ISSN 2409-9066. Sci. innov. 2016, 12(3): 43—55 doi: http://dx.doi.org/10.15407/scine12.03.043

Karpov[1], P.A., Demchuk[1], O.M., Britsun[2], V.M., Lytvyn[1], D.I., Pydiura[1], N.O., Rayevsky[1], A.V., Samofalova[1], D.A., Spivak[1], S.I., Volochnyuk[2], D.M., Yemets[1], A.I., and Blume[1], Ya.B.

[1] Institute of Food Biotechnology and Genomics, the NAS of Ukraine, Kyiv
[2] Institute of Organic Chemistry, the NAS of Ukraine, Kyiv

NEW IMIDAZOLE INHIBITORS OF MYCOBACTERIAL FtsZ: THE WAY FROM HIGH-THROUGHPUT MOLECULAR SCREENING IN GRID UP TO *in vitro* VERIFICATION

Within the framework of virtual organization CSLabGrid, high-throughput molecular screening has been performed for new antituberculosis compounds. Using the FlexX program installed on the Institute of Food Biotechnology and Genomics (IFBG) Cluster and models of four promising ligand binding sites on the surface of FtsZ protein from Mycobacterium tuberculosis, virtual screening has been done for the database containing 2886 compounds synthesized in the Institute of Organic Chemistry of the NAS of Ukraine. Based on the LE and ΔG scores, the docking scores of CCDC Gold, and the results of molecular dynamics, a group of Mycobacterial FtsZ inhibitors has been selected. In vitro validation have revealed 6 compounds with the highest inhibition of GTPase activity of FtsZ. Also, based on in vitro experiment, three of selected compounds demonstrste strong inhibition of FtsZ polymerization together with inhibition of its GTPase activity.

Keywords: tuberculosis, structural biology, bioinformatics, high-throughput screening (HTS), and in vitro.

Tuberculosis is one of the most widespread infection diseases in the world. In 2011, the WHO reported 8.7 million new cases of disease and 1.4 million fatal outcomes caused by *Mycobacterium tuberculosis* [1, 2]. In Ukraine, an outbreak of tuberculosis was recorded in 1995, and, unfortunately, the situation has not been improved yet [3—6]. It should be noted that the curing of tuberculosis is complicated by many factors, including one of the most important ones, a genetic variability of *M. tuberculosis* proteins targeted by drugs [7]. To this end, the most advisable strategy is to design new drugs targeting the most conservative proteins. The majority of existing antituberculosis drugs are known to act at the stage of synthesis of cell walls, proteins or fat acids [8—10]. Among anti-TB compounds, there are such benzimidazole derivatives as VRT-125853 and VRT-752586 [11, 12] inhibiting the bacterial topoisomerases II and IV [11, 13].

At the same time, as the number of *M. tuberculosis* strains resistant to the existing drugs grows, it is necessary to search new molecular targets and compounds in order to guarantee success in fighting the tuberculosis pestilence [14]. Undoubtedly, the highest potential can be showed by the proteins constituting the cell division mechanism [15], first of all, the bacterial homologs of tubulin, namely, FtsZ (Filamental temperature-sensitive Z)-proteins [12, 16, 17]. The globular parts of bacterial FtsZ-proteins and eukaryotic tubulin show a quite high similarity of spatial structure [2]. To-

gether they form a common protein family that significantly differs from other GTP-binding proteins [17]. Due to certain structural similarity of FtsZ-proteins and tubulins, selective interaction between the ligands and Mycobacterium FtsZ should be caused by certain differences in amino acid composition of respective sites of this protein as compared with the human tubulin. According to the literature data and the results of our own research the imidazole derivatives are considered very promising candidates for inhibitors of FtsZ-protein polymerization [2, 13, 18]. *Firstly*, different imidazole derivatives show a certain specificity to various β-tubulins at the level of individual kingdoms [19, 20]. *Secondly*, as exemplified by the benzimidazole derivatives, it is possible to cause destruction of bacterial cytoskeleton [21—23].

The main requirements for methodological approaches to be used for studying the substances capable of effecting the FtsZ-protein polymerization are their high sensitivity and an approximated native ability to polymerize *in vitro*. At the beginning of prokaryotic cell division, the FtsZ-proteins form a cytokinetic ring in the center of the cell, which acts as scaffold for association of other proteins of cell division. The formation of so called *Z-ring* plays a crucial role in proper localization of division plane. The dynamic assembly of FtsZ-ring is regulated by a group of proteins depending on the type of bacteria [24]. For instance, the ZapA, ZipA, and FtsA proteins are stabilizing factors, whereas the SulA, EzrA, and MinCD are typical destabilizers [25]. The overexpression of stabilizing factors leads to the formation of abnormal extended structure of the FtsZ-ring, while that of destabilizers can entail destruction of FtsZ-polymeric formations [26]. The SepF protein stimulates the assembly of FtsZ-protein in critical concentration and creates stable complexes with its monomers. Among negative regulators of the *M. tuberculosis* Z-ring formation, the SulA, EzrA, and MinCD proteins should be mentioned[4]. The SulA protein is bound directly to FtsZ-protein and depending on concentration can inhibit either polymerization or GTF activity of the latter. The EzrA protein is able to destabilize newly created FtsZ-ring thereby preventing the creation of Z-ring in nonspecific loci of the cell. Finally, the MinCD[4] proteins prevent the creation of anomalous Z-rings on the cell poles [27].

The specific features of FtsZ-protein polymerization have been studied *in vitro*, therefore the formation of primary super-molecular structures such as straight and curved protofilaments, filament sheets as well as beams and tubes of the filaments have been described in detail depending on the composition of components of polymerization buffer, nucleotide concentration, and additional proteins involved in the reaction. The FtsZ protofilament architecture under the natural conditions has not been studied well yet, although electron cryotomography (ECT) on *Caulobacter crescentus* cells enable to assume Z-ring as a result of binding of relatively short primary protofilaments rather than of extensive assembly [28].

Hence, the bacterial FtsZ-proteins are undoubtedly among the molecular targets of priority pharmacologic importance and should be studied very carefully. Recently, *I. Ojima* group [2] have showed that 3-substituted benzimidazole derivatives are capable for efficientl inhibiton of mycobacterial FtsZ-protein. These data and our experience in structural bioinformatics and experimental study of FtsZ-proteins, synthesis of new compounds of imidazole group [13, 23], and development of algorithms for the application in Grid-technologies for searching and selecting new compounds with anti-tubulin effect [29—32] hit upon an idea of making experiment with the use high-throughput molecular screening for imidazole compounds effectively inhibiting the polymerization of FtsZ-proteins. In particular, the authors of this research from the Institute of Organic Chemistry of the NAS of Ukraine have created a chemical library of imidazole derivatives numbering in 2886 individual compounds. This library has been used by us as framework for a virtual library for Grid-based virtual screening.

In 2011, a virtual laboratory CSLabGrid (http://ifbg.org.ua/uk/cslabgrid; http://www.youtube.com/user/CSLabGrid) was launched based on Grid-cluster of the Institute for Food Biotechnology and Genomics of the NAS of Ukraine (http://ifbg.org.ua) [29–32]. CSLabGrid is aimed at joining computational resources and professionals for solving theoretical and applied tasks of molecular and cell biology research of cytoskeleton. Therefore, the selection of FtsZ *M. tuberculosis* inhibitors is an example of biologically active compound screening that fully complies with the conception of CSLabGrid, in particular, a practical combination of Grid computations with further laboratory testing. Hence, this research is aimed at implementing the modern drug design algorithm *chemical synthesis → virtual screening → laboratory confirmation of biological activity* as exemplified by benzimidazole series compounds capable to inhibit the FtsZ polymerization.

METHODOLOGICAL FRAMEWORK OF RESEARCH

3D modelling of *M. tuberculosis* FtsZ-protein

The complete amino acid sequence of *M. tuberculosis* FtsZ-protein (P64170) [33, 34] was obtained from the UniProt (www.uniprot.org/) database [35]. Upon the results of PDB-blast search, crystallographic structures that are most similar to *M. tuberculosis* FtsZ-protein have been selected from RCSB Protein Data Bank (http://www.rcsb.org): 2Q1X (2,35 Å), 2Q1Y (2,30 Å), 1RLU (2,08 Å), 1RQ2 (1,86 Å) i 1RQ7 (2,6 Å). The quality of PDB-structures has been analyzed using «DeepView-Swiss-PdbViewer4.0.3» (http://www.expasy.org/spdbv/) [36].

