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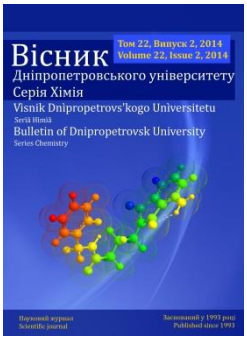

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MOLECULAR DOCKING OF ZSTK474 DERIVATIVES AS POTENTIAL PI3Kδ INHIBITORY AGENTS

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Abstract

The phosphatidylinositol 3-kinase delta (PI3Kδ) controls a range of cellular processes. Its overexpression is found in many human tumors. PI3Kδ inhibitors are potential anticancer agents and anti-inflammatory agents for treatment of rheumatoid arthritis. Derivatives of ZSTK474, an effective inhibitor of PI3Kδ, were screened virtually by computational docking for inhibitory activity towards PI3Kδ. Some of modeled compounds showed better docking energies than ZSTK474 indicating that the former could be potent enzyme inhibitors. Additional binding energy was provided by extra ligand-protein interactions. Substituents in morpholine and benzimidazole rings cause increase and decrease of ligand-protein binding, respectively. Energetically favorable ZSTK474 derivatives satisfy Lipinski's Rule of five which testifies to their druglikeness (absorption, distribution, metabolism and excretion) and possible pharmacological activity.

Keywords: molecular docking; binding energy; binding site; inhibitors.

МОЛЕКУЛЯРНЫЙ ДОКИНГ ПРОИЗВОДНЫХ ZSTK474 КАК ПОТЕНЦИАЛЬНЫХ ИНГИБИТОРОВ PI3Kδ

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Аннотация

Фосфатидилинозитол-3-киназа дельта (PI3Kδ) контролирует ряд клеточных процессов. Ее избыточная экспрессия встречается во многих видах опухолей человека. Ингибиторы PI3Kδ являются потенциальными противоопухолевыми агентами и противовоспалительными агентами для лечения ревматоидного артрита. Молекулярный докинг производных ZSTK474, эффективного ингибитора PI3Kδ, был проведен для проверки их ингибиторной активности в отношении PI3Kδ. Некоторые из смоделированных соединений показали лучшие энергии образования комплекса лиганд – белок, чем ZSTK474, что свидетельствует о потенциально более сильном ингибировании фермента. Большая энергия связывания обусловлена дополнительными взаимодействиями лиганд – белок. Заместители в морфолиновых и бензимидазольном циклах вызвают соответственно увеличение и уменьшение связывания лиганд – белок. Применение правила пяти Липинского к энергетически наиболее выгодным производным ZSTK474 для оценки подобия лекарственным веществам (абсорбция, распределение, метаболизм и выделение) не показало ни одного отклонения от правила, которое определяет фармакологическую активность лекарственного вещества в организме.

Ключевые слова: молекулярный докинг; энергия связывания; центр связывания; ингибитор.

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МОЛЕКУЛЯРНИЙ ДОКІНГ ПОХІДНИХ ZSTK474 ЯК ПОТЕНЦІЙНИХ ІНГІБІТОРІВ PI3Kδ

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Анотація

Фосфатидилинозитол-3-кіназа дельта (PI3Kδ) контролює ряд клітинних процесів. Її надлишкова експресія зустрічається в багатьох пухлинах людини. Інгібітори PI3Kδ є потенційними протипухлинними агентами і протизапальними агентами для лікування ревматоїдного артриту. Молекулярний докінг похідних ZSTK474, ефективного інгібітора PI3Kδ, був проведений для перевірки їх інгібіторної активності щодо PI3Kδ. Деякі зі змодельованих сполук показали кращі енергії утворення комплексу ліганд – білок, ніж ZSTK474, що свідчить про потенційно більш сильнее інгібування ферменту. Більша енергія зв'язування обумовлена додатковими взаємодіями ліганд – білок. Замісники у морфолінових і бензімідазольному циклах викликають відповідно збільшення і зменшення зв'язування ліганд – білок. Застосування правила п'яти Ліпінського до енергетично найбільш вигідних похідних ZSTK474 для оцінювання подібності до лікарських речовин (абсорбція, розподіл, метаболізм і виділення) не показало жодного відхилення від правила, яке визначає фармакологічну активність лікарської речовини в організмі.

