Integration of Structure-Based Drug Design and SPR Biosensor Technology in Discovery of New Lead Compounds

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At present time the pathway of drug discovery consists of some basic steps from idea to market: (i) disease selection, (ii) target selection, (iii) lead compound identification, (iv) lead optimization, (v) preclinical trial evaluation, (vi) clinical trials and (vii) drug manufacturing. All this process is highly time- and money-consuming and cost-saving of the last three steps is practically impossible owing to strict state standards and laws. Therefore, researchers try to reduce R&D costs of earlier stages which result to lead compounds discovery and optimization. Scientists paid special attention to increase the efficiency of lead compounds development using *in silico* technologies (bioinformatics and structure-based drug design) integrated with new *in vitro* experimental methods. Recently these approaches have been merged into platform “From Gene to Lead Compound” [1-3].

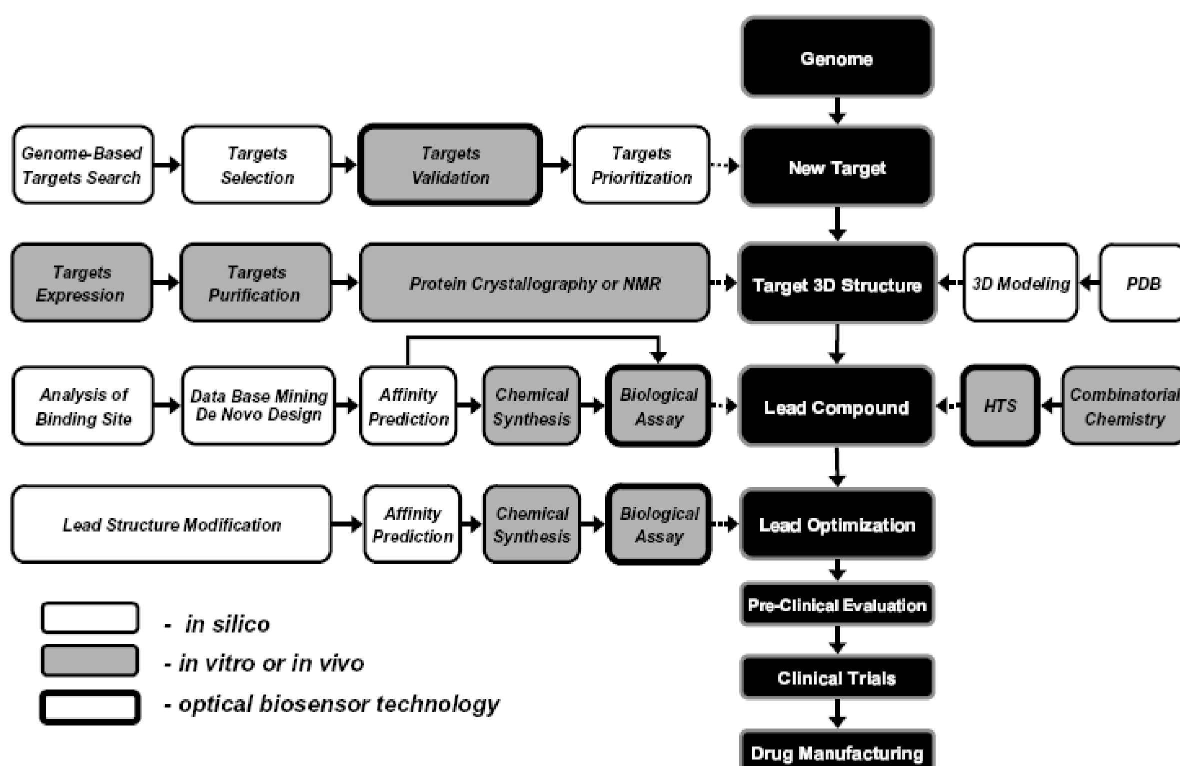

Fig. 1. A schematic illustration of pipeline “from gene to drug”: integration of virtual and real experiments.

Several steps of this platform include computer 3D modeling of target protein complexes with potential lead compounds, virtual screening of chemical databases using molecular docking procedures, affinity prediction, etc. Such bioinformatics technologies can reduce the list of potential lead compounds that must be further tested by up to hundreds times. At the same time, these approaches cannot completely replace the real experiments. The purpose of computer methods is to generate highly probable hypotheses about new ligands that must be tested later in real experiments.

