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Development of receptor-based protein kinase Cβ (PKCβ) pharmacophore model for the search of inhibitors with potential activity against acute respiratory distress syndrome (ARDS)

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Aim. Development of receptor-based pharmacophore model for protein kinase *Cβ* (PKCβ). **Methods.** Pharmacophore modeling was performed with Discovery Studio Visualizer 4.0 and PharmDeveloper software. **Results.** The resulted pharamacophore model consisted of five pharamacophore features, two aromatic features without vectors, two acceptors of hydrogen bonds, one donor of acceptor bond, was generated and validated. **Conclusion.** This pharmacophore model will be used for virtual screening of compound collection in order to identify potential inhibitors of PKCβ.

K e y w o r d s: protein kinase ***Cβ***, PKCβ, pharmacophore model, pharmacophore features.

Introduction

According to WHO statistics 2021, COVID-19 is the first deadliest infectious killer in the world. As of February 2022 in Ukraine there were proved more than 2.3 million cases of SARS-CoV-2 virus infection and more than 103,000 deaths (https://covid19.gov.ua/). Acute respiratory distress syndrome (ARDS) is observed in 42 % of people suffering from pneumonia caused by this virus and in more than 61 % of people needing intensive therapy. ARDS is fatal in more than 26 % of cases in intensive care patients, and in more than 65 %

patients connected to ventilators [1, 2]. One of the main pathogenetic mechanisms of ARDS during COVID-19 is an activation of neutrophilic granulocytes with the formation of neutrophil extracellular traps (NETs) [1, 3]. It was shown that in patients with ARDS, the number of NETs in bronchial aspirates correlates with the severity of disease. Therefore, nowadays the development of novel drugs inhibiting molecular targets involved in NETs formation is of urgent need. Protein kinase ***Cβ (PKCβ)*** is considered as a promising target since it is activated by induction of neutrophil receptors (a number of toll-like receptors (TLRs), CD-14, *etc.*) and is implicated in signaling pathways of NETosis [4–7]. Noteworthy, the activation of PKC***β*** is quite specific in the induction of NETosis. The model can be used for virtual screening in order to find specific small-molecular inhibitors of this protein kinase with potential activity against acute respiratory distress syndrome (ARDS).

Materials and Methods

Receptor-based protein kinase ***Cβ (PKC β)*** pharmacophore model was built with the program Discovery Studio Visualizer 4.0 [8]. The pharmacophore model was developed based on two co-crystal structures of PKC***β*** with bisindolylmaleimide inhibitor (PDB accession code: 2I0E) [9] and phosphoaminophosphonic acid-adenylate ester (PDB ID: 3PFQ) [10]. A primary pharmacophore model was constructed on basis of intermolecular interactions between the amino acid residues of PKCβ and the ligands in the complexes. The complexes were superimposed. The radii of pharmacophore features were set by default: for aromatic features — 1.2 Å, for hydrophobic features — 1.0 Å, for donors and acceptors of hydrogen bond — 0.9 Å.

The pharmacophore model built with the program Discovery Studio Visualizer 4.0 was saved in .CHM format. Using our in-house program PharmDeveloper [11, 12], the file was converted from .CHM format to .QUERY format. The pharmacophore features at a distance of no more than 0.5 Å were averaged in one feature with PharmDeveloper software. The excluded volumes were added using Discovery Studio Visualizer 4.0. The validation screenings were performed with the program PharmDeveloper.

Results and Discussion

The primary pharmacophore model of PKC***β*** was built based on the spatial structures of the complexes of catalytic subunit of this protein kinase with bisindolylmaleimide inhibitor (PDB ID: 2I0E) and phosphoaminophosphonic acid-adenylate ester (ANP800) (PDB ID: 3PFQ). All aromatic rings of two indole and one maleimide heterocycles of the bisindolylmaleimide inhibitor form a series of hydrophobic interactions with the amino acid residues in the ATP-binding site of protein kinase PKCβ such as Ala369, Val356, Leu348, Val423 and Ala483. Considering these interactions we generated three aromatic pharmacophore features without vectors with the centers localized in the geometric centers of inhibitor aromatic heterocycles. We added two acceptors of hydrogen bonds on keto groups of maleimide which correspond to hydrogen bonds with Val423 and Thr404. Also, we generated donor of hydrogen bond on nitrogen atom of maleimide heterocycle which forms hydrogen bond with Glu421 (Fig. 1, a).

The adenine fragment of ANP800 forms a number of hydrophobic interactions with amino acid residues in the adenine-binding region of PKC*β* ATP-binding site such as Val356, Lys371, Leu348, Ala369, Ala483, Glu421, Tyr422 and Val423. Therefore, we generated aromatic feature without vector with the center localized in the geometric center of adenine of phosphoaminophosphonic acid-adenylate ester. We built two hydrogen bond acceptor pharmacophore features on nitrogen atom which forms hydrogen bond with Val423. Also, we built hydrogen bond donor pharmacophore features on amino group of adenine ring which forms hydrogen bond with Glu421 and on oxygen atom of the first phosphoryl group which forms hydrogen bond with Lys371 (Fig. 1, b).