The protein homological structure was modelled using Modeller 9v8 (http://salilab.org/modeller/) [37] software and selected template structures from RCSB Protein Data Bank (www.rcsb.org). Two specific methods have applied: *1*) using a template *x*-Ray structure (Modeller) and *2*) using a set of templates in accordance with I-TASSER server algorithm [38, 39]. A

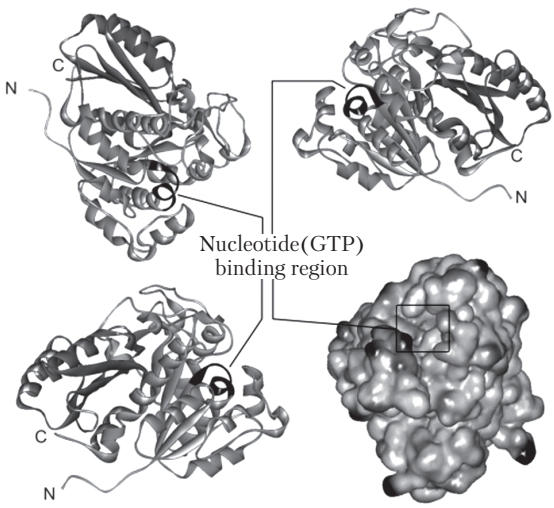


Fig. 1. Full atom model of *M. tuberculosis* FtsZ-protein. It was built by the homology modelling method using *x-Ray*-structures of *M. tuberculosis* (2Q1X (2.35 Å), 2Q1Y (2.30 Å), 1RLU (2.08 Å), 1RQ2 (1.86 Å), and 1RQ7 (2.60 Å) and on the basis of modelling results obtained with the help of I-TASSER server

3D-model of fragment, from Asn6 up to Asp313, has been built with the help of homology modelling method.

Using a I-TASSER tool (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) five full-atomic models (job id S119158): Model 1 (C-score=–0.85); Model 2 (C-score=–1.68); Model 3 (C-score=–2.11); Model 4 (C-score=–2.43) i Model 5 (C-score=–3.04) have been developed (Fig. 1). According to the server protocol, modelling in I-TASSER was based on 10 leading structures (Table 1): 2rhlB_A (2.45 Å) from *Bacillus subtilis*; 4dxdA_A (2.01 Å) from *Staphylococcus aureus*; 1fszA (2.8 Å) from *Methanocaldococcus jannaschii*; 2vamA (2.50 Å) from *Bacillus subtilis*; 2rhlB_B (2.45 Å) from *Bacillus subtilis*; 4dxdA_B (2.01 Å) from *Staphylococcus aureus*; 1w5fA (2.0 Å) from *Thermotoga maritima*; 2rhlB (2.45 Å) from *Bacillus subtilis*; 1rluA (2.08 Å) from *Mycobacterium tuberculosis* and 2vawA (2.9 Å) from *Pseudomonas aeruginosa* (Fig. 1).

The ultimate full-atomic model of *M. tuberculosis* FtsZ-protein was built by homology modelling using the PDB data and results obtained

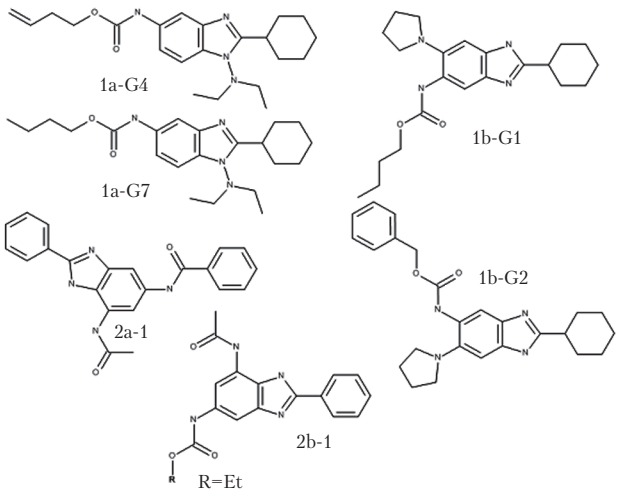


Fig. 2. Structures of 3-substituted benzimidazoles (synthesized by Prof. *I. Ojima et.al*) used for studying potential affinity of benzimidazoles to 4 studied sites: 1a-G4, 1a-G7, 1b-G1, 1b-G2, 2a-1, and 2b-1 [2]

with the help of I-TASSER tool. The final model was assembled using EasyModeller 4.0 [40]. The molecular dynamics of the model was implemented in aqueous environment using Gromacs (IFBG Cluster) package. After solvation and 10 ns relaxation with the use of amber99 force field (GROMACS v. 4.5.5), model geometry was confirmed with ANOLEA (Atomic Non-Local Environment Assessment) (www.protein.bio.puc.cl) server [41], ProCheck program [42], and MolProbity server [43]. The final verification of results was made using Verify3D [44]. The final visualization of structures, structural alignment, and analysis of respective amino acid residues were made using Accelrys Discovery Studio Visualizer 3.1 (Accelrys Software Inc.-http://accelrys.com/), and PyMOL (http://www.pymol.org/) software (Fig. 2).

Library of Low-Molecular Compound

The library of ligands for molecular screening and docking was based on the collection of imidazole compounds synthesized in the Institute for Organic Chemistry of the NAS of Ukraine. Totally, it embraces 2886 individual compounds in the following formats: *.sd/*.mol, *.mol2. The 3-substituted benzimidazole derivatives synthesized by the team of Prof. Ojima group [2] (Fig. 3) were used as reference structures. Considering conformers built using JChem 5.12.0 (ChemAxon, 2012, www.chemaxon.com), the total number of ligands exceeds 5000. The topology files were obtained using SwissParam (www.swissparam.ch) server [45].

Search of Predicted Binding Sites of Heterocyclic Compounds at the Surface of *M. tuberculosis* FtsZ-Protein

Since there are no complexes of heterocyclic compounds of imidazole group with FtsZ-proteins, which are confirmed by crystallographic methods, we search for the most probable binding sites of these compounds at the surface of protein molecules using I-TASSER server (Predicted Binding Site module) and built theoretical complexes with ligands from matrix structure: GSP (1rluA), SO4 (2rhhA i 2rhjA), PEPTIDE (1rq20), MG (3e22C i 1z2bC), T13 (3hkeB), HOS (3du7C), and TZT (3e22C) (Fig. 1).

The modelling was made for the following binding sites: GSP (5'-guanosine-diphosphate-monothiophosphate), T13 (2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl) benzene sulfonate amid), HOS (phomopsine A), and TZT-1027 (sobllidotine). In addition, a model of predicted binding site of taxol has been designed upon the results of structural alignment of FtsZ-protein and tubulin. The PDB 2WBE, 3DCO, 2P4N, 2HXH, and 1JFF structures were used as reference. With the help of LeadIT program, models for the four binding sites: GTP/GSP; TZT-1027; HOS/T13 and Taxol have been developed using the reference ligand method (Fig. 4). The models were required for molecular docking purposes. They were added to the repository of CSLabGrid virtual laboratory for their further use within the CSLabGrid.

