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Evaluation of 4H-4-chromenone derivatives as inhibitors of protein kinase CK2

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Protein kinase CK2 (Casein Kinase 2) is a ubiquitous serine/threonine protein kinase involved in various cell signal transduction pathways. Thus, CK2 is a new perspective target for anticancer drugs. The receptor-based virtual screening of 2000 compounds from combinatorial library of 4H-4-chromenones has been carried out in search for CK2-inhibitors. 90 compounds have been chosen for biological testing based on the score values calculated by DOCK 4.0 software. It has been revealed, that 3-(4-chloro-3,5-dimethylphenoxy)-7-(4-methoxyphenylcarbonyloxy)-4-oxo-4H-chromene (12) and 7-(4-fluorophenylcarbonyloxy)-4-oxo-3-(4-phenylphenoxy)-4H-chromene (14) inhibit CK2 activity with IC_{50} = 18.8 μM and IC_{50} = 22.4 μM, respectively.

Key words: receptor-based design, CK2 kinase inhibitor, 4H-4-chromenone, chromone.

Introduction. Protein kinases are one of the largest enzymes family committed to the catalysis of protein phosphorylation, the most general and frequent mechanism controlling diverse aspects of cell life [1, 2].

Protein kinase CK2 (Casein Kinase 2) is a ubiquitous protein serine/threonine kinase participating in the regulation of cell growth and proliferation.

The negative regulation of the CK2 in cell control mechanisms (anti-apoptotic protecting function) has been also proven [3, 4]. Its activity is elevated in rapidly proliferating tissues as well as in the variety of tumors. CK2 may substantially contribute to carcinogenesis through its direct interaction with the cell-survival circuitry [3—8]. Hence, CK2 is a promising target for anticancer drugs.

Isoflavonoids are a large group of natural phenolic compounds distributed in the plant kingdom. The biological activity of isoflavonoids is related to their antioxidative effects [9—11] and their action on tumor cell proliferation, differentiation and apoptosis [12—16]. The antiproliferative activities of isoflavonoids include inhibition of protein tyrosine kinase [17—20], DNA topoisomerase I and 2 [21, 22], protein kinase C, phosphoinositol kinase [23—25] and cyclin-dependent kinases [26]. The CK2 inhibition has been also described [27]. Among flavonoids the most effective CK2 inhibitor is 3,3',4',7-tetrahydroxyflavone (Fisetin, IC_{50} = 0.35 μM) (Fig. 1) [27].

Molecular modeling techniques are intensively used in modern drug research and development. Strategies of the molecular modeling are based on the searching for structure-activity relationships of the chemical compounds for selection of promising compounds against a biological target. Receptor-based approach uses methods of molecular docking, molecular dynamics, energy minimization and molecular structure optimization to estimate ligand binding affinities to the receptor. The receptor spatial structure, obtained from X-ray, NMR or homology modeling data is necessary for this approach. Six spatial structures of the inhibitor—CK2 complexes have been determined by X-ray technique [28, 29, 41, 42].

The core of receptor-based approaches is a mo-

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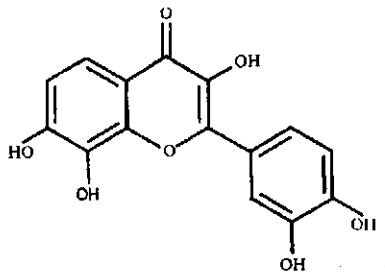


Fig. 1. Inhibitor of CK2 kinase — Fisetin

lecular docking. Docking procedure consists of generation of the receptor-ligand complexes and estimation of favorable interactions by analyzing contributions of each component of the complex into total free energy of binding. Recently DOCK software was successfully applied in a number of screening activities [30—33, 42]. So, for virtual screening combinatorial library of 4H-4-chromenones we have used the system based on DOCK 4.0 package.

Materials and Methods. *Software background.* To predict the affinity of diverse sets of compounds we have used our in-house screening system (Fig. 2). It uses the results on GAMESS AM1 semi-empirical calculation [34], geometry optimization by GROMACS [35], and docking output of DOCK package [36].

