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QSAR MODELING AND MOLECULAR DOCKING STUDIES ON BENZIMIDAZOLE DERIVATIVES AS ANTICANCER AGENTS

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Abstract. The triple-negative breast cancer cell line MDA-MB-231 has been known as one of the most tenacious cancer cancer cells and paid attention by many researchers. A two-dimension quantitative structure-activity relationship (2D-QSAR) model of 131 benzimidazole derivatives was developed to relate the chemical–biological interactions and predicted the half maximal inhibitory concentration (IC_{50}) against MDA-MB-231 cell line. The 2D-QSAR model was obtained satisfactory internal and external validation parameters such as the square of correlation coefficient R^2 = 0.904 and concordance correlation coefficient CCC = 0.867. The model was and applied on 35 synthesized benzimidazoles to predict IC_{50} values. The results showed that benzimidazoles with IC_{50} less than 50 µM displayed a quite similarity between predicted and experimental IC_{50} values (R_a^2 = 0.924). The molecular docking study was investigated to clarify the binding mode of the most potential synthesized benzimidazoles (**BLMM**, and **BL3H**) into topoisomerase I-DNA complex. The docking results revealed that they intercalated and interacted to crucial amino acids in the binding site of complex by hydrogen bonds and hydrophobic interactions and compared to standard drug camptothecin.

Keywords: Benzimidazole, QSAR, anticancer, MDA-MB-231, molecular docking, topoisomerase I.

Classification numbers: 1.2.4, 1.2.5, 4.8.5

1. INTRODUCTION

Breast, lung, and colorectal cancers have been the most common causes of cancer death for woman in recent years [1]. The studies, which mention the methods to inhibit cancer cell line or reduce the number of deaths related to cancer, has been finding and updating day by day.

Among the methodologies for breast cancer treatments such as chemotherapy, surgery, radiotherapy, immunotherapy, and hormone therapy, the chemotherapy is the most popular one [2].

Drug discovery is an arduous process that requires a large investment of money and time. It takes 12 years or much longer from the stage of target identification to approval for marketing [3,4]. Therefore, *in silico* screening in drug discovery has been introduced and saved a large amount of time and money [5]. Such methods have combined the experiment database with analysis tools supported by the computer to discover and optimize a molecule with affinity to a target in short time [6, 7]. The quantitative structure-activity relationship (QSAR) is one of the most famous virtual screenings, it facilitates the design and finding out the new targets of new compounds and makes the biological test more directional. The interesting points in a QSAR model are that the molecular structure is represented by chemical descriptors and the relationship between chemical structures and biological activity is quantitated in term of numbers [8].

Benzimidazole scaffold is well-known because of its multi biological activities [9 - 11], and as a breast anticancer inhibitor [12 - 15]. Therefore, in this study, we constructed the 2D-QSAR model to calculate the correlation of benzimidazole structures and their anticancer activity against MDA-MB-231 cell line that is famous for highly aggressive and invasive character. After the validation process, the model was applicated to evaluate the anticancer activity of synthesized benzimidazoles which we published before [14, 15] to prove the ability of prediction.

Human DNA topoisomerase I (TopI) has an important effect on different cancer cell lines and regulates almost the action of DNA such as replication, translation, transcription, etc., by the relaxation of supercoiled DNA [16]. Additionally, camptothecin (CPT) – a representative TopI inhibitor – intercalates the TopI-DNA complex to beget a ternary complex of CPT and Top1-DNA [17]. Besides, benzimidazole framework is considered as TopI-DNA inhibitor and some derivatives are listed in **Fig. 1** [18 - 22]. In order to develop our previous work, the molecular docking study was carried out between the most potential benzimidazoles and TopI-DNA complex and the result was compared with the intercalated CPT model to clarify the mechanism of anticancer action of benzimidazoles we published.

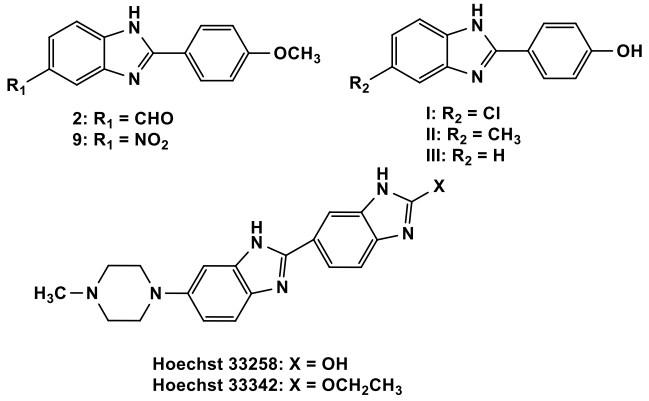

Figure 1. Structure of benzimidazoles as TopI-DNA inhibitors.