Further, binding sites were assessed using a local version of LeadIT v.2.1.3 package and results of low-molecular ligand docking obtained with the help of CCDC GOLD package. While using

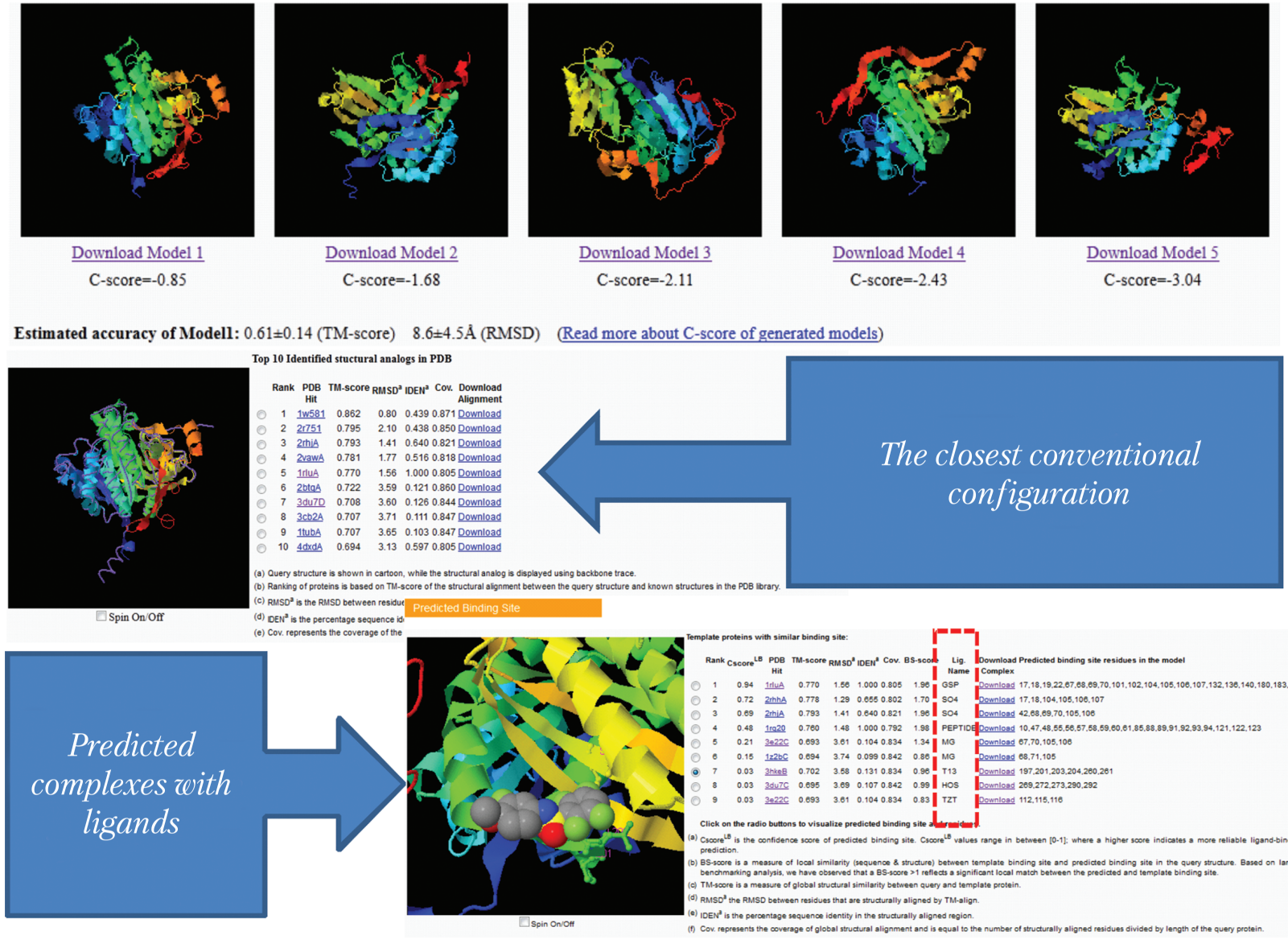


Fig. 3. Modelling of spatial structure of *M. tuberculosis* FtsZ and reconstruction of ligand-protein complexes (*Predicted Binding Site*) using I-TASSER tool

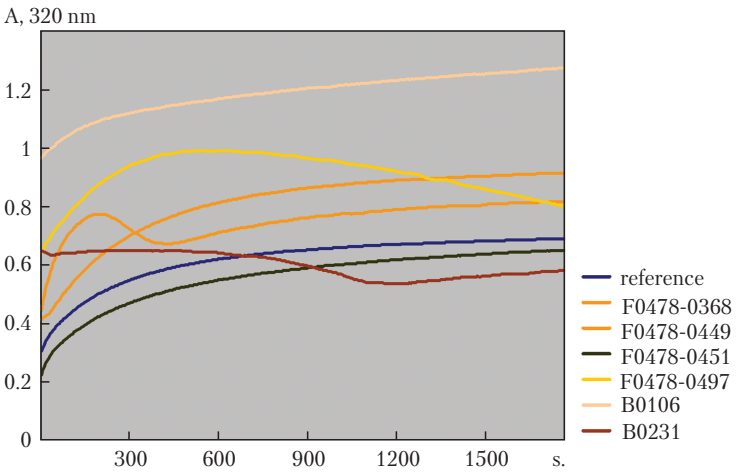


Fig. 6. Dynamics of FtsZ polymerization in the presence of compounds having no effect of inhibitor: F0478-0368, F0478-0449, F0478-0451, F0478-0497, B0106, and B0231

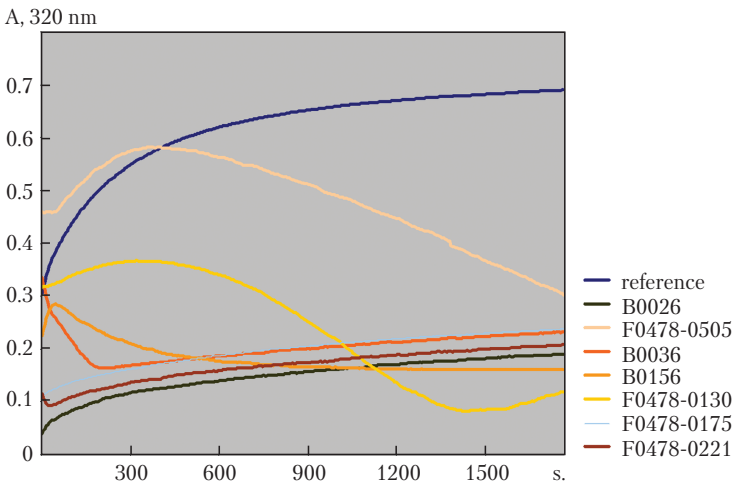


Fig. 7. Dynamics of FtsZ polymerization in the presence of compounds having a strong effect of inhibitor: B0026, F0478-0505, B0036, B0156, F0478-0130, F0478-0175, and F0478-0221

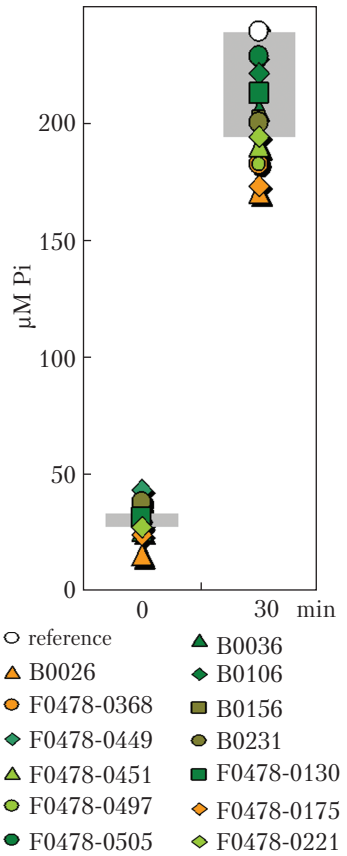


Fig. 8. Inhibition of FtsZ GTPase activity by compounds studied

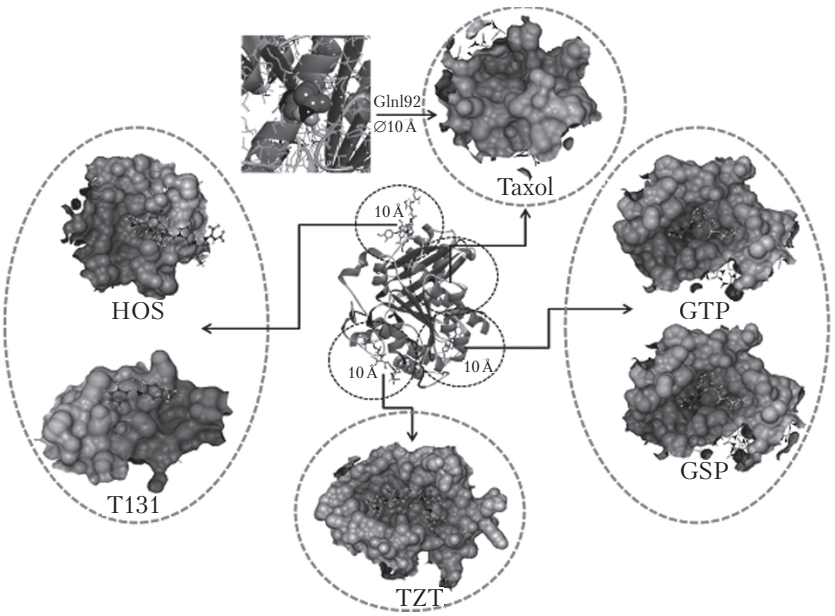


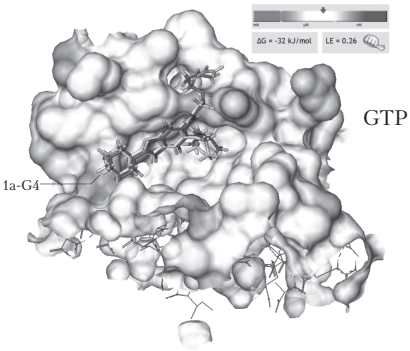
Fig. 4. Modelling of predicted sites of imidazoles. Bindin the reference ligand method with a distance of 10 Å, with the help of LeadIT software, models of four most perspective of predicted sites have been designed: 1) GTP/GSP, 2) TZT-1027, 3) HOS/T13, and 4) Taxol