Additionally, we added excluded volumes on the all atoms of amino acid residues in the ATP-binding site of PKC*β* within a radius of 5 Å around the ligand. The radius of the excluded volumes was set to 1.2 Å, which corresponds to the van der Waals radius of the hydrogen atom.

In obtained raw model (Fig. 2, a) the pharmacophore features located at a distance less than 0.5 Å were geometrically averaged with PharmDeveloper. As a result, a primary pharamacophore model consisted of seven pharamacophore features — three aromatic features without vectors, three acceptors of hydrogen bonds, one donor of acceptor bond, and 158 excluded volumes was generated (Fig. 2, b). In order to make clear presentation of the pharmacophore model the excluded volumes are not visualized since they cover the pharmacophore features. The model was complex. Therefore, simpler derived models were obtained from it by combining features using PharmDeveloper. The total number of pharmacophore features in the derived models was set as five. All features except the two acceptors and one donor directed to the hinge

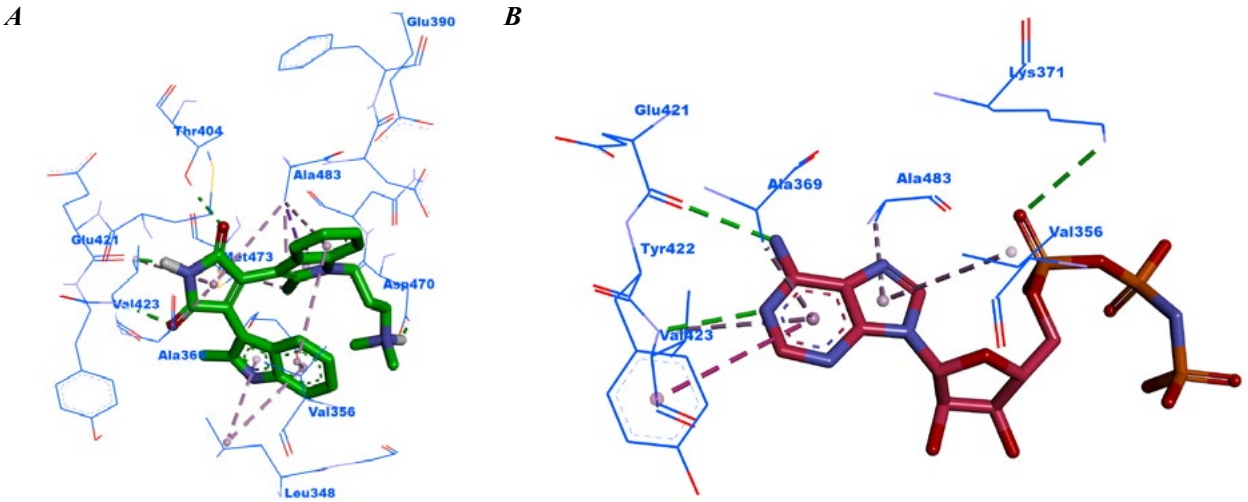

Fig. 1. The complexes of bisindolylmaleimide (a) and ANP800 (b) with the amino acid residues in ATP-binding site of PKC*β*. The hydrogen bonds are presented by green dashed lines and hydrophobic interactions are shown by magenta dashed lines.

