INSTRUCTIONAL DESIGN AND ASSESSMENT

Laboratory Exercises to Teach Clinically Relevant Chemistry of Antibiotics

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Submitted January 14, 2013; accepted August 26, 2013; published March 12, 2014.

Objectives. To design, implement, and evaluate student performance on clinically relevant chemical and spectral laboratory exercises on antibiotics.

Design. In the first of 2 exercises, second-year pharmacy students enrolled in an integrated laboratory sequence course studied the aqueous stability of β -lactam antibiotics using a spectral visual approach. In a second exercise, students studied the tendency of tetracycline, rifamycins, and fluoroquinolones to form insoluble chelate complexes (turbidity) with polyvalent metals.

Assessment. On a survey to assess achievement of class learning objectives, students agreed the laboratory activities helped them better retain important information concerning antibiotic stability and interactions. A significant improvement was observed in performance on examination questions related to the laboratory topics for 2012 and 2013 students compared to 2011 students who did not complete the laboratory. A 1-year follow-up examination question administered in a separate course showed >75% of the students were able to identify rifamycins-food interactions compared with <25% of students who had not completed the laboratory exercises.

Conclusion. The use of spectral visual approaches allowed students to investigate antibiotic stability and interactions, thus reinforcing the clinical relevance of medicinal chemistry. Students' performance on questions at the 1-year follow-up suggested increased retention of the concepts learned as a result of completing the exercises.

Keywords: drug interactions, β -lactam antibiotics, medicinal chemistry, pharmacy practice laboratory, spectral and chemical visual approaches

INTRODUCTION

The switch to the all doctor of pharmacy (PharmD) curriculum enforced more emphasis on pharmaceutical sciences teaching in the context of applications to pharmacy practice and clinical pharmacy.^{1,2} The Accreditation Council for Pharmacy Education (ACPE) Guidelines for 2011 state in Standard 11 that the college of pharmacy must integrate teaching and learning methods that foster the development and maturation of critical thinking and problem-solving skills.² Additionally, the Educational Outcomes for Medicinal Chemistry issued by the Center for the Advancement of Pharmaceutical Education reinforce the concept of comprehending, analyzing, and evaluating information about the chemical structure of drugs in order to implement, monitor, and evaluate pharmaceutical care plans that are patient-specific and evidence based. Clinical pharmacy entails the clinical application

of knowledge of drug chemistry across pharmacological classes.³ In fulfilling the University of Louisiana at Monroe School of Pharmacy's goal to integrate teaching and learning methods, an integrated laboratory class involving active-learning strategies focusing on Medicinal Chemistry and Clinical Pharmacy of antibiotics was developed.

Medicinal chemistry offers pharmacy students the foundational concepts of drug mechanisms of action, structure-activity relationships, acid-base/physicochemical properties, and absorption, distribution, metabolism, excretion, and toxicity profiles.⁴⁻⁶ Understanding chemical and structural information enables pharmacists to rationally justify drug actions, interactions, routes of administration, and best indications. Therefore, medicinal chemistry knowledge provides pharmacy students with critical thinking and evidence-based problem-solving skills for appropriate clinical and therapeutic decisions.⁶⁻⁸ Medicinal chemistry's indispensable role in pharmacy education is best illustrated in the area of infectious diseases.⁹ Pharmacy students can justify the specific indications, spectrum of activity, route of administration, drug-antibiotic and food-antibiotic interactions, stability, and other clinically

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relevant information based on medicinal chemistry knowledge of antibiotics. An example of clinical importance of chemistry knowledge is in the area of β -lactam antibiotics where the student should be able to identify oral or parenteral use, β -lactamases resistance or sensitivity, potency and spectrum of activity, bioavailability, and duration of activity by carefully looking at the chemical structure.

