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DESCRIPTIVE REPORT

Molecular docking studies of tyrosine kinase inhibitors: Exemplified protocol to advance pharmaceutical education in medicinal chemistry

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Abstract

Background: One of the essential goals of pharmacy education is to extend professional learning to pharmacists, pharmacy students and educators. Medicinal chemistry is a major component of this professional learning. Nowadays, computer-aided drug design (CADD) is considered a cornerstone in drug discovery platforms. However, this computational drug design is not crystal-clear to pharmacy students and pharmacists. In this study, a molecular docking process was established to advance pharmaceutical education in the medicinal chemistry field. **Method:** CADD is available in the syllabus of Iraqi pharmacy schools however the software packages are not available for the students to practice the docking process. Five different compounds were designed and docked by employing the Genetic Optimisation of Ligand Docking (GOLD) software and the scores were recorded. **Results:** Pharmacy students would use this exemplified docking to enhance their understanding of the medicinal chemistry module. **Conclusion:** This educational docking study was successfully performed to advance the pharmacy education.

Introduction

The principal goal of pharmacy education is to offer pharmacy students, pharmacists, and pharmacy educators the knowledge and skills to render them capable of demonstrating professional skills in their working environments (Salter *et al.*, 2014). As pharmaceutical sciences encompass various disciplines, medicinal chemistry is considered an indispensable cornerstone of the pharmacy education curriculum. Medicinal chemistry and pharmaceutical drug design enhance pharmacy students' knowledge and awareness of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles (Alam *et al.*, 2021). Furthermore, a medicinal chemistry module enhances the understanding of other pharmaceutical sciences. For instance, medicinal chemistry can reveal how the drug molecule changes (pharmacodynamics) or is altered (pharmacokinetics). This, in turn, allows

the pharmacy student, pharmacists, and the pharmacy educator to answer difficult questions in a unique way among other healthcare professionals as chemists besides being healthcare providers (Alsharif *et al.*, 1999; Khan *et al.*, 2011). Nowadays, computer-aided drug design (CADD) has a large impact on drug discovery and development (Yu *et al.*, 2022). Hence, CADD can assist pharmacy students to understand how an entirely new drug candidate can be designed and, thus, learn about how CADD enhances the education process. Unfortunately, CADD is not universally taught in pharmacy curriculums. For instance, in Iraq, undergraduate pharmacy student education is not supported with software packages even when CADD is included in the coursework. Whereas in south India, CADD is not included in the course syllabi (Sivajothi *et al.*, 2011).

One of the major applications of CADD is the chemotherapeutic field (i.e., antimicrobial and

anticancer drugs) in terms of design, synthesis, and development (Ismaeel *et al.*, 2020; Salih & Salih, 2020; Muhsin *et al.*, 2021). Cancer is a leading cause of death in many countries (Bray *et al.*, 2021; Koczwara *et al.*, 2021; Soerjomataram & Bray, 2021). Soerjomataram and Bray (2021) concluded the incidence of cancer will double in the next 50 years. Targeting cancer is one of the major platforms that researchers are working on worldwide. One of the well-recognized druggable targets in cancer cells is the tyrosine kinase (TK) enzyme. Designing an inhibitor to the TK enzymes is an ongoing global work. Some TK inhibitors (TKI) have been recognized and therapeutically approved for various types of cancer (Saraon *et al.*, 2021).

In this work, the authors demonstrate how a step-by-step molecular docking study of TKI enriches and advances pharmaceutical education in medicinal chemistry.

Method

How to perform a docking process

The following actions can be utilised as educational guidance, which is required to conduct molecular docking of a small molecule (a.k.a ligand) with a macromolecule (e.g. enzyme). System requirements include: Computer properties required for such work are Core (TM) i7-7700HQ, Intel (R), the CPU @ 2.80GHz, 2.81 GHz, and RAM of 16.0 GB.