Receptor preparation. A receptor molecule has been minimized in water with GROMACS molecular dynamics simulation package (GROMACS force field, steepest descent algorithm, 1000 steps, em_tolerance = 100, em_step = 0,001). Active site spheres were calculated with DOCK *sphgen* software. 31 spheres from the biggest cluster of 37 spheres were selected to fill receptor active site. Six spheres were deleted manually since their positions were outside of the active site cavity. Connolly MS (http://www.net-sci.org/Science/Compchem/feature14.html) and Grid programs from DOCK package were used to generate receptor Connolly surface and energy grids. Surface and grid calculations were performed with parameter settings as in [37], except for grid spacing that was set to 0.3.

Ligand database processing. Ligand molecules have been processed with SCREENER in-house software (the preprocessing of input ligand database file, converting 2D structures to 3D), GROMACS MD package (fast energy minimization of the ligands by GROMOS 96 force field), and GAMESS QM package (complete energy minimization by AM1 semi-empirical method, calculation of partial charges). Our own program TOPBUILDER has been used to generate GROMACS-formatted molecular topologies, control ligand energy minimization in GROMACS, and assign atom partial charges calculated in GAMESS.

Flexible docking. DOCK program has been used for receptor-ligand flexible docking. DOCK input parameters have been set as in [37] with some exceptions to increase the calculations accuracy: the minimum of heavy atoms in the anchor was set to 6, the maximum number of orientations was set to 1000, and the «all atoms» model has been chosen. The docking scores have been obtained in the range from −14 up to −51 kcal/mol and used for selection of potential inhibitors. The compounds with scores less than −42 kcal/mol have been chosen as promising. The 90 best-scored candidates have been inspected for sterical clashes and unfavorable contacts and taken for the kinase assay analysis.

Chemical synthesis of the library of 4H-4-chromenone derivatives has been performed by modification of the reported methods [38—40]. Structure and purity of the synthesized compounds have been confirmed by ^{1}H NMR spectroscopy. The spectra have been obtained with Varian VXR-300 NMR spectrometer at 300 MHz.

Biological testing. The selected compounds have been tested using the kinase in vitro assay. The volume of reaction was 30 μl (buffer: 20 mM Tris-HCl, pH 7.5 at 25 °C; 50 mM KCl; 10 mM $MgCl_2$). Each reaction contained: 1 μg of peptide substrate, nearly 500 μM final; 10 units of CK2 human, recombinant, from «BioLabs» (USA), concentration 500000 unit/ml supplied in water buffered solution (0.02 μl of purchased solution was added for 1 reaction point).

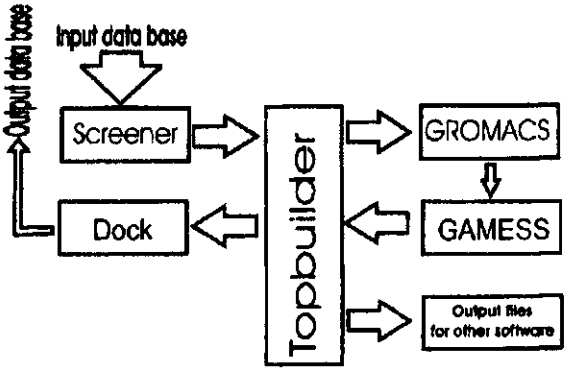
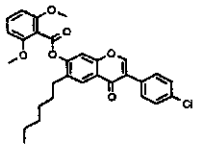
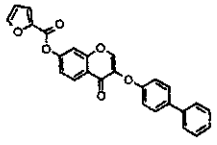
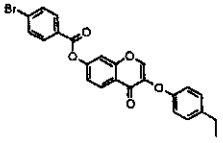
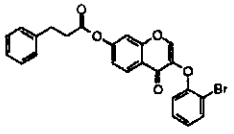
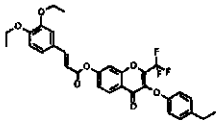
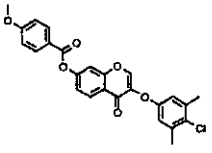
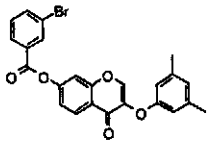
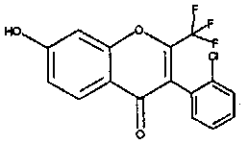
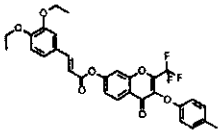
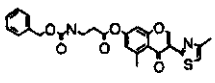
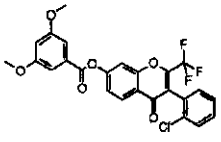
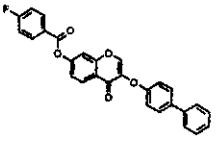
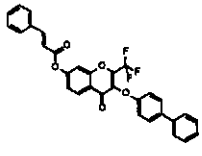
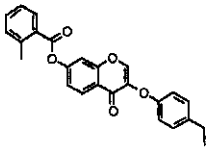