From our point of view, the optical biosensors based on effect of surface plasmon

resonance (SPR) are more useful for solving different experimental tasks in analyzing intermolecular interactions. It is necessary to highlight the universal character of SPR technology, which allows sensitive real-time monitoring of association and dissociation reactions between molecules regardless of their chemical nature and binding forces, therefore providing important kinetic and thermodynamic information on molecular interactions. It can be used for analysis of protein interaction with small ligands with MW is up to 100 Da.

The given report is aimed to show two examples from our works in lead compounds discovery utilized integration of virtual and SPR biosensor technologies.

In silico and in vitro screening of HIV protease dimerization inhibitors.

The main function of HIVp is the slicing of viral preprotein to mature proteins. The enzyme also aggravates AIDS by damaging the host cell proteins. Many rather effective competitive inhibitors of HIVp are known and some of them are used now in AIDS therapy. Their systematic application as drugs, however, inevitably promotes the generation of viral strains that are resistant both to the inhibitor used and to most of its structural analogs.
The drug-resistant strains are a result of HIVp mutational modifications. HIVp operates in dimeric form, consisted of two identical subunits. The main interface region in the homodimer represents the antiparallel four-strand β-sheet, which involves the C- and N-terminal peptides of both subunits.

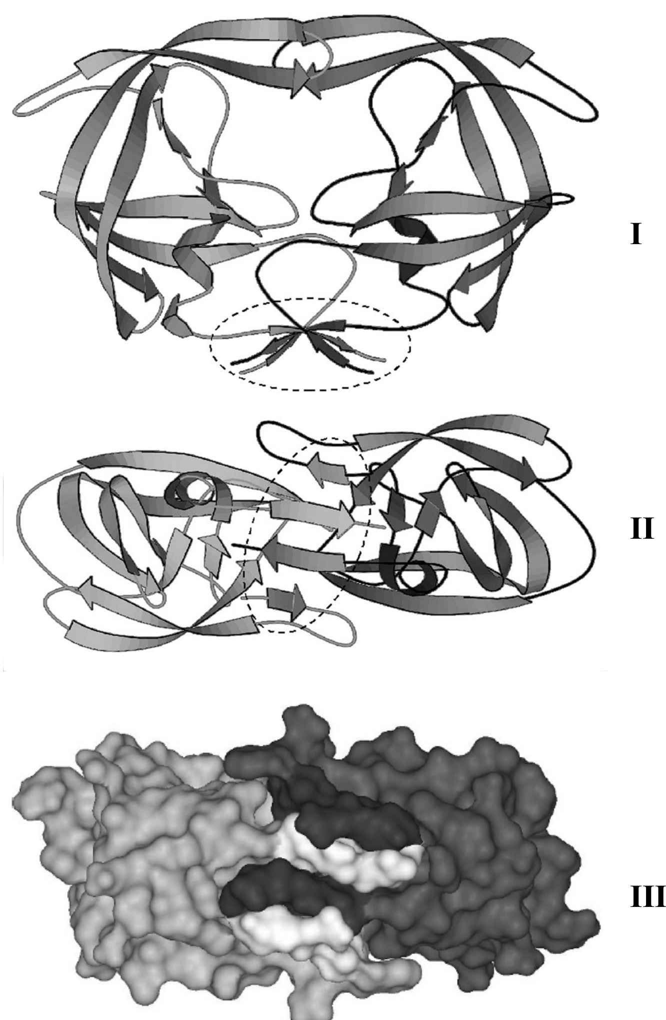

Fig. 2. Schematic structure of HIVp homodimer
I – front view
II – bottom view
The dotted line leads around the area of the main HIVp dimerization interface, formed by C– and N–terminal sites of both subunits (III - colored by white and black).
Coordinates of HIVp structure were taken from PDB (hiv 10nci).