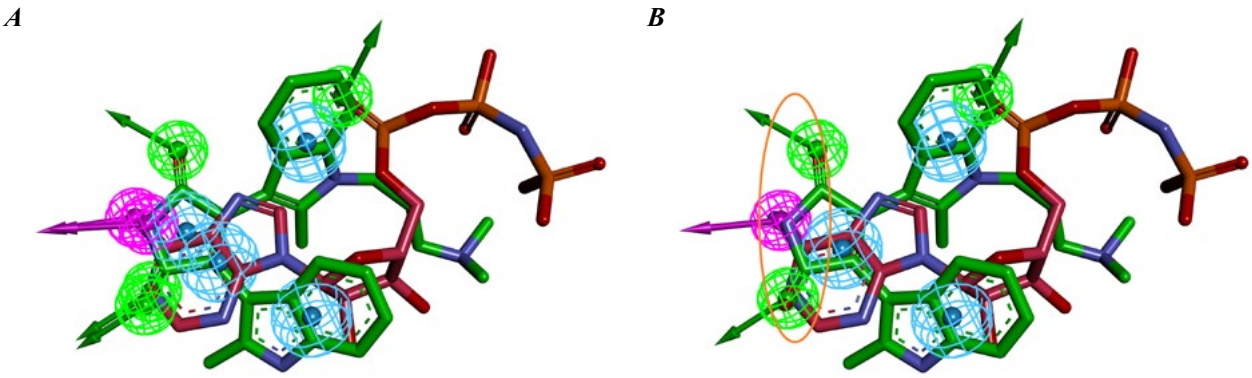

Fig. 2. The receptor-based raw pharmacophore model of protein kinase C***β*** inhibitors (a) and this model (primary) after averaging of features located at a distance less than 0.5 Å (b). Aromatic pharmacophore features without vectors are labeled with blue color, acceptors of hydrogen bond pharmacophore features with vectors are shown with green color, donor of hydrogen bond pharmacophore feature with vector is presented by magenta color. The orange circle indicates the group of pharmacophore features defined for combination.

region of the protein kinase and aromatic feature near them were set as required by default. Acceptors, donor and aromatic feature formed a combination group, from which two features were selected for each derived model. As a result, six derived models were generated for pharmacophore screening.

The chemical structures of known protein kinase C***β*** inhibitors (PKC***β***) collected from the ChEMBL database and from the literature were used to validate the receptor-based pharmacophore models. There were 731 compounds in total. The best results showed a model with donor and acceptor without aromatic feature from combining group — this model predicted activity of compounds in three times more accurately than other models (Fig. 3). This model selected 73 compounds from test set. Among them 21 compounds had the inhibitory activity toward PKC***β*** with IC_{50} values less than 100 nM and 48 compounds — with IC_{50} values less than 1000 nM.

Conclusion

The obtained receptor-based pharmacophore model of PKC***β*** will be used for virtual screening of the compound collection in order to find

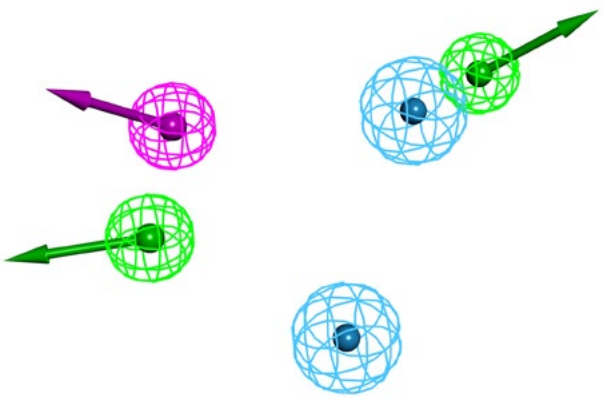

Fig. 3. Resulted pharmacophore model of protein kinase C***β*** inhibitors for pharmacophore screening. Aromatic pharmacophore features without vectors are labeled with blue color, acceptors of hydrogen bond pharmacophore features with vectors are shown with green color, donor of hydrogen bond pharmacophore feature with vector is presented by magenta color.

inhibitors with potential activity against acute respiratory distress syndrome.

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Розробка рецепторно-орієнтованої фармакофорної моделі протеїнкінази С бета (PKCβ) для пошуку інгібіторів з потенційною активністю проти гострого респіраторного дистрес синдрому

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Мета. Розробити рецепторно-орієнтовану фармакофорну модель протеїнкінази С бета (PKCβ). **Методи.** Фармакофорне моделювання здійснювали за допомогою програм Discovery Studio Visualizer 4.0 і PharmDeveloper. **Результати.** Розроблено та валідовано фармакофорну модель, що складалася з п'яти фармакофорних точок — двох ароматичних точок без векторів, двох акцепторів водневих зв'язків та одного донора водневого зв'язку. **Висновки.** Одержана фармакофорна модель буде використана для віртуального

скринінгу колекції сполук з метою пошуку інгібіторів PKCβ.

Ключові слова: протеїнкіназа С бета, PKCβ, фармакофорна модель, фармакофорні точки.

Разработка рецепторно-ориентированной фармакофорной модели протеинкиназы С бета (PKCβ) для поиска ингибиторов с потенциальной активностью против острого респираторного дистресс-синдрома

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Цель. Разработать рецепторно-ориентированную фармакофорную модель протеинкиназы С бета (PKCβ). **Методы.** Фармакофорное моделирование осуществлялось с помощью программ Discovery Studio Visulizer 4.0 и PharmDeveloper. **Результаты.** Разработана и валидирована фармакофорная модель, состоящая из пяти фармакофорных точек — двох ароматических точек без векторов, двох акцепторов водородных связей и одного донора водородной связи. **Выводы.** Полученная фармакофорная модель будет использована для виртуального скрининга коллекции соединений для поиска ингибиторов PKCβ.

Ключевые слова: протеинкиназа С бета, PKCβ, фармакофорная модель, фармакофорная точка.

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