Several models have been published that highlight the clinical relevance and application of medicinal chemistry to pharmacy education.⁴⁻⁸ An example of these is the incorporation of cognitive and affective learning and case studies using representative drug classes through collaboration between experienced pharmacy faculty scholars.⁵ The instructional model used integration of Bloom's cognitive and Krathwohl's affective taxonomies, which significantly improved student performance compared to that in prior years.⁶ This model also improved overall student enthusiasm and offered better understanding of the value of medicinal chemistry to clinical pharmacy practice. Another relevant model attempted the integration of process-oriented guided-inquiry learning and team-based activities to a single-semester medicinal chemistry course.⁸ The implementation of self-selected teams with guided-inquiry exercises significantly improved students' examination scores, grade distribution, and the classroom environment, and offered prompt feedback to the instructor concerning student-knowledge deficiencies compared with traditional teacher-centered lectures. Another model used problem-based learning in which precourse and post-course examinations were given to 2 groups of students: before and after conducting problem-based learning and before and after receiving classroom instruction.¹⁰ Students in the problem-based learning group performed the same on higher-order thought questions as did the non-problem-based learning group (those who received classroom instruction), suggesting that learning occurred in both cohorts. The medicinal chemistry of particular drug classes, including antihyperlipidemics and angiotensin converting enzyme inhibitors, was taught using various learning methods and strategies designed to aid students in quickly identifying the most critical aspects of the particular drug class and practicing required critical-thinking and analysis skills.^{11,12} Various medicinal chemistry teaching examples were also published, including the use of a "Who Wants to be a Medicinal Chemistry Millionaire" learning game to reinforce the importance of chemistry to pharmacy practice,¹³ a model to enhance the knowledge and ability of pharmacy students to better predict the acid-base reactivity and degree of ionization and to convey better understanding of drug actions *in vivo* and *in vitro*,¹⁴ and the use of crossword puzzles as educational aids to enhance pharmacy student learning of anti-ulcer drugs.¹⁵

The current study presents a novel approach that used experimental, chemical, and spectral visual approaches to improve students' learning of antibiotic stability and interactions with food and drugs, with the goal of highlighting the clinical relevance of medicinal chemistry. We believed this unique active-learning strategy would help students understand core concepts of medicinal chemistry and how they can relate this basic science information to their clinical knowledge. Appendix B of the ACPE Guidelines for 2011 provide guidance on the science foundation for the curriculum and recommend the incorporation of laboratory experiences and patient-care simulations into the curriculum.²

Chemistry is a visual science.¹¹ The use of a visual experimental approach can improve knowledge (recalling learned material), comprehension (meaning and understanding), and application (using rules, methods, concepts, principles, laws or theories in new situations).^{6,11} Thus, this study used experimental chemical and spectral visual application approaches to enhance student knowledge and comprehension of the subject with the goal of increasing retention. Student retention was evaluated by the performance measures of examination questions in the lecture-based course associated with these experiments and compared with the 2011 class performance without the benefit of the laboratory exercise. Retention was also assessed with the use of an in-class question in the Pulmonary Module 1 year after the laboratory exercise. To date, no other clinically relevant medicinal chemistry active-learning exercise on antibiotics has been published in the literature. The laboratory exercise is an example of how a medicinal chemistry instructor could incorporate clinical relevance into a practice laboratory.

DESIGN

The β -lactam antibiotics are among the most commonly prescribed drugs, grouped together based on a sharing of the β -lactam ring as the main pharmacophore.⁹ The β -lactam ring is necessary for the bactericidal activity and is highly sensitive to nucleophilic and hydrolytic interactions. Co-administration of β -lactam antibiotics and any other basic nitrogen-containing drugs or antibiotics will result in the hydrolysis and chemical degradation of the β -lactam ring and subsequent inactivation (Figure 1).⁹

Tetracyclines, rifamycins, and fluoroquinolones can chelate polyvalent metal ions (Ca^{2+} , Fe^{2+} , Mg^{2+} , Zn^{2+} , Al^{3+} , Bi^{3+}) to form less water-soluble complexes, thereby considerably reducing their bioavailability and, consequently, their therapeutic effectiveness.⁹ Therefore, concomitant use of these antibiotics and antimicrobials with milk products, vitamins containing minerals, hematinics, and antacids rich in the abovementioned polyvalent

Figure 1. Aqueous and nucleophilic degradation of benzyl penicillin (PG).⁹

ions, can result in significantly reduced therapeutic effects. Pharmacists' knowledge of this information will enable effective patient counseling and better therapeutic outcomes.