the Hyde (LeadIT) module proceeding from the ligand efficiency of reference benzimidazoles 1a-G4 (5-But-3-enoxycarbonylamino-2-cyclohexyl-6-N,N-dimethylamino-1H-benzo[d]imidazole (ΔG) and LE, 1a-G7 (5-butoxycarbonylamino-2-cyclohexyl-6-N,N-dimethylamino-1H-benzimidazole), 1b-G1 (5- butoxycarbonylamino-2-cyclohexyl-6-(pyrrolidine-1-yl)-1H-benzo[d]imidazole), 1b-G2 (5-benzyloxycarbonylamino-cyclohexyl-6-(pyrrolidine-1-yl)-1H-benzo[d] imidazole), 2a-1 (7-acetylamino-5-etoxycarbonylamino-2-phenyl-1H-benzo[d]imidazole) and 2b-1 (ethyl-n-(5-acetamido-1-hydrogen-2-phenyl-3a,6-dihydro-1H-1,3-benzimidazole-6-yl)-n-hydrogen carbonate) (Figs. 3 and 5) the possibility of 3-substituted benzimidazoles in the four above mentioned sites has been established. The results of the further verification based on the analysis of their molecular docking in CCDC GOLD package have showed that in terms of GoldScore, ChemScore, and ASP, GTP/GSP binding site is in the priority for reference compounds [46, 47]. This conclusion has been confirmed by the results of molecular dynamics in Gromacs (charmm27). Thus, the further virtual screening of tested compounds was made for the GTP-binding site.

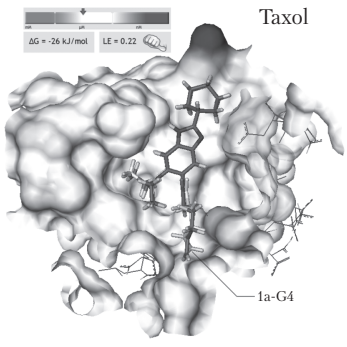
Virtual Screening in Grid Using FlexX Algorithm and Further Verification of Leading Compounds Using CCDC Gold and Molecular Dynamics

The high-throughput virtual screening of benzimidazole derivatives was made using the molecular docking method and FlexX software installed on the IFBG Cluster [32, 48]. The two personal academic licenses each valid for 1 month were used: Pavel Karpov-SN: 0BS080F1E97114A3B62 E417780A8752D6F3; Ozeredov Sergey-SN: 0BS 61DE9C1DFA4CD32BABB9DC02452EFF0B. The computations were made with the help of HP ProLiant AV340A (8 cores) (CSLabGrid) server. The model of GTP-binding site of *M. tuberculosis* FtsZ-protein as target and the library of compounds synthesized in the IOC were used.

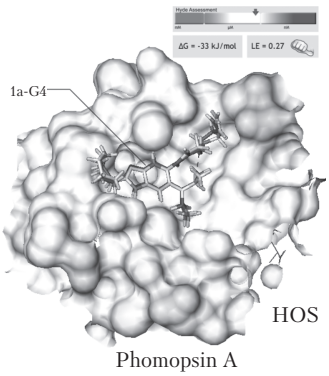
The complexes of imidazole group heterocyclic compounds with FtsZ-protein were assessed on the basis of LeadIT evaluation functions: *Score*, *Match*, *Lipo*, *Ambig*, *Clash*, *Rot*, *RMSD*, *Simil*, and *Match*, as well as the ligand affinity evaluation functions ΔG (Gibbs free energy/Binding affinity) and LE (Ligand efficiency) of the Hyde module (http://www.biosolveit.de) [49, 50]. Upon results of comprehensive verification, 50 perspective compounds were selected.



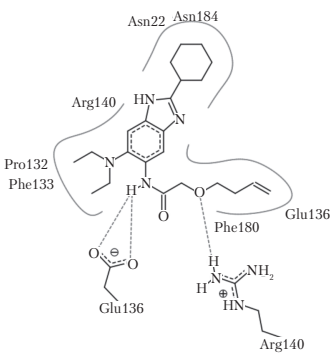
GTP and GSP

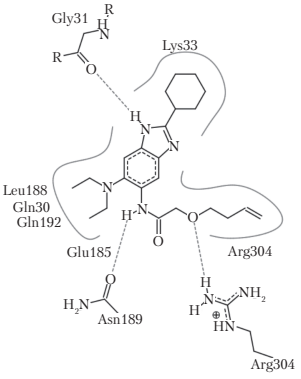


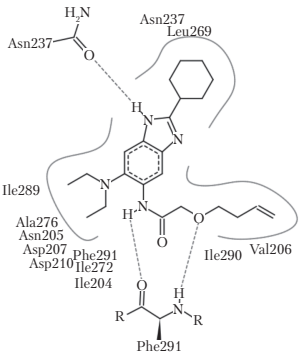
TAXOL

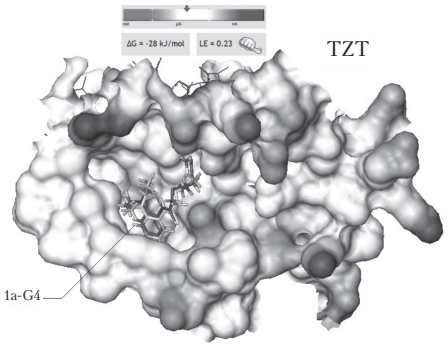


Phomopsin A
and T13 (2,3,4,5,6-pentafluoro-
N-(3-fluoro-4-methoxyphenyl)
benzenesulfonamide)









TZT-1027 (SOBLIDOTIN)

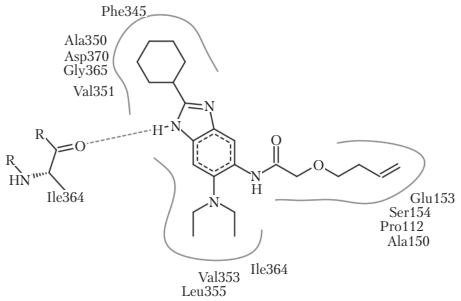
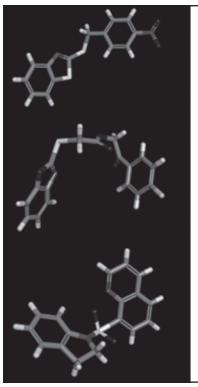


Fig. 5. On the basis of binding energy (ΔG) and LE (Ligand Efficiency) established using Hyde module (LeadIT software package) the above mentioned sites have been evaluated and a group of leading compounds has been selected for further docking with the use of genetic algorithm in CCDC GOLD. Example for the reference compound 1a-4G (*Ojima Research Group*) is given [2]

The next stage was the docking of the 50 selected compounds in GTP-binding site of *M. tuberculosis* FtsZ-protein using CCDC GOLD 5.2.2 (www.ccdc.cam.ac.uk) [46, 47]. In order to control the docking, the 3-substituted benzimidazoles synthesized by Prof. Ojima's team [2]: 1a-G4, 1a-G7, 1b-G1, 1b-G2, 2a-1, and 2b-1 were used. The Gold genetic algorithm enabled selection of the most perspective compounds based on calculations of binding energy and number of hydrogen bonds. Using GoldScore, ChemScore and ASP evaluation functions the number of compounds was reduced to 26.

