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 ongoing need to synthesize and discover novel drugs to combat the diseases that follow infection. If it were not for the collaborations between scientific disciplines, the production of effective novel 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 into 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 nucleus of penicillin antibiotics is the chemical compound (+) 6-aminopenicillanic 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.

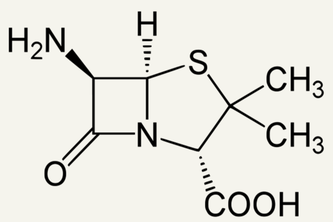

Figure 1: Chemical structure of (+)-6-aminopenicillanic acid (6-APA1); penicillin "head group".

Educational Goals:

This experiment illustrated the benefits of performing open-ended research to create new possible antibiotics and their subsequent biological testing to visualize the antimicrobial efficacy of each antibiotic on bacterial strains through an interdisciplinary undergraduate collaboration between organic chemistry and microbiology courses.

Overall, this experiment gave students in each course the chance to teach and share their newly learned expertise with their peers, to make scientific connections across disciplines, and to address an authentic, open-ended research problem through cooperative learning.

Experimental Development:

To implement this educational experiment into the existing curriculum, an original experiment was designed and tested in the fall of 2014 to develop a synthetic experimental procedure and biological assay that could be used by organic chemistry and microbiology students in the following spring.

Experimental Development (continued):

The synthetic experimental portion had to be completed within a three hour laboratory period, yet provide enough versatility for each set of students to attempt to synthesize different penicillin compounds by varying the acyl tails attached to the penicillin head group. Once the penicillin compounds were synthesized, the organic chemistry student's prepared brief presentations to explain the chemistry behind their syntheses to the microbiology students, who aided in their biological testing, allowing students to visualize the antimicrobial efficacy of their antibiotic on bacterial strains. Microbiology students collaborated in the biological analysis by teaching the chemistry students how to perform a disc diffusion assay and interpret possible susceptibility that the antibiotics may have had on gram-negative and gram-positive bacterial strains.

Synthesis:

The highly reactive beta lactam ring allowed organic chemistry students to synthesize a penicillin compound as part of a parallel combinatorial synthesis. Acyl chloride choice was given to the students that would perform an acylation reaction with 6-APA, enabling the synthesize of a penicillin derivative (Figure 2).[1]

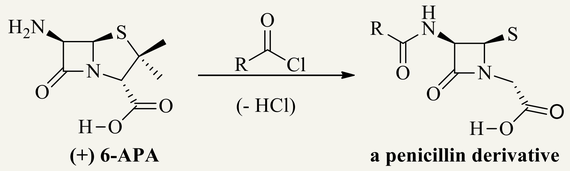

Figure 2: Acylation of the amine functional group of 6-APA.

Methods: Synthesis of penicillin derivatives

(+)-6-aminopenicillanic acid (1.08 g, 0.005 mol) was dissolved in acetone (3 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 into 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 cold 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 slug of glass wool loosely packed into a Pasteur pipet to remove the drying agent prior to adding 2-ethylhexanoate (2ml, 50% w/v in 1-butanol) to induced crystallization of the potassium salt of the penicillin derivative. The crystals were vacuum filtered, washed with acetone (1 mL), and dried for 24 hours.

An IR spectrum, melting point, and percent yield aided in characterization of the synthesized product.

Results: Synthesis of penicillin derivatives

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.**

Acyl Chloride	Bacterial Susceptibility	Salt/ Acid	MP (°C)	Recovered Solid (g)	Percent Yield	Measured Zone of Inhibition (799 IU)
o-acetylsalicyloyl chloride	no	A	112-114	0.074	4%	0 mm (G+/-)
benzyl chloride	no	K+	NA	0	0%	0 mm (G+/-)
p-nitroacetyl chloride	no	K+	NA	0	0%	0 mm (G+/-)
hydrocinnamoyl chloride	yes/yes	K+/K+	NA/ NA	0.2349/ 0.6266	14%/16%	2 mm (G+)/ 16 mm (G+)
penacetyl chloride	yes	A	181-191	0.1482	9%	17 mm (G+), 22 mm (G-)
p-anisoyl chloride	yes/no	A/A	171-185/ NA	1.7219/ 0.9695	66%/50%	8 mm (G+)
3,5-dinitro benzoyl chloride	yes/yes	K+/A	NA/ 180	0.1649/1.65/ 4	8%/79%	1.1 /11 mm (G+)
cinnamoyl chloride	yes	K+	NA	0.4743	27%	1.1 mm (G+)
benzoyl chloride	yes/yes	K+/K+	NA/ NA	0.1534/ 0.0881	10%/6%	17 mm (G+)* 0 mm (G+/-)
p-nitro benzoyl chloride	yes	A	119-123	0.075	NA	0 mm (G+/-)
diphenylacetyl chloride	no	NA	NA	0	0%	0 mm (G+/-)
4-(heptyloxy) benzoyl chloride	NA	NA	NA	0.0527	3%	0 mm (G+/-)
cyclopentane carbonyl chloride	yes	K+	NA	NA	NA	20 mm (G-)*