It is hypothesized that specific ligands binding with dimerization interface can interfere with enzyme dimerization. If the binding site coincides or overlaps with the interface

region, all the mutations that diminish subunit affinity to a ligand will be also affect negatively inter-subunit interactions. As a result, the mutant protease formed will be less stable and, consequently, less active dimers. At least two highly specialized and synchronous mutations are necessary for generation of drug resistant strain with high inter-subunit affinity. It is obvious that such mutations are highly improbable.

Our results of discovery of HIVp dimerization inhibitors [3, 4] are presented below.

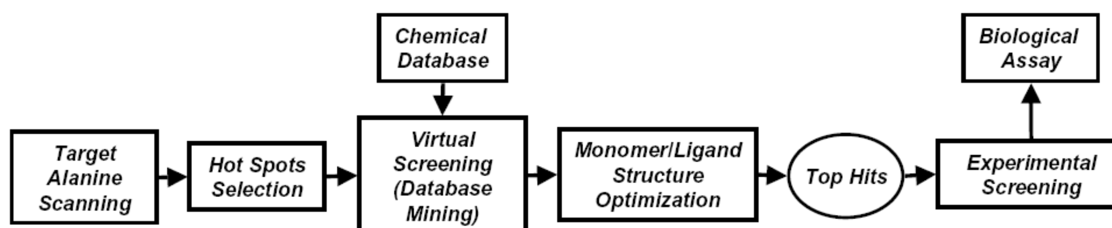

Fig. 3. Strategy of screening of HIVp dimerization inhibitors: integration of *in silico* and *in vitro* technologies [3].

The computational alanine scanning was carried out with HIVp as target. Four hot spots have been found playing important role in HIVp dimerization.

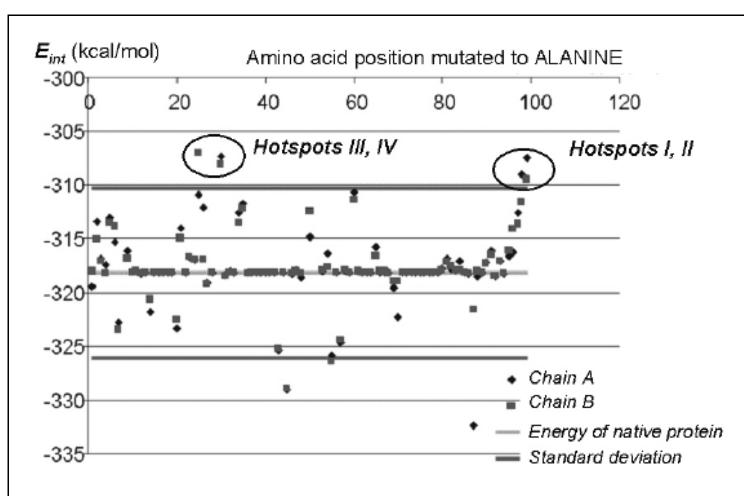

Fig. 4. Computational alanine scanning of HIVp for dimers stability.

Further NCI database (National Cancer Institute, USA), containing about 250 000 commercially available compounds, was used for virtual screening of inhibitors of HIVp dimerization. Database mining was carried out using molecular docking software AutoDock. As a result, a list of about 10,000 potential lead compounds was obtained. At the next step, all hypothetical molecular complexes were structurally and energetically optimized using molecular modeling software Sybyl. The final top list of about 1000 probable lead compounds was formed. Prioritization of these hypothesis gave us 42 structures (top hits) for subsequent experimental testing.

It was necessary to develop biological assay for direct *in vitro* analysis of interactions of lead compounds with the interface site of HIVp monomer. This assay was created based on SPR biosensor Biacore 3000. HIVp dimmers were immobilized in 2 channels of optical chip CM5. Further dimers in channel #1 were stabilized by chemical cross-linking, while in channel #2 - were dissociated up to monomers. The testing is carried out by sequential injections of solutions of analyzed substances into both biosensor channels. Binding of a tested compound only with HIVp monomers immobilized in channel #2 is considered as a positive result.

