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5-Benzylidenerhodanine and 5-benzylidene-2-4-thiazolidinedione based antibacterials

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ABSTRACT

Herein we outline the antibacterial activity of amino acid containing thiazolidinediones and rhodanines against Gram-positive bacteria *Staphylococcus aureus* ATCC 31890, *Staphylococcus epidermidis* and *Bacillus subtilis* ATCC 6633. The rhodanine derivatives were generally more active than the analogous thiazolidinediones. Compounds of series **5** showed some selectivity for *Bacillus subtilis* ATCC 6633, the extent of which is enhanced by the inclusion of a non-polar amino acid at the 5-position of the core thiazolidinediones and rhodanines scaffolds. SAR data of series **8** demonstrated improved activity against the clinically more significant *Staphylococci* with selectivity over *Bacillus subtilis* ATCC 6633 induced by introduction of a bulky aryl substituent at the 5-position of the core scaffolds.

Thiazolidinediones and rhodanines of type **1** (Fig. 1) are an important and versatile class of compounds that show a wide variety of biological activities.[1–9] For example, compounds possessing these scaffolds have recently been shown by us to inhibit cholesterol esterase.[3] In addition, examples are known to have antifungal,[5] antibacterial,[6,7] antiviral,[8] antitumor,[9] and antidiabetic potential.[10] Antibacterial activity is of particular importance given the dramatic rise of drug-resistant bacteria and the paucity of new agents currently in development.[11–14] One report on benzylidenethiazolidinediones identifies the key requirements for antibacterial activity to be an NH at the 3-position, a heteroatom at the 1-position and a substituted phenyl group at the 5-position.[15] This is typified by structure **2a** (Fig. 1) that is reported to show activity against clinically important *Staphylococcus aureus*.[15] Herein, we report a study to extend this new class of antibiotic. Our aim was to study and develop a more chemically versatile and diverse series of thiazolidinediones and rhodanines that contain an amino acid attached to the aryl substituent to explore a wider structural space,[3] with the generation of associated SAR data.

With this in mind we prepared thiazolidinediones and rhodanines **5a–k** by simple amination of **3a** and **3b** with an N-protected amino acid in the presence of EDCI and HOBt according to our earlier report[3] (Scheme 1).[16,17] We also prepared the literature derivative **2a**[15] and the new rhodanine analogue **2b** for comparative activity studies. Both thiazolidinedione and rhodanine derivatives were prepared and assayed to more fully investigate this series. An N-terminal propylphenyl group was incorporated into **5a–k** to allow direct comparison to **2**.

Our series of amino acid containing thiazolidinediones and rhodanines (**5a–k**) have clear advantages over the earlier literature structures of type **2** due to (i) ease of synthesis; (ii) an opportunity to introduce a variety of optically active amino acids and or related groups; and (iii) the constituent amino acid providing a potential

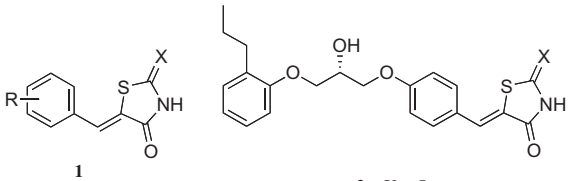

Figure 1. Thiazolidinedione and rhodanine structures.

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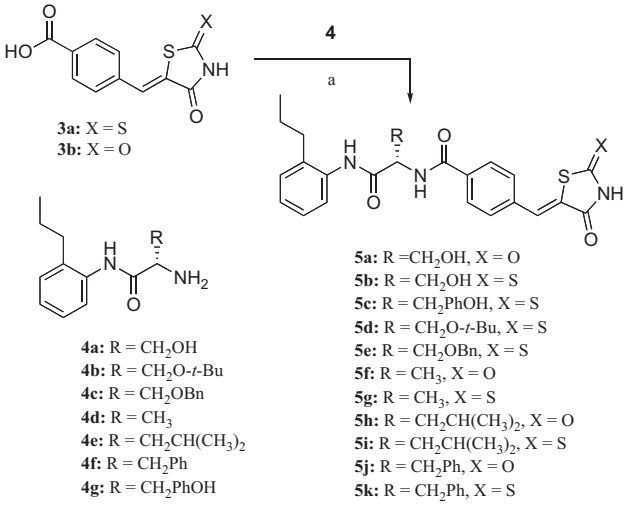


Scheme 1. Reagents and conditions: (a) EDCI, HOBt, DMF, (*i*Pr)$_2$NEt, rt.

