Interdisciplinary research-based learning in organic chemistry and microbiology laboratories: Synthesis and biological testing of novel penicillin derivatives.

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Background

The ability of bacteria to gain antibiotic resistance has strengthened the urgency in synthesizing and discovering new drugs to combat the diseases that follow infection. If we were not for the collaborations between scientific disciplines, the production of effective new drugs such as penicillin would not be the same. To encourage undergraduate students to make real-world connections across disciplines, the development of an interdisciplinary organic chemistry-microbiology laboratory experiment was implemented at the Linfield College Organic Chemistry Laboratory. By utilizing discovery-based, authentic research to intentionally encourage student collaboration and improve retention of knowledge gained, a pedagogical experiment involving students from both organic chemistry and microbiology was designed to meet these goals.

Penicillin belongs to the β-lactam class of antibiotics and is effective against most gram-positive bacteria and some gram-negative cocci. The common molecule of penicillin antibiotics is the chemical compound (+)-6-amino penicillinic acid (6-APA), which is capable of being chemically modified (Figure 1). Undergraduate organic chemistry students were able to synthesize 13 penicillin derivatives by performing an amidation reaction on 6-APA (Table 1) using different acyl chlorides, followed by the testing of their antibiotic efficacy in collaboration with microbiology students.

Mechanistically, penicillin functions by inhibiting the formation of peptidoglycan crosslinks within bacterial cell walls, ultimately causing cell lysis through osmotic pressure arising on the cell membrane.

![Figure 2: Acylation of the amine functional group of 6-APA.](image)

**Figure 2:** Acylation of the amine functional group of 6-APA.

**Methods:** Synthesis of penicillin derivatives

(+)-6-amino penicillinic acid (1.08 g, 8.005 mmol) was dissolved in acetone (5 mL) and sodium bicarbonate (20 mL, 1.07 M) was added while stirring until everything was dissolved. Separately, the students each dissolved the acyl chloride of their choice (0.01 mol) in acetone (1-4 mL) and slowly added it to their reaction flask over a period of 5 minutes and then stirred for an additional 40 minutes. The amount of acetone and stirring times varied depending on the solubility of each acyl chloride.

After the reaction was complete, the mixture was extracted with room temperature n-butyl acetate (3x, 6 mL) to remove any unreacted acyl chloride. Cold n-butyl acetate was added to the remaining aqueous phase and made acidic (pH 2) by the slow addition of sulfuric acid (2 mL, 5 M). The organic layer, now containing the penicillin derivative, was separated from the aqueous layer, washed with cold DI water (5 mL), and dried over anhydrous sodium sulfate (15 min).

The dried solution was filtered through a plug of glass wool loosely packed into a Pasteur pipet to remove the drying agent prior to adding 2-ethylhexanoate (2 mL, 1.07 M) while stirring until everything was dissolved. Then the solution was dried for 24 hours. The dried solution was filtered through a plug of glass wool loosely packed into a Pasteur pipet to remove the drying agent prior to adding 2-ethylhexanoate (2 mL, 1.07 M) while stirring until everything was dissolved. Then the solution was dried for 24 hours.

**Results:** Synthesis of penicillin derivatives

A total of 10 penicillin derivatives were synthesized and recovered from the available 13 acyl chlorides (Table 1). Melting points were taken from the 5 penicillin derivatives that crashed out of solution with the addition of sulfuric acid (SM), ranging from the low melting point of n-acetylbenzylpenicillin chloride at 112-114 °C to the slightly higher melting point of penecymat chloride at 181-191 °C. Many of the recovered solids had low percent yields, however, amidation of 6-APA with p-anisoyl chloride produced two samples of recovered solid with a percent yield of 66% and 50% respectively. A total of 9 penicillin derivatives were depurinated into the salt that aided in the chemical solubility when making serial dilutions for antimicrobial susceptibility testing.

**Methods: Antimicrobial Susceptibility**

A disc diffusion method of assaying antimicrobial susceptibility was used to test the different synthesized penicillin derivatives on Staphylococcus aureus. Acylation of 6-APA with cinnamoyl chloride and 3,5-dinitrobenzoyl chloride produced zones of inhibition ranging from 0.5-1.1 mm at high concentrations (Table 1), any diameter less than 29 mm suggests resistance towards the antibiotic however, increased bacterial susceptibility was demonstrated at high concentrations of derivatives testing hydrocinnamyl chloride and phenylacetyl chloride, producing diameters ranging from 12-17 mm. Zones of inhibition were depicted in both S. aureus and Enterococcus faecalis (Figure 4, image left to right respectively) with measured zones of inhibition ranging from 11-17 mm for benzoyl chloride (Figure 3 left image) toward S. aureus.

Remarkably, the penicillin derivative utilizing cyclohexanone carbonyl chloride (Figure 3 right image) yielded the greatest bacterial susceptibility toward E. faecalis, with a measured zone of inhibition of 20 mm at high concentrations. A zone of inhibition greater than 15 mm in this species suggests strong susceptibility towards this antibiotic. Furthermore, it should be noted that phenylacetyl chloride was the only penicillin derivative that caused susceptibility towards the gram-negative E. coli species, resulting in zones of inhibition reaching 22 mm in both medium and high concentrations.

**Educational Results:** Student quotes in response to the study

“Working with partners on an experiment with an unknown outcome was very interesting as I had to collaborate and come together as a lab to work towards answering a research question.”

“Overnight suspensions of TSB broth (TSB) and bacteria to be tested were adjusted to a concentration of 10⁹ organisms/mL, with a 0.5 McFarland Standard. Standardized bacterial broth suspensions were served as the medium for the bacterial “lawn” that is essential for measuring zones of inhibition (mm). Any zone of inhibition greater than 15 mm in this species suggests strong susceptibility towards this antibiotic however, increased bacterial susceptibility was demonstrated at high concentrations of derivatives testing hydrocinnamyl chloride and phenylacetyl chloride, producing diameters ranging from 12-17 mm. Zones of inhibition were depicted in both S. aureus and Enterococcus faecalis (Figure 4, image left to right respectively) with measured zones of inhibition ranging from 11-17 mm for benzoyl chloride (Figure 3 left image) toward S. aureus.

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**References:**


For further information For further information, please email eatkins @linfield .edu,

![Figure 3: Recovered penicillin derivitives as white and yellow solids.](image)

**Figure 3:** Recovered penicillin derivatives as white and yellow solids.

**Table 1:** Qualitative and quantitative collection of data from syntheses and antimicrobial susceptibility testing of penicillin derivatives.

<table>
<thead>
<tr>
<th>Acyl Chloride</th>
<th>Bacterial Species</th>
<th>Zone of Inhibition (mm)</th>
<th>S. aureus</th>
<th>E. feacalis</th>
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<tr>
<td>Penacetyl chloride</td>
<td>S. aureus</td>
<td>22</td>
<td>17</td>
<td></td>
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<tr>
<td>Phenylacetyl chloride</td>
<td>S. aureus</td>
<td>19</td>
<td>18</td>
<td></td>
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<tr>
<td>Hydrocinnamyl chloride</td>
<td>S. aureus</td>
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<td>19</td>
<td></td>
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<td>Benzoyl chloride</td>
<td>S. aureus</td>
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</tr>
<tr>
<td>Cinnamoyl chloride</td>
<td>S. aureus</td>
<td>20</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>3,5-Dinitrobenzoyl chloride</td>
<td>S. aureus</td>
<td>15</td>
<td>14</td>
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</tbody>
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