

A PROBLEM-BASED LEARNING APPROACH TO INTRODUCE THE ENZYME INHIBITORS BLIND SCREENING TO UNDERGRADUATE BIOCHEMISTRY STUDENTS

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Abstract

Learning styles based on inquiry, discovery, and problem-based approaches can promote students' problem-solving skills, critical thinking, and self-confidence development. Our Educational Innovation Group TR4BIOCHEM (PIE22-067) is interested in implementing new inquiry-based biochemistry laboratory experiments focused on the last course-chemistry and biochemistry undergraduate students. The high throughput blind screening of enzyme inhibitors, one of the most widely used strategies in pharmacology for the discovery of new drugs, is the subject of a new problem-based learning (PBL) activity developed at the University of Malaga in the last few years. Within the subject "Pharmacological Biochemistry", 4th year-biochemistry undergraduate students must face a situation that resembles the real scenario encountered by a professional working in medicinal chemistry. Working in groups of 4-5 students, and guided by a challenging driving question, students get involved in a meaningful learning process which leads them to propose solutions and carry out the practical identification of acetylcholinesterase inhibitors through enzymatic analysis. The hands-on *in vitro* studies allow students to put into practice in the laboratory much of the knowledge that they have acquired throughout their studies and face, for the first time in some cases, some practical issues such as the reagents selection and protocols optimization. The implementation of this PBL has been very satisfactory in terms of academic performance. As for the students' perception, they appreciate the opportunity to apply concepts in a real-world context, considering that these experiences can better prepare them for their future professional scenarios.

Keywords: *Problem-based learning, drug discovery, blind screening, laboratory experiments, undergraduate.*

1. Introduction

Traditionally based in a "cookbook" style of expository instruction, laboratory practices in science laboratories at the university level, often ignore the principles of the scientific method. Sometimes considered of minor relevance in a teaching-learning process that is mainly based in the use of theoretical lectures in which students are at risk of playing a passive role, their objectives are often limited to the development of certain technical skills. Being aware that the laboratory teaching at our university demands a change to make students become protagonists of their own learning process, our Educational Innovation Group TR4BIOCHEM (PIE22-067) has been working for several years in the implementation of new inquiry-based laboratory projects (García Ponce et al., 2019 and 2021, García Caballero et al., 2022).

Under the umbrella of the educational innovation projects funded by the University of Malaga, our group is interested in transforming the laboratory teaching of last course-chemistry and biochemistry undergraduate students into cross-disciplinary inquiry-based laboratory projects that could make students formulate questions, discuss protocols, collect and analyze data, and finally, draw conclusions (Ronennbeck et al., 2016). This could make them play a more active role in their learning, not limited to the acquisition of new technical competences, but also to improve their critical thinking skills. In the following sections of this chapter, we present one of these approaches, aimed to illustrate some principles of drug discovery to the last course undergraduate biochemistry students.

2. Topic of the study: The early drug discovery based on the *in vitro* blind screening of enzyme inhibitors

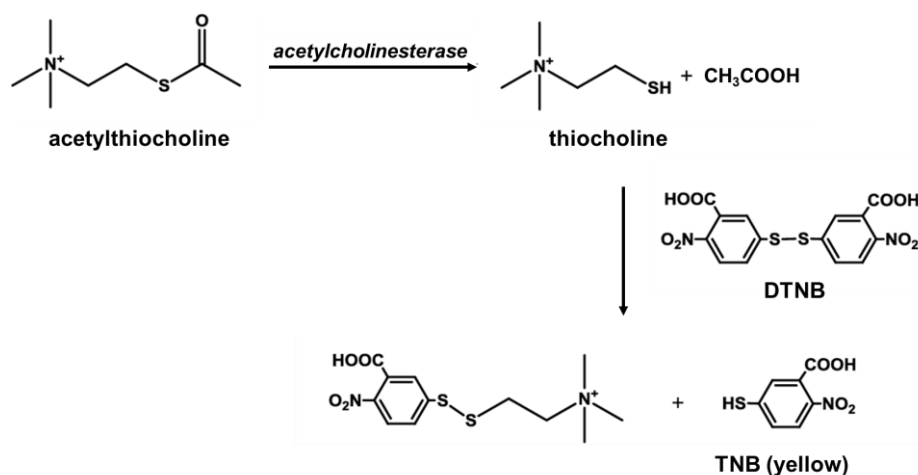
The development of a new drug is a costly and complex process that can take 12–15 years. Preclinical stages of the drug discovery process include target identification and validation, assay development, high throughput screening (HTS) of a chemical library, hit identification, lead optimization, and finally, the selection of a candidate molecule for further clinical development (Hughes et al., 2011).