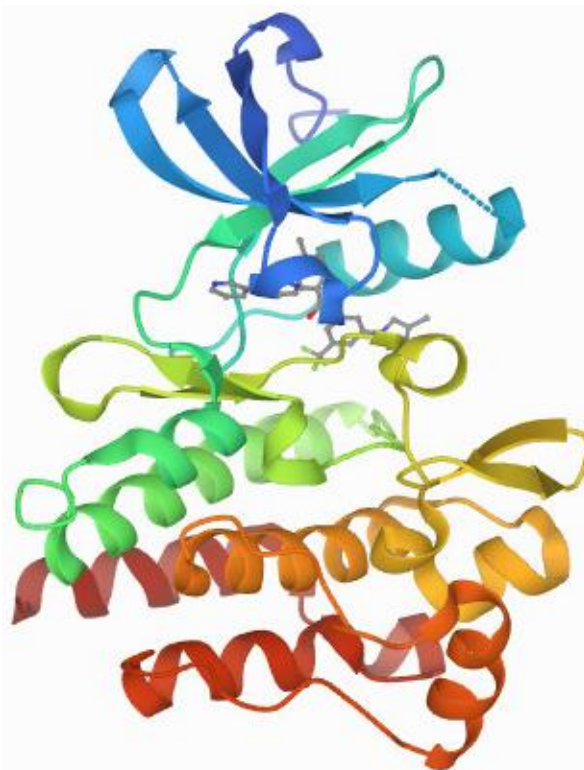
Notably, computational chemistry, molecular docking, quantitative structure-activity relationship (QSAR), CADD, and other related topics exist in the curricula for third, fourth-, and fifth-year pharmacy students in Iraq. Nevertheless, the above system requirements and the software packages are not available for practical training.

Molecular docking studies

How to perform a protein setup

Students are guided in the careful selection (when applicable, choose human protein with high resolution) of the molecular target protein. The chosen protein file is then downloaded from the [Protein Data Bank \(PDB\) website](https://www.rcsb.org/). When last visited, this freely accessed website contained more than 191,328 biological macromolecular structures. Accordingly, the tyrosine kinase (PDB ID 3CS9, shown in Figure 1) was downloaded as a PDB file and saved. The PDB ID 3CS9 is exemplified for educational purposes. This downloaded file can be opened in a docking software, Genetic Optimisation of Ligand Docking (GOLD),

version 2021.2.0. This is a licensed programme that can be obtained from [Cambridge Crystallographic Data Centre \(CCDC\)](https://www.ccdc.cam.ac.uk/) website. When the GOLD programme is open, the wizard box is clicked. Thereafter, a GOLD setup page is opened from which the downloaded protein can be launched via the "load protein" box. It may take a while till the protein is fully loaded, depending on the size of the protein (the number of amino acid residues) and the processor of the utilised computer. When protein loading is finished, the protein setup is performed with the following three steps; The first step is the addition of hydrogens, the second step is the deletion of water molecules, and the third step is the extraction of ligand molecules (if any). Further steps will be revealed in the docking section.



This structure was obtained with the x-ray diffraction method with a resolution of 2.21 Å

Figure 1: The crystalline structure of the Human ABL kinase in complex with nilotinib (PDB ID 3CS9)

How to perform a ligand setup

The preparation of ligands is a crucial process before performing the docking procedure. Herein, the authors selected five imatinib analogues as examples of ligands. These ligands were carefully designed with the employment of knowledge and skills acquired from medicinal chemistry modules. For instance, the employment of isosteres and the Lipinski rule of five are examples of the knowledge and skills of a medicinal

chemist. The designed molecules and the reference standard (the prototype imatinib) can be sketched as two-dimensional structures with ChemDraw Professional version 19.1.0.8. These structures can be saved as "mol" files. Sketching the structures by the students would enhance their understanding of drug molecules, stressing the pharmacophores and what drug moiety is essential for activity. Thereafter, the ChemBio3D Ultra version 19.1.0.8 (or ChemDraw Professional version 19.1.0.8) can be opened, and the structures can be loaded one by one. Each structure should minimise energy as the minimised energy structure is the one that would reach the receptor to bind to it with this exact pose. The step of energy

minimisation can be performed by pressing the "calculation" tab, then selecting the "MM2" drop list and choosing the "minimise energy" option. A new page would open to select the "Job Type" as "Minimise Energy" and the "Minimum RMS Gradient" of 1% (or 0.01). Then, click the "Run" box to activate the energy minimisation process. This may take a few seconds to a few minutes, depending on the chemical structure that is loaded and the properties of the utilised computer. When completed, the energy-minimised three-dimensional structure is then saved from being utilised in the docking process. Figure 2 shows the structure of Imatinib and the designed molecules.

