, 4.87.

3.1.2.2.2. 2-[5-(3-Hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid[7] ***3b***. Yellow solid; yield 82%; mp 292–295 °C decomp. Anal. Calcd. for $C_{12}H_9NO_4S_2$ (295.33): C, 48.80; H, 3.07; N, 4.74. Found: C, 48.98; H, 3.25; N, 4.94.

3.1.2.2.3. 2-[5-(4-Hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid[7,21] ***3c***. Yellow solid; yield 82%; mp > 300 °C (lit. >300 °C[21]). Anal. Calcd. for $C_{12}H_9NO_4S_2$ (295.33): C, 48.80; H, 3.07; N, 4.74. Found: C, 48.89; H, 2.94; N, 4.66.

3.1.2.2.4. 2-[5-(5-Chloro-2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid[6,9] ***3d***. Yellow solid; yield 78%; mp 248.5–251 °C decomp. (lit. 243–247 °C[6]). Anal. Calcd. for $C_{12}H_8ClNO_4S_2$ (329.78): C, 43.70; H, 2.45; N, 4.25. Found: C, 43.79; H, 2.19; N, 4.44.

*3.1.2.2.5. 2-[5-(5-Bromo-2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid **3e***. Yellow solid; yield 81%; mp 231–234 °C decomp. IR (ATR): 3270, 3050, 2982, 2943, 1729, 1703, 1687, 1585, 1484, 1416, 1401, 1392, 1366, 1329, 1292, 1239, 1193, 1134, 1100, 1060, 960, 918, 893, 817, 746, 720, 690, 673 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 13.16 (1H, s, COOH), 11.51 (1H, s, OH), 7.88 (1H, s, -CH═), 7.52–7.46 (2H, m, H4, H6), 6.94 (1H, d, *J* = 8.7 Hz, H3), 4.69 (2H, s, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ 193.63, 167.44, 166.61, 156.86, 135.54, 132.09, 128.56, 122.33, 122.18, 118.68, 110.82, 45.47. Anal. Calcd. for $C_{12}H_8BrNO_4S_2$ (374.23): C, 38.51; H, 2.15; N, 3.74. Found: C, 38.69; H, 2.11; N, 3.93.

3.1.2.2.6. 2-[4-Oxo-5-(pyridin-2-ylmethylene)-2-thioxothiazolidin-3-yl]acetic acid[11] ***3f***. Yellow solid; yield 97%; mp 257–259.5 °C (lit. 260–261 °C[11]). Anal. Calcd. for $C_{11}H_8N_2O_3S_2$ (280.32): C, 47.13; H, 2.88; N, 9.99. Found: C, 46.98; H, 3.00; N, 9.84.

*3.1.2.3. Synthesis of 2-[5-(2-acetoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid **4***. Compound **3a** (0.4 mmol) was suspended in 4 mL of Ac_2O and then 2 drops of concentrated sulphuric acid was added. The mixture was refluxed for 2 h, and then poured in cold water. The resulting precipitate was filtered off and washed with a small volume of cold water to provide pure crystals of **4**.

*3.1.2.3.1. 2-[5-(2-Acetoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetic acid **4***. Yellow solid; yield 78%; mp 137.5–138.5 °C. IR (ATR): 2917, 1746, 1720, 1432, 1399, 1364, 1318, 1240, 1220, 1208, 1125, 1103, 1038, 980, 880, 857, 778, 761, 721 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 13.46 (1H, s, COOH), 7.72 (1H, s, —CH═), 7.62–7.57 (2H, m, H4, H6), 7.47 (1H, td, *J* = 7.6 Hz, *J* = 1.2 Hz, H5), 7.35 (1H, dd, *J* = 7.6 Hz, *J* = 1.4 Hz, H3), 4.73 (2H, s, CH_2), 2.36 (3H, s, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 193.34, 169.13, 167.39, 166.24, 150.03, 132.63, 129.19, 127.20, 126.76, 125.79, 124.66, 124.00, 45.24, 20.85. Anal. Calcd. for $C_{14}H_{11}ClNO_5S_2$ (337.37): C, 49.84; H, 3.29; N, 4.15. Found: C, 49.72; H, 3.05; N, 4.10.