Fig. 2. Schematic representation of inhouse docking-based viritual screening system

The pre-screening data of the CK2 inhibition at compound concentration of 33 μM (the data show the structures with IC_{50} < 33 μM)

Structure of tested compound	Rest of CK2 activity, %	Structure of tested compound	Rest of CK2 activity, %	Structure of tested compound	Rest of CK2 activity, %
 1	30	 6	41	 11	35
 2	32	 7	41	 12	7
 3	33	 8	43	 13	35
 4	41	 9	31	 14	5
 5	37	 10	35		

The reaction master mix was prepared without ATP and aliquoted in 1.5 ml tubes at room temperature.

The stock solutions of inhibitors were prepared in DMSO, the concentration of inhibitor was 5 mM. The concentration of DMSO in the reaction did not exceed 3 %. At a higher concentrations, DMSO inhibits the CK2 activity more than 10 %.

ATP solution was prepared separately. For each sample 0.05 mCi of γ-[P^{32}]ATP was taken (specific activity of 100 μCi/μM).

The total concentration of labeled and unlabeled ATP was 40 μM. The reaction was started with adding ATP mix. The time of reaction was 20 min at 30 °C. The reaction was stopped by adding 20 μl of 0.5 M orthophosphoric acid, reaction mixture was loaded on the 20 mm filter discs of the cellulose phosphate paper («Whatman», Great Britain). Filters were washed three times with 0.075 M orthophosphoric acid at room temperature and dried.

For detection of products, dried filters were counted by Cherenkow's method on the LKB gamma-counter. 1 μl of DMSO was added to the reaction volume instead of the inhibitor stock solution for a positive control (blank). As a negative control we used Quercetine, the known inhibitor of CK2, in final concentration of 0.55 μM to inhibit the CK2 activity to 50 %. The sorption control was used from time to time as a reaction mix without enzyme, the non-specific sorption did not exceed 5 % of the lowest counts.

Results and Discussion. We have carried out the computer receptor-based virtual screening of 2000 compounds of combinatorial library of 4H-4-chromenone derivatives for design of CK2-inhibitors based on DOCK, GAMESS, GROMACS, and TOPBUIL-

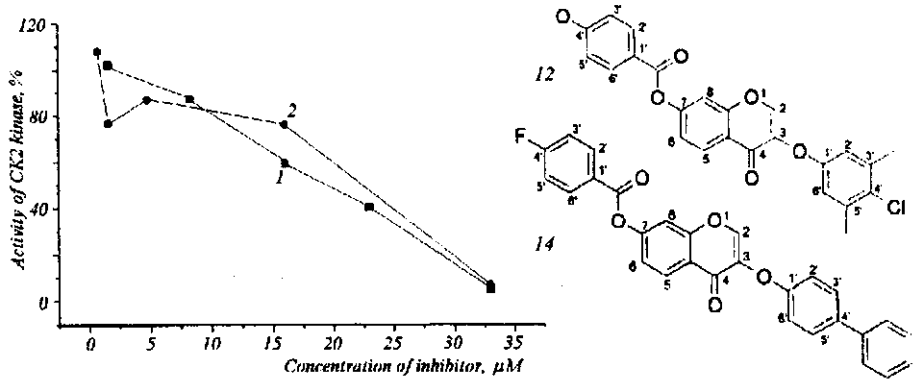


Fig. 3. Inhibition of CK2 activity by compounds 12, 14: inhibitor 12 IC_{50} = = 18.8 μM (*1*); 14 — IC_{50} = 22.4 μM (*2*)

