SEARCH OF PROTEIN KINASE CK2 INHIBITORS BASED ON PURINE-2,6-DIONES DERIVATIVES

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This work is aimed to the search of protein kinase CK2 inhibitors among the purine-2,6-dione derivatives by molecular docking and biochemical tests. It was found that the most active compound 8-[2-[(3-methoxyphenyl)methylidene]hydrazine-1-yl]-3-methyl-7-(3-phenoxypropyl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione inhibited protein kinase CK2 with IC_{50} value of 8.5 µM in vitro in kinase assay. Biochemical tests and computer simulation results allowed determining the binding mode of the most active compound and structure-activity relationships.

K e y w o r d s: protein kinase CK2, inhibitor, purine-2,6-dione, xanthine, virtual screening.

P rotein kinases is a family of enzymes that catalyze the phosphorylation of proteins. Since the regulation of most physiological and pathological functions depends on the level of the protein substrate phosphorylation, protein kinases are key targets for the drugs development [1].

Casein kinase II (CK2) is a pleiotropic (has more than 500 protein substrates [2]), serine-threonine protein kinase. This enzyme uses both ATP and GTP molecules as a phosphate donor and can be able to phosphorylate the tyrosine residue [4]. This enzyme has a tetrameric structure and consists of two catalytic (α, α and/or α') and two regulatory (β) subunits [3]. Catalytic subunits are encoded by genes that are localized in different chromosomes [5, 6]. It has been shown that high expression of α subunit causes suppression of apoptosis [7].

Protein kinase CK2 is involved in the development of a wide range of diseases. Viruses use this enzyme as a phosphorylating agent for their own proteins [8]. Inflammatory reactions are regulated by a number of transcription factors [9] activated by CK2. Overexpression or increased activity of this enzyme is observed in malignant neoplasms: lung cancer, hepatocellular cancer, colorectal cancer, breast cancer, leukemia, prostate cancer and other [10, 11]. It has been shown that CK2 is involved in the development of neurodegenerative diseases [12]: Alzheimer's disease [13], Parkinson's disease [14, 15], schizophrenia [16], Guam-Parkinson's dementia, progressive supranuclear palsy and Pick's disease [15]. Recently, the involvement of protein kinase CK2 in angiogenesis-related diseases was established [17]. Since some protein fibers are substrates for CK2 (transcription factors, troponin, myosin light chain and skeletal muscle proteins), it is obvious that this protein kinase may be involved in the development of diseases affecting skeletal muscles, bone and muscle tissues [18].

Due to the fact that protein kinase CK2 is involved in the regulation of a significant number of metabolic and pathological processes, the search for new inhibitors is promising both for pharmaceutical use and for application as a research tool in molecular biology.

Derivatives of purine-2,6-dione (xanthine) are purine alkaloids. Some of the xanthines are widely used in medicine [19]. For example, sodium caffeine benzoate and eufilin are used as stimulants of the central nervous system, cardiotonics and for spasm of blood vessels. Theobromine and theophylline are spasmolytic (vasoconstrictor, bronchodilator) and diuretic agents. Diprophylline and xanthine nicotinate are used to improve peripheral and cerebral circulation, respectively. Pentoxifylline is used for treatment of disorders of peripheral circulation, atherosclerotic disorders, ischemic states after a heart attack, in ophthalmology and hearing disorders.

It is known that purine-2,6-dione derivatives are inhibitors of BRAF protein kinase [20], Caf1 human poly (A) selective ribonuclease [21], 5-HT1A receptor [22], adenosine receptor [23], adenosine A2B receptor [24] and HM74A receptor [25]. Therefore, we decided to search for CK2 inhibitors among xanthine derivatives.

Materials and Methods

The synthesis of compounds was carried out at the Department of Biological Chemistry of Zaporozhye State Medical University under the leadership of Dr. Pharm. Professor M. I. Romanenko. *Molecular docking.* In order to find CK2 inhibitors we have performed virtual screening of 1943 organic compounds using AutoDock 4.2.6 program targeting ATP-binding site of enzyme. Ligands for the docking were prepared with Vega ZZ (command line) [27] and MGL Tools 1.5.6 [26]. Calculations of ligand geometry were performed using AUTODOCK force field.

Docking was carried out in catalytic subunit of CK2. The crystal structure of human protein kinase CK2 was obtained from the Brookhaven Protein Data Bank (PDB ID: 3NSZ) [28].