Ключові слова: молекулярний докінг; енергіяз в'язування; центр зв'язування; інгібітор.

Introduction

The PI3Kδ (phosphatidylinositol 3-kinase delta) is a lipid kinase related to class I PI3Ks and mainly presents in leukocytes [1]. It has a physiological role in B-cell signaling, development and survival. PI3Kδ is a heterodimer consisting of p110δ catalytic subunit and p85 regulatory subunit that catalyzes the ATP-dependent phosphorylation of phosphoinositide substrates [2]. The resulting second messengers can regulate a variety of physiological processses, such as metabolism and cell survival [3]. Deregulation of p110δ subunit by activation or transforming mutations was implicated in cancer, rheumatoid arthritis and asthma [4–6]. Consequently, the selective inhibition of PI3Kδ is a promising therapeutic strategy [7]. PI3Kδ was extensively studied and exploited as a target for cancer drugs [8–22]. Earlier developed small-molecule ATP-competitive PI3K inhibitors had low affinity, instability, nonselectivity and toxicity that limited their clinical use [8]. Further chemical modification significantly improved their pharmacological properties. Search of new inhibitors for PI3K has got a great attention and was directed to minimize undesired and often poorly understood toxic side effects, enhance potency, selectivity and pharmacological properties. A lot of structures, including TGX286 (8-(1-anilinoethyl)-6-methyl-2-pyridin-4-ylchromen-4-one), PIK39 (5-chloro-3-(2-methoxyphenyl)-2-(7H-purin-6-ylsulfanylmethyl)-quinazolin-4-one), IC87114 (2-[(6-aminopurin-9-yl)methyl]-5-methyl-3-(2-methylphenyl)quinazolin-4-one), CAL101 (5-fluoro-3-phenyl-2-[(1S)-1-(7H-purin-6-ylamino)propyl]quinazolin-4-one), ZSTK474 (4-[4-[2-(difluoromethyl)benzimidazol-1-yl]-6-morpholin-4-yl-1,3,5-triazin-2-yl]morpholine), NVP-BEZ235 (2-methyl-2-[4-(3-methyl-2-oxo-8-quinolin-3-ylimidazo[4,5-c]quinolin-1-yl)phenyl]propanenitrile), and IPI-145 (8-chloro-2-phenyl-3-[(1S)-1-(7H-purin-6-ylamino)ethyl]isoquinolin-1-one) have been reported and some of them have already entered clinical trials [9–18].

The new selective PI3Kδ inhibitor **ZSTK474** strongly inhibits the growth of tumor cells in human cancer xenografts [16; 18]. It inhibits tumor cell proliferation via G1 arrest of the cell cycle without inducing apoptosis *in vitro* and *in vivo* as long as it is administered, and could be used for months as maintenance therapy for patients with advanced cancers [19]. **ZSTK474** was demonstrated to have potential *in vitro* antimetastatic effects on PC3 cells *via* dual mechanisms: inhibition of metastatic processes including cell migration, invasion and adhesion, and antiangiogenesis *via* blockade of VEGF (vascular endothelial growth factor) secretion [20]. The structure of the complex of **ZSTK474** with PI3Kδ has been experimentally determined. It was revealed that **ZSTK474** is noncovalently bound inhibitor and occupies the ATP binding site of protein [21]. The present study focuses on search of substituents which will increase the binding of **ZSTK474** with PI3K. We believe that further chemical modifications of **ZSTK474** helped to improve its drug-like properties.

Computational Methodology

The three dimensional structure of PI3K protein [PDB: 2WXL] was obtained from Protein Data Bank. ATP and **ZSTK474**, the pan-inhibitor of PI3K, were used as the ligands and were retrieved from NCBI PubChem Compound database (compound ID: 5957 and 11647372, respectively). **ZSTK474** analogs were simulated by insertion of different substituents (alkyl, hydroxy, alkoxy, amino, halogen, nitro, cyano) to its structure followed by optimiza-