At the final stage of evaluation, the molecular dynamics of ligand-protein complexes applied using GROMACS (v. 4.5.5) [51, 52] and force field charmm27 (www.charmm.org) [53]. The ligand topology for charmm27 was taken from the SwissParam database [45]. The computations were made for solvatated environment (*editconf* and *genbox* modules). The volume of aqueous environment was generated automatically. The long-range electrostatic interactions were taken into consideration using PME (Particle Mesh Ewald) method [54]. The negative charge of obtained molecular systems was neutralized using the *genion* module, by substituting the water molecules for sodium and chloride ions in physiological concentration (0.15 mole/l). The geometry of molecular systems was optimized by minimization of potential energy (*grompp* and *mdrun*) using steepest descent algorithms at a maximum number of steps of 1000 and a gradient of 0.1. The energy was minimized using force field charmm27 [53] as well. The next procedure of restrained molecular dynamics (molecular restraining) was made using the above mentioned modules at a temperature of 310 K during 0.2 ps. Also, molecular dynamics was carried out for 50 ns using the *grompp* and *mdrun* modules. For simulating the bulk solvent, the periodical boundary conditions were applied. The system temperature was kept at 310 K by the Berendsen thermostat with time of interaction of 0.1 ps. The constant pressure was kept by external barostatic function. The length of bonds involving hydrogen atoms was fixed at an equilibrium level using the Lincs algorithm [55]. The molecular dynamics results for ligand/FtsZ-protein complexes were analyzed using the *genergy* module enabling to evaluate the Coulomb (electrostatic) and Lennard–Jones (LJ) interactions of ligands both in the complex with protein and in free state in aqueous environment.



B0026
2[(4-nitrophenyl) methylthio]-1, 3-benzothiazol

F0478-0175
[2-(4-fluorophenyl) -2-oxo-ethyl]2-(1, 3-benzoxazole-2-ylthio) acetate

F0478-0221
8-indoline-1-ylsulfonylquinolin

Fig. 9. Leading compounds selected on the basis of molecular docking and molecular dynamics data and upon the results of experimental verification of ability to inhibit polymerization and GTPase activity of *Micobacterium tuberculosis* FtsZ protein *in vitro*

Energy of ligand/protein binding was calculated by the formula [56]:

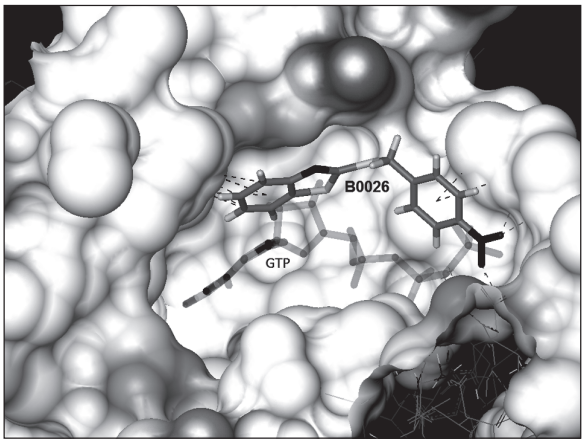
$$\Delta G_{bind} = \alpha(\langle V_{l\text{-}s\ LJ}\rangle_p - \langle V_{l\text{-}s\ LJ}\rangle_w) + \beta(\langle V_{l\text{-}s\ el}\rangle_p - \langle V_{l\text{-}s\ el}\rangle_w), \quad (1)$$

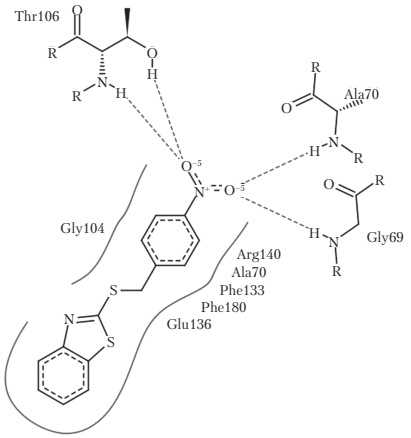
where $\langle V_{l\text{-}s\ LJ}\rangle_p$ is LJ component of ligand/protein interaction; $\langle V_{l\text{-}s\ LJ}\rangle_w$ is LJ component of ligand/water interaction; $\langle V_{l\text{-}s\ el}\rangle_p$ is electrostatic component of ligand/protein interaction; $\langle V_{l\text{-}s\ el}\rangle_w$ is electrostatic component of ligand/water interaction.

The coefficient values were taken from [57] by default and equal to $\alpha = 0.18$ and $\beta = 0.50$.

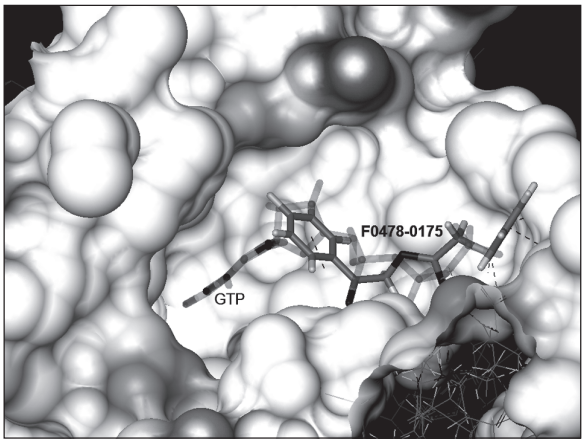
Upon the results of molecular dynamics calculations, the 6 compounds F0478-0451, F0478-0505, F0478-0368, B0026, F0478-0175, and F0478-0221 have been concluded to have the highest potential affinity to Mycobacterial FtsZ-protein.

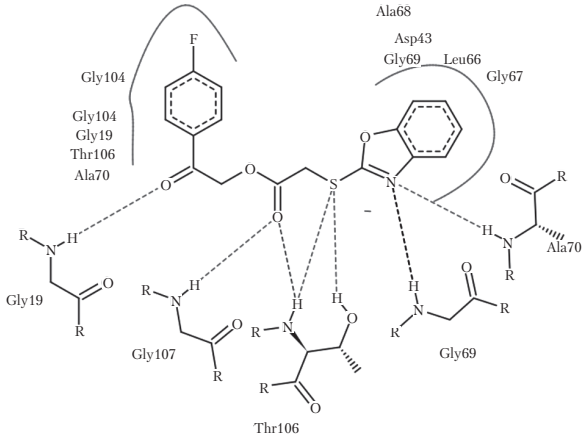
B0026 (2[(4-nitrophenyl)methylthio]-1,3-benzothiazole)



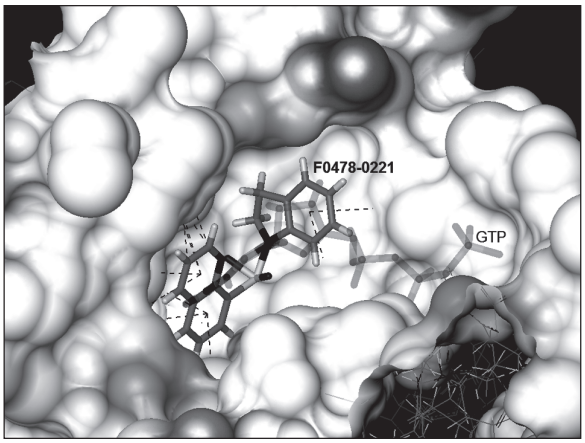


F0478-0175 ([2-(4-fluorophenyl)-2-oxo-ethyl]2-(1,3-benzoxazole-2-ylthio)acetate)





F0478-0221 (8-indoline-1-ylsulfonylquinoline)



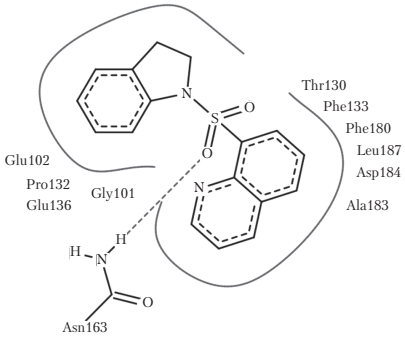


Fig. 10. Peculiarities of ligand-protein interaction of the leading compounds (B0026, F0478-0175, F0478-0221) with *M. tuberculosis* FtsZ-protein. Ligands position in GTP-exchange site of FtsZ-protein (*left*) and pharmacophoric maps of ligand-protein interaction (*right*). Reference – GTP molecule