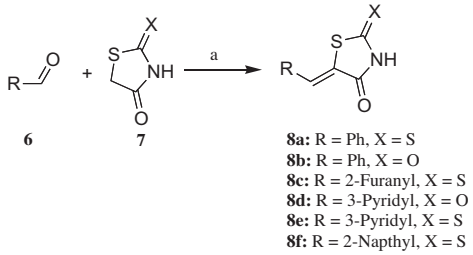
hydrogen bond donor and acceptor, which literature suggests is important for activity.[15]

The minimum inhibitory concentrations (MICs) were determined for all the prepared compounds against three Gram-positive bacteria; *S. aureus* ATCC 31890, *S. epidermidis* and *Bacillus subtilis* ATCC 6633, as per NCCLS protocol[18] using Mueller Hinton broth (Becton Dockson, USA). Contrary to literature,[15] the thiazolidinedione derivative **2a** was only active against *B. subtilis* in our assays that used a slightly different strain of *S. aureus*, see Table 1. Interestingly, the previously untested rhodanine analogue **2b** was active against all three bacteria. A polar amino acid within our new series (**5**) is not tolerated, with the (*S*)-serine containing thiazolidinedione **5a** and rhodanine **5b** derivatives being inactive against all three bacteria. This observation is consistent with the (*S*)-tyrosine analogue **5c** also being inactive against *S. aureus* and *S. epidermis.* However, this derivative did show some activity against *B. subtilis*, as did the benzyl ether protected analogue of **5b**, see **5e**. Significantly, the *tert*-butyl protected rhodanine **5d** was activate against *B. subtilis*, *S. aureus* and *S. epidermidis*.

The introduction of a non-polar amino acid, for example (*S*)-alanine or (*S*)-leucine, gave *B. subtilis* selective compounds in both the rhodanine and thiazolidinedione series (see derivatives **5g**/**5h** and **5i**). Increasing steric bulk with the inclusion of an (*S*)-phenylalanine did not further improve potency (see **5j/5k**), with the rhodanine derivative **5k** being devoid of activity in this case. With the exception of **5d**, all the bioactive examples of **5** are *B. subtilis* selective.

Table 1
Minimum inhibitory concentrations of 2,4-thiazolidinediones and rhodanines

Compds	R	X	MIC (μg/mL)		
			S. aureus	*S. epidermis*	*B. subtilis*
2a	—	O	>64	>64	8–16
2b	—	S	16	32	16
5a	CH_2OH	O	>64	>64	>64
5b	CH_2OH	S	>64	>64	>64
5c	$CH_2C_6H_5OH$	S	>64	>64	8
5d	CH_2O-*t*-Bu	S	16–32	16–32	8
5e	CH_2OBn	S	>64	>64	8
5f	CH_3	O	>64	>64	>64
5g	CH_3	S	>64	>64	32
5h	$CH_2CH(CH_3)_2$	O	>64	>64	8
5i	$CH_2CH(CH_3)_2$	S	>64	>64	32
5j	CH_2Ph	O	>64	>64	8
5k	CH_2Ph	S	>64	>64	>64



Scheme 2. Reagents and conditions: (a) Piperidine, ethanol, 80 °C.

Table 2
Minimum inhibitory concentrations of simple 2,4-thiazolidinediones and rhodanines

Compds	R	X	MIC (μg/mL)		
			S. aureus	*S. epidermis*	*B. subtilis*
3a	4-Carboxy phenyl	O	>64	>64	>64
3b	4-Carboxy phenyl	S	>64	>64	>64
8a	Ph	O	>64	>64	>64
8b	Ph	S	2	4	16
8c	2-Furanyl	S	16–32	16–32	>64
8d	3-Pyridyl	O	>64	>64	>64
8e	3-Pyridyl	S	>64	>64	>64
8f	2-Naphthyl	S	0.5	0.5	>64

We next investigated the antibacterial activity of the thiazolidinedione and rhodanines containing synthetic intermediates **3a,b** and also the aryl analogues **8a,b** and **f**, and the heterocyclic analogues **8c–e** in order to gain further SAR data on this series. These derivatives were synthesized in good yield via Knoevenagel condensation of the relevant aldehydes with **7** as outlined in Scheme 2.[17]

The MICs of **3a**,**b** and **8a**–**f** against *S. aureus*, *S. epidermidis* and *B. subtilis* were determined as shown in Table 2. Interestingly, derivatives **8b**, **8c** and **8f** were more potent than **5** against the clinically important *Staphylococci*, with these compounds being the first examples to show selectivity for this bacterium over *B. subtilis.* Interestingly the rhodanine **8b** displayed broad activity against all three bacteria, with MIC values less than or equal to 16 μg/mL in all cases. The corresponding thiazolidinedione **8a** was inactive, which is consistent with our earlier observation that rhodanines are generally more active than the corresponding thiazolidinediones (see Table 1). The presence of a carboxyl group on the aryl substituent, as in the thiazolidinedione **3a** and rhodanine **3b** is not tolerated, see Table 2. A π-excessive heterocycle is accommodated at this position, with the 2-furanyl rhodanine **8c** being active against *S. aureus* and *S. epidermidis.* A π-deficient 3-pyridyl group is however not tolerated, with the thiazolidinedione **8d** and rhodanine **8e** both being devoid of activity. Interestingly, the introduction of a large naphthyl group (see **8f**) resulted in good potency against *S. aureus* and *S. epidermidis*, but not *B. subtilis*.