2. MATERIALS AND METHODS

2.1. Biological data

In this research, the data set of 131 benzimidazole derivatives were collected from the previous reports [12, 23 - 33]. Moreover, benzimidazole derivatives that we synthesized and published [14, 15], were calculated the predicted and experiment values as the application of the 2D-QSAR model. *In vitro* biological activities (IC_{50}, µM) were displayed in the minus logarithmic (-log) scale (pIC_{50}). The IC_{50} values of the compounds used in this paper were selected from the same testing cytotoxic method which is MTT assay against the MDA-MB-231 cell line using CPT as the reference drug [15]. The structures, experiment and predicted IC_{50}, pIC_{50} values of compounds were detailed in the supplementary information (Tables S1 and S2).

2.2. Preparation of the data sets and 2D molecular descriptor of 2D-QSAR model

The chemical structures of 131 benzimidazole were built by Chemdraw 19.1 [34] and Molecular Operating Environment (MOE) 2015.10 software [35]. The energy optimization of structures were performed by the function Energy Minimize in MOE 2015.10. The data set was split and separated randomly for five time into 20 % for the test set and 80 % for the training set. To calculated 2D-descriptors, the structures of derivatives were standardized by command Depict2D and then calculated 206 2D-descriptors in MOE 2015.10 software.

2.3. Process of the 2D-QSAR study

The process of 2D-QSAR study follows the previous report [36] with the modification and is illustrated in the Fig. 2. They include seven main steps:

Step No.1, 2 and 3 were mentioned in the section 4.2 below; Step No.4: the descriptors were selected using RapidMiner 5.3.013 [37] and Weka 3.8 [38] software, the useless descriptors were removed with intercorrelation greater than 0.8. The multi linear regression algorithm (MLR) was used to optimize the selection with modification that included the limitation of descriptors and kept more than one best subset of descriptors, they were validated by Leave One Out (L.O.O) cross-validation; Step No.5: the model was built using MLR and validated the predictive ability by L.O.O; Step No.6: the model was validated by test set; Step No.7: The model was applied to predict the IC_{50} values in the application set.

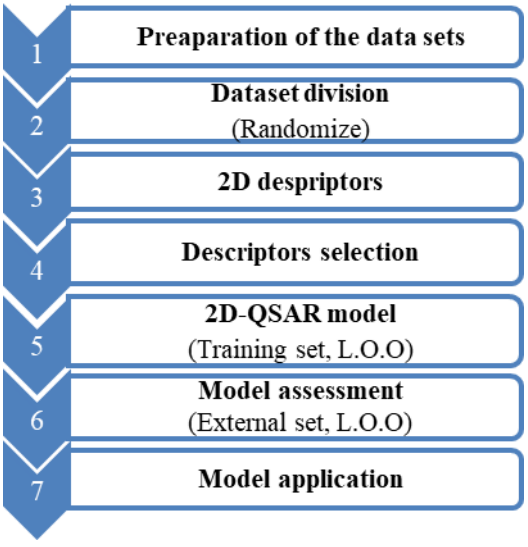

Figure 2. Process of 2D-QSAR study *(L.O.O = Leave One Out).*

2.4. Molecular docking study

The 2D and 3D chemical structure of ligands **BLMM**, **BL3H**, and **CPT** were built using software ChemDraw 19.1 and MOE 2015.10, respectively. The ligand structures were optimized by the Energy Minimization and Molecular Dynamic functions in Sybyl-X 1.1 [39]. In energy minimization process, the methods were used Conj Grad and Gasteiger-Huckel charges and the process was stopped when a minimum energy change was 0.001 kcal.mol^{-1} with the maximum number of iterations was set to 10,000. Additionally, the Simulated Annealing method was used in this process in which the ligands were heated at 700 K in 1000 femtoseconds and then cooled down to 200 K in the same period to obtain their final conformations with the stable states. This process was undergone five cycles to achieve the minimum energy of final conformations of ligands.