Water molecules, ions, and ligand were removed from the PDB file of receptor.

Ligands were prepared by Vega ZZ (command line) and MGL Tools 1.5.6. The incoming formats of receptor and ligands data were converted into PDBQT-format with Vega ZZ in AUTODOCK force field to carry out calculation with the aid of Autodock program. This format contains the coordinates of the atoms and partial charges. Hydrogen atoms were removed from nonpolar atoms. The receptor was prepared using MGL Tools and AutoGrid.

For docking calculation, we have used parameters that reported earlier [29].

Visual analysis of the best-scored complexes was performed using Discovery Studio Visualizer 4.0 [30].

Biochemical testing. Compounds were tested using *in vitro* kinase assay [31]. Each test was done in triplicate in a reaction volume of 30 μl, containing 6 μg of peptide substrate RRRDDDSDDD (New England Biolabs); 10 units of recombinant human CK2 holoenzyme (New England Biolabs); 50 μM ATP and γ-labeled 32P ATP, diluted to specific activity 100 μCi/μM; CK2 buffer (20 mM Tris-HCl, pH 7.5; 50 mM KCl; 10 mM $MgCl_2$) and inhibitor in varying concentrations. Incubation time was 20 min at 30 °C. The reaction was stopped by adding an equal volume of 10% o-phosphoric acid and the reaction mixture was loaded onto 20-mm discs of phosphocellulose paper (Whatman). Disks were washed three times with 1% o-phosphoric acid solution, air-dried at room temperature, and counted by the Cherenkov method in a beta-counter (LKB). As negative control an equal volume of DMSO was added to the reaction mixture. Percent inhibition was calculated as ratio of substrate-incorporated radioactivity in the presence of inhibitor to the radioactivity incorporated in control reactions, i.e. in the absence of inhibitor. Serial dilutions of inhibitor stock solution were used to determine its IC_{50} concentration.

Results and Discussion

To identify candidate inhibitors of CK2 we have performed structure-based virtual screening using molecular docking calculations. The virtual screening was performed using ATP-binding site of enzyme as the target and the collection of 1943 purine-2,6-dione derivatives.

After docking calculations, the best-ranked molecules based on predicted binding energy, were visually inspected in the complexes with amino acid residues of CK2 ATP-binding site.

The selected compounds were used for investigation of their inhibitory activity toward CK2 in vitro using a direct radiometric method [31]. The residual activity of CK2 at 10 μM compound concentration is presented in Table 1 (the chemical structures of compounds were drawn in the program MarvinSketch Chemaxon [32]).

Accordingly to the results of the biochemical assay, it was found that compound 1-8-[2-[(3-methyloxyphenyl) methylidene]hydrazine-1-yl]-3-methyl-7-(3-phenoxypropyl)-2,3,6,7 -tetrahydro-1H-purine-2,6-dione (compound **1**) was the most active