*3.1.2.4. Synthesis of 2-(5-arylmethylidene-4-oxo-2-thioxothiazolidin-3-yl)-N-(4-chlorophenyl)acetamides **5***. *N*-(4-Chlorophenyl)-2-(4-oxo-2-thioxothiazolidin-3-yl)acetamide **1b** (0.5 mmol) together with 1 equivalent of anhydrous sodium acetate were dissolved in glacial acetic acid (5 mL) followed by addition of 1.1 equivalents of substituted benzaldehyde/pyridine-2-carbaldehyde. The reaction mixture was refluxed for 5 h. After cooling down of the mixture, resulting crystals were filtered off, washed with a small volume of cold water and dried to give products **5a-d**. If necessary, they were recrystallized from ethyl acetate/*n*-hexane.

*3.1.2.4.1. N-(4-Chlorophenyl)-2-[5-(2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetamide **5a***. Buff solid; yield 83%; mp 195–198 °C decomp. IR (ATR): 3295, 3040, 1725, 1678, 1593, 1530, 1489, 1454, 1403, 1375, 1356, 1311, 1264, 1242, 1201, 1187, 1175, 1123, 1092, 1036, 1011, 991, 941, 825, 808, 764, 749, 739, 694, 669 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 10.86 (1H, s, OH), 10.56 (1H, s, NH), 8.04 (1H, s, —CH═), 7.59–7.55 (2H, m, H2, H6), 7.41–7.35 (4H, m, H3, H5, H4′, H6′), 7.00–6.95 (2H, m, H3′, H5′), 4.88 (2H, s, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ 194.20, 166.98, 163.62, 157.90, 137.53, 133.50, 129.99, 129.70, 128.96, 127.43, 120.90, 120.70, 120.20, 120.01, 116.55, 46.87. Anal. Calcd. for $C_{18}H_{13}ClN_2O_3S_2$ (404.88): C, 53.40; H, 3.24; N, 6.92. Found: C, 53.68; H, 3.36; N, 7.13.

*3.1.2.4.2. 2-[5-(5-Chloro-2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-N-(4-chlorophenyl)acetamide **5b***. Yellow-orange solid; yield 85%; mp 221–224 °C. IR (ATR): 3296, 3194, 3118, 3076, D2975, 2930, 1739, 1687, 1661, 1619, 1600, 1539, 1492, 1403, 1359, 1325, 1256, 1219, 1198, 1176, 1108, 1094, 1045, 1013, 974, 958, 881, 833, 782, 730, 702 cm^{-1}. ^1H NMR (500 MHz, MSO-d_6): δ 11.26 (1H, s, OH), 10.56 (1H, s, NH), 7.91 (1H, s, —CH═), 7.59–7.54 (2H, m, H2, H6), 7.42–7.35 (4H, m, H3,

H5, H4′, H6′), 6.98 (1H, d, J = 8.7 Hz, H3′), 4.87 (2H, s, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ 193.93, 166.83, 163.55, 156.44, 137.51, 132.75, 129.20, 128.96, 128.56, 127.44, 123.44, 122.58, 121.60, 120.89, 118.26, 46.90. Anal. Calcd. for $C_{18}H_{12}Cl_2N_2O_3S_2$ (439.33): C, 49.21; H, 2.75; N, 6.38. Found: C, 48.95; H, 3.00; N, 6.32.