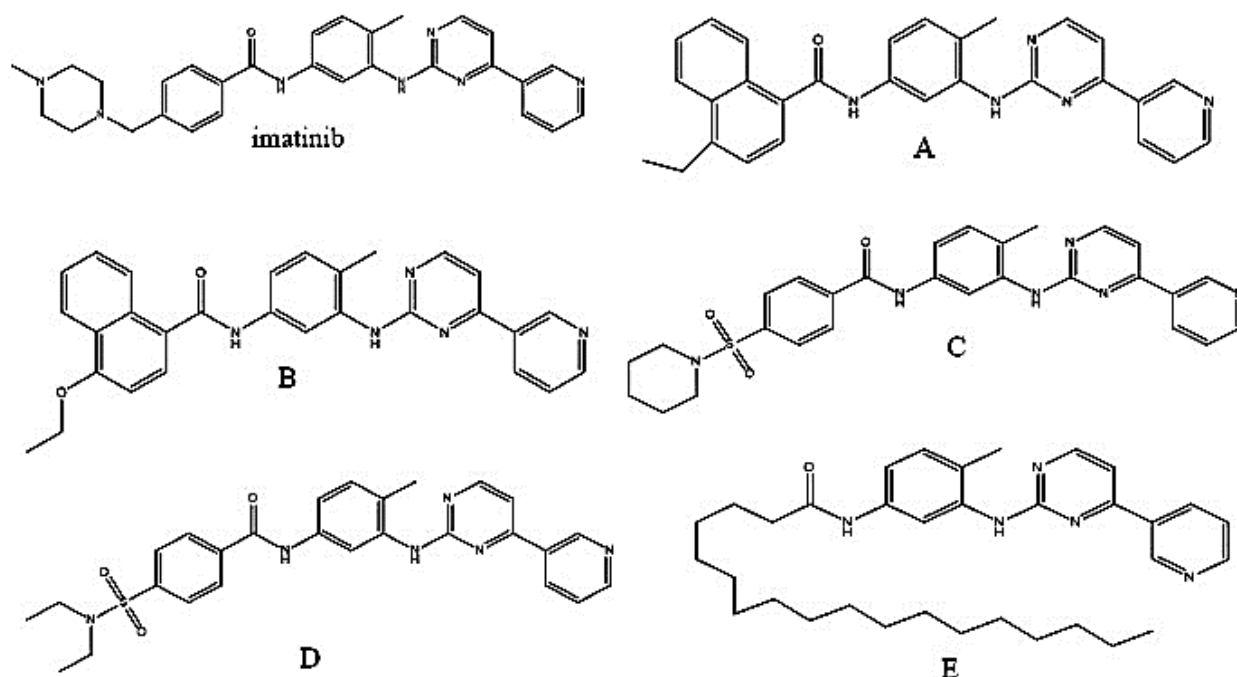


Figure 2: The chemical structures of Imatinib and the designed analogues (A-E)

Performing molecular docking process utilising the GOLD programme

After the protein setup procedure detailed above, the next button can be clicked to reach the "Define the binding sites" new page. Thereafter, a list of residues defining the binding sites, can be loaded as a text file. Clicking "next" brings an optional selection page of available templates from which "chemscore_kinase" can be chosen. Again, the "next" button can be clicked to reach the "Select ligands" page. The five three-dimensional saved structures (labelled A-E) can be loaded and the reference ligand (Imatinib) can also be loaded each on their specified boxes. Moreover, the "next" button can be clicked to lead to the "Choose a fitness function" page. On this page, the "CHEMPLP" scoring function can be chosen and the "next" button

can be clicked. This can lead to the "Genetic algorithm search options" page in which the slow (most accurate) option can be chosen. Clicking the next button brings the "Finish basic GOLD configuration" page. After that, a "Run GOLD" box would appear in the middle of the page. Pressing this box leads to the save options dialogue box. Choosing the folder to save the docking data will end with performing the docking process. When the docking process is finished, a view solution box will be activated. Pressing this box leads to the visualisation of the docking scores. A list of these scores with the corresponding pose will be reachable. This, in turn, would assist the student's understanding of how the pose is connected with the score and would render the theoretical part that they have learned to an easy, visualised, and manageable application.