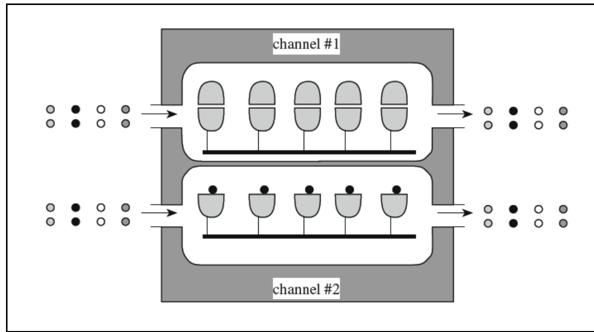

Fig. 5. The scheme of the differential biosensor test system for the analysis of interaction of potential dimerization inhibitors with dimers and monomers of HIVp.

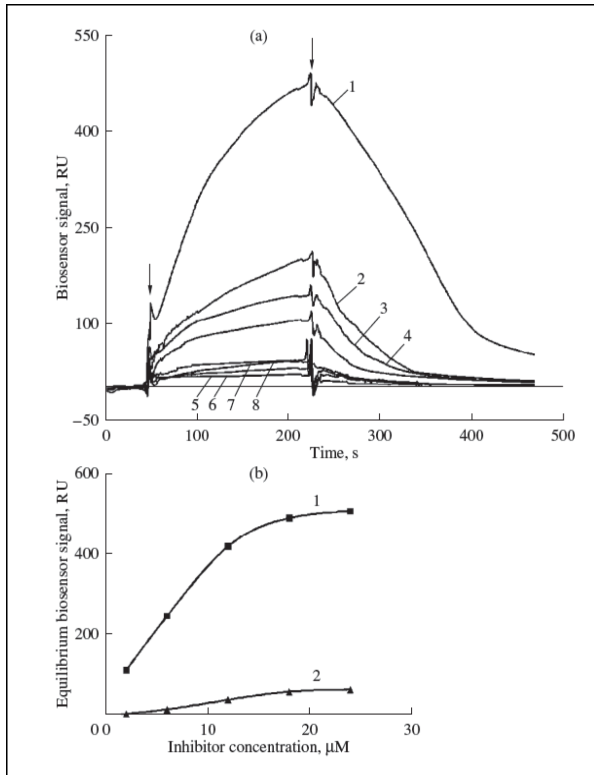

Fig. 6. Validation of the differential biosensor tests system for *in vitro* screening of potential inhibitors of HIVp dimerization.

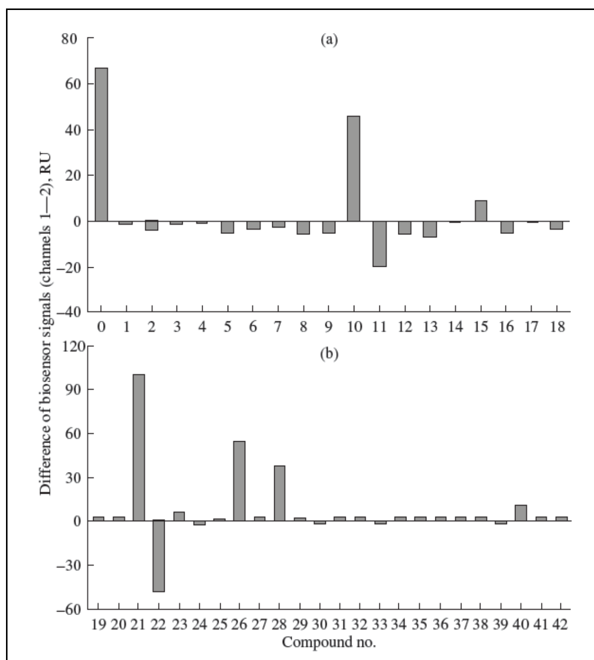

Fig. 7. Results of the *in vitro* biosensor screening of potential inhibitors of HIVp dimerization.
Compound 0 – positive control (known peptide inhibitor).

Verification of assay was carried out using known peptide inhibitor of HIVp

dimerization. It is visible that the control inhibitor actually has bond only with monomeric form of HIVp, which indicates that it interacts selectively with subunits interface.

Using the developed test system we have performed screening of most perspective candidate substances found by the method of virtual screening. As the result of this study we have found several compounds, which preferentially interacts with HIVp monomers. Biochemical analysis of inhibitory activity of these substances towards enzymatic activity of HIVp has shown that some of them inhibit HIVp with the IC50 value of about 10^{-6} M.