Stability and Drug Interactions of β -Lactam Antibiotics

Sixty-one second-year pharmacy students were enrolled in the fourth of six 1-credit-hour courses in an integrated laboratory sequence. The integrated laboratory courses were intended to reinforce the knowledge and skills taught in all of the other courses in which the students were enrolled. The course consisted of a 2.5-hour laboratory section offered weekly. The lecture-based courses within the curriculum, also known as modules, are developed based on disease state(s). Each module integrates basic science and clinical pharmacy instruction into the course, and has been termed an "integrated modular curriculum." This curriculum is different from traditional programs that group together all basic science courses followed by all clinical science courses. The goal of the integrated laboratory sequence within the integrated modular curriculum was to encourage teaching and learning techniques that promoted knowledge-based development; integration, application, and assessment of principles; critical thinking and problem solving; and professionalism as outlined in ACPE accreditation standards.² This laboratory session was designed to teach clinically relevant medicinal chemistry of antibiotics using chemical and spectral experimental approaches. The specific objectives for this laboratory course were for the second-year pharmacy student to be able to use: (1) nuclear magnetic resonance (NMR) testing as an example of analytical tools to assess the stability and common interactions of antibiotics as they relate to the β -lactam pharmacophore stability, (2) thin layer chromatography (TLC) analysis as it relates to the stability of antibiotics, and (3) spectrophotometry to identify the chemical interaction between tetracycline and metal cations. We hypothesized that the

pharmacy students would: (1) perform better on their medicinal chemistry examination based on their hands-on learning in the laboratory, (2) have a better understanding of drug-drug and drug-food interactions and antibiotic stability, and (3) understand the purpose of using selected models of analytical techniques to simulate certain clinical approaches associated with the chosen antibiotics.

Students in each laboratory section were divided into 2 groups of 15 students each. Each group completed either the penicillin stability or the turbidimetry experiment first. After completing the first exercise, the 2 groups switched and conducted the other experiment. Group 1 investigated the aqueous stability of β -lactam antibiotics represented by benzyl penicillin at room temperature and after heating. Stability also included the nucleophilic interaction and chemical incompatibility between benzyl penicillin and other basic nitrogen-containing antibiotics represented by aminoglycosides.

Intact β -lactam ring is essential for effective bactericidal activity.⁹ Beta-lactam antibiotic stability correlates with their interactions with some bacterial β -lactamases, which nucleophilically attack, open, and inactivate the β -lactam pharmacophore, imparting β -lactam antibiotic bacterial resistance (Figure 1).⁹ Similarly, the β -lactam antibiotics can be attacked by other drugs containing basic functional groups by opening the β -lactam ring, resulting in activity loss. Benzyl penicillin's aqueous stability and interactions with streptomycin (representing antibiotics with basic amine groups) and NaOH solutions (representing nucleophiles including bacterial β -lactamases) were monitored by TLC and ¹H NMR spectroscopy.

The group of 15 students was subdivided into groups of 5 students to help the laboratory session flow and ensure everyone was able to participate in small-group discussions with a teaching assistant or instructor. Each group weighed 2 sets of 25 mg benzyl penicillin sodium salt in dry 1-dram vials. These sets were then dissolved in 700 μ L D₂O. A starting line was drawn by a pencil, 0.5 cm