Outcomes of docking

When the previously listed requirements are provided by the pharmacy faculties, the learning outcomes regarding the medicinal chemistry module would be touchable. The observed docking scores for this exemplified learning are listed in Table I. As revealed in Table I, compounds D and E show the best docking scores, which reflects the best configuration that fits into the kinase enzyme active site. The scores are

superior to the utilised reference standard (i.e. Imatinib), indicating the potential of developing a more potent tyrosine kinase inhibitor. When performed as mentioned above, the docking of the Imatinib and its derivatives (compounds A-E) can be observed, as shown in Figure 3. The performance of these steps allows the student to understand the practical utility of CADD. In a satisfying way, the student applies the theoretical learning from the lecture to design a new and theoretically improved drug molecule.

Table I: Labelling code, IUPAC names, docking scores of the reference standard (RS) Imatinib, and five designed derivatives

No	Code	IUPAC Name	Docking scores	Amino acid involved in short binding	Docking visualisation
RS	Imatinib	N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-4-((4-methylpiperazin-1-yl)methyl)benzamide	99.82	TYR253, GLU286, VAL289, VAL299, THR315.	Fig. 2-Imatinib
1	A	4-ethyl-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-1-naphthamide	92.39	GLU286, VAL289, VAL299, THR315.	Fig. 2A
2	B	4-ethoxy-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-1-naphthamide	93.30	VAL299, TYR253, ILE313, GLU286, VAL289, VAL299, THR315.	Fig. 2B
3	C	N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)-4-(piperidin-1-ylsulfonyl)benzamide	95.41	VAL256, GLU286, VAL289, VA299, THR315, MET318, LEU370	Fig. 2C
4	D	4-(N,N-diethylsulfamoyl)-N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)benzamide	101.31	LEU248, TYR253, GLU286, VAL289, ILE313, THR315	Fig. 2D
5	E	N-(4-methyl-3-((4-(pyridin-3-yl)pyrimidin-2-yl)amino)phenyl)stearamide	115.9	LEU248, TYR253, GLU286, VAL289, ILE313, THR315, PHE317	Fig. 2E

Discussion

To connect these results with advancing pharmacy education, the Pharmacy Education Journal website was searched. Only three published papers on medicinal chemistry education were found over all the published issues; one paper dealt with cancer, one with diabetic neuropathy, and the last one dealt with a potential inhibitor of SARS-CoV-2 ("IAI CONFERENCE: In silico screening of mint leaves compound (Mentha piperita L.) as a potential inhibitor of SARS-CoV-2," 2021; Muttaqin *et al.*, 2022; Nisa *et al.*, 2022). Furthermore, only a single study revealed the history of pharmacy education in Iraq since 1936. The authors, while discussing the barriers to pharmacy education in Iraq, proposed that there is a lack of new electronic teaching methodologies (Al-Iela *et al.*, 2014). This finding may highlight this significant area of pharmacy education concerning the utilisation of modern software and other electronic tools to advance pharmacy education in Iraq and other similar countries like India (Sivajothi *et al.*, 2011). Herewith, the molecular docking studies are explained in a step-by-

step manner so that they can be reproduced by other medicinal chemistry instructors. Consequently, this will enhance and advance pharmacy education in the medicinal chemistry discipline.

Conclusion

A step-by-step molecular docking study was performed to show how designing five tyrosine kinase inhibitors can advance pharmaceutical education in medicinal chemistry. Access to these advanced software platforms and supporting hardware can help pharmacy students understand the practical application of CADD.

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