Preparation of protein. The X-ray crystallographic structure of human dsDNA Top I co-crystallized in complex with reference drug CPT was taken from the Protein Data Bank (PDB ID: 1T8I) and used as the receptor model. The 3D structure of protein was added hydrogen, protonated and deleted the unbound water by QuickPrep tool in MOE 2015.10. Then, this structure was imported to the BiosolveIT LeadIT 2.1.8 software [40]. The active site was prescribed by the reference ligand (EHD) with the radius sphere of 6.5 Å centered by the EHD ligand.

Evaluation of docking results. The redocking was conducted to validate the docking protocol. The co-crystallized reference ligand EDH was exported from the crystallographic structure and re-docked into the active site of the human dsDNA Top I complex. The successful docking protocol relates the Root Mean Squared Deviation (RMSD) between the native conformation and the best re-docked one, the RMSD value of redocking less than 2.0 Å proves a good docking protocol [41,42]. The docking process used BiosolveIT LeadIT 2.1.8 which was set the parameters as follows: the maximum number of solutions per iteration (1000), the maximum number of solutions per fragmentation (200), the number of poses to keep for interaction analysis (1 – Top 1). These posed were scored and the best conformation with the most minus docking score (free energy of binding) was further analyzed. The 3D poses of ligands with human dsDNA Top I complex were visualized by Discovery Studio 4.0 client software [43].

3. RESULTS AND DISCUSSION

3.1. 2D-QSAR model by MLR

The compounds which studied in this research possessed the similar core as benzimidazole framework. According to the IC_{50} values, 131 benzimidazoles in the data sets were displayed in the Fig. 3 as two types including below 50 µM (88 %) and 50 - 100 µM (12 %).

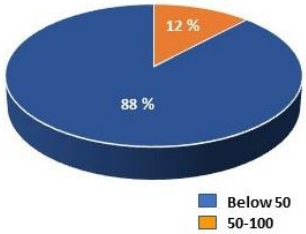

Figure 3. The distribution (%) of data sets based on IC_{50} value (µM).

The 2D-QSAR equation was built by MLR as below:

$$pIC_{50} = 4{,}62894 + 0{,}04823 * BCUT\_PEOE\_1 + 0.186364 * rings - 0.285714 * PEOE\_VSA + 2 + 0.423591 * PEOE\_VSA + 3 + 0.386351 * a\_hyd - 0{,}09953 * SlogP\_VSA1$$

There were six descriptions that were selected among 206 of 2D-structure descriptions in the step Descriptors Selection. The detail of molecular descriptors are showed in Table 1.

Table 1. The descriptors of 2D-SAR model.

No.	QSAR Descriptor	Description
1	BCUT_PEOE_1	The BCUT descriptors are calculated from the eigenvalues of a modified adjacency matrix.
2	Rings	The number of rings.
3	PEOE_VSA+2	PEOE: Sum of vi where q_i is in the range [0.10, 0.15).
4	PEOE_VSA+3	PEOE: Sum of vi where q_i is in the range [0.15, 0.20).
5	A_hyd	Number of hydrophobic atoms.
6	SlogP_VSA1	Subdivided Surface Areas: Sum of v_i such that L_i is in (−0.4, −0.2].

3.2. Model assessment method

For 2D-QSAR validation, many parameters were used to evaluate the model such as the square of correlation coefficient (R^2 and r^2 for training and testing set, respectively), the root mean square deviation (RMSE), standard errors of training and test sets [44]. The results of internal validation process showed that R^2 = 0.904 (> 0.5), RMSE = 0.082 (< 0.5). In combination of L.O.O method, the model validation expressed R^2 = 0.896 and RMSE = 0.098. Moreover, the external validation was carried out to evaluate the predictive power of this model.

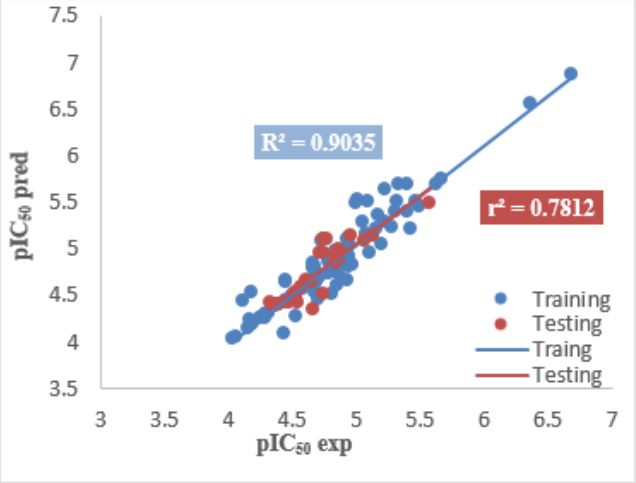

Figure 3. The relationship between experiment (pIC_{50} exp) and predicted (pIC_{50} pred) data from 2D-QSAR model. The compounds of training set in blue dots and red dots as the ones of test set.