In vitro Verification of the Leading Compounds

The samples of 6 leading compounds, as well as of 7 ones that upon the screening results did not show respective affinity to FtsZ-protein were brought from the IOC of the NAS of Ukraine. The tests on influence of studied compounds on FtsZ-protein polymerization were made using the two methods: *1*) the spectrophotometric identification of FtsZ-protein polymerization dynamics at 320 nm and *2*) the colorimetric identification of GTP hydrolysis in the reaction with benzaldehyde green. The FtsZ-protein sample was manufactured by *Cytoskeleton Inc*. The lyophilic protein preparation was re-suspended in buffer (100 mM MES-NaOH, $pH = 6.5$, 1 mM EGTA, 10 mM $CaCl_2$) up to a concentration of 1 mg/ml. Further, the sample was incubated on ice for 30 min. The protein solution was centrifuged at 14 000 rpm, with supernatant taken for the further tests. The ultimate concentration of protein in the reaction mix was checked by the spectrophotometric method and it reached 88 mg/ml. The FtsZ-protein polymerization dynamics and GTP hydrolysis by FtsZ-protein were evaluated at the presence of heterocyclic compounds selected during the screening, *in silico,* at a concentration of 50 μM. The reaction mixes with respective dimethyl sulfoxide content were used as reference.

The spectrophotometric study of *in vitro* FtsZ-protein polymerization dynamics was made using *Specord* 210 spectrophotometer (*AnalytikJena*, Germany) in *double-beam* mode, at a wavelength of 340 nm (4 nm detector, 5 s scanning pitch) for 30 min in the cuvette holder thermostated by Peltier elements at 37 °C. The polymerization was induced by adding GTP to the reaction mix up to 1 mM. A buffer for polymerization without FtsZ-protein, which contained respective analyte was introduced to the reference cell in the double-beam mode.

While analyzing the influence of 13 heterocyclic compounds on the FtsZ polymerization dynamics, two discrete groups having different impact on the process were distinguished. Six compounds, namely F0478-0368; F0478-0449; F0478-0451; F0478-0497; B0106; and B0231 did not significantly inhibit the polymerization; some of them showed a polymerization level that was higher than the reference one (Fig. 6). Among this group, two compounds (B0106 and B0231) showed the values close to the reference one, while the rest of them (F0478-0368, F0478-0449, F0478-0451, F0478-0497) and especially B0106 exceeded the reference. This can be explained by capability of these compounds to trigger the formation of protein aggregates, which, in its turn, enhanced optical absorption.

The other compounds (B0026, F0478-0505, B0036, B0156, F0478-0130, F0478-0175, F0478-0221) can be characterized as potential inhibitors of FtsZ polymerization (the polymerization either had minimum dynamics (B0026, B0036, B0156, F0478-0175, F0478-0221) or was instable and ended with FtsZ de-polymerization (F0478-0505, F0478-0130)) (Fig. 7).

In vitro Study of FtsZ GTPase Activity

The GTPase activity of FtsZ-proteins was studied using PhosFree™ Phosphate Assay Biochem Kit (BK050, *Cytoskeleton Inc*., USA) based on colorimetric reaction of phosphate with benzaldehyde green. The changes in phosphate ion concentrations were compared in boundary time of FtsZ polymerization dynamics: 0 min (immediately after addition of GTP, with a 15 s delay of the measurement start with method manipulations taken into account) and 30 min. The reaction took place in water bath, at 37 °C. The reaction was terminated by perchlorate acid preparation. The optical absorption was measured at 650 nm. The content of PO_4^{3-} releasing during the GTP hydrolysis in micro-molecular range was determined using a calibration curve built by titration of reference phosphate solution.

The evaluation results showed that all studied compound samples demonstrated GTP hydrolysis level lower than that of the reference one (239 μM PO_4^{3-} for polymerization of 24.8 μM FtsZ in the presence of 50 mM GTP). All samples

inhibited GTPase activity of FtsZ-protein with different efficiency (from 4 to 28%). However, in the case of the six leading compounds the inhibition of GTPase activity exceeded 18%: B0026 (28%), F0478-0368 (24%), F0478-0451 (20%), F0478-0497 (24%), F0478-0175 (27%), and F0478-0221 (19%) (see Fig. 8).

Having compared the results of the two experimental approaches for the studied compound samples, three compounds, namely, B0026 (2[(4-nitrophenyl)methylthio]-1,3-benzothiazole), F0478-0175([2-(4-fluorophenyl)-2-oxo-ethyl]2-(1,3-benzoaxole-2-ylthio)acetate), and F0478-0221 (8-indoline-1-yl sulfonyl quinoline) were determined to have the highest inhibitory effect (maximum inhibition of polymerization and GTPase activity of FtsZ-protein) (Fig. 9). Therefore, proceeding from the obtained results these compounds can be recommended for the further study of their influence on *M. tuberculosis* fission.

These properties of above mentioned compounds have been showed for the first time. Currently, they are considered perspective FtsZ inhibitors and will be further studied for establishing their pharmacological capacity as new antituberculosis drugs. In addition, the established specific features of interaction between the leading compounds and *M. tuberculosis* FtsZ-protein give us information on ligand-protein interaction (Fig. 10). The obtained information is very important for the further design and synthesis of more effective inhibitors of mycobacterial division.

CONCLUSIONS

Within the framework of implementing the objectives of CSLabGrid virtual laboratory, new compounds with antituberculosis activity have been selected. A method for effective high-throughput screening of imidazole compounds has been developed combining the structural bioinformatics methods, Grid computations, and *in vitro* tests. Respective libraries of ligands and molecular targets for the further studies using CSLabGrid have been created. Upon the results of 2886-compound ligand library screening, as well as bio-informational and biochemical analyses, three compounds have been identified as maximally inhibiting both polymerization and GTPase activity (18—28%) of *Mycobacterium tuberculosis* FtsZ-protein, namely: B0026 (2[(4-nitrophenyl)methylthio]-1,3-benzothiazole), F0478-0175([2-(4-fluorophenyl)-2-oxo-ethyl]2-(1,3-benzoaxole-2-ylthio)acetate), and F0478-0221 (8-indoline-1-yl sulfonyl quinoline).

The research was supported by the project of target program for fundamental research of the NAS of Ukraine «Fundamental Problems of Creating New Chemical Substances and Materials» (2012—13): «Search and Evaluation of Antituberculosis Action of New Heterocyclic Series Compounds on the Basis of Structural Biological Docking and Design», (reg. no. 0112U003389). Partly, the research is directly related to the creation of databases and use of Grid-computations within the framework of the government target R&D program for implementing Grid-technologies for 2009—13 project «Use of Grid Technology in the Fundamental and Applied Cytoskeleton Research by Creating and Developing CSLabGrid Virtual Laboratory» (2012, reg. no. 0112U004000).

REFERENCES

1. World Health Organization. Global tuberculosis report. Kyiv, 2012. http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf.
2. Kumar K., Awasthi D., Berger W.T., Tonge P.J., Slayden R.A., Ojima I. Discovery of anti-TB agents that target the cell-division protein FtsZ. *Future Med Chem.* 2010, 2(8): 1305—1323.
3. *Osnovni zasady organizacii' medychnoi' dopomogy hvorym na tuberkul'oz* (posibnyk z organizacijno-metodychnoi' roboty). Ju. I. Feshhenko, V. M. Mel'nyk, V. G. Matusevych, I.O. Novozhylova, V.O. Juhymec', M.I. Lynnyk. Za red. Ju. I. Feshhenka, V. M. Mel'nyka. Elektron. dani. Kyiv, 2012. http://www.ifp.kiev.ua/ftp1/original/2012/feschenko2012-1.pdf [in Ukrainian].
4. Levyc'ka N.A., Bazhora Ju.I., Nikolajevs'kyj V.V., Asmolov O.K. Medykamentozna rezystentnist' mikobakterij tuberkul'ozu, shho buly vydileni vid hvoryh v mykolai'vs'kij oblasti Ukrai'ny protjagom 2000—2002. *Ukrai'ns'kyj pul'monologichnyj zhurnal.* 2003, 4: 17—20 [in Ukrainian].

