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The Synthesis and SAR of Rhodanines as Novel Class C β-Lactamase Inhibitors

Eugene B. Grant,* Deodialsingh Guiadeen, Ellen Z. Baum, Barbara D. Foleno, Haiyong Jin, Deborah A. Montenegro, Erin A. Nelson, Karen Bush and Dennis J. Hlasta

Antimicrobial Agents Research, The R.W. Johnson Pharmaceutical Research Institute, Route 202, PO Box 300, Raritan, NJ 08869, USA

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Abstract—β-Lactam antibiotics such as the cephalosporins and penicillins have diminished clinical effectiveness due to the hydrolytic activity of diverse β-lactamases, especially those in molecular classes A and C. A structure–activity relationship (SAR) study of a high-throughput screening lead resulted in the discovery of a potent and selective non-β-lactam inhibitor of class C β-lactamases.

Bacterial resistance to β-lactam antibiotics can result from their overuse in treating mild infections and from patient failure in following a prescribed dosing regimen. The surviving bacteria often become resistant due to the β-lactam hydrolyzing enzymes known as β-lactamases.[1] To circumvent this resistance, bacterial infections have been treated by administering a β-lactamase inhibitor with the β-lactam antibiotic.

β-Lactamases are divided into four classes based on their molecular structure. Class C and class A β-lactamases, with an active site serine, represent the majority of all β-lactamases.[2] Commercially available inhibitors such as clavulanic acid (**1**), tazobactam (**2**, TZB), and sulbactam (**3**) inactivate most common class A β-lactamases, but are ineffective clinically against class C β-lac-tamases (Fig. 1).[3] Chromosomally-encoded class C β-lactamases rapidly degrade cephalosporins, and they are generally not inhibited by the existing β-lactamase inhibitors. The most active broad-spectrum β-lactamase inhibitor that is effective against class A and C β-lactamases is BRL 42715 (**4**). However, this compound was not developed as a clinical agent.[4]

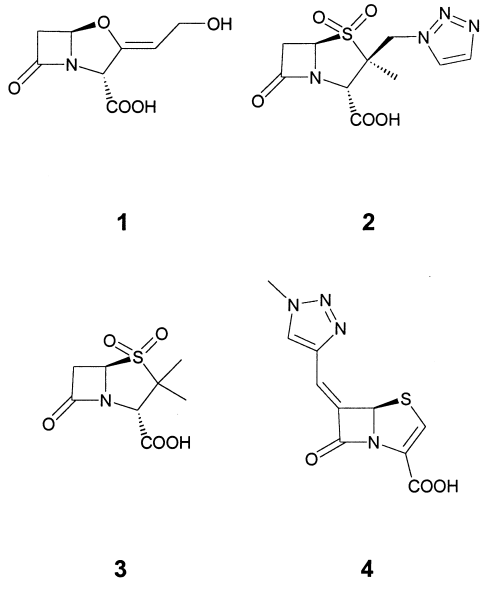

Figure 1.

The class C cephalosporin-hydrolyzing β-lactamase class is an attractive target for drug discovery. As a dominant resistance mechanism in Gram-negative bacteria, it is expected to become more widespread in the absence of a commercially available inhibitor.

High-throughput screening of our chemical library identified rhodanine **5** as a promising lead inhibitor candidate (Fig. 2). P99, isolated from *Enterobacter cloacae*, and TEM-1, isolated from *Escherichia coli*, were used in these studies as prototypical class C and class A β-lactamases.[5] Rhodanine **5** is a non-β-lactam with an IC_{50} of 2.6 μM against the class C enzyme P99 and an IC_{50} of 8.7 μM against the class A enzyme TEM-1. This paper describes our efforts to discover a

*Corresponding author. Tel.: +1-908-218-6589; fax: +1-908-203-8109; e-mail: egrant@prius.jnj.com

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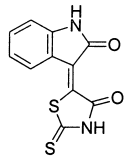
rhodanine β-lactamase inhibitor which is selective for P99 over TEM-1.



IC_{50} (μM)	
P99	TEM-1
2.6	8.7

5

Figure 2.