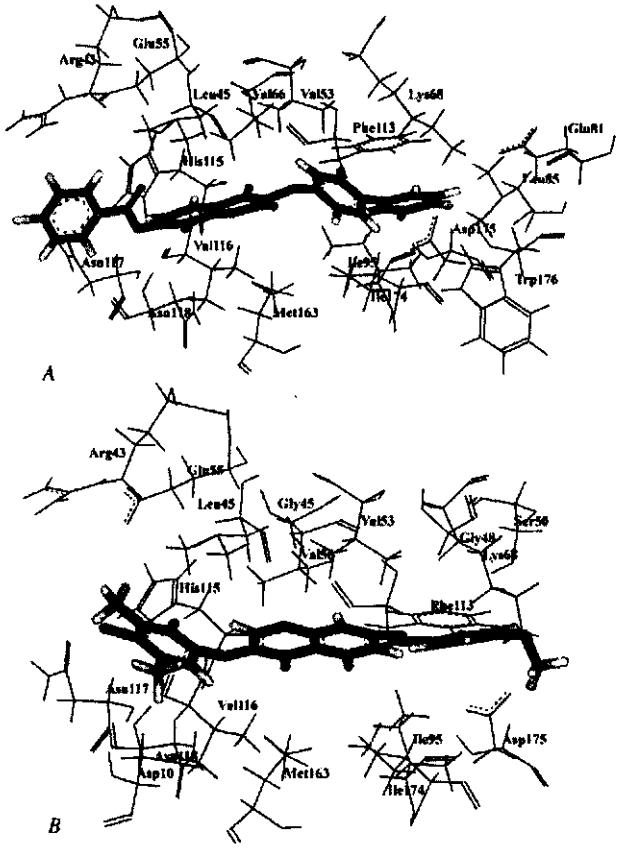


Fig 4. Binding modes of the active compounds 14 (*A*) and 12 (*B*) into the CK2 ATP-binding site

DER. 90 compounds have been chosen for biological testing based on the score values calculated by DOCK 4.0. The *in vitro* testing demonstrated, that 14 from them (15 % of selected compounds) inhibit CK2 activity with $IC_{50} < 33$ μM. The results of pre-screening the most active compounds 1—14 (selected data with permanent activity of CK2 kinase < 45 %, inhibitor concentration — 33 μM) are shown in Table.

The compounds 3-(4-chloro-3,5-dimethylphenoxy)-7-(4-methoxyphenylcarbonyloxy)-4-oxo-4H-chromene (12) and 7-(4-fluorophenylcarbonyloxy)-4-oxo-3-(4-phenylphenoxy)-4H-chromene (14) inhibit the CK2 activity for more than 90 %, and they have been selected for the testing at the five concentrations. The compound 12 demonstrates IC_{50} = = 18.8 μM ($18.8 \cdot 10^{-6}$ M), the compound 14 — IC_{50} = = 22.4 μM ($22.4 \cdot 10^{-6}$ M) (Fig. 3).

We performed visual inspection of obtained complexes and tried to indicate interactions caused activity of the inhibitors. The ligand-receptor hydrogen bonding that usually makes major contributions into ligand affinity have not been observed in both complexes. But it must be noted, that active site of rigid receptor does not structurally optimal for the binding of such type of ligands, and hydrogen bonds can be formed upon ligand-receptor complex fluctuations in solvent. Nevertheless, DOCK software selected these ligands as promising on the base of hydrophobic contacts. So it is reasonably to suppose that hydrophobic interaction makes the most important contribution to the stabilization of the inhibitors 12 and 14 in the CK2 active site (Fig. 4). The main hydrophobic contact for the inhibitor 14 (Fig 4, *A*) is a clamping of 4'-phenylphenilen residue into hydrophobic cave formed by amino acid residues Phe113, Ile95, Ile174, Trp176. The additional contribition into complex stability is a stacking between 4'-phenyl residue of 3-hydroxyphenyl group of ligand and residue Phe113. Isoflavone core and oxygen of its carboxyl group are also involved in weak hydrophobic and electrostatic interactions with residues Asn118, Met163 and Leu45.