Take a look on Fig. 3, the model exhibited the ability of prediction as well as the relationship between observed and predicted data of training set and test set, respectively. The values R^2 and r^2 which are 0.9035 and 0.7812 of training set and test set, respectively, confirmed the good correlation between the predicted and experimental data and the reliability of model as well.

Additionally, Roy *et al* proposed several new metrics such as [$\overline{r_m^2}$ (> 0.5), Δr_m^2 (< 0.2)] and CCC (> 0.85) as the external validation of QSAR model [45,46]. The result of Roy validation, which is 0.669, 0.196, and 0.867 of $\overline{r_m^2}$, Δr_m^2, and CCC values, respectively, proves that the model is affordable to predict. The equations for calculation of those metrics can be found in the supplementary information.

3.3. Application of 2D-QSAR model for calculation of the anticancer activity of synthesized benzimidazoles

Benzimidazole derivatives, which we synthesized, were classified as five sets based on their IC_{50} values and calculated the IC_{50} values as the same process descripted to clarify the able prediction of 2D-QSAR model. As we can see in the Table 2

Table 2. The result of 2D-QSAR model application.

Set No.	Compound number	IC_{50} value (μM)	R^2
1	7	Below 50	0.9249
2	9	50 - 100	0.0409
3	8	100 - 150	0.3523
4	7	150 - 200	0.0603
5	4	Above 200	0.0064

R^2 value of five sets ranges from 0.0064 to 0.9249 and the bad correlation (R^2 < 0.5) belongs to the sets **2**, **3**, **4** and **5** which has IC_{50} values larger than 50 μM. On the other hand, IC_{50} values of set **1** is less than 50 μM, while the correlation coefficient of set **1** (0.9249) was the best and expresses the powerful predicted possibility of the model. This can be explained that the IC_{50} values of the input database almost was smaller than 50 μM (82 % of the input database), the model is able to calculate the compounds which possess the IC_{50} value below 50 μM, efficiently.

3.4. Molecular docking study

Among our synthesized benzimidazoles, the derivatives **BLMM** and **BL3H** were chosen as the most potential compounds against MDA-MB-231 (IC_{50} < 15 μM) (Table S2) to perform the molecular docking and compared to the intercalated CPT mode. The analyses of the docking scores and the interaction between residues which are in the binding site of TopI-DNA and the most potential compounds was carried out to clarify the binding modes. After the binding site was established, reference drug (CPT) was redock into the binding site and the result of redock process showed RMSD = 1.5686 Å (< 2 Å). Docking score of CPT was -29.51 $kcal.mol^{-1}$ and it interacted with dt 10 and Asn722, Asp533 which are famous for the sensitive amino acids toward to CPT in the binding site of complex [17,18], via hydrogen bonds (Fig. 5A, B). After

performing the interactive simulations with two potential benzimidazoles, the docking scores, IC_{50} values (µM), and interacted residues were showed in the Table 3.

Table 3. Docking score of the most potential benzimidazoles and CPT against MDA-MB-231 cell. Italic symbols denoted the residues interacted by hydrogen bond and normal ones denotes the residues interacted by hydrophobic interactions.

Cps.	**IC_{50} value (µM)**	**Docking score (kJ.mol^{-1})**	**Residues interacted**
BLMM	10.82	-25.86	*Asp533*; dt10, Tgp11, Arg364, dg12, and da113
BL3H	14.90	-22.46	*Asp533*; dt10, Arg364, Tgp11, dg12, and da113
CPT	1.35	-29.51	*Asp533*, *Asn722* and *dt10*; da113, dc112, Tgp11, and Thr718