The blind screening of enzyme inhibitors is one of the most widely used strategies in pharmacology for the discovery of new drugs. Many marketed drugs today function through inhibition of enzymes mediating disease phenotypes. In "Pharmacological Biochemistry", a subject offered to 4th year-biochemistry undergraduate students at the University of Malaga, students get familiar with the main types of drugs and their mechanism of action. With a number of students ranging 30-40 per year, its syllabus also includes a chapter devoted to the basic principles of drug discovery, based on the identification of new inhibitors of a target enzyme. This chapter includes those considerations to be considered when designing a high throughput enzymatic assay focused to the *in vitro* identification of new inhibitors, as well as the use of *in silico* modeling tools for the characterization and improvement of the drug interaction with its molecular target. Our approach to teaching this subject has evolved over the years, adding a series of activities aimed at helping the students to put into practice what they have learned in theory classes and better assimilate these concepts. Among others, these activities include the *in silico* drug-molecular target studies, or the design of a robust enzymatic assay for HTS applications.

Some critical issues that need to be addressed when developing an enzymatic assay for HTS purposes are, among others, pharmacological relevance of the assay (the assay is capable of identifying compounds with the desired potency and mechanism of action), source of enzyme (use of a relevant isoenzyme, assayed in physiological conditions), reliability and reproducibility (positive and negative controls of inhibition), cost (the use of microtiter plates minimizes the costs of the assay and allows automatization), effect of the solvent used to store the chemical libraries, concentration(s) at which compounds will be tested, or threshold to be reached for a compound to be considered a hit (Copeland 2005, Hughes et al., 2011). All these issues are discussed by the teacher in the theoretical class. However, these concepts are internalized to different extents by the students, depending on whether or not they have been reinforced with some additional active learning experiences focused to put them into practice, as will be explained in the following sections.

As for the target enzyme, we have worked with acetylcholinesterase, since inhibitors of this enzyme are currently used for the treatment of Alzheimer patients, and they can be easily identified *in vitro* by means of a quite inexpensive colorimetric method (Figure 1) (Ellman et al., 1961). Other target enzymes that we have used in this experience are mammalian dihydrofolate reductase and bacterial β -lactamase (for the discovery of new antitumor drugs or antibiotics, respectively)

Figure 1. Ellman's method, used for the *in vitro* detection of acetylcholinesterase inhibitors.



3. Developing a new PBL activity aimed to illustrate the early stages of drug discovery

As a practical tool to teach this topic, we have implemented a new PBL activity, intended to be a hands-on introduction to the early stages of the drug discovery process. Under the instructor's guidance,

students, working in groups, are involved in a meaningful learning process focused on proposing solutions and carrying them out in a practical way, both *in vitro* and *in silico*.

The sequence of activities of this PBL is:

1. After a theoretical introduction in the classroom by the teacher on the bases of the drug discovery process and the strategies for a blind screening of enzyme inhibitors, students are asked to form groups of 4-5 people. Groups will work as independent pharmaceutical laboratories that receive a letter stating the objective "To find new acetylcholinesterase inhibitors as drug candidates for the treatment of Alzheimer's disease".
2. After searching for information in the bibliographic databases, students propose solutions to the driving question and design protocols. The experimental development includes a bibliographic search on the Alzheimer's disease and current therapies, the use of acetylcholinesterase inhibitors for the treatment of patients, and those enzymatic assays that could be used for the identification of new inhibitors of this enzyme. This search brings students to select the above mentioned Ellman's procedure (Figure 1). Concerning the enzyme employed in the *in vitro* HTS, cost and commercial availability considerations make *T. californica* acetylcholinesterase the best choice. Thereafter, groups summarize their findings in a report, explaining what reagents and instrumentation are needed to carry out the *in vitro* screening for inhibitors of this enzyme.
3. During the laboratory activities phase, students design the experimental protocol, make calculations of how the reagents are prepared, optimize the experimental protocols in the laboratory and finally perform the screening test. As a result of this process, they identify an inhibitor of the enzymatic activity of the acetylcholinesterase among a group of unlabeled compounds provided by the instructor, facing a situation that resembles a real scenario found in the area of medicinal chemistry.
4. Once the *in vitro* part of this PBL is performed, groups accomplish the *in silico* studies. After an introductory lecture in the classroom, introducing the drug design rationale using computational techniques and the main available informatic tools, groups carry out *in silico* docking studies and explore the drug-protein interactions using PyRosetta, a package containing the Python-bindings of the Rosetta v3.0 source code, in Jupyter notebooks. The digital format of Jupyter Notebooks allows students to practice with interactive coding exercises, make molecular visualization movies and embed images to study drug-protein interactions.
5. At the end of the PBL, students prepare both a final report and an oral presentation about the different stages of the project and the results obtained.