Further microbiology testing was performed to better assess the spectrum of activity and also a possible mechanism of action for this class. All compounds were assayed against Gram-negative *Escherichia coli* K12 and an efflux deficient *tolC* mutant strain[19] to define antibacterial spectrum. The more promising entries were also tested against *Pseudomonas aeruginosa* PAO1, *Mycobacterium tuberculosis* H37Rv and BCG and the pathogenic fungi *Candida albicans* SC5314. All compounds were inactive against Gram-negative *E. coli*, suggesting a narrow antibacterial spectrum against Gram-positive species. Noteworthy exceptions include **8f**, which was active against efflux-deficient *E. coli* (MIC 8 μg/mL) but not wildtype K12, and **8e** with anti-*Candida* activity (MIC 10 μg/mL). An assay addressing *S. aureus* cell viability in the initial phase of growth was performed with compounds **2b** and **8b**. The numbers of live

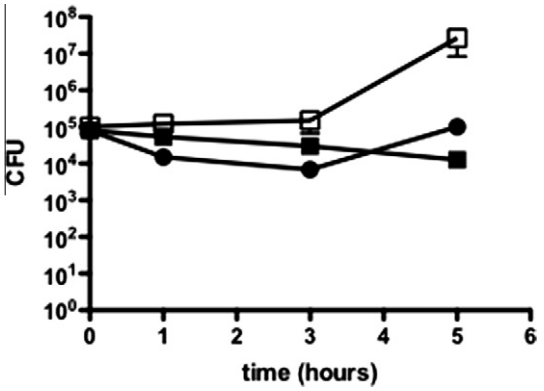


Figure 2. Time course of bacteriostatic action. The numbers of viable *S. aureus* bacteria was assessed after treatment with **2b** (closed circles) or **8b** (closed squares). A DMSO vehicle control (5%) is included (open squares).

bacteria remained constant throughout the 6 h of treatment, demonstrating that the mechanism of action was bacteriostatic (Fig. 2).

In this Letter we report a series of thiazolidinedione and rhodanine compounds **5**, examples of which show good selectivity for *B. subtilis* over other Gram-positive bacteria. Further studies identified related compounds that showed selectivity for *S. aureus* over *B. subtilis*. The scope and possible mechanism of action of this class is also addressed for the first time. The compounds are easy to prepare and their versatile design is amenable to the introduction of a range of different substituents. Thus these compounds represent a particularly versatile addition to the growing list of biologically active thiazolidinedione and rhodanine derivatives with potential, in this case, as antibacterial agents. It appears that rhodanines are generally more active than the thiazolidinediones and non-polar substituents on the aryl group and π-excessive heterocycles are favored. We are currently further investigating the activity of this series against a variety of bacteria.

Acknowledgments

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16. *General procedure for Knoevenagel reaction*: To a solution of respective 2,4-thiazolidinedione or rhodanine (1 equiv) in anhydrous ethanol (20 mL/1 g of 2,4-thiazolidinedione or rhodanine), the respective aldehyde (1 equiv) and piperidine (0.1 equiv) were added in a single portion and heated under reflux under nitrogen for 8 h. The reaction was cooled to room temperature, diluted with water (10 mL) and precipitated with glacial acetic acid. The mixture was filtered and washed with cold water (2×10 mL) followed by ethanol (2×10 mL). The precipitates were dissolved in toluene and concentrated in vacuo to yield the desired pure products **3a**/**b** and **8a**–**f**.[3]
17. *General procedure for amide synthesis*: To a solution of the respective 2,4-thiazolidinedione or rhodanine (1 equiv) and respective amine (1 equiv) in anhydrous DMF (10 mL/1 g of 2,4-thiazolidinedione or rhodanine) under nitrogen was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1.2 equiv), hydroxybenzotriazole (1.2 equiv) and *N*,*N*-diisopropyl ethylamine (4.75 equiv) in a single portion and stirred for 16 h. The solution was diluted with 1 M hydrochloric acid (50 mL) and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with 1 M hydrochloric acid (2×50 mL) followed by brine (2×50 mL) and dried (Na_2SO_4), filtered and the volatiles removed in vacuo. The crude residue was purified by column chromatography to furnish the desired pure products **5a**–**k**.[3]
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