from the bottom of normal phase Si gel G₂₅₄ plates (5 x 10 cm) and 3 tracks were marked at equal distances. About 50 μ L of this solution was spotted at the first track, which was considered the 0-time or reference benzyl penicillin sodium solution. Each solution was transferred to a 5 mm glass NMR tube. The ¹H NMR spectrum was acquired for 1 of these benzyl penicillin solutions using the available JEOL Eclipse-400 spectrometer (Figure 2). This spectrum represented the control benzyl penicillin solution before reactions. A second group of 5 students performed the NMR data acquisitions facilitated by a teaching assistant to keep the students focused on the clinical relevance of the experiment and to minimize class time spent on spectrometer training. About 100 μ L of either 2N NaOH or streptomycin 25 mg/mL solution in D₂O was added to an NMR tube containing benzyl penicillin sodium solution. Each reaction was then gently heated on a preheated water bath at 80°C for 10 minutes. This reaction can be completed at room temperature in a few hours; however, to keep the experiment within the allotted class time, heating was used to reduce the reaction time to a few minutes. The benzyl penicillin-NaOH and benzyl penicillin-streptomycin resultant solutions were then spotted on TLC chromatograms tracks 2 and 3. The ¹H NMR spectrum of each solution was also recorded (Figure 2). TLC chromatogram was developed ascendingly using methanol-acetone-methylene chloride-ammonia solution (3:2:2:2) as a mobile phase following a 20-minute jar saturation with the mobile system.¹⁶ The polarity of this solvent system was optimal to get the R_f value (distance travelled by benzyl penicillin/distance travelled by solvent) of benzyl penicillin around 0.5. This allowed better and easier detection of any more or less polar degradation products. The plates were dried using a hot air gun after complete development. Students then visualized the plates under an ultraviolet (UV) lamp at λ 254 nm (optimal wave length for ultraviolet activity of benzyl penicillin's aromatic chromophore) and marked the UV-active spots using a 0.5 mm pencil. Students also used chemical visualization by spraying the TLC with 1% 4-dimethylaminobenzaldehyde in 10% methanolic HCl and heating the chromatogram with a heating gun for 2-minute at 100°C to give a distinct orange color with benzyl penicillin and a yellow color with its degradation products. (This spray reagent was discovered by the instructor using trial and error because reported spray reagents, like I₂ and soluble starch, take a considerable amount of time to show effective spot colors.¹⁶)

The instructor elaborated on general stability of β -lactam antibiotics as it correlates with shelf storage, concomitant use with other antibiotics, and β -lactamases sensitivity as the students were performing the experiment. Chemical instability of benzyl penicillin toward

nucleophiles and penicillinases was reinforced from lecture material with an emphasis on mechanisms and degradation products (Figure 1).⁹ Penicilloic and penilloic acids identified as prompt degradation products of penicillin, followed by the slow transformation to penilloaldehyde and D-penicillamine (Figure 1).⁹ In the pre-class presentation, the instructor took 5 minutes to remind students of the applications of NMR spectroscopy and the spin-spin coupling concept. No time was invested on the theory and basic aspects of this technique. The instructor also showed the students pictures of the laboratory tools and equipment to be used in class, which saved time and gave the students confidence to run the experiments.¹⁷

The simplicity of the ¹H spectrum of benzyl penicillin in D₂O (Figure 2) significantly shortened the time required for the students to recall the concept. Students were able to identify most of the benzyl penicillin's ¹H NMR signals (Figure 2). This provided the opportunity for the instructors to explain the concept of bioisostere protection of the β -lactam ring against β -lactamases using various chemical strategies and a 3D molecular model to explain geometrical requirements in various β -lactamases-resistant antibiotics. Visually, students were able to confirm the transformation of benzyl penicillin to 2 different degradation products by comparing the original ¹H NMR spectrum of benzyl penicillin with its spectrum after heating with NaOH solution (Figure 2). This was based on the existence of 2 pairs of C-3 methyl singlets (δ 0.82-1.36), different in chemical shift values from those of the original benzyl penicillin signals (δ 1.30 and 1.36, Figure 2). They were also able to draw the same conclusion through observation of the changes in chemical shifts of other benzyl penicillin proton signals or replacement by other signal sets. This conclusion was further confirmed by the TLC analysis of benzyl penicillin solution in D₂O before and after heating with NaOH through the replacement of the original benzyl penicillin spot by 2 more polar spots with much smaller R_f values.