5. Cheren'ko C. Tuberkul'oz — hvoroba social'na. UNIAN. *Zdorov'ja*. 2008, 77: 9—18 [in Ukrainian].
6. de Colombani P., Veen J. (Ed.) Review of the National Tuberculosis Programme in Ukraine. 10—22 October 2010, WHO Regional Office for Europe. *World Health Organization*: 2011.
7. Musser J.M., Amin A., Ramaswamy S. Negligible genetic diversity of mycobacterium tuberculosis host immune system protein targets: evidence of limited selective pressure. *Genetics*. 2000, 155(1): 7—16.
8. Hudson A., Imamura T., Gutteridge W., Kanyok T., Nunn P. The current anti-TB drugre search and development pipeline. *TDR/PRD/TB/03.1W*. 2003: http://www.who.int/tdr/publications/documents/anti-tb-drug.pdf.
9. Tripathi R.P., Tewari N., Dwivedi N., Tiwari V.K. Fighting tuberculosis: an old disease with new challenges. *Med Res Rev*. 2005, 25(1): 93—131.
10. Pavan F.R., Sato D.N., Higuchi C.T., Santos A.C.B., Vilegas W., Leite, C.Q.F. *In vitro* anti-*Mycobacterium tuberculosis* activity of some Brazilian «Cerrado» plants. *Revista Brasileira de Farmacognosia*. 2009, 19: 204—206.
11. Mani N., Gross C.H., Parsons J.D., Hanzelka B., Müh U., Mullin S., Liao Y., Grillot A.L., Stamos D., Charifson P.S., Grossman T.H. *In vitro* characterization of the antibacterial spectrum of novel bacterial type II topoisomerase inhibitors of the aminobenzimidazole class. *Antimicrob Agents Chemother*. 2006, 50(4): 1228—1237.
12. Grossman T.H., Bartels D.J., Mullin S., Gross C.H., Parsons J.D., Liao Y., Grillot A.L., Stamos D., Olson E.R., Charifson P.S., Mani N. Dual targeting of GyrB and ParE by a novel aminobenzimidazole class of antibacterial compounds. *Antimicrob Agents Chemother*. 2007, 51(2): 657—666.
13. Brycun V.N., Karpov P.A., Emec A.I., Lozinskij M.O., Bljum Ja.B. Protivotuberkuleznye svojstva proizvodnyh imidazola i benzimidazola. *Zhurnal org. ta farm. himii*. 2011, 9(3, 35): 3—14 [in Russian].
14. Koch A., Mizrahi V., Warner D.F. The impact of drug resistance on Mycobacterium tuberculosis physiology: what can we learn from rifampicin? *Emerging Microbes & Infections*. 2014, 3(e17): doi:10.1038/emi.2014.17.
15. Loose M., Mitchison T.J. The bacterial cell division proteins FtsA and FtsZ self-organize into dynamic cytoskeletal patterns. *Nature Cell Biology*. 2014, 16: 38—46.
16. Chen Y., Anderson D.E., Rajagopalan M., Erickson H.P. Assembly dynamics of Mycobacterium tuberculosis FtsZ. *J Biol Chem*. 2007, 282(38): 27736—27743.
17. White E.L., Ross L.J., Reynolds R.C., Seitz L.E., Moore G.D., Borhani D.W. Slow polymerization of Mycobacterium tuberculosis FtsZ. *J Bacteriol*. 2000, 182(14): 4028—4034.
18. Kapoor S., Panda D. Targeting FtsZ for antibacterial therapy: a promising avenue. *Expert Opin Ther Targets*. 2009, 13(9): 1037—1051.
19. MacDonald L.M., Armson A., Thompson A.R., Reynoldson J.A. Characterisation of benzimidazole binding with recombinant tubulin from *Giardia duodenalis*, *Encephalitozoon intestinalis*, and *Cryptosporidium parvum*. *Mol Biochem Parasitol*. 2004, 138(1): 89—96.
20. Robinson M.W., McFerran N., Trudgett A., Hoey L., Fairweather I. A possible model of benzimidazole binding to beta-tubulin disclosed by invoking an inter-domain movement. *J. Mol. Graph. Model*. 2004, 23(3): 275—284.
21. Sambanthamoorthy K., Gokhale A.A., Lao W., Parashar V., Neiditch M.B., Semmelhack M.F., Lee I., Waters C.M. Identification of a novel benzimidazole that inhibits bacterial biofilm formation in a broad-spectrum manner. *Antimicrob Agents Chemother*. 2011, 55(9): 4369—4378.
22. Küçükbay H., Durmaz R., Okuyucu N., Günal S., Kazaz C. Synthesis and antibacterial activities of new bis-benzimidazoles. *Arzneimittelforschung*. 2004, 54(1): 64—68.
23. Karpov P.A., Demchuyk O.M., Blume Ya.B., Britsun V.M., Volochnyk D.M. Discovery of new anti-TB compounds that target Mycobacterial FtsZ: highthroughput screening and molecular docking. *Moscow Conference on Computational Molecular Biology (MCCMB'13)*. Moscow, Russia July 25—28. 2013: 223—224.
24. Adams D.W., Errington J. Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nat. Rev. Microbiol*. 2009, 7: 642—653.
25. Blaauwen T.D.E.N., Buddelmeijer N., Hameete C.O.R.M., Nanninga N. Timing of FtsZ Assembly in Escherichia coli. *Journal of bacreriology*. 1999, 181: 5167—5175.
26. Goehring N.W., Beckwith J. Diverse Paths to Midcell: Assembly of the Bacterial Cell Division Machinery. *Current Biology*. 2005, 15(13): R514—R526.
27. Hong W., Deng W., Xie J. The Structure, Function, and Regulation of Mycobacterium FtsZ. *Cell Biochem Biophys*. 2013, 65(2): 97—105.
28. Król E., Scheffers D. FtsZ Polymerization Assays : Simple Protocols and Considerations. *Journal of Visualized Experiments*. 2013, 81: 1—13.
29. Pydiura N., Karpov P., Blume Ya. Design of specific cytoskeleton related biological database and data management environment for bioinformatical cytoskeleton investigation and collaboration within virtual Grid-organisation. Proceedings of the International Moscow Conference on Computational Molecular Biology (MCCMB'11). July 21—24, 2011, Moscow, Rossia: 297—298.
30. Pydiura N., Karpov P., Blume Ya. Hardware environment for CSLabGrid: Reaching maximum efficacy of computations in structural biology and bioinformatics. *Second International Conference «Cluster Computing» CC 2013* (Ukraine, Lviv, June 3—5, 2013), Ukraine, Lviv; 06/2013: 191—194.
31. Pydiura N., Karpov P., Blume Ya. On the Efficiency of CPU and Hybrid CPU-GPU Systems in Computational

