INHIBITION OF *ESCHERICHIA COLI* TEM-2 β-LACTAMASE BY THE SULFATED COMPOUNDS IZUMENOLIDE, PANOSIALIN AND SODIUM DODECYL SULFATE

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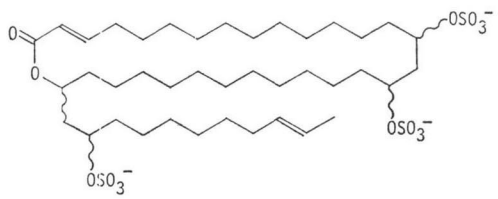
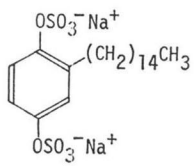
Izumenolide (**I**), which was isolated from *Micromonospora chalcea* subsp. *izumensis*,[1] has recently been reported to be a specific inhibitor of β-lactamase, especially those enzymes from Gram-negative bacteria[2]. In studies with TEM-2 β-lactamase from *Escherichia coli*, incubation of izumenolide and enzyme at molar ratios greater than ten resulted in a complete, and irreversible, loss of activity. This specificity is greater than that observed with clavulanic acid which requires about 115 moles of inhibitor per mole of enzyme before the TEM-2 β-lactamase is inactivated[3].

In contrast to other naturally-occurring β-lactamase inhibitors such as clavulanic acid[4] and the olivanic acids[5~8], izumenolide is not a β-lactam, but a macrolide containing sulfate ester groups[9]. Because the specificity of the molecule appears to be associated with the sulfate ester functionality[2], other enzyme inhibitors containing sulfate groups were studied. Panosialin (**II**), which is widely distributed among *Streptomyces* strains (WELLS and SYKES, unpublished observations), is a mixture of compounds reported to inhibit sialidase, acid phosphatase and polygalacturonase[10]. SDS (sodium dodecyl sulfate, **III**) has long been recognized both as a general enzyme inhibitor[11] and as an inhibitor of β-lactamases[12]. Both II and III are active presumably because of their detergent-like properties. In order to determine whether izumenolide resembles SDS and panosialin, the mode of action of these compounds was studied with respect to their inhibition of TEM-2 β-lactamase.



(I)



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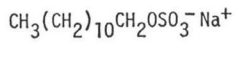


(III)

TEM-2 β-lactamase was purified to homogeneity using a modification of the method described by MELLING and SCOTT[13]. Experimental procedures, including details regarding the spectrophotometric assay of β-lactamase activity, are described elsewhere[2]. SDS was obtained from Sigma, panosialin and izumenolide were isolated from our screening program.

As seen in Table 1, SDS and panosialin behave as pure competitive inhibitors of TEM-2 β-lactamase, indicating that these anionic detergents bind at the β-lactamase active site. However, initial reaction rates in the presence of izumenolide exhibited either mixed kinetic behavior or hyperbolic competitive kinetics[2], suggesting that the binding site for izumenolide may extend beyond the enzymatic active site.

Unlike izumenolide and other β-lactamase inactivators[3] no evidence was observed with either SDS or panosialin for progressive inhibition of the enzyme. Thus, the inhibitory characteristics of the two detergent molecules are significantly different from those of izumenolide.

Multiple inhibition studies were conducted using carbenicillin (a poor substrate) and either SDS or izumenolide, according to the procedure described by YONETANI and THEORELL[14]. As seen in Fig. 1 parallel plots for control enzyme and SDS-treated enzyme indicate that carbenicillin and SDS bind at the same site, whereas the intersecting lines for control enzyme and izumenolide-treated enzyme show that nonidentical binding sites are involved for izumenolide and carbenicillin. These results are consistent with the kinetic studies, which show that SDS binds at the catalytic center whereas izumenolide binding involves another site on the enzyme.

Together, these studies indicate that the TEM-2 β-lactamase recognizes sulfate-containing molecules such as SDS and panosialin at the catalytic

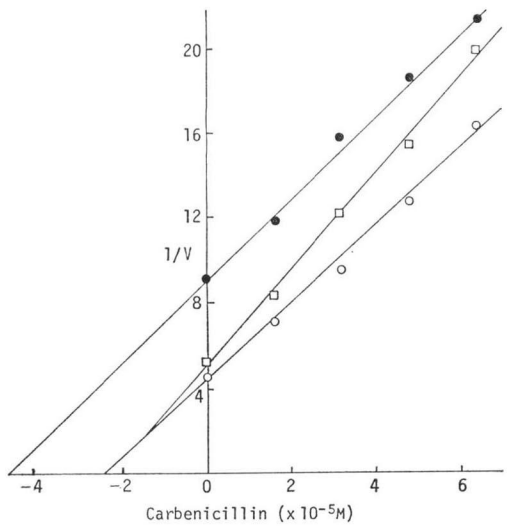
Table 1. Inhibition characteristics for sulfate-containing inhibitors of TEM-2 β-lactamase.

Compound	Inhibition	I_{50} (μM) after 10 min. preincubation[a]	Kinetic pattern of initial reaction	Ki (μM)
SDS	Nonprogressive	54	Competitive[b]	20
Panosialin	Nonprogressive	0.23	Competitive[b]	0.1
Izumenolide	Progressive	0.01	Mixed[b]	N.D.[d]
			Hyperbolic competitive[c]	N.D.

[a] Ampicillin as substrate.
[b] Benzylpenicillin as substrate.
[c] Nitrocefin as substrate.
[d] Not determined.

Fig. 1. Multiple inhibition of TEM-2 β-lactamase by SDS or izumenolide in the presence of carbenicillin.

A 1.05-ml solution of cephaloridine (1.0 mM) in 0.1 M phosphate buffer, pH 7.0, was mixed with either buffer (○—○), 29 μM SDS (●—●) or 1.6 μM izumenolide (□—□) in the presence of varying concentrations of carbenicillin. Assays were initiated by the addition of 10 μl of TEM-2 β-lactamase. Duplicate determinations were performed throughout. Enzyme was 0.4 nM.



site. However, the binding of izumenolide, which is highly specific for β-lactamases from Gram-negative organisms, must involve a second site on the enzyme, although this secondary site may overlap or interact with the active site. The action of izumenolide on TEM-2 β-lactamase as a result of this behavior is quite different from that of an anionic detergent.

Acknowledgment

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