Interaction of Tetracyclines, Rifamycins, and Fluoroquinolones With Polyvalent Cations

The other experiment, which group 2 conducted first, involved the tendency of soluble forms of tetracycline, rifamycins, and fluoroquinolones to form insoluble chelate complexes (turbidity) with polyvalent metal ions including Ca²⁺, Fe²⁺, Mg²⁺, Zn²⁺, Al³⁺, Bi³⁺, usually present in dairy products, multivitamins, hematinics, and antacids. Four successive dilutions of tetracycline base (0.1, 0.2, 0.6, and 0.9%) were prepared first by sonication of each solution for 5 minutes, followed by gentle heating at 60°C, and mixing using a magnetic stirrer bar on a stirrer hotplate. About 2 mL of each solution was treated with 0.1 mL 20%

Figure 2. (A) ¹H NMR spectrum of PG in D₂O. (B) ¹H NMR spectrum of PG's degradation products (penilloic and penicilloic acids) after heating with nucleophiles (operation frequency 400 MHz).

aqueous CaCl₂ solution in 10-mL glass culture tubes, and the reaction was allowed to stand for 5 minutes at room temperature. Because of the formation of an insoluble chelate complex (Figure 3), a light precipitate spontaneously developed causing concentration-dependent turbidity, which was quantitatively measured as percentage transmittance using a spectrophotometer. The concentration of CaCl₂ solution and reaction time were optimized by the instructors prior to the laboratory session to determine the most reproducible readings. Students used graph paper to draw a standard calibration curve. An unknown tetracycline solution (0.3%) was provided to students for determination of its concentration using the standard calibration curve. Rifampicin and ciprofloxacin (1-2%) aqueous solutions (representing rifamycins and fluoroquinolones, respectively) were subjected to the same turbidimetric evaluation for demonstration.

Following completion of the experiments, the remaining 30 minutes of the laboratory session were reserved for the medicinal chemistry and clinical laboratory coordinators to summarize and discuss the results and elaborate on the experiments. Faculty members involved in facilitating the laboratory sessions included the medicinal chemistry course coordinator, clinical laboratory coordinator, and 4 teaching assistants familiar with the laboratory experiments. Table 1 provides a list of resources that can be used to reproduce the same laboratory exercise.

EVALUATION AND ASSESSMENT

Each laboratory exercise was worth 5 points. Students were given 5-points credit for correctly determining the unknown tetracycline concentration and 5 points for determining the time point at which penicillin G was maximally degraded by heat with NaOH in D₂O solution.

Figure 3. Chelation of tetracycline, fluoroquinolones, and rifampicin by interaction with polyvalent metal ions.⁹

The laboratory objectives were assessed both quantitatively and qualitatively. Quantitative assessment involved conducting a postlaboratory Likert-scale survey and the addition of 3 questions pertaining to the 2 laboratory exercises on the examination for the Infectious Disease module. Qualitative assessment included obtaining student comments on the postlaboratory survey instrument and from the end-of-semester course evaluation.

Table 1. Resources Needed for the Medicinal Chemistry Laboratory Exercise

Resources	
Equipment	
	NMR Spectrometer (JEOL ECS-400)
	Spectrophotometer (Thermo Scientific Genesys 20)
	Glass sprayer
	Spectroline Model CM-10 fluorescent analysis cabinet
	UV lamp, λ 254 nm
	Working chemistry hood
	TLC Spray cabinet II (CAMAG)
Supplies	
	1. Deutrated water (D_2O , 100 mL)
	2. 4-Dimethylaminobenzaldehyde (5 g)
	3. 5 mm NMR sample tubes
	4. Culture tubes
	5. Micropipettes
	6. Graph paper
	7. TLC chromatograms (E-Merck)
	8. HCl (100 mL)
	9. Methanol (500 mL)
	10. 2-mL disposable pipette tips
	11. 10 mm Acrylic disposable spectrophotometer cuvettes
Medications	
	12. 5 g Benzyl penicillin sodium
	13. 2 g Rifampicin, tetracycline base, streptomycin, and ciprofloxacin
	14. 10 g Calcium chloride
Support Staff	
	Four teaching assistants
	Facilitate the use of NMR spectrometer
	Facilitate the turbidity experiments and use of spectrophotometer

Abbreviations: NMR=nuclear magnetic resonance; TLC=thin layer chromatography.