4. Students' achievement of the learning goals

Students' knowledge about several issues regarding drug discovery was evaluated in the final exam of the subject by using a rubric in which some key points that students should mention were identified. These concepts had been taught by the teacher in his/her introductory lecture class, delivered to all groups of students and were related to the following topics:

1. Therapeutic relevance of the target enzyme
2. Need of a HTS assay
3. Use of multiwell plates
4. Need of fixing an inhibition threshold
5. Hit criteria (< 1%)
6. Need of a positive control (known inhibitor)
7. Compounds are assayed at a fixed concentration (typically 1-10 μM)
8. Enzyme characteristics (isolated from natural sources, recombinant...)
9. Initial velocity is measured (continuous recording or fixed time measurement)
10. Enzyme concentration
11. Use of physiological conditions in the assay
12. Check the effect of the vehicle (solvent in the compounds solutions)
13. Substrate concentration (near K_m)
14. Reagents addition order. Preincubate with the inhibitor
15. From Hit to Lead (check positive in a secondary screening assay)
16. ADME/Tox /Clinical assays
17. *In vivo* assays are needed to confirm positives.

As mentioned, although initially the teaching of this topic was limited to a theoretical exposition by the teacher and an unrelated *in silico* exercise of drug-protein modelling, lately it has been transformed into a more complete sequence of related activities.

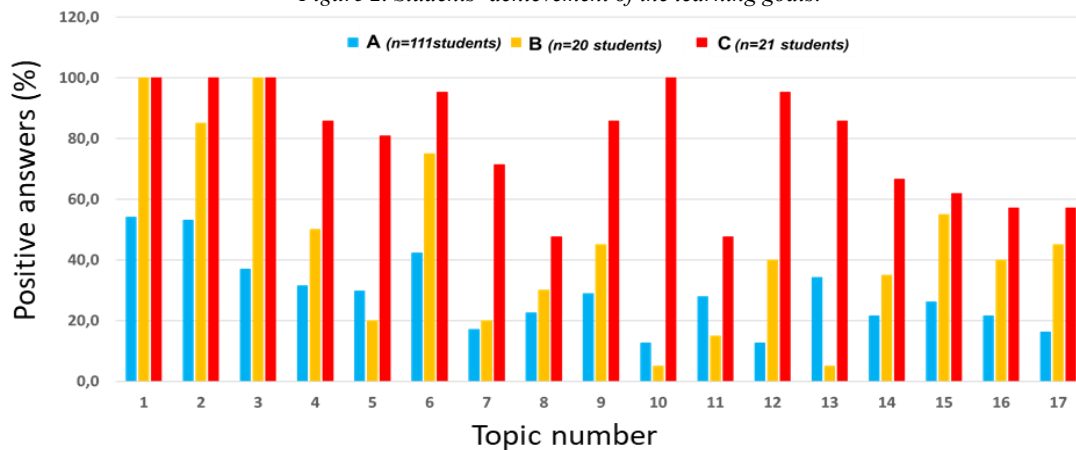
Figure 2 show the achievement of the learning goals by students belonging to three different groups of students that learn this subject using different approaches:

Group A. The principles of blind screening were just explained by the teacher (expositive lecture) (n=111 students).

Group B. Besides the expositive lecture by the teacher, blind screening was included in a PBL activity that excluded the laboratory work (students had to design the in vitro assay, but did not put it into practice in the laboratory) (n=20 students).

Group C. Besides the expositive lecture by the teacher, blind screening was included in a PBL activity that resembles the full process, including protocol optimization and drug selection in the laboratory (as described in section 3 of this chapter) (n= 21 students).

Figure 2. Students' achievement of the learning goals.