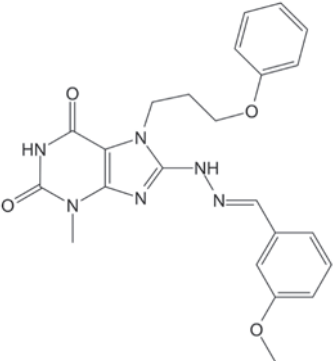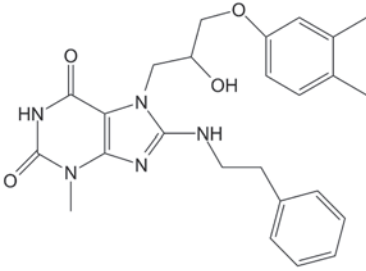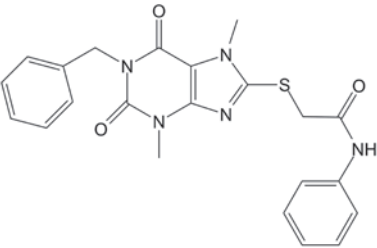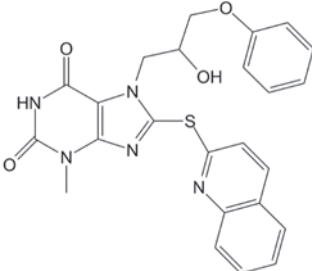*Structure and inhibitory activity of purine-2,6-dione derivatives toward CK2*
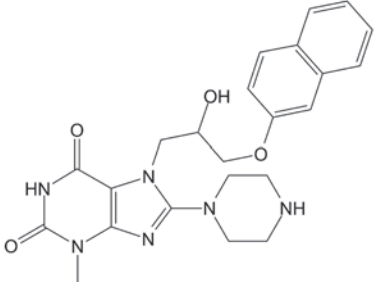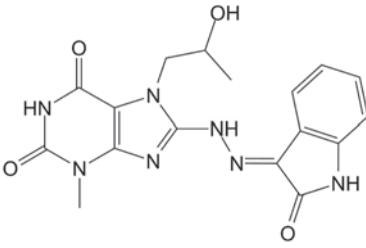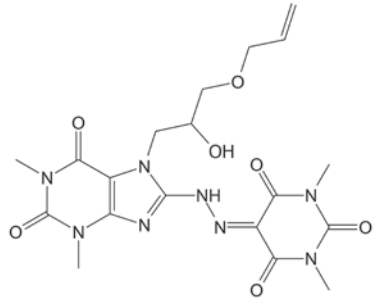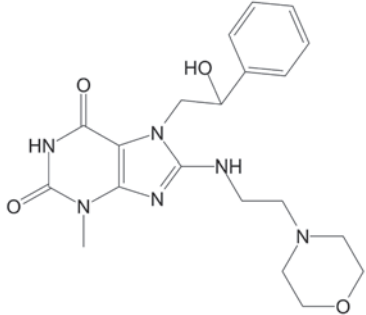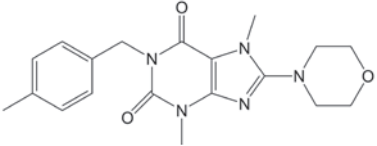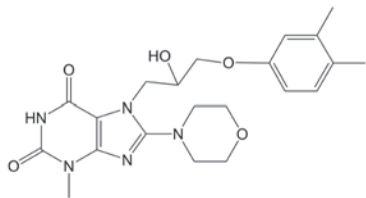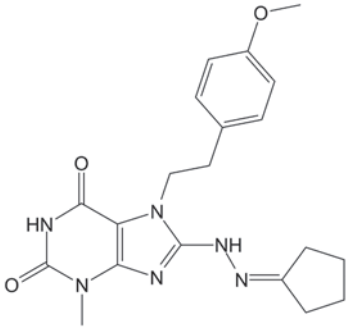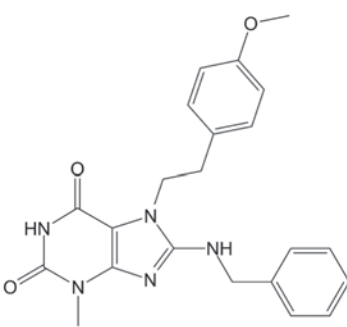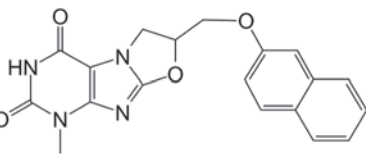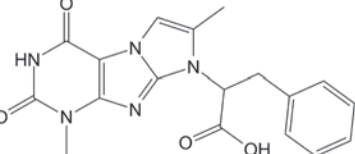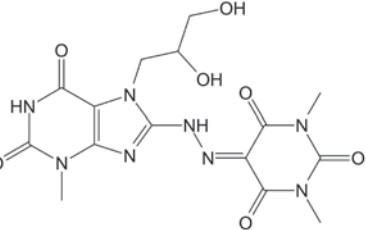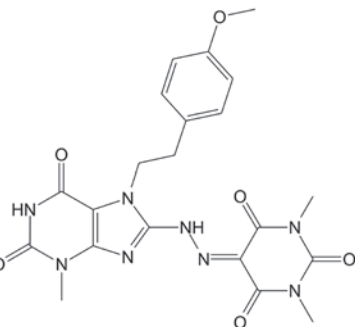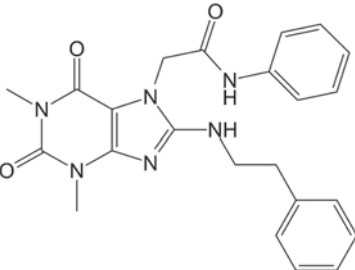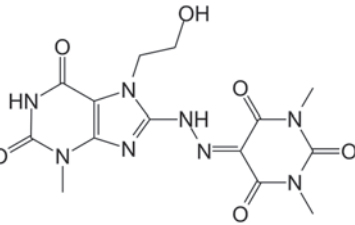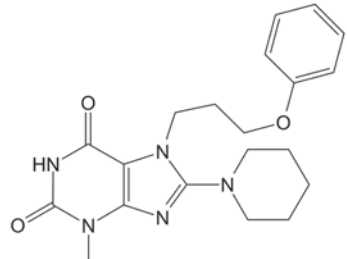
#	Structure	Residual activity at 10 µM, %	#	Structure	Residual activity at 10 µM, %
1		22.34 (IC_{50} = 8.5 µM)	2		59.48
3		60.49	4		69.75
5		71.05	6		78.67
7		78.75	8		80.49
9		81	10		81.9