The postlaboratory survey identified the degree to which students felt that they understood the purpose of the technique that was used in the experiments and how it related to stability and interactions between antibiotics, and their confidence in retaining this information (Table 2). Students indicated their level of understanding using a Likert scale ranging from strongly agree to strongly disagree, and a “not sure” column. A percentage was calculated based on the number of responses to each category. The first 3 assessment questions were chosen to identify student understanding of the 3 main clinical concepts presented within the laboratory session (drug-drug interactions, drug-food/dietary supplement interactions, and antibiotic stability). The next 3 questions pertained to the laboratory equipment used to test the clinical concepts noted above. The students needed to understand the purpose of the medicinal chemistry analytical techniques as they related to the clinically relevant chemical interactions. The last question tested students’ awareness and retention of information concerning a common drug interaction between tetracyclines, fluoroquinolones, and rifamycins with food and polyvalent metal cations in some medications. The students performed the tetracycline interaction experiment using the spectrophotometer to measure percent transmittance (interaction effect); however, because of time limitations, the students were shown examples of fluoroquinolone and rifamycin solution interactions with calcium chloride solution as the source of metal cation instead of performing the experiment themselves. Ninety-seven percent of students in 2012 agreed (somewhat agreed to strongly agreed) that the laboratory session helped them to better understand drug-drug interactions, drug-food interactions, and β -lactam antibiotic stability. This percentage increased in 2013 to 100% of students.

In written comments on the survey instrument, students with prior experience with these same experimental techniques in prepharmacy or undergraduate course work finally understood the importance and purpose of the NMR, spectrophotometry, and chromatography as they related to medications. Additionally, many commented that the hands-on experiments helped them better understand the material being presented in class and/or it was a fun way to grasp the information. Eighty-five percent of

Table 2. Postlaboratory Student Survey of the Medicinal Chemistry Laboratory Session^a

Item	Strongly Disagree, %		Disagree, %		Somewhat Agree, %		Agree, %		Strongly Agree, %		Not Sure, %	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Laboratory session helped me better understand drug-drug interactions.	0	0	0	0	11	9	38	40	51	51	0	0
Laboratory session helped me better understand drug-food/dietary supplement interactions.	0	0	2	0	8	15	41	35	49	51	0	0
Laboratory session helped me better understand β -lactam antibiotic stability.	0	0	1	0	7	1	31	27	61	72	0	0
I understood the purpose and use of NMR testing as it relates to the stability of antibiotics.	0	0	0	0	15	7	26	23	59	71	0	0
I understood the purpose and use of thin layer chromatography testing as it relates to the stability of antibiotics.	0	0	2	0	11	8	33	24	54	68	0	0
I understood the purpose of spectrophotometry as it relates to the chemical interaction between tetracycline and metal cations (dairy products).	0	0	0	1	11	4	23	28	66	65	0	1
I will remember the drug interaction between tetracycline and food/dairy products better because of this laboratory.	0	0	3	0	0	7	33	24	64	68	0	1

^a Number of students responded: 2012=61 students (84%), 2013=75 students (90%).

the students in 2012 and 100% of the students in 2013 understood the purpose of the NMR testing as it related to stability of antibiotics. Eighty-nine percent of the students in 2012 and 100% of the students in 2013 understood the purpose of the TLC and spectrophotometry as it related to stability of antibiotics and chemical interactions, respectively. Ninety-seven percent of students in 2012 and 99% of students in 2013 agreed that they would remember the drug interaction between tetracycline and food/dairy products because of this laboratory exercise.

