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Scaffold Hopping Computational Approach for Searching Novel β-Lactamase Inhibitors

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Abstract—We present a novel computational ligand-based virtual screening approach with scaffold hopping capabilities for the identification of novel inhibitors of β-lactamases which confer bacterial resistance to β-lactam antibiotics. The structures of known β-lactamase inhibitors were used as query ligands, and a virtual in silico screening a database of 8 million drug-like compounds was performed in order to select the ligands with similar shape and charge distribution. A set of numerical descriptors was used such as chirality, eigen spectrum of matrices of interatomic distances and connectivity together with higher order moment invariants that showed their efficiency in the field of pattern recognition but have not yet been employed in drug discovery. The developed scaffold-hopping approach was applied for the discovery of analogues of four allosteric inhibitors of serine β-lactamases. After a virtual in silico screening, the effect of two selected ligands on the activity of TEM type β-lactamase was studied experimentally. New non-β-lactam inhibitors were found that showed more effective inhibition of β-lactamases compared to query ligands.

Keywords: beta-lactamases, virtual screening, scaffold hopping, ligands, inhibitors, antibiotic resistance
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INTRODUCTION

The aim of drug discovery is the identification of novel compounds active against selected protein targets. Many hit compounds identified by high throughput screening or virtual screening cannot be developed further due to their poor metabolic and/or physicochemical properties, high toxicity or low oral bioavailability [1]. In this case new compounds with similar biological activity and certain similarity in shape, pharmacophore features or electrostatic potentials with respect to the reference compound, could be of high interest even if they belong to different chemical classes. The identification of compounds with similar biological activity but different core elements of their structure is known as a scaffold hopping [2, 3]. During last decade the technique has become widely used in drug discovery today, and the amount of dedicated publications has increased rapidly [4, 5].

One of the outstanding challenges in virtual screening is the development of a fast and robust algorithm to search large molecular databases and identify hit compounds with desired biological activity. Until now, different ligand-based virtual screening methods with scaffold hopping capabilities have been proposed. These methods can be separated into alignment-free, descriptor vector-based methods, fragment matching and reduced graphs, fragment replacement and methods generating ligand alignments in three-dimensional (3D) space [1]. The (USR) [6], spherical harmonics [7] and auto-correlation vectors (alignment-free descriptor vector-based methods include those based on fingerprints [8–11]), shape descriptors [10].

The most widely used fingerprint-based methods characterize molecules by their 2D fragment substructures [8]. The fragments can be defined based on a dictionary (BCI fingerprints) [1], systematically extracted connection paths (Daylight fingerprints) [1, 11], and circular substructures such as extended connectivity fingerprints (ECFPs) [1, 9]. The similarity between two molecules is computed by the number of identical substructural fragments [1]. Despite the low computational costs, fingerprint representations have limited capacity, do not scale well with increasing compound complexity, depend strongly on a set of predefined features, and have poor scaffold-hopping performance [8, 12].

As the affinity of a ligand to a given protein is defined by interactions in 3D space, various methods making use of 3D features have been proposed [13–15]. In contrast to the descriptor vector-based methods, the 3D-methods capture conformational flexibility of the ligands, their shape and functional features, required for the biological interaction. Of particular interest is the potential of such methods to provide scaffold hopping—the identification of molecules based on a different chemical sub-structure, but making similar interactions with the target [16]. Many 3D similarity methods implement virtual screening through alignment of ligands in three-dimensional space. These approaches describe molecules by a set of descriptors, among which the most common ones are field-based (GRID) [17], shape-based (ROCS) [14, 15] and pharmacophore-based (FEPOPS) [18] representations of the molecules. Optimization methods are subsequently applied to find the best overlay of molecular structures [19].

The available 3D similarity methods require a high computational power due to the complexity associated with the ligand flexibility and with determination of the optimal 3D alignment [19]. It has been reported that few hundreds of conformers are needed to cover efficiently biologically active conformations of a ligand [19, 20]. Efficient computational approaches applicable to databases with millions of small-molecule compounds are urgently required.