T a b l e. (Continuation)

#	Structure	Residual activity at 10 μM, %	#	Structure	Residual activity at 10 μM, %
11		82.35	12		84.85
13		85.3	14		86.31
15		91.36	16		93.04
17		94.61	18		96.26
19		97.13			

among the studied (IC_{50} = 8.5 μM). Complex of compound **1** with ATP-binding site CK2 was obtained using the program Autodock 4.2.6 (Figure).

The xanthine fragment of compound **1** is located in the adenine-binding site of the CK2 ATP-binding pocket and forms hydrogen bonds with Glu114 and Val116. Xanthine heterocycle is involved in hydrophobic π-alkyl interactions with Ile174 and Val66. Methyl in the third position is directed to the hydrophobic region II. 3-phenoxypropyl is oriented towards the active site and forms hydrogen bond with Lys68.

The substitution of hydrogen atom with benzyl or 4-methylbenzyl in the first position of compounds **3** and **9**, respectively, leads to the loss of inhibitory activity toward CK2. It can be explained by displacement of xanthine cycle from the adenine-binding site with the loss of hydrogen bonds with the amino acid residues Glu114 and Val116.

Substituents containing flexible linkers in the seventh position of compounds **2**, **4**, **7**, **8**, **10**, **15**, **19**, **11**, **12** lead to deterioration of stability of hydrogen bond with Lys68 and as a consequence to decrease of CK2 inhibitory activity.

Since the search of new inhibitors was conducted with the aim of further development of drugs on their basis, it is important to take into account the physico-chemical properties of the studied compounds. One of these parameters is LogP. Under Lipinski's rule of five LogP value must not exceed 5. For compound **1** the value of cLogP was 2.9. Often, in order to improve the activity of compounds (especially in relation to protein kinase CK2), additional hydrophobic substituents are required. The inhibitory activity of compound **1** can be greatly improved by this approach.