Students' performance on 3 Infectious Disease module examination questions concerning the topics covered in the laboratory and administered after this laboratory class were evaluated and compared with the 2011 students who did not complete this laboratory session (Table 3). The questions administered in all 3 years were identical. In the first question, students were asked to identify which antibiotic class's bioavailability would be adversely affected by forming chelate complexes with food and dairy products. In the second question, 5 different representative classes of antibiotics, including an aminoglycoside and penicillin G, were presented and students were required to identify the chemically incompatible antibiotic classes. In the third question, 5 different fluoroquinolone structures were presented with 1 missing the C-3 carboxy group. Students were asked to identify the fluoroquinolone structure that would not interact with polyvalent metal cations. The percentage of correct answers for each year is noted in Table 3. All 3 years of students were taught by the same instructor using identical teaching methodology and under the same curriculum. Students did not have access to questions after the 2011 and 2012 examination; therefore, 2012 and 2013 students had no advantage or prior knowledge of questions.

A paired *t* test was used to compare the overall percentage scores in 2011 vs 2012 and 2013 to determine if a significant difference resulted from participation in the laboratory session. The students who completed the laboratory exercises in 2012 performed much better than the students from the previous year who had not ($p=0.047$). In 2013, a significant improvement was noted in examination scores ($p=0.054$).

A year after taking this laboratory class, >75% of the 2012 students were able to identify rifamycins-food interactions in an antimycobacterial medicinal chemistry class, compared with <25% of students in the previous year who had not completed the laboratory exercise described, suggesting improved retention of knowledge of the concept.

DISCUSSION

The manuscript provides an example of a pharmacy practice laboratory session that emphasizes the clinical relevance of medicinal chemistry of antibiotics. Based

Table 3. Comparison of Students' Performance on Examination Questions^a

Class	Tetracycline-food	Aminoglycoside-Beta-lactam	Fluoroquinolone-metal cations
	No. of Correct Answers/Total Student, Ratio (%)	No. of Correct Answers/Total Student, Ratio (%)	No. of Correct Answers/Total Student, Ratio (%)
2011	32/45 (71.1)	41/45 (91.1)	36/45 (80)
2012	67/73 (91.8)	73/73 (100)	70/73 (95.9)
2013	81/83 (97.5)	81/83 (97.5)	78/83 (94)

^a In the 2011 class, 45 students did not take the medicinal chemistry laboratory class, unlike the 2012 (73 students) and 2013 class (83 students).

on their experience in the laboratory session, we hypothesized that the pharmacy students would perform better on their medicinal chemistry examination in the corresponding course module, and have a better understanding of certain antibiotic stability and interactions with food and other drugs as well as of the laboratory equipment used to conduct the experiments. Course objectives for this exercise were to enable the students to use analytical techniques (NMR, chromatography, and spectrophotometry) as they correlated with the stability and interactions of certain antibiotics. Based on the response to the in-class questions, examination scores, and student comments, the short-term course objectives were met. Instructors are planning to use more clinically oriented questions for assessment in the future.

Turbidimetric experiments provided students with additional virtual evidence and documentation of the interaction of polyvalent cations in food, vitamins, hematinic, antacids, and many drugs with certain 1,3-keto-enol pharmacophore-containing antibiotics including tetracyclines, fluoroquinolones, and rifamycins. The instructor elaborated on the chemistry and the nature of the irreversibly formed metal chelate complex and how its poor water solubility can preclude gastrointestinal absorption and adversely affect the therapeutic activity of some antibiotics (Figure 3). In-depth discussions with students on the importance of counseling their patients about these important drug interactions and examples of various foods, vitamins, antacids, etc, were reinforced through open discussion with the students as they were performing the experiment. Student groups were asked to draw a standard calibration curve. Individually, students were asked to determine the concentration of an unknown tetracycline solution using the calibration curve, providing hands-on virtual and analytical experience. The students also noted that the higher the concentration of the tetracycline, the more precipitate formed, thus a more pronounced drug interaction. Additionally, instructors elaborated on the clinical relevance of experiments and how they correlated with β -lactam antibiotics use for β -lactamase-producing infections and interactions with other drugs containing basic functional groups when concomitantly used.