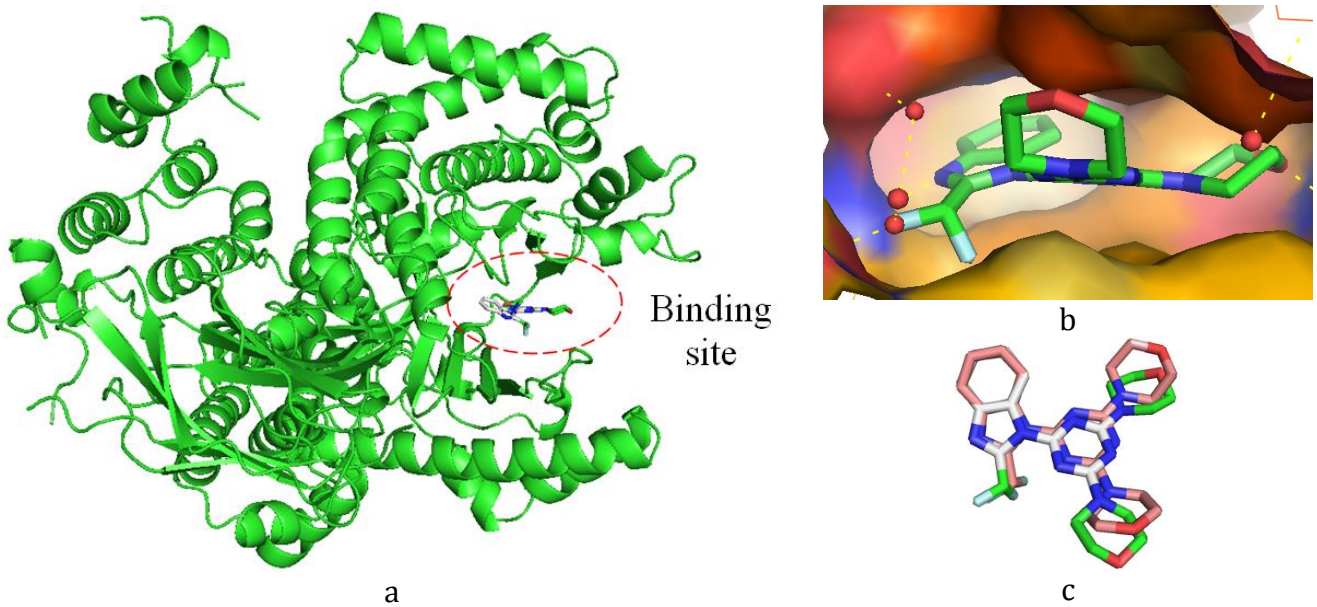

Fig. 1. Structure of PI3K with docked ZSTK474: the whole view (a), the binding pocket in PI3K (b), experimentally obtained (light grey) and calculated (dark grey) conformation of ZSTL474 in binding site of PI3K (c)

tion of the structures at the B3LYP/6-31+G* level using Gaussian 09 [22]. The Graphical User Interface program AutoDock Tools 1.5.4 was used to simulate the protein and ligands. The grid boxes size was set at 90, 86 and 126 Å (x, y, and z) to include all the amino acid residues that present in a binding site. The spacing between grid points was 1.0 Å. The AutoDock Vina 4.0 was used to run and analyze the rigid docking simulations [23]. During the docking process, a maximum of 9 conformers was considered. Autodock results were analyzed to study interactions and binding energy of the docked structure. Hydrogen bonds and hydrophobic binding distance between atoms were measured for the best conformers. The results were visualized by PyMol [24]. Molecular descriptors were calculated using Virtual computational chemistry laboratory [25].

Results and Discussion

At the beginning of our study we docked **ZSTK474** with PI3K and compared the structure of the obtained cluster with available experimental data [1] to verify the accuracy of AutoDockVina program. Docking for **ZSTK474** led to a cluster (Fig. 1a, b) with binding energy of –9.5 kcal/mol. It has two hydrogen bonds and about twenty hydrophobic interactions. The hydrogen bonds were formed between morpholine oxygen atom of **ZSTK474** and NH group of aminoacid VAL828 through a distance of 2.8 Å (N–H…O), and between benzimidazole nitrogen atom of **ZSTK474** and amino group of aminoacid LYS779 with a distance of 3.1 Å (N–H…N). The hydrophobic bonds were formed between carbon atoms of **ZSTK474** and aminoacids MET752, TRP760, THR833, ILE777, TYR813, ILE825, ILE910, ASP787, ASP911, and PHE908. The obtained calculated and experimental structures are similar (Fig. 1c). The fact confirms AutoDock Vina reproduces structure of **ZSTK474**-PI3K complex quite accurately and might be used for the present study.