3.1.2.4.3. 2-[5-(5-Bromo-2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-N-(4-chlorophenyl)acetamide ***5c***. Yellow-orange solid; yield 77%; mp 215.5–217 °C decomp. IR (ATR): 3261, 3058, 1686, 1670, 1583, 1546, 1491, 1416, 1403, 1369, 1358, 1335, 1292, 1254, 1187, 1136, 1093, 1062, 1015, 984, 957, 847, 830, 817, 750, 712, 693, 669 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.47 (1H, s, OH), 10.56 (1H, s, NH), 7.88 (1H, s, —CH═), 7.59–7.54 (2H, m, H2, H6), 7.50–7.47 (2H, m, H4′, H6′), 7.39–7.35 (2H, m, H3, H5), 6.90 (1H, d, J = 9.4 Hz, H3′), 4.87 (2H, s, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ 194.01, 172.16, 166.87, 163.58, 137.52, 135.61, 132.31, 128.95, 127.42, 122.24, 122.04, 120.89, 118.97, 110.27, 46.88. Anal. Calcd. for $C_{18}H_{12}BrClN_2O_3S_2$ (483.78): C, 44.69; H, 2.50; N, 5.79. Found: C, 44.60; H, 2.75; N, 6.01.

3.1.2.4.4. N-(4-Chlorophenyl)-2-[4-oxo-5-(pyridin-2-ylmethylene)-2-thioxothiazolidin-3-yl]acetamide ***5d***. Yellow-green solid; yield 84%; mp 293–296 °C decomp. IR (ATR): 3244, 3044, 2934, 1709, 1672, 1661, 1611, 1594, 1544, 1492, 1433, 1402, 1377, 1326, 1301, 1251, 1227, 1211, 1198, 1125, 1096, 1053, 1014, 979, 951, 910, 827, 783, 743, 728, 705, 692 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.67 (1H, s, NH), 8.83 (1H, d, J = 4.9 Hz, H6′), 8.00–7.89 (3H, m, —CH═, H3′, H4′), 7.60–7.56 (2H, m, H2, H6), 7.50–7.45 (1H, m, H5′), 7.39–7.34 (2H, m, H3, H5), 4.89 (2H, s, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ 200.08, 166.87, 163.67, 151.19, 149.73, 137.92, 137.58, 129.33, 128.94, 128.59, 127.39, 126.68, 124.50, 120.90, 46.46. Anal. Calcd. for $C_{17}H_{12}ClN_3O_2S_2$ (389.87): C, 52.37; H, 3.10; N, 10.78. Found: C, 52.70; H, 3.12; N, 10.92.

3.2. Pharmacology

3.2.1. Antimycobacterial activity

The antimycobacterial activity against *Mycobacterium tuberculosis* (*Mtb.*) 331/88 (H_{37}Rv; dilution of this strain was 10^{-3}), *Mycobacterium avium* 330/88 (dilution of 10^{-5}), and two strains of *Mycobacterium kansasii*, namely 235/80 (dilution of 10^{-4}) and the clinically isolated strain 6509/96 (dilution of 10^{-5}) was evaluated using a previously described method.[22] The following concentrations were used: 1000, 500, 250, 125, 62.5, 32, 16, 8, 4, 2, and 1 μM. MIC is the lowest concentration at which complete inhibition of mycobacterial growth was observed. INH was chosen as a reference compound. The experiments were prepared in quadruplicates and the determination was repeated twice.

3.2.2. Antibacterial activity

The antibacterial activities were assayed against a panel of eight Gram-positive and Gram-negative strains: *Staphylococcus aureus* CCM 4516/08, methicillin-resistant *Staphylococcus aureus* H 5996/08 (MRSA), *Staphylococcus epidermidis* H 6966/08, *Enterococcus* sp. J 14365/08; *Escherichia coli* CCM 4517, *Klebsiella pneumoniae* D 11750/08, extended-spectrum β-lactamases (ESBL) positive *Klebsiella pneumoniae* J 14368/08, and *Pseudomonas aeruginosa* CCM 1961.