One of the first examples of successful application of scaffold hopping is a discovery of tramadol, a potent analgesic, which works similar to its parent molecule morphine, but shows reduced side effects [3, 21]. The possibility for discovering structurally novel compounds from known active drugs is of high interest for treatment of a growing number of the microbial infections—such as pneumonia, tuberculosis and salmonellosis; and to combat antibiotic resistance [22].

Microbial antibiotic resistance represents a global threat and serious challenge for modern medicine [23, 24]. Modification of antibiotic molecules is one of the most widely-spread bacterial mechanisms of antibiotic resistance [25, 26]. The most used β-lactam antibiotics such as penicillins, cephalosporins and carbapenemes loose their effectiveness due to the production of bacterial β-lactamase enzymes that hydrolyse antibiotic β-lactam ring thus making it inactive [27]. One of the main routes to combat antibiotic resistance is to design novel beta-lactamase inhibitors and use them in combination with antibiotics [28]. First inhibitors (clavulanic acid, sulbactam, tazobactam) binding covalently to the β-lactamase active site serine are themselves β-lactams [29, 30]. These inhibitors are actively used in clinical practice, but their number is limited and they have narrow specificity for β-lactamases [31].

In addition, the widespread use of inhibitors led to the emergence of mutant forms of β-lactamases, capable of deacylating of enzyme complexes with an inhibitor, and as a result becoming resistant to their action. A new strategy is currently being developed for the search for inhibitors acting on the active center of enzymes but not containing the β-lactam ring [32]. Compounds of the class of diazabicyclooctanes (avibactam and relebactam) were found to be the most effective, they form carbamyl-enzyme complexes with a catalytic serine, which then undergo slow reversible recycling with the release of the inhibitor molecule [33, 34]. Derivatives of boronic acids (vaborbactam), which are inhibitors of the transition state of the complex of the enzyme with the antibiotic, are also actively studied as new inhibitors of β-lactamases [35–37]. However, the structure of these compounds is still based on the structure of known antibiotics, and resistance to these inhibitors has already been discovered [38, 39].

There is a clear pressing need for the discovery of novel non-β-lactam inhibitors. We note that only a small number of non-β-lactam inhibitors of various types of β-lactamases have been discovered till now [40, 41].

The aim of this work was to search for analogues of known allosteric inhibitors using a new approach for computational screening. We attempted to mimic in silico an inverse process to the one nature uses in vivo throughout the evolution of antibiotic resistance. Given the fact that bacteria mutate β-lactamase enzymes to hydrolyse different β-lactam antibiotics more effectively, we set out to mutate the inhibitors so that their shape and charge distribution remain roughly preserved while the scaffold may change thus allowing to find new inhibitors for the given target enzyme. We describe a newly developed scaffold hopping approach for 3D ligand-based virtual screening and present its application for a search of novel non-covalent β-lactamase inhibitors. Known allosteric inhibitors of class A β-lactamases were investigated as starting molecules. Ligands selected on the basis of virtual screening were experimentally studied using recombinant TEM type β-lactamases of molecular class A.

MATERIALS AND METHODS

Database of Molecular Structures of Potential Ligands for Virtual Screening

An in-house database contains 8 million commercially available drug-like compounds from various chemical suppliers (Amri, Asinex, Bionet, Chemblock, Chembridge, ChemDiv, Chemti, Enamine, Florida, Fluorochem, Matrix, Maybridge, Nanosyn, Oakwood, Otava, Specs, TimTec, Ukrorgsynth). The size of the molecules varies from 5 to 100 non-hydrogen atoms. The database was created at the Chemical Biology Core Facility (EMBL, Heidelberg, Germany).