As a result of our work we found a number of inhibitors of protein kinase CK2 among deriva-
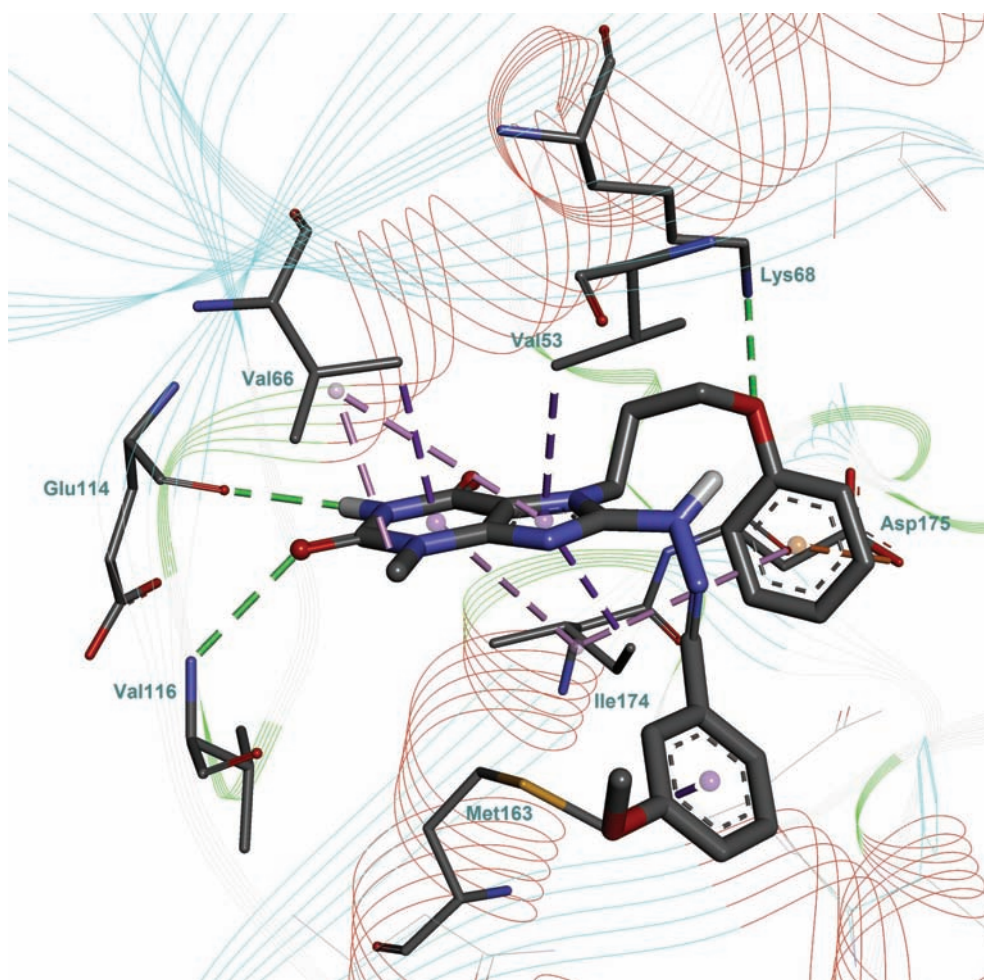
*The complex of compound **1** with ATP-binding site of human protein kinase CK2. Hydrogen bonds are indicated by green dotted lines, hydrophobic bonds – by purple dotted lines*

tives of purine-2,6-dione. IC_{50} of the most active compound 8-[2-[(3-methoxyphenyl)methylidene]hydrazine-1-yl]-3-methyl-7-(3-phenoxypropyl)-2,3,6,7-tetrahydro-1H-purine-2,6-dione is 8.5 μM. Summarizing analysis of SAR of the purine-2,6-dione derivatives, the importance of the substituents in the first and seventh position should be emphasized. The substitution of hydrogen atom in the first position with other substitutions and in the seventh position on large flexible substitutions has a negative effect on the inhibitory activity of the compounds toward CK2.

ПОШУК ІНГІБІТОРІВ ПРОТЕЇНКІНАЗИ СК2 СЕРЕД ПОХІДНИХ ПУРИН-2,6-ДІОНУ

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Робота присвячена пошуку інгібіторів протеїнкінази СК2 серед похідних пурин-2,6-діону за допомогою молекулярного докінгу та біохімічного тестування. Встановлено, що 8-[2-[(3-метилоксифеніл)метиліден]гідразин-1-іл]-3-метил-7-(3-феноксипропіл)-2,3,6,7-тетрагідро-1Н-пурин-2,6-діон інгібував активність протеїнкінази СК2 з IC_{50} 8,5 мкМ. Під час порівняння результатів біохімічного тестування та комп'ютерного моделювання визначено залежність інгібувальної активності сполук від їхньої структури та встановлено положення лігандів у сайті зв'язування.

К л ю ч о в і   с л о в а: інгібітори протеїнкінази СК2, ксантин, пурин-2,6-діон, віртуальний скринінг.

ПОИСК ИНГИБИТОРОВ ПРОТЕИНКИНАЗЫ СК2 СРЕДИ ПРОИЗВОДНЫХ ПУРИН-2,6-ДИОНА

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Работа посвящена поиску ингибиторов протеинкиназы СК2 среди производных пурин-2,6-диона с помощью молекулярного докинга и биохимического тестирования. Установлено, что 8- [2 - [(3-метилоксифенил) метилиден] гидразин-1-ил]-3-метил-7-(3-феноксипропил)-2,3,6,7-тетрагидро-1Н-пурин-2,6-дион ингибировал активность протеинкиназы СК2 с IC_{50} 8,5 мкм. При сопоставлении результатов биохимического тестирования и компьютерного моделирования определена зависимость ингибиторной активности соединений от их структуры и установлено расположение лигандов в сайте связывания.

К л ю ч е в ы е   с л о в а: ингибиторы протеинкиназы СК2, ксантин, пурин-2,6-дион, виртуальный скрининг.

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