The microdilution broth method modified according to standard M07-A07 in Mueller-Hinton broth (HiMedia Laboratories, Mumbai, India) adjusted to pH 7.4 (±0.2) was used. The tested compounds were dissolved in DMSO to final concentrations ranging from 500 to 0.49 μM. Bacitracin (BAC) was used as a reference drug. A bacterial inoculum in sterile water was prepared to reach 0.5 on the McFarland scale (1.5×10^8 CFU/mL). The minimum inhibitory concentrations were assayed as a reduction in growth of at least 95% (IC_{95}) compared with the control. The results were analysed visually. The MIC values were determined after 24 and 48 h of incubation in the dark at 35 °C (±0.1) in a humid atmosphere.[22]

3.2.3. Antifungal activity

The antifungal properties were evaluated against four *Candida* strains (*Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, and *Candida glabrata* 20/I), *Trichosporon asahii* 1188 and three strains of filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, and *Trichophyton mentagrophytes* 445).

The microdilution broth method was used according to the CLSI M27-A3 and M38-A2 guidelines in RPMI 1640 with glutamine (KlinLab, Prague, Czech Republic) buffered to pH 7.0 with 0.165 mol of 3-morpholino-propane-1-sulphonic acid (Sigma-Aldrich, Darmstadt, Germany). DMSO served as a diluent for all the compounds. In yeast, the final size of the inoculum was $5 \times 10^3 \pm 0.2$ CFU/mL, and in the case of the moulds, the final size of the inoculum was $0.5–5 \times 10^4$ CFU/mL. Fluconazole (FLU) was involved as a comparative drug. The MIC values for yeasts and filamentous fungi were assayed as a reduction of growth of at least 80% (IC_{80}) or of at least 50% (IC_{50}) compared with the control, respectively. The results were analysed visually and/or spectrophotometrically at 540 nm. The MIC values were determined after 24 and 48 h of incubation in the dark at 35 °C (±0.1) in a humid atmosphere, but for *T. mentagrophytes*, the final MIC values were determined after 72 and 120 h of incubation.[23]

4. Conclusions

Twenty-four derivatives of rhodanine-3-acetic acid were evaluated as potential antimicrobial agents. Amides and esters were taken over one of our previous work,[4] condensates of RAA with aromatic aldehydes and "mutual conjugates" of *N*-(4-chlorophenyl)-2-(4-oxo-2-thioxothiazolidin-3-yl)acetamide and aromatic aldehydes were synthesized originally. All of these derivatives underwent *in vitro* assays against mycobacteria, eight bacterial and fungal strains.

All of the derivatives were active against mycobacteria. Among the amides and esters, only the *N*-(4-chlorophenyl)amide exhibited a potent antimycobacterial activity (MIC against *Mtb.* of 32–62.5 μM) and only 4-(trifluoromethyl)phenyl ester showed an inhibition of Gram-positive bacteria comparable to bacitracin at low concentrations starting from 15.62 μM. Salicylaldehyde-based rhodanine C-5 condensates produced a more efficient and consistent inhibition of mycobacteria. An additional substitution of salicylaldehyde does not improve antibacterial and antimycobacterial properties significantly with an exception of *S. epidermidis*. Isomeric 3- and 4-hydroxybenzaldehydes and pyridine-2-carbaldehyde-based derivatives lack the activity. Based on these results, the conjugates combining both these pharmacophores were designed. *N*-(4-Chlorophenyl)-2-[5-(2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]acetamide was identified as the most active agent against *Mtb.* with MIC of 8–16 μM. These derivatives were also able to affect the growth of INH-resistant atypical mycobacteria, thus indicating no cross-resistance. Gram-negative bacteria and most of the fungi tolerate the majority of the investigated rhodanines. In comparison, only fungus *Trichophyton mentagrophytes* was inhibited more considerably, although at higher concentrations.

Importantly, all of the reported rhodanines **1**–**5** meet the requirements of Lipinski's rule of five (MW < 500, no more than five hydrogen bond donors and ten hydrogen bond acceptors, log P not > 5).

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Declaration of interest

The authors declare no conflict of interest.

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