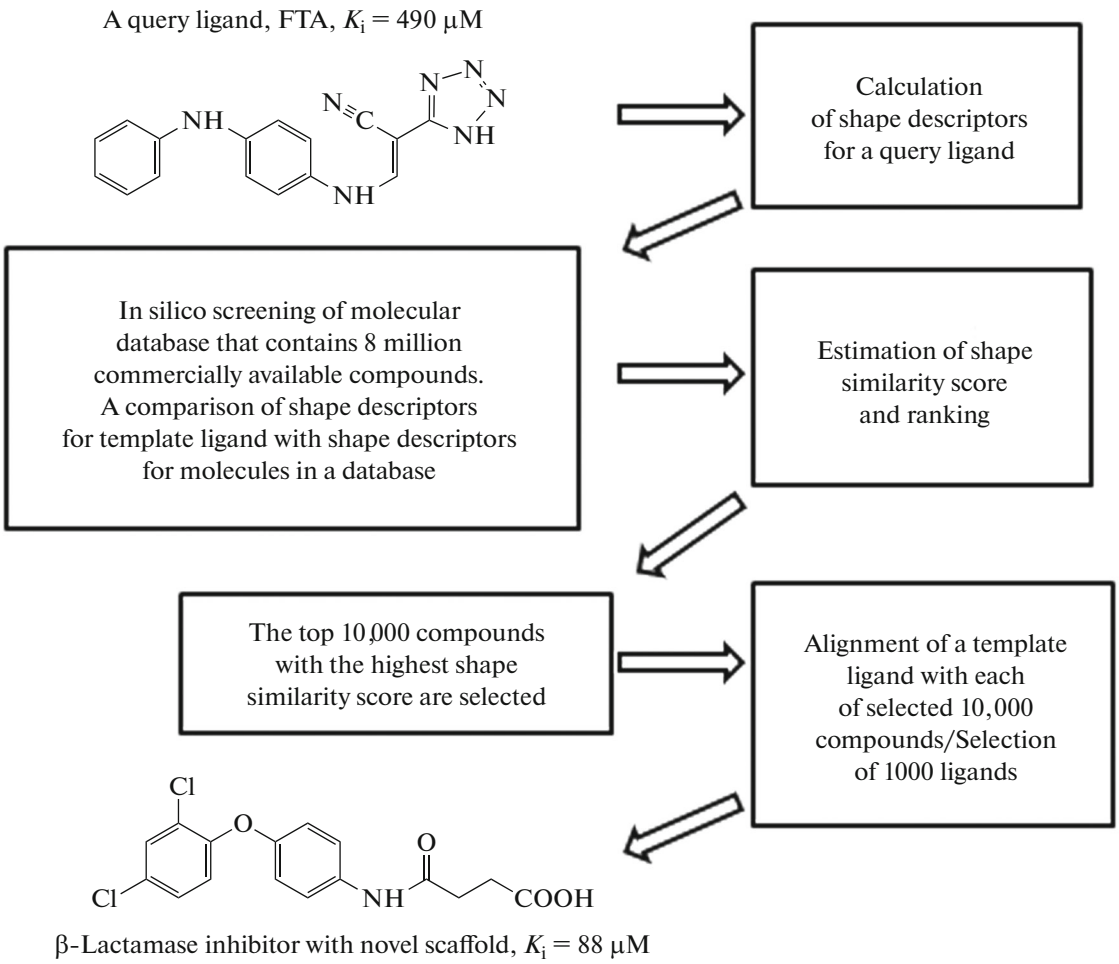


Fig. 1. The flowchart of the scaffold hopping algorithm approach.

Scaffold Hopping Screening Approach

Numerical values of the descriptors were calculated for the ligands contained in the database, up to 200 different conformations were considered for each structure. The set of numerical descriptors (Table 1) includes general characteristics of the molecule, chirality, moments of one-dimensional distribution of distances between atoms, and moments of a higher order that were not previously used to search for ligand structures.

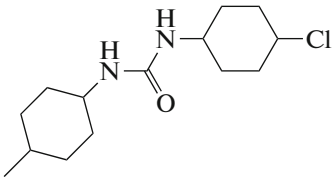
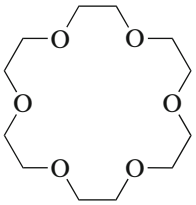
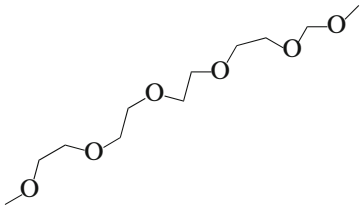
The algorithm of a scaffold hopping approach is presented in Fig. 1. The input is a set of shape descriptors for a query ligand compound. They are computed and compared to those for all compounds in the database. A shape similarity score is computed for each database compound in its best-matching conformation. This forms the basis of the pattern-recognition technique employed: if a ligand from the database yields features similar to those computed from a query compound, the atomic structure of the ligand and its shape can be regarded as similar to that of the query compound. The ligands with the highest shape similarity scores are selected and output.