The simplest modification of **5** was to excise the pyrrolidone ring and to construct analogues by condensation of substituted aromatic aldehydes with 2-thio-thiazolidi-4-none (Fig. 3). In general, synthesis of these alkylidene rhodanines was facile, and only the *Z* isomer was isolated.[6]

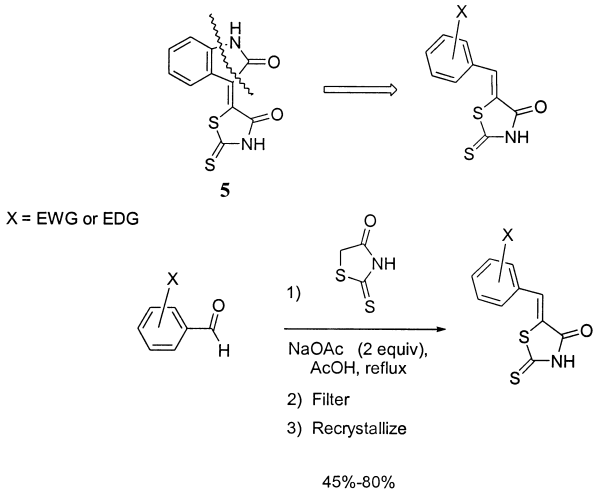


Figure 3.

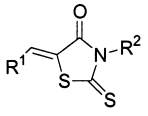
A wide variety of rhodanines was prepared and tested as inhibitors of P99 and TEM-1 β-lactamases. Examining the activity of selected rhodanines, two general trends emerged. First, the most selective compounds for P99 lacked a substituent at the ring nitrogen (Table 1). Second, the analogues with a nitro group on the benzylidene phenyl ring had superior activity.

The relatively weak but selective activity of these analogues encouraged us to extend the sidechain with a two-carbon spacer in order to mimic the cephalosporin substrate of class C β-lactamases. The addition of the two-carbon spacer led to improved IC_{50}'s, while maintaining essentially the same trends observed for the simpler analogues (Table 2). Interestingly, the activity of the saturated analogue **19** was superior to unsaturated **17**, however the selectivity for P99 was diminished. Preparation of the E isomer at C3 of **17** was attempted, but was unsuccessful.

Finally, we decided to examine heterocyclic sidechains. Substitution of an electron withdrawing triazole for the double bond yielded a moderately active analogue, **20**, while the imidazole analogue **21** was inactive. Replacement of the phenyl group with a furan to give analogue **23** further increased the activity. The most active analogue in this study was the 5-nitrofuran analogue **24**. Rhodanine **24** was 200-fold more selective for P99 than TEM-1, as compared to our original rhodanine **5** which was only threefold more selective.

We next examined **24** for synergy with the β-lactam piperacillin in a six-organism panel using TZB as a positive control (Table 3). This panel includes bacterial strains producing class A enzymes and isogenic strains of *S. marcescens* and *P. aeruginosa* producing class C β-lactamases in an inducible or constitutive state. As demonstrated in this panel, piperacillin MICs were lowered against *S. marcescens* and *P. aeruginosa* strains

Table 1. Inhibitory activity of substituted rhodanines[7] against P99 and TEM-1[8]



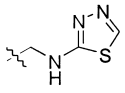
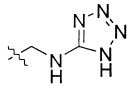
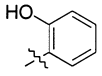
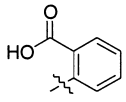
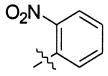
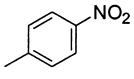
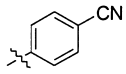
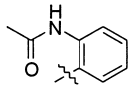
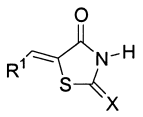
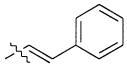
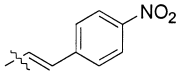
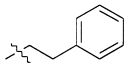
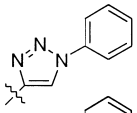
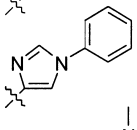
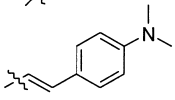
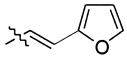
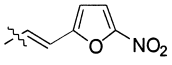
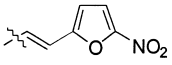
Compound	R^1	R^2	IC_{50} (μM) P99	IC_{50} (μM) TEM-1
6	Phenyl	H	455	>700
7	Phenyl	Ethyl	240	432
8	Phenyl		>700	>700
9	Phenyl		246	138
10	Phenyl		435	>700
11		H	335	>700
12		H	>700	>700
13		H	290	>700
14		H	43	>700
15		H	500	>700
16		H	>700	190