Since **ZSTK** is placed in ATP-binding pocket of PI3K it was interesting to compare binding energy of **ZSTK** and ATP with the protein. Our calculation results in binding energy of –6.4 kcal/mol for ATP-PI3K cluster. This confirms ZSTK is a ATP-competitive inhibitor of PI3K with higher affinity as compared to ATP.

Further docking of structures, modeled on the base of **ZSTK474**, was performed with PI3K. Obtained clusters have similar structure with **ZSTK474**-PI3K cluster: modeled compounds are placed in ATP-binding pocket of the protein and have the same type of interaction with protein as **ZSTK474**. Docking results for some **ZSTK474** derivatives showed binding energy of –9.9 – –11.1 kcal/mol. These structures contain substituents in morpholine rings. Some of them are shown in Fig. 2.

While introduction of substituents in benzimidazole ring led to decrease of binding energy. The observed reducing protein affinity is in accordance with experimental evidence about decreased potency for benzimidazole ring substituted compounds as compared with **ZSTK474** [26]. More binding energy of protein-ligand complex in case of structures **Z1**–**Z4** was provided by additional molecular interactions formed between **Z1** and SER754, MET752; **Z2** and ILE910; **Z3** and

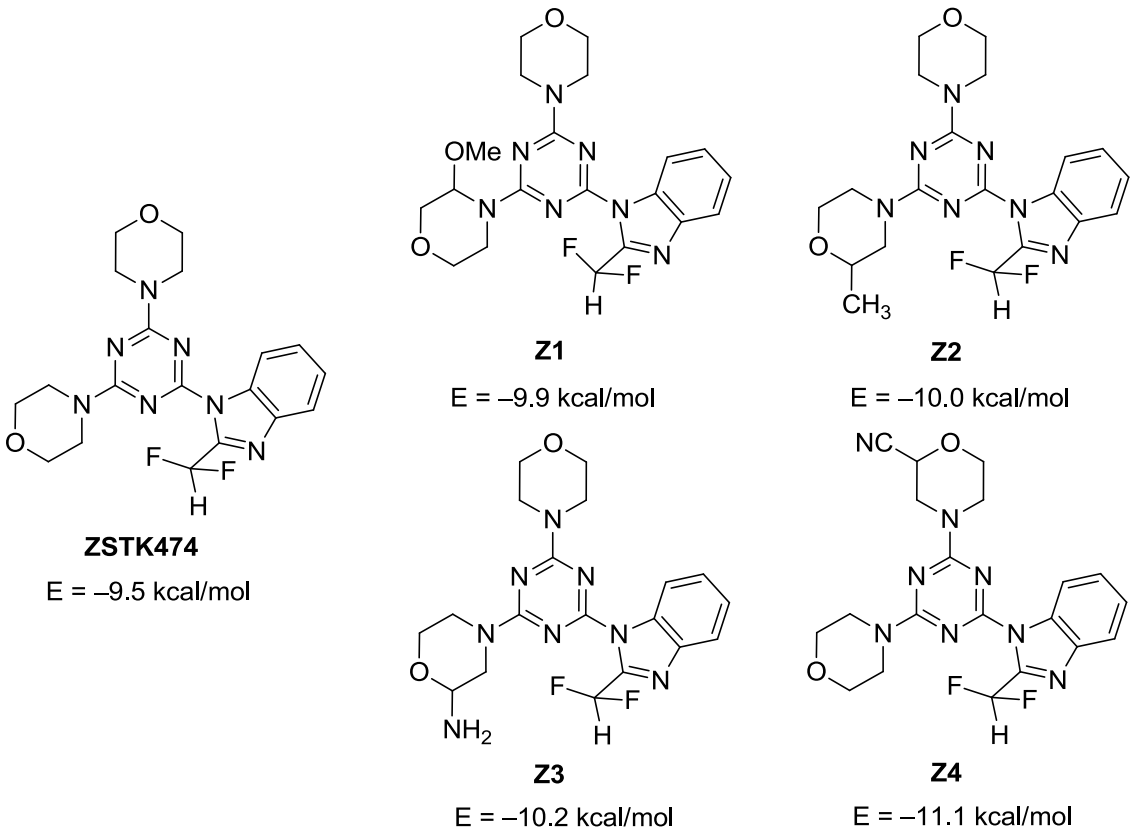

Fig. 2. ZSTK474 and modeled molecules with the lowest binding energies

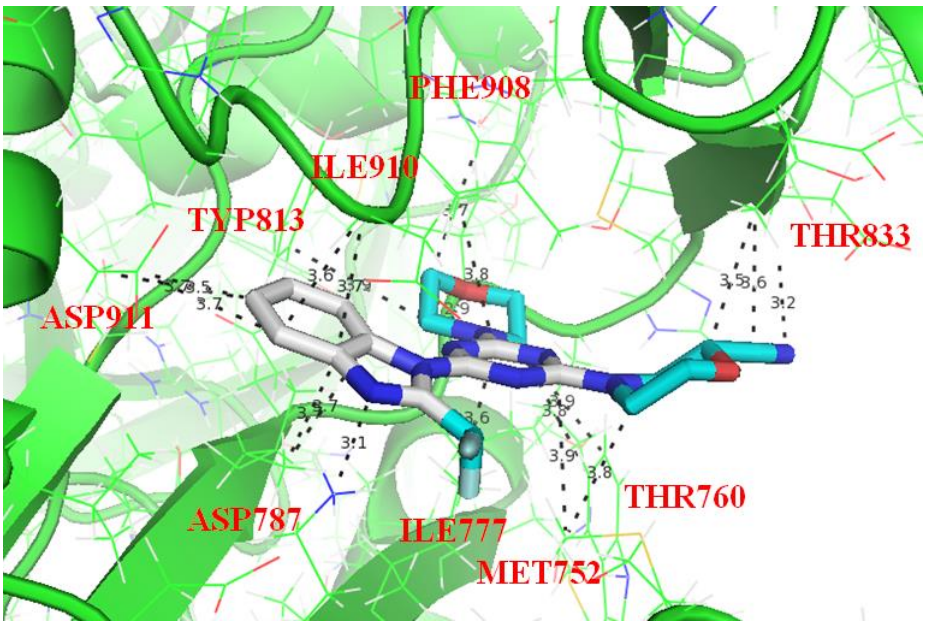

Fig. 3. The minimized energy pose of Z4 in PI3K

SER831, VAL828; **Z4** and THR833. The most energy favourable structure of potential inhibitor–PI3K complex along with intermolecular interactions is displayed in Fig. 3.