Shape Descriptors

We use a number of numerical descriptors of molecular shape and topology (Table 1). Table 1 contains the examples of descriptor values for three chemical compounds (ligands 1, 2, 3) with an equal number of atoms and different molecular structures (linear, planar and cyclic). Some of these descriptors have been used within the pattern recognition tools implemented in the ARP/wARP software for building of macromolecular structures in crystallographic and cryo-electron microscopy density maps [43–46]. These were adapted for shape description of ligand molecules considered as drug-like compounds. The descriptors based on the connectivity matrix were implemented for their specific application for scaffold-hopping.

The first two features, F1 and F2, describe overall characteristics of the molecule—the number of atoms

Table 1. Molecular descriptors used

Descriptor	Molecular structures/Descriptor value		
	Ligand 1 	Ligand 2 	Ligand 3 
F1, Radius of gyration normalised by the number of atoms in the molecule [56]	0.225	0.184	0.301
F2, Number of atoms	18	18	18
F3, Chirality index [43]	1.150	0.000	4.689
F4–F5, Third (skewness) and fourth (kurtosis) central moments of the distances between atoms and the centre of the molecule [47]	1.250 1.679	1.022 1.056	1.256 1.685
F6, eigenvalues of connectivity matrices [48, 49]			
1st eigenvalue	1.399×10^{-3}	3.350×10^{-3}	8.937×10^{-4}
2nd eigenvalue	7.0513×10^{-3}	3.350×10^{-3}	3.547×10^{-3}
3rd eigenvalue	1.666×10^{-2}	1.299×10^{-2}	7.881×10^{-3}
4th eigenvalue	1.835×10^{-2}	1.299×10^{-2}	1.376×10^{-2}
5th eigenvalue	2.632×10^{-2}	2.778×10^{-2}	2.101×10^{-2}
6th eigenvalue	2.632×10^{-2}	2.778×10^{-2}	2.941×10^{-2}
7th eigenvalue	3.337×10^{-2}	4.591×10^{-2}	3.870×10^{-2}
8th eigenvalue	3.741×10^{-2}	4.591×10^{-2}	4.861×10^{-2}
9th eigenvalue	5.263×10^{-2}	6.520×10^{-2}	5.882×10^{-2}
10th eigenvalue	5.905×10^{-2}	6.520×10^{-2}	6.904×10^{-2}
11th eigenvalue	7.895×10^{-2}	8.333×10^{-2}	7.894×10^{-2}
12th eigenvalue	7.895×10^{-2}	8.333×10^{-2}	8.824×10^{-2}
13th eigenvalue	9.821×10^{-2}	9.811×10^{-2}	9.663×10^{-2}
14th eigenvalue	0.107	9.811×10^{-2}	0.104
15th eigenvalue	0.113	0.108	0.110
16th eigenvalue	0.121	0.108	0.114
17th eigenvalue	0.125	0.111	0.117
F7–F16 Third order moment invariants [43, 52]			
F7	0.301	8.944×10^{-2}	0.314
F8	−0.294	4.789×10^{-2}	−0.314
F9	-1.531×10^{-3}	-2.395×10^{-10}	-6.151×10^{-3}
F10	-1.717×10^{-3}	-1.745×10^{-9}	-8.243×10^{-3}
F11	4.148×10^{-4}	2.705×10^{-11}	2.046×10^{-3}
F12	-1.214×10^{-4}	-1.771×10^{-11}	-5.979×10^{-4}
F13	-6.814×10^{-5}	4.753×10^{-11}	-4.109×10^{-4}
F14	1.631×10^{-6}	3.192×10^{-20}	2.966×10^{-5}
F15	-1.689×10^{-7}	-1.843×10^{-20}	-2.564×10^{-6}
F16	6.552×10^{-8}	1.369×10^{-20}	1.289×10^{-6}
F17	3.962×10^{-8}	-3.424×10^{-20}	1.215×10^{-6}