Table 2. Inhibitory activity of substituted rhodanines against P99 and TEM-1



Compound	X	R^1	IC_{50} (μM) P99	IC_{50} (μM) TEM-1
6	S		455	>700
17	S		76	>700
18	S		4.2	100
19	S		9.7	32
20	S		83	>700
21	S		>700	>700
22	S		8	>100
23	S		62	100
24	S		0.45	100
25	O		>50	>50
2		TZB	7.7[a]	0.06

[a]Average IC_{50} of tazobactam in 10 experiments.

Table 4. Antimicrobial activity of rhodanines **24** and **25**[a]

Bacteria	Enzyme	MIC (μg/mL) **24**	MIC (μg/mL) **25**
E. coli OC 4075	TEM-1	2	1
E. cloacae OC 4080	P99	2	0.5
E. cloacae OC 4092	E2 (ind)	32	4
E. cloacae OC 4081	E2 (const)	32	4
P. aeruginosa OC 4270	AmpC (ind)	32	32
P. aeruginosa OC 4290	AmpC (const)	32	32

[a]See Table 3 footnotes for abbreviations.

with inducible or constitutive production of class C cephalosporinases. Because of the inherent antibiotic activity of **24**, it was impossible to evaluate the synergistic potential at concentrations greater than its MIC.[10]

The nitrofuran moiety of **24** was suspected to be associated with the antibacterial activity since 2-nitrofurans, such as nitrofurantoin, are known antibacterials.[12] To test this hypothesis, thiazolidinedione **25** was prepared and tested in the biological assays (Table 4). Thiazolidinedione **25** had better MICs against *E. cloacae* isolates as compared to **24**, but **25** lacked inhibitory activity at the concentrations tested (IC_{50} >50 μM). Therefore, it is likely that the nitrofuran was not responsible for inhibition of P99 in **24**, but is responsible for the antibacterial activity of **24**.

In conclusion, the rhodanines represent a novel class of β-lactamase inhibitors that can be modified to show selectivity for class C β-lactamases. The rhodanines differ chemically from traditional β-lactamase inhibitors such as clavulanic acid and tazobactam, but do exhibit inhibitory activity against both class A and class C β-lactamases.

Table 3. Susceptibility testing of **24** in combination with piperacillin

Bacteria	Enzyme	Molecular class	MIC (μg/mL)[a] Piperacillin	PIP/TZB[b]	**24**	PIP/**24** (1)[c]	PIP/**24** (4)[c]
E. coli OC 4075	TEM-1	A	>64	8	2	>64	NA[d]
E. cloacae OC 4080	P99	C	>64	64	2	>64	NA
S. marcescens OC 4101	AmpC (ind)[e]	C	8	4	8	**2**	**2**
S. marcescens OC 4294	AmpC (const)[f]	C	>64	32	8	32	**8**
P. aeruginosa OC 4270	AmpC (ind)[e]	C	8	8	32	4	4
P. aeruginosa OC 4290	AmpC (const)[f]	C	64	32	32	**16**	**8**

[a]MICs were determined using standard methods according to NCCLS.[11]
[b]PIP, piperacillin; TZB, tazobactam at 4 μg/mL.
[c]Inhibitor concentration in μg/mL shown in parentheses.
[d]NA, not applicable.
[e]Inducible β-lactamase production.
[f]Constitutive β-lactamase production.

References and Notes

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7. All new compounds had satisfactory ^{1}H NMR and mass spectral data.
8. Inhibitor or buffer was incubated with β-lactamase for 10 min. Reaction was initiated by addition of the chromogenic cephalosporin nitrocefin (200 μM final concentration) and monitored spectrophotometrically using a Molecular Devices ThermoMax Microplate Reader, at a wavelength of 490 nm. The IC_{50}, the concentration at which 50% inhibition of the β-lactamase activity is achieved, was determined graphically.[9]
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12. McOsker, C. C.; Fitzpatrick, P. M. *J. Antimicrob. Chemother*. **1994**, *33*, 23.