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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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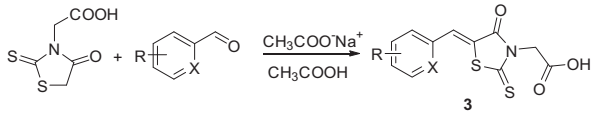


Scheme 1. Synthesis of 5-arylidenerhodanine-3-acetic acids **3** (X = CH, N; R = H, 2-OH, 3-OH, 4-OH, 2-OH-5-Br, 2-OH-5-Cl).

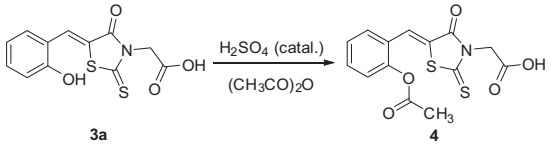
C-5 substituted rhodanine-3-acetic acids were synthesized by heating of parent RAA together with 1.1 equivalents of appropriate hydroxybenzaldehydes (Scheme 1, X = CH) and pyridine-2-carbaldehyde (X = N, R is missing) in glacial acetic acid in the presence of sodium acetate. This procedure takes 5 h 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

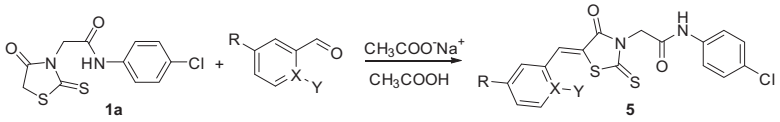
2.2.1. Antimycobacterial activity

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).