Biophysical Interactions of a Novel, Multi-Substituted β-Lactone Antimicrobial

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Abstract

Antibiotic resistance poses a continuing threat to global health as pathogenic bacteria adopt defenses against conventional β-lactam based therapies, often by the expression of β-lactamase enzymes. Large molecule drugs such as obafluorin, oxazolomycin, and curromycin feature the β-lactone functional group and have been successfully employed as antibiotics but are challenging to synthesize or isolate from natural sources.

Synthesized easily from a ketene heterodimer, a novel, small molecule β-lactone, (3S,4Z)-4-[1-(4-chlorophenyl)ethylidene]-3-methyloxetan-2-one) shows antimicrobial activity against Gram-positive *M. luteus*, Gram-negative *E. coli* K-12, as well as the fungi *S. cerevisiae* and *C. albicans* in disk diffusion assays. Binding affinity of the compound to calf thymus DNA and bovine serum albumin were investigated to characterize interactions with major biomolecules.

The broad-spectrum, intermediate intensity of action of the easily synthesized small molecule support further investigation toward identifying a mechanism of action and consideration of incorporating the β-lactone moiety into future antimicrobial agents.

Introduction

Many current antibiotics featuring the β-lactam group have experienced reduced efficacy or lost antibiotic properties altogether due to resistance mechanisms mounted by bacteria specifically acting on the β-lactam ring. The threat of unchecked infection and increased infection-related deaths call for antibiotics with structures that can evade current bacterial defenses.

The closely related β-lactone group has been found in a natural antibiotic, obafluorin, produced by the bacteria *Pseudomonas fluorescens.* Although the exact mechanism of action of obafluorin is unknown, it is proposed to interfere with protein translation[1]. Obafluorin features a multi-substituted β-lactone with two chiral centers and like penicillin and other analogs, has a peptide bond[2].

Using a simpler, novel β-lactone obtained through collaboration with the Kerrigan Group (formerly of Oakland University) our group has discovered its antimicrobial properties against Gram-positive *Micrococcus luteus,* Gram-negative *Escherichia coli* K-12, as well as antifungal action against *S. cerevisiae* and the more pathogenic *C. albicans* showing favorable properties as a broad-spectrum antimicrobial.

Because of the positive antimicrobial activity observed, biophysical assays were performed to determine the compounds interactions with major biological molecules using calf-thymus DNA (CT-DNA) and bovine serum albumin (BSA) as human analogs.

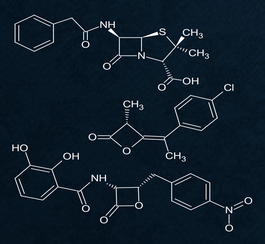

Figure 1. Penicillin (top), ((3S, 4Z)-4-[1-(4-chlorophenyl)ethylidene-3-methyloxetan-2-one) (middle), and obafluorin (bottom)

Methods

BSA stoichiometry was obtained by Uv-vis spectrophotometry by the method of continuous variation of mole fractions of BSA and ligand. Binding was also determined by Uv-vis spectrophotometry by holding BSA at 20 μM and varying ligand concentrations from 16-128 μM. Similarly, ligand held at 20 μM was titrated against CT-DNA varying from 20 - 140 μM. Viscometry was performed at 25°C with a semi-micro Ostwald viscometer with 16 μM CT-DNA against ligand at 2-32 μM.

Results

Serum albumin is a major blood transport protein and can be used to carry biomolecules and drugs. BSA was used as an analog of human serum albumin to determine the binding affinity of the β-Lactone to BSA. A 1:1 stoichiometry was shown by continuously varying the mole ratio of ligand and protein while keeping the total moles constant. The ligand dissociation constant was found to be 600 μM showing a moderate affinity for BSA (Figure 2).

As a possible drug target, DNA binding of the ligand was evaluated by titrating the ligand with CT-DNA. A hyperchromic shift (Figure 3) was observed accompanied by a dissociation constant of 265 μM (Figure 3, inset). The hyperchromic shift suggests denaturing of the strand not necessarily lengthening; viscometric data also did not suggest strand lengthening and when combined with spectral data ruled out intercalation as a binding mode.

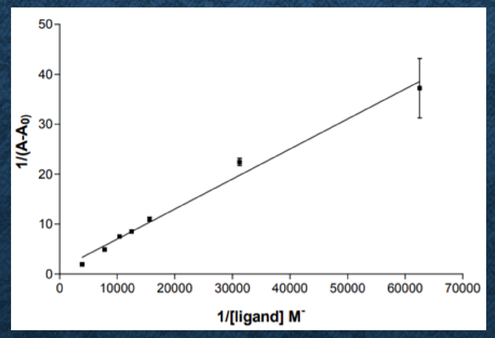

Figure 2. BSA binding to β-lactone. Spectral absorbance data was transformed by the Benesi-Hildebrand method to determine K_D

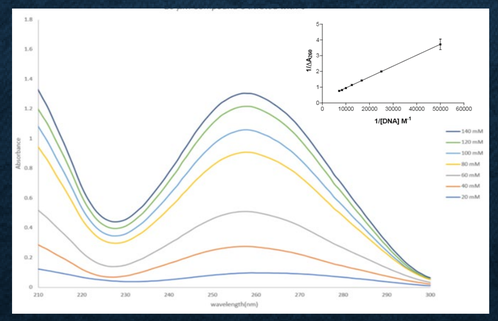

Figure 3. DNA binding to β-lactone. Hyperchromicity suggests possible denaturation; viscosity did not increase suggesting groove binding. Inset: Benesi-Hildebrand transformation to determine K_D.

Using the binding constant, K_b, free energies of binding, $\Delta G°_b$ were calculated as -4123 kJ/mol for ligand binding to BSA and -9365 kJ/mol for CT-DNA.

Discussion

The novel β-lactone demonstrates broad-spectrum antimicrobial behavior and measurable, moderate interactions[3] with the major biomolecules of serum albumin and DNA.

Moderate binding to BSA suggests that the protein can sufficiently transport the compound yet not bind it so tightly that it is irreversibly bound and therefore less bioavailable.

The spectrophotometric and vicometric data for CT-DNA interactions are supportive of major or minor groove binding although a covalent binding model has not been ruled out due to the possibility acylation or alkylation of nucleophilic sites on DNA[4]. The stoichiometric ratio of ligand to CT-DNA remains undetermined by UV-vis spectroscopy due to nearly identical λ_{max} (ligand = 258 nm, CT-DNA = 260 nm).

While the molecule possesses favorable characteristics such as a low molecular weight, intermediate polarity, and relatively easy synthesis, a mode of action and molecular target remain undetermined. To expand on the data obtained thus far, future studies include log *P*, determination of minimum inhibitory concentrations, toxicological profile, and binding to peptidoglycan and ergosterol, the major components in bacterial and fungal cell walls, respectively.

The demonstrated properties of the novel β-lactone studied here have shown that it merits further study for use as a tool in the fight against antimicrobial resistance to existing therapies.

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