As can be observed in this figure, our results indicate that, in general, PBL approaches (groups B and C) improves the student's achievement of the learning goals, when compared to students that only received a theoretical class. In addition, those students who completed the entire PBL (group C) assimilated practical aspects better (topics 4-14).

5. Students' perception of the teaching methodology

In order to compare the students' perception of the PBL methodology, groups B and C students were asked through of a post-course mixed questionnaire, using 1 to 5 Likert-type scale questions. Results are presented in Table 1.

Table 1. Perception of the teaching methodology by those students who carried out the PBL (groups B and C).

| Statement | B | | C | | p value (t test) |
|---|------|------|------|------|------------------|
| | mean | sd | mean | sd | B versus C |
| The learning methodology (PBL) learning) used in this activity is innovative with respect to that used in other subjects of the degree. | 2,90 | 1,45 | 4,76 | 0,44 | 1,45E-05 |
| I think the work dynamic has been efficient and satisfactory. | 2,85 | 0,81 | 4,33 | 0,86 | 1,393E-06 |
| I did not like working autonomously, without the teacher being directly responsible for my learning. | 3,20 | 1,01 | 1,57 | 0,75 | 1,154E-06 |
| Sometimes I have been confused about how I should approach the resolution of the problem posed. | 4,50 | 0,69 | 2,86 | 0,91 | 1,159E-07 |
| With this methodology (PBL) I felt especially involved in my learning. | 3,20 | 0,89 | 4,62 | 0,50 | 7,86E-07 |
| With this methodology (PBL) I did not learn more than through the traditional way of studying. | 3,40 | 1,14 | 1,14 | 0,36 | 1,948E-08 |
| I think I/we managed to work well in order to solve the proposed case. | 3,20 | 1,06 | 4,19 | 0,87 | 0,0023703 |
| This work methodology (PBL) did not require more work and preparation on my part than in others of the same or other subjects. | 1,60 | 1,10 | 2,52 | 1,36 | 0,021614 |
| The solution of the PBL case took me too much time, sometimes incompatible with the workload I had to devote to other subjects and assignments. | 4,50 | 1,24 | 2,14 | 0,85 | 3,836E-08 |
| In my opinion, the weight of the PBL activity in the evaluation of the course grade was too low for the time I spent on the task. | 4,20 | 0,95 | 1,86 | 0,85 | 4,778E-10 |
| In this PBL I was confronted with situations similar to those I may encounter in my future professional development. | 3,75 | 1,12 | 4,76 | 0,44 | 0,0008906 |
| With this PBL I learned how to plan my own laboratory experiments. | 2,90 | 1,21 | 4,81 | 0,40 | 7,576E-07 |
| Global perception of this experience | 2,70 | 0,80 | 4,60 | 0,58 | 3,848E-10 |

Results presented in Table 1 show that the students' perception was significantly more positive for those students who carried out the complete PBL, including the laboratory practical work (group C), with a global satisfaction grade of 4.6 versus 2.7 (group B) with this experience.

Those students who had been able to fulfill the identification of an inhibitor of acetylcholinesterase by using the enzymatic analysis method designed by themselves (group C), found the experience much more gratifying. In this regard, they perceived the learning methodology as innovative and the work dynamic efficient. In addition, they considered that although this activity had taken them more time than others of the same or other subjects, this extra-effort had been worthwhile, allowing them to face and solve situations that they could find in their future professional development. This positive perception was not shared to the same extent by those students who completed a PBL in which the laboratory sessions had been removed (group B).

All these results suggest that this PBL is composed by a series of steps that fit together to give meaning to the whole. Additionally, it makes the students get involved in the activity, assuming the roles derived from the gamification of the activity. For this reason, the completion of the PBL with a hands-on laboratory work that could resemble a real-world scenario, could be essential for the students to enjoy and recognize the advantages of this educational experience.

6. Conclusion

Drug discovery at early stages provides an excellent scenario to simulate real-world problems that could make science undergraduate students take more active roles in their learning process. Although inquiry-based approaches are more demanding in terms of effort and time, they are very positively perceived by students, who become more actively involved in their learning process and find this type of research experience very rewarding. Making the experience to resemble as much as possible the real-world situation contributes to engage students in their learning process. In this sense, gamification may help students to gain confidence in their capability to apply their knowledge to solve specific problems, by having replicated situations that they will most probably face in their next professional careers.

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