Further molecular descriptor analysis was performed to identify druglikeness of the **ZSTK474** derivatives **Z1**–**Z4** by Lipinski's Rule of five, which describes molecular properties important for a drug pharmacokinetics in the human body. According to the obtained results (Table 1) the derivatives **Z1**–**Z4** obey Lipinski's Rule.

Table 1

Calculated molecular descriptors for Z1–Z4

Compound	Molecular weight	log P	H-bond donors	H-bond acceptors
Z1	447	2.57	–	10
Z2	431	2.85	–	9
Z3	432	1.77	2	10
Z4	442	2.15	–	10
Lipinski's Rule	≤500	≤5	≤5	≤10

Conclusion

Modeling interaction between **ZSTK474** and PI3K using AutoDock Vina results in a cluster with a binding energy of –9.5 kcal/mol. The value is 3 kcal/mol lower than energy of ATP-PI3K complex formation. That computationally confirms the experimental evidence of effective role of **ZSTK474** as ATP competitive inhibitor of PI3K. Docking structures modeled on base of **ZSTK474** demonstrated influence of substitutes on a binding energy. Obtained results show that the introduction of methyl, amino, methoxy, and cyano groups in morpholine ring leads to increased binding of ligand with protein. While substituted benzimidazole ring causes decrease of binding. Four structures **Z1**–**Z4** with the best protein affinities (–9.9 – –11.1 kcal/mol) satisfy Lipinski's Rule of five, so, they could be potent more effective enzyme inhibitors than **ZSTK474** and, thus, potentially more effective anticancer agents. We think during clinical treatment the suggested compounds will display increased potency and favorable pharmacokinetics relative to the parent compound.

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