Screening of novel ligands for human cytochrome P450(51)

The aim of this research was to apply the integration of *in silico* and *in vitro* technologies in screening of novel ligands for human cytochrome P450(51). This enzyme catalyzes the oxidative removal of the 14α-methyl group (C-32) of the lanosterol, which is an essential reaction in the biosynthetic pathway for cholesterol, and is thus a useful target for anti-atherosclerosis drug design.

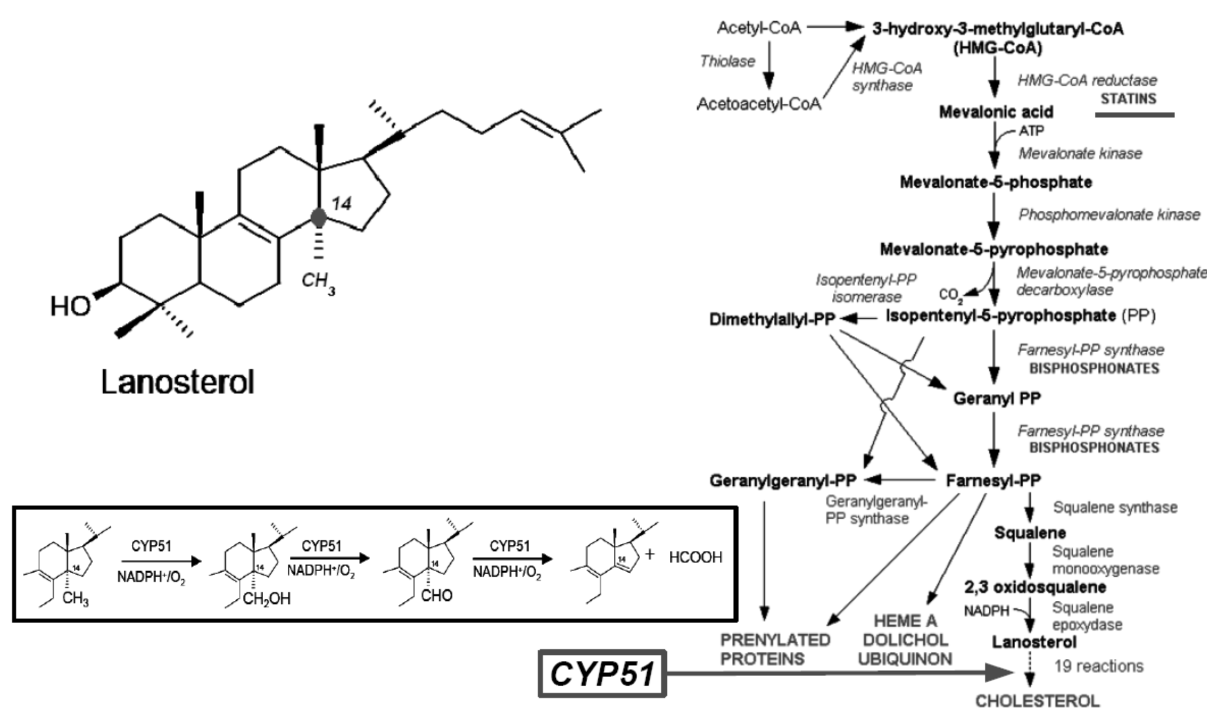

Fig. 8. Human cytochrome P450(51) (CYP51, 14alpha-demethylase) catalyzed reaction in biosynthesis of cholesterol.

Virtual screening was carried out by using molecular docking program Dock 6.0, which docks ligands with flexible conformation into the rigid active site of enzyme. Crystal structure of human P450(51) (PDB code 3I3K) and inside database of steroid and triterpenoid compounds available in our laboratory were used. Known P450 inhibitors (azole derivatives) were utilized as positive control. Virtual screening have resulted in obtaining the list of mostly perspective potential ligands. Some compounds from the list were included into the set of 22 compounds for experimental screening.

Experimental screening was carried out by using surface plasmon resonance (SPR) biosensor Biacore T-100. CYP51 was immobilized on the surface of optical chip CM5 by covalent coupling. Verification of assay was carried out using known P450 inhibitors: ketoconazole, Liarozole, Doconazole and Parconazole. It is visible that not all control inhibitors actually has bond with human CYP51.