and the radius of gyration. The latter reflects the spread of atoms relative to the center of mass of the molecule and may be regarded as the compactness of the molecular structure. The chirality index, F3, is one of the chiral invariants of the molecule, indicates a dissimilarity between the molecule and its mirror image, is related to its optical chirality and implemented as described in [44]. The chirality index is equal to 0 if the object is non-distinguishable from its mirror image (as for the case of Ligand 2), and increases with increasing dissimilarity between object and its mirror image (as for Ligand 1 and Ligand 3). Features F4 (skewness) and F5 (kurtosis) are the third and the forth central moments of a one-dimensional distribution of the Euclidean distances between the atoms and the centre of the molecule [47]. Features F6 provide information about atomic connectivity. The topology of a molecule is defined by how the atoms are connected to each other and it can be used to infer the chemical properties of the molecule [16, 48, 49]. The topology can be represented as an undirected and unweighted simple graph. The graph, in turn, can be mathematically represented using a set of different matrices, whose eigenspectra provide some information about their structure. Here we used a Laplacian connectivity matrix to enumerate the extent to which a molecular graph differs at one vertex from its values at nearby vertices. The number of non-zero eigenvalues of a connectivity matrix is equal to the number of atoms minus the number of not-connected clusters [50]. For example, a graph for 10 atoms with all of them connected will have nine non-zero eigenvalues, while for 10 disconnected atoms all eigenvalues will be zero.

The variations in the shape of the objects can be exploited by the use of moments, since a well behaved probability density function can be uniquely described by exactly one infinite set of spatial moments. The second- and third-order three-dimensional central moments can be transformed to moment invariants [51] using group-theoretic techniques [52]. The 11 third-order moment invariants (features F7–F17) were effectively applied to distinguish the shape of the objects in three-dimensional electron density maps [43, 52] and are used in the present work for comparison of the atomic ligand molecules.

Ranking Molecules

The shape-similarity score for a query ligand and a candidate compound from a database is estimated as:

$$\text{Shape Similarity Score} = \sum_{i}^{N} w_i \left(F_i^q - F_i^c\right)^2,$$

where i runs over all the N features, F_i^q represents the ith feature for the query ligand, F_i^c is the ith feature for the candidate molecule from the database. After screening the whole database and estimation of shape similarity scores, the software returns a list of 10,000 top ranking of compounds according to the scores.

Experimental Testing of the Selected Compounds

Chemical compounds selected as potential β-lactamase inhibitors were purchased from: Asinex (www.asinex.com, USA), Chembridge, USA; ChemDiv, USA and Bionet (www.keyorganics.net), UK. Recombinant β-lactamase TEM-171 was expressed and purified as described in [53]. In brief, recombinant enzyme was isolated from bacterial periplasm using osmotic shock procedure and purified further by anion-exchange and size exclusion chromatography.

Inhibition of β-lactamase TEM-171 by the compounds was determined as reduction in the rate of enzymatic hydrolysis of chromogenic substrate CENTA (β-Lactamase Substrate—CAS 9073-60-3—"Calbiochem" [54]). Stock solutions of compounds of potential inhibitors (10 mM) were prepared in 100% dimethyl sulfoxide (DMSO). Assay conditions were as follows: 50 mM sodium phosphate buffer pH 7.0 with 0.05% pluronic F-127, enzyme concentration was of 6.25 nM, CENTA concentration of 100 μM. The reaction was start by the adding of substrate solution to the mixture of enzyme and compound tested in buffer. An increase in the optical density of the enzymatic hydrolysis product was recorded at a wavelength of 405 nm. The degree of inhibition was assessed by the slope of the accumulation curve of the colored reaction product, as well as by the absorption values of the product after 30 minutes. Data were normalized to positive (10 μM tazobactam) and negative (DMSO) controls. Compounds with inhibitory activity at or greater 20% were progressed for IC_{50} determination. For the best ligands, the equilibrium inhibition constants K_i were determined.

RESULTS AND DISCUSSION

Virtual Selection of Analogues of β-Lactamase Inhibitors by Scaffold Hopping Approach

As search query molecules we selected four known low-affinity non-β-lactam inhibitors of class A β-lactamases:

FTA, 3-(4-phenylamino-phenylamino)-2-(1H-tetrazol-5-yl)-acrylonitrile—inhibitor of β-lactamase TEM-1 [40];

CBT, *N,N*-bis(4-chlorobenzyl)-1H-1,2,3,4-tetraazol-5-amine—inhibitor of β-lactamase TEM-1 [40];

1CE, 3-(1H-tetrazol-5-ylmethyl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one—inhibitor of β-lactamase CTX-M-9 [41];

F13, 3-fluoro-*N*-[3-(1H-tetrazol-5-yl)phenyl]benzamide—inhibitor of β-lactamase CTX-M-9 [41].