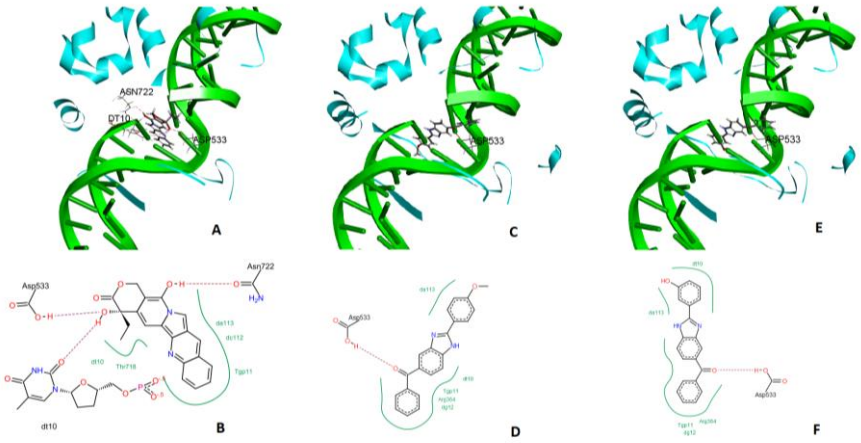

Figure 5. 3D and 2D intercalated models of TopI-dsDNA with **CPT** (A and B), compound **BLMM** (C and D), and compound **BL3H** (E and F). dsDNA shows in green, TopI as cyan ribbons, the hydrogen bonds are displayed as dash line and the green curves lines exhibits the hydrophobic interactions.

With attention to correlation between the docking scores and the inhibition of MDA-MB-231 cell line, the less the docking score was, the better the cytotoxic activity expressed. The docking score of CPT (-29.51 kJ.mol^{-1}) was smaller than that of **BLMM** and **BL3H** which are -25,86 kJ.mol-1 and -22,46 kJ.mol^{-1}, respectively. The same fashion took place in their cancer activity (**CPT** > **BLMM** > **BL3H**). Moreover, the interaction of **CPT**, **BLMM** and **BL3H** with amino acids in the binding site of TopI-DNA complex had reflected their anticancer activity. Compounds **BLMM** and **BL3H** interacted uniquely with amino acid Asp533 via hydrogen bond (**Fig. 4C, D** and **Fig. 4E, F**, respectively), while **CPT** displayed three hydrogen bonds with Asp533, Asn722 and dt10. **CPT** interacted and intercalated at the site of dsDNA cleavage via dt10 to generate the ternary complex between **CPT** and TopI-DNA [17]. As a result, the cytotoxicity of **BLMM** and **BL3H** is worse than that of CPT. The other residues in the binding site of complex interacted by hydrophobic interactions with **CPT** (da113, dc112, Tgp11, and Thr718), **BLMM** (dt10, Tgp11, Arg364, dg12, and da113), and **BL3H** (dt10, Arg364, Tgp11, dg12, and da113) as well. Based on these results, we hypothesis that inhibiting TopI-DNA is one of the mechanisms of anticancer action of **BLMM** and **BL3H**. In short, besides the docking score, the ability of formation of hydrogen bonds with crucial amino acids strongly affects the

inactivation of TopI-DNA complex and associated with the anticancer possibility on the MDA-MB-231.

4. CONCLUSIONS

In summary, the 2D-QSAR analysis of the structures of benzimidazole and anticancer activity against MDA-MB-231 cell line was built with 131 derivatives. The trustworthiness of the model was potent with R^2 = 0.904, r^2 = 0.7812, RMSE = 0.08 and CCC = 0.867 as the metrics of internal and external validation. The model was applied for calculation of IC_{50} values of benzimidazoles (five sets) that was published by our group, the compounds of set **1** that had IC_{50} value below 50 μM were calculated perfectly with R^2 = 0.9249. The reason of those cases is that the IC_{50} values in the constructed database account for 88 %. It proves the 2D-QSAR model could be used to *in silico* screening to find out new breast anticancer agents possessed benzimidazole frameworks. The most potential benzimidazoles (**BLMM** and **BL3H**) against MDA-MB-231 were proposed the mechanism of cytotoxic action as inhibiting TopI-DNA complex and clarified by the molecular docking study, compared to **CPT**. Their IC_{50} values shows a linear relationship both of docking scores and interaction with essential residues Asp533, Asn722 and dt10 in the binding site of TopI-DNA complex. This molecular docking model can be developed for screening the perspective TopI-DNA inhibitors as well as the 2D-QSAR model for finding the new potential anticancer agents against MDA-MB-231 cells with IC_{50} value lower than 50 μM in the further study.

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Credit authorship contribution statement. Hoang-Phuc Nguyen: methodology, data curation, formal analysis, software, validation, visualization, writing-original draft, writing-review & editing; Khac-Minh Thai: software, visualization, and writing-review & editing; Thi-Kim-Chi Huynh and Thi-Kim-Dung Hoang: conceptualization, methodology, resources, data curation, formal analysis, investigation, software, supervision, validation, visualization, writing-original draft, writing-review & editing; All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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