The performance of 2011 students who did not complete this laboratory session and 2012 and 2013 students

who did was evaluated using examination questions covering the laboratory topics (Table 3). These topics covered the identification of tetracyclines and fluoroquinolones as the antibiotic/antimicrobial entities that will encounter inhibition of gastrointestinal absorption if concomitantly used with milk, dairy products, hematinics, or bismuth-containing antacids (Table 3). The performance of 2012 students significantly improved by 15.9%-20.7% in these questions, compared to 2011 students and improved by 14%-26.4% in 2013, compared to 2011 students. This clearly shows the positive impact of this experimental and virtual experience on improving the knowledge base of the students. Another question topic addressed the chemical incompatibility of aminoglycosides and β -lactam antibiotics. There was an 8.9% performance improvement for the 2012 class and a 6.4% improvement for the 2013 class vs the 2011 class (Table 3), which further defines the importance of experimental approach on the learning ability of students. Student retention of information was assessed a year later during a medicinal chemistry class on antimicrobial agents. More than 75% of students were able to recall the food and polyvalent cation-rifampicin interactions. Only 25% of students who did not take this integrated laboratory sequence course responded positively to the same question with the same instructor in the same class the previous year. Teaching medicinal chemistry using active-learning strategies and hands-on experiments within the integrated laboratory class can be an effective approach to improve student learning without decreasing the amount of factual content the student is receiving in the corresponding lecture-based course.¹⁰ This approach is different from any previously published active-learning strategies.¹⁸ Although students did not perform patient counseling in this laboratory session, another pharmacy practice laboratory session the following semester allowed students to counsel on a tetracycline interaction with antacids. Although, no formal assessment was done to compare students' performance in the 2 sessions, they had no trouble remembering what they had learned about antibiotic interactions and successfully counseling their patient.

Despite every effort to minimize problems during the laboratory instruction, a few issues came up and were documented for improvement in future laboratory sessions. The

¹H NMR testing took the longest time, and this created a backup of students waiting to use the spectrometer. In a subsequent laboratory session held in 2013, instead of each student performing individual NMR acquisitions, instructors subdivided the group of 15 students to work in groups of 3 or 4. Instructors also discovered an issue with the stability and reproducibility of the results for tetracycline salts. The tetracycline solutions were prepared a few days prior to the laboratory exercise and tested; thus, this issue was resolved before the first laboratory exercise by the use of free tetracycline base. Free base has less water solubility, but proved to be more stable and experimentally reproducible. Some of the antibiotics were initially on backorder and delivery had to be rushed. Based on medication availability, we recommend ordering all supplies well in advance of the laboratory session. Instructors had planned to meet with the students for 30 minutes following the experiments to summarize the results and elaborate on the laboratory activity. Because of the extended length of time used to run individual NMR samples, the postlaboratory discussion in 2012 was relatively short. Several students suggested having a longer concluding presentation and discussion following the laboratory experiments. By improving time management, we were able to implement this in the 2013 laboratory and the longer discussion period will be continued in future laboratory exercises.

CONCLUSION

This study demonstrates the use of experimental approaches to emphasize the clinical relevance of medicinal chemistry and reinforce students' knowledge and skills of certain antibiotics. The understanding of these important antibiotic interactions is necessary for effective future counseling in the area of antibiotics and antimicrobials with regard to food and drug interactions. This was achieved by implementing new visual teaching and learning techniques that promoted knowledge-based development and reinforcement of medicinal chemistry concepts with clinical relevance. This laboratory exercise may motivate other medicinal chemistry instructors to design and incorporate medicinal chemistry exercises with clinical relevance into integrated laboratory settings.

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