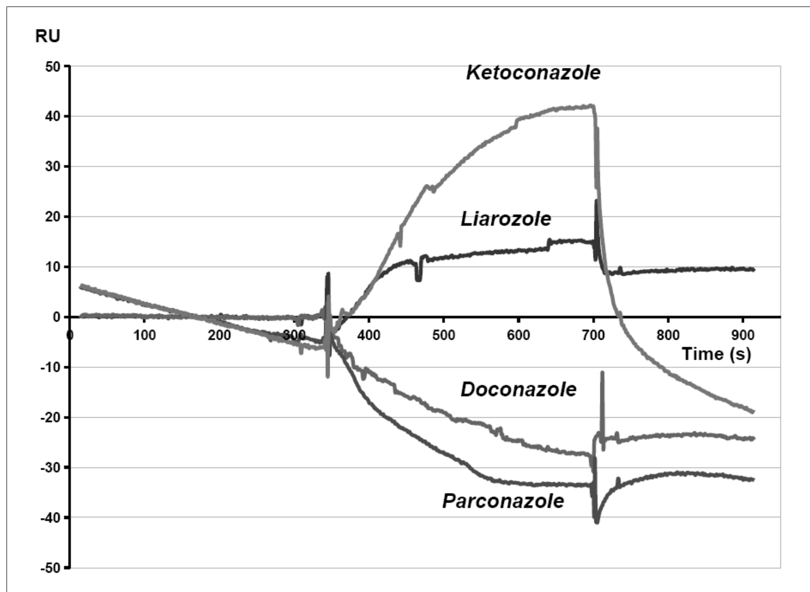

Fig. 9. Human CYP51 biosensor assay validation.

SPR screening of human CYP51-ligand interaction yields 12 potential ligands, which bound with target protein (light gray bars in Fig. 10). Among these ligands the subsequent biochemical analysis revealed 4 compounds with micromolar Kd and IC50 values including one (compound 12) which is listed in pharmacopeia.

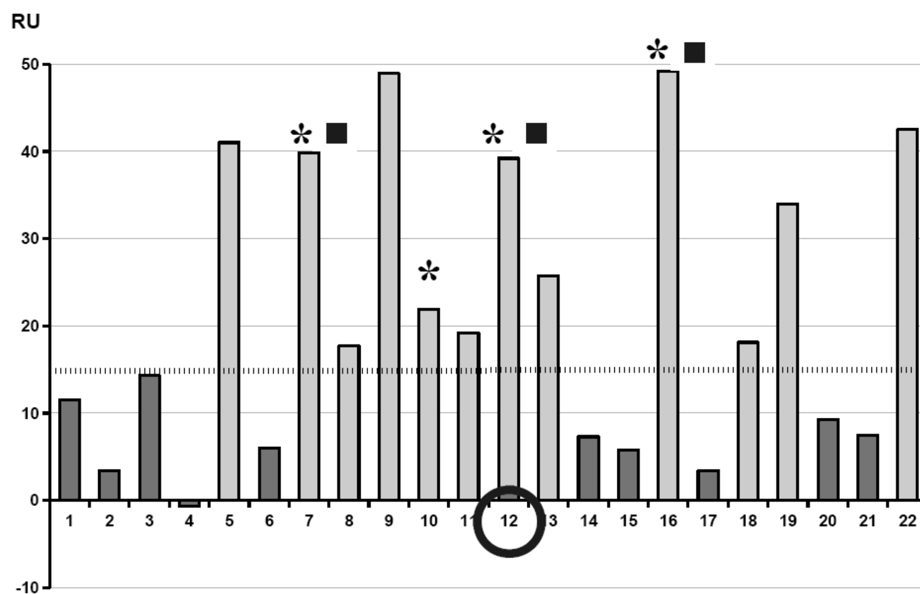

Fig. 10. Results of SPR screening of human CYP51-ligand interaction, spectral titration and biochemical testing.

Conclusion. Integration of virtual and SPR biosensor screening technologies has allowed us to find some new potential ligands for target proteins.

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