Biology Tasks. *Computer Science and Applications*. 2014, 1(1): 48—59.
32. Karpov P.A., Bryrsun V.M., Rayevsky A.V., Demchuk O.M., Pydiura N.O., Ozheredov S.P., Samofalova D.A., Spivak S.I., Yemets A.I., Kalchenko V.I., Blume Ya.B. High-throughput screening of new antimitotic compounds based on CSLabGrid Virtual Organization. *Sci. Innov.* 2015, 11(1): 85—93.
33. Cole S.T., Brosch R., Parkhill J., Garnier T., Churcher C.M., Harris D.E., Gordon S.V., Eiglmeier K., Gas S., Barry C.E. III, Tekaia F., Badcock K., Basham D., Brown D., Chillingworth T., Connor R., Davies R.M., Devlin K., Barrell B.G. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature*. 1998, 393: 537—544.
34. Fleischmann R.D., Alland D., Eisen J.A., Carpenter L., White O., Peterson J.D., DeBoy R.T., Dodson R.J., Gwinn M.L., Haft D.H., Hickey E.K., Kolonay J.F., Nelson W.C., Umayam L.A., Ermolaeva M.D., Salzberg S.L., Delcher A., Utterback T.R., Fraser C.M. Whole-genome comparison of *Mycobacterium tuberculosis* clinical and laboratory strains *J. Bacteriol.* 2002, 184: 5479—5490.
35. The UniProt Consortium. The Universal Protein Resource (UniProt). *Nucl. Acids Res.* 2008, 36: 190—195.
36. Guex N., Peitsch M.C. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis*. 1997, 18(15): 2714—2723.
37. Eswar N., Marti-Renom M.A., Webb B., Madhusudhan M.S., Eramian D., Shen M.Y., Pieper U., Sali A. Comparative Protein Structure Modeling With MODELLER. *Cur Prot in Bioinform, John Wiley & Sons, Inc*. 2006. Sup.15: 5.6.1—5.6.30.
38. Roy A., Kucukural A., Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocols*. 2010, 5: 725—738.
39. Roy A., Yang J., Zhang Y. COFACTOR: An accurate comparative algorithm for structure-based protein function annotation. *Nucl. Acids Res*. 2012, 40: W471—W477.
40. Kuntal B.K., Aparoy P., Reddanna P. EasyModeller: A graphical interface to MODELLER. *BMC Res Notes*. 2010, 3(226). doi: 10.1186/1756-0500-3-226.
41. Melo F., Feytmans E. Assessing protein structures with a non-local atomic interaction energy. *J. Mol. Biol.* 1998, 277: 1141—1152.
42. Laskowski R.A., MacArthur M.W., Moss D.S., Thornton J.M. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Cryst*. 1993, 26: 283—291.
43. Chen V.B., Arendall W.B., Headd J.J., Keedy D.A., Immormino R.M., Kapral G.J., Murray L.W., Richardson J.S., Richardson D.C. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D. Biol. Crystallogr*. 2010, 66(Pt.1): 12—21.
44. Eisenberg D., Lüthy R., Bowie J.U. VERIFY3D: assessment of protein models with three-dimensional profiles. *Methods Enzymol*. 1997, 277: 396—404.
45. Zoete V., Cuendet M.A., Grosdidier A., Michielin O. SwissParam: a fast force field generation tool for small organic molecules. *J. Comput. Chem*. 2011, 32(11): 2359—2368.
46. Verdonk M.L., Cole J.C., Hartshorn M.J., Murray C.W., Taylor R.D. Improved protein-ligand docking using GOLD. *Proteins*. 2003, 52(4): 609—623.
47. Hartshorn M.J., Verdonk M.L., Chessari G., Brewerton S.C., Mooij W.T., Mortenson P.N., Murray C.W. Diverse, High-Quality Test Set for the Validation of Protein-Ligand Docking Performance. *J. Med. Chem*. 2007, 50: 726—741.
48. Huang S.-Y., Zou X. Advances and Challenges in Protein-Ligand Docking. *Int. J. Mol. Sci*. 2010, 11: 3016—3034.
49. Schneider N., Lange G., Hindle S., Klein R., Rarey M. A consistent description of HYdrogen bond and DEhydration energies in protein—ligand complexes: methods behind the HYDE scoring function. *J. Comput. Aided. Mol. Des*. 2013, 27(1): 15—29.
50. Schneider N., Hindle S., Lange G., Klein R., Albrecht J., Briem H., Beyer K., Claußen H., Gastreich M., Lemmen C., Rarey R. Substantial improvements in large-scale redocking and screening using the novel HYDE scoring function. *J. Comput. Aided. Mol. Des*. 2012b. 26: 701—723.
51. Hess B., Kutzner C., van der Spoel D., Lindahl E. GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J. Chem. Theory Comput*. 2008, 4(3): 435—447.
52. Pronk S., Páll S., Schulz R., Larsson P., Bjelkmar P., Apostolov R., Shirts M.R., Smith J.C., Kasson P.M., van der Spoel D., Hess B., Lindahl E. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics*. 2013, 29(7): 845—854.
53. Vanommeslaeghe K., Hatcher E., Acharya C., Kundu S., Zhong S., Shim J., Darian E., Guvench O., Lopes P., Vorobyov I., Mackerell A.D.Jr. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J. Comput. Chem*. 2009, 31(4): 671—690.
54. Essmann U., Perera L., Berkowitz M.L., Darden T., Lee H, Pedersen L.G. A smooth particle-mesh Ewald potential. *J. Chem. Phys*. 1995, 103(19): 8577—8592.
55. Hess B., Bekker H., Berendsen H.J.C., Fraaije J.G.E.M. LINCS: a linear constraint solver for molecular simulations. *J. Comput. Phys*. 1997, 18: 1463—1472.
56. Almlöf M., Brandsdal B.O., Aqvist J. Binding affinity prediction with different force fields: examination of the linear interaction energy method. *J. Comput. Chem*. 2004, 25(10): 1242—1254.
57. Stacklies W., Seifert C., Graeter F. Implementation of force distribution analysis for molecular dynamics simulations. *BMC Bioinformatics*. 2011, 12(101): 1—5.

П.А. Карпов [1], О.М. Демчук [1], В.М. Брицун [2], Д.І. Литвин [1], М.О. Пидюра [1], О.В. Раєвський [1], Д.О. Самофалова [1], С.І. Співак [1], Д.М. Волочнюк [2], А.І. Ємець [1], Я.Б. Блюм [1]

[1] Інститут харчової біотехнології і геноміки НАН України, Київ
[2] Інститут органічної хімії НАН України, Київ

НОВІ ІМІДАЗОЛЬНІ ПОХІДНІ ЯК ІНГІБІТОРИ FtsZ-БІЛКІВ МІКОБАКТЕРІЙ: ВІД ВИСОКОПРОПУСКНОГО МОЛЕКУЛЯРНОГО СКРИНІНГу В Ґрід ДО ЕКСПЕРИМЕНТАЛЬНОГО АНАЛІЗУ *in vitro*

У рамках реалізації головної мети створеної в УНГ віртуальної організації CSLabGrid було виконано пошук нових протитуберкульозних сполук. З використанням встановленої на IFBG Claster програми FlexX і моделі чотирьох перспективних сайтів зв'язування лігандів на поверхні FtsZ-білка *Mycobacterium tuberculosis* було виконано скринінг бази даних, що містила 2886 сполук, синтезованих в Інституті органічної хімії НАН України. На підставі показників LE і G, результатів докінгу в програмі CCDC Gold і обрахунку молекулярної динаміки комплексів було відібрано групу перспективних інгібіторів FtsZ. Під час експериментальної перевірки *in vitro* шість речовин проявили найвищу ефективність інгібування ГТФ-азної активності FtsZ-білка. Також за результатами експериментальної оцінки *in vitro* було обрано три речовини, які водночас проявляють максимальне пригнічення полімеризації та ГТФ-азної активності FtsZ-білка.

Ключові слова: туберкульоз, структурна біологія, біоінформатика, віртуальний скринінг, *in vitro*.

П.А. Карпов[1], О.Н. Демчук[1], В.Н. Брыцун[2], Д.И. Литвин[1], Н.А. Пыдюра[1], А.В. Раевский[1], Д.А. Самофалова[1], С.И. Спивак[1], Д.Н. Волочнюк[2], А.И. Емец[1], Я.Б. Блюм[1]

[1]Институт пищевой биотехнологии и геномики НАН Украины, Киев
[2]Институт органической химии НАН Украины, Киев

НОВЫЕ ИМИДАЗОЛЬНЫЕ ПРОИЗВОДНЫЕ КАК ИНГИБИТОРЫ FtsZ-БЕЛКОВ МИКОБАКТЕРИЙ: ОТ ВИСОКОПРОПУСКНОГО МОЛЕКУЛЯРНОГО СКРИНІНГА В Ґрід ДО ЭКСПЕРИМЕНТАЛЬНОГО АНАЛИЗА *in vitro*

В рамках реализации главной цели созданной в УНГ виртуальной организации CSLabGrid был выполнен поиск новых противотуберкулезных соединений. С использованием установленной на IFBG Claster программы Flex и модели четырех перспективных сайтов связывания лигандов на поверхности FtsZ-белка *Mycobacterium tuberculosis* было выполнено скрининг базы данных, которая содержала 2886 соединений, синтезированных в Институте органической химии НАН Украины. На основании показателей LE и ΔG, результатов докинга в программе CCDC Gold и обсчета молекулярной динамики комплексов была отобрана группа перспективных ингибиторов Fts. Во время экспериментальной проверки *in vitro* шесть веществ проявили высочайшую эффективность ингибирования ГТФ-азной активности FtsZ-белка. Также по результатам экспериментальной оценки *in vitro* было избрано три вещества, которые одновременно проявляют максимальное угнетение полимеризации и ГТФ-азной активности FtsZ-белка.

Ключевые слова: туберкулез, структурная биология, биоинформатика, виртуальный скрининг, *in vitro*.

Received 17.02.16