In complex with the inhibitor 12 (Fig 4, *B*) the stacking with Phe113 is not observed. But stability of the complex is probably achieved due to 4H-4-chromenone ring which situated deeper in the cleft

and three methyl groups that enforce fixation of the ligand. The selectivity of the compounds is doubtful. There is no any strongly marked interaction with the key CK2 residues Val66 and Ile174 that would cause selectivity of effective CK2 inhibitors [25].

On a basis of obtained data some conclusions were made. The presence at C-3 position of the 4H-4-chromenones of high hydrophobic group is favorable, and its replacing with the 2'-bromophenoxy-, 4'-methoxyphenoxy-, 3',4'-dimethoxyphenyloxy-, 2'-isopropylphenyloxy-, 4'-biphenyloxy moieties resulted in the loss of the CK2 inhibitor activity. The addition of two methoxy groups into positions 3'- and 5'- of 7-(4'-methoxybenzoyl)- of the compound 12 also resulted in the activity decrease. The introduction into C-7 position of the compound 14 of the 4'-nitrobenzoyl-, 4'-methoxybenzoyl-, 2'-methoxybenzoyl-, 3',4'-dimethoxybenzoyl-, 2',6'-dimethoxybenzoyl-, benzoyl-, 2'-furoyl- instead of 4'-fluorbenzoyl-decreased the CK2 kinase inhibitor activity. Further studies will be performed on the optimization of the active structures 12, 14.

Conclusions. The 90 compounds from the combinatorial library of 2000 4H-4-chromenones have been selected using computer receptor-based virtual screening. It is revealed, that 14 from them (15 % of selected compounds) inhibit CK2 activity with $IC_{50} < < 33\ \mu M$. The two compounds 3-(4-chloro-3,5-dimethylphenoxy)-7-(4-methoxyphenylcarbonyl oxy)-4-oxo-4H-chromene (12) and 7-(4-fluorophenylcarbonyloxy)-4-oxo-3-(4-phenylphenoxy)-4H-chromene (14) inhibit the CK2 activity with $IC_{50} = 18.8\ \mu M$ and $IC_{50} = 22.4\ \mu M$, respectively.

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Пошук інгібіторів протеїнкінази CK2 серед похідних 4H-4-хроменонів

Резюме

Протеїнкіназа CK2 є убіквітальною багатосубстратною кіназою, що залучена до багатьох сигнальних шляхах клітинного росту та проліферації. CK2 кіназа розглядається як перспективна мішень для створення протиракових ліків. Для пошуку інгібіторів протеїнкінази CK2 нами проведено комп'ютерний рецептор-орієнтований віртуальний скринінг 2000 сполук комбінаторної бібліотеки 4H-4-хроменону. На основі розрахованих програмою DOCK 4.0 оціночних показників вільної енергії взаємодії відібрано 90 сполук для біологічного тестування. Знайдено, що дві сполуки пригнічують активність CK2 кінази з $IC_{50} = 18,8$ мкМ та $IC_{50} = 22,4$ мкМ

Ключові слова: рецептор-орієнтований пошук, інгібітори CK2 кінази, 4H-4-хроменон, хромон.

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Поиск ингибиторов протеин киназы CK2 среди производных 4H-4-хроменонов

Резюме

Протеин киназа CK2 — убиквитальная многосубстратная киназа, участвующая ко многих сигнальных путях клеточного роста и пролиферации. CK2 киназа рассматривается как перспективная мишень для создания противоопухолевых лекарств. Для поиска ингибиторов протеин киназы CK2 нами проведен компьютерный рецептор-ориентированный виртуальный скрининг 2000 соединений комбинаторной библиотеки 4H-4-хроменона. На основании рассчитанных программой DOCK 4.0 оценочных показателей свободной энергии связывания отобраны 90 соединений для биологического тестирования. Выявлено, что два соединения ингибируют активность CK2 киназы с $IC_{50} = 18,8$ мкМ и $IC_{50} = 22,4$ мкМ.

Ключевые слова: рецептор-ориентированный поиск, ингибиторы CK2 киназы, 4H-4-хроменон, хромон.

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