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 (5M), ranging from the low melting point of o-acetylsalicyloyl chloride at 112-114 °C to the slightly higher melting point of penacetyl chloride at 181-191 °C. Many of the recovered solids had low percent yields, however; acylation 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 deprotonated into the salt that aided in the chemicals 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 both Escherichia coli and Staphylococcus aureus bacterial species.[2] Discs containing known amounts of antibiotics were prepared from serial dilutions of a single derivative, initially making a high concentration solution composed of 0.020 g of the antibiotic and 1000 uL of Millipore DI water (20.0mg/mL: 799 IU). 500 mL of the high concentration solution was mixed with 500 mL of Millipore DI water in order to produce a medium concentration solution (10.0mg/mL; 399.5 IU). An additional 500 mL of medium concentration solution was mixed with 500 mL of Millipore DI water; serving as the low concentration solution (1.0mg/mL; 39.95 IU). A total of 32 paper discs were loaded with 25 uL of each concentration in increasing order of IU, allowing each disc to dry for 24 hours.

Methods: Antimicrobial Susceptibility (continued)

Overnight suspensions of T-soy broth (TSB) and bacteria to be tested were adjusted to a concentration of 10^8 organisms/mL with a 0.5 McFarland Standard[2]. Standardized bacterial broth suspensions served as the medium for the bacterial "lawn" that is essential for measuring zones of inhibition (mm). 150 mm plates of Mueller-Hilton Agar (MHA) allowed for suitable placement of each concentration of antibiotic along with a positive Penicillin G disc, and a disc containing water or ethanol as a negative control depending on the solubility of the antibiotic. Bacterial plates were incubated for 24 hours at 37 °C, allowing bacterial susceptibility to be measured by the diameter of zones of inhibition.

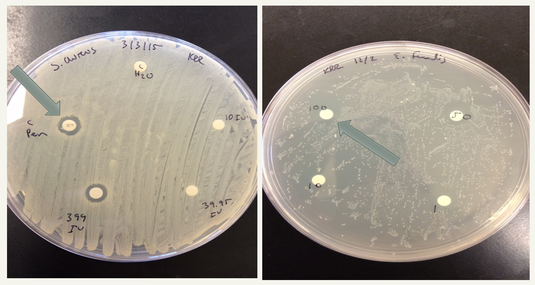

Figure 4: Gram-positive bacterial susceptibility towards synthesized penicillin derivatives

Results: Antimicrobial Susceptibility

Measured zones of inhibition illustrated the antimicrobial efficacy of synthesized penicillin derivatives on *Syaphylococcous aureus*. Acylation of 6-APA with cinnamoyl chloride and 3,5-dinitro benzoyl chloride produced zones of inhibition ranging from 0.5-1.1 mm at high concentrations (Table 1), any diameter less than 29[2] mm suggests resistance towards the antibiotic however. Increased bacterial susceptibility was demonstrated at high concentrations of derivatives testing hydrocinnamoyl chloride and phenylacetyl chloride, producing diameters ranging from 12-17 mm. Zones of inhibition were depicted in both *S. aureus* and *Enterococcus feacalis* (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 cyclopentane carbonyl chloride (Figure 3 right image) yielded the greatest bacterial susceptibility toward *E. feacalis*, 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[2]. 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 answer was very interesting! It was nice to collaborate and come together as a lab to work towards answering a research question."

"This experiment was highly enjoyable due to the fact that it was research oriented and success was not guaranteed."

"I enjoyed the lab research nature instead of following previously optimized protocols. I felt like this lab above all others, I focused on precision and careful documentation of all observations compared to any other lab."

Acknowledgements:

I would like to thank Dr. Elizabeth J.O. Atkinson, Dr. Anne Kruchten, Heather Long, and the Linfield Biology and Chemistry departments.

Reference:

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2. Linfield College Microbiology Lab Manual, (Accessed 11-8-14)
3. Whitaker, R.D., *et.al. J. Chem. Ed.*, 87, 6, 2010, 634-636.

For further information

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