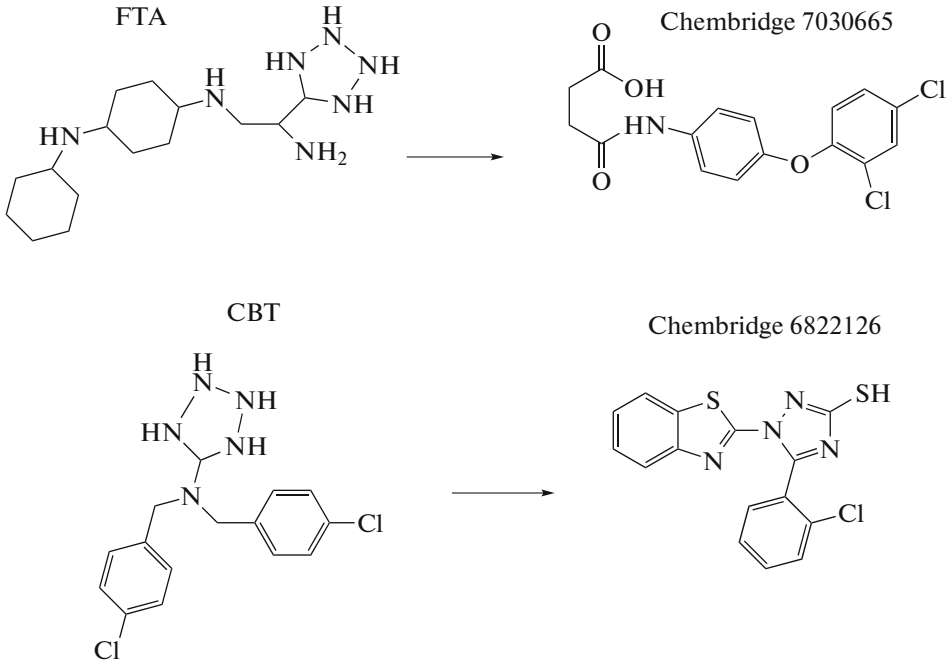


Fig. 2. Chemical structures of template molecules, FTA and CBT, and a novel non-β-lactam inhibitors, Chembridge 7030665 and Chembridge 6922126, outputted by the scaffold hopping approach.

All query molecules were used in their active conformations obtained from their co-crystallized structures with the protein targets in protein data bank (pdb ID: 1pzp, 1pzo, 3g34, 3g35).

For starting ligands, sets of descriptors were calculated, comparing them with a set of ligand descriptors from a database containing 8 million compounds, and shape similarity parameters were calculated for each of compounds in the optimal conformation. As a result of screening, about 10,000 compounds were selected with the maximum value of the shape similarity parameter. Then, 3D alignment was performed for a set of selected structures with the structure of each query ligand. Based on the best similarities, 1000 compounds were selected.

Testing the Ligands-Potential β-Lactamase Inhibitors Using Recombinant TEM Type β-Lactamase

For experimental confirmation of the results of virtual screening, the inhibition of recombinant β-lactamase TEM-171 by the selected compounds was investigated. As a control reaction, the hydrolysis of the chromogenic substrate CENTA by β-lactamase was used. To increase the productivity of the analysis, the reaction was carried out in 96-well plates. Reagent concentrations were selected so that the linearity of the initial portion of the curve of colored product accumulation was maintained for at least 30 minutes at room temperature. The action of potential inhibitors was evaluated by changing the initial slope of the kinetic curve of roduct accumulation, as well as by estimation of the changein the product absorption values in 30 minutes after the reaction start. The end point and kinetic raw data were normaized to the positive (tazobactam at 10 μM) and negative (DMSO) control to generate the inhibition data. Using the hit list from the kinetic analysis, compounds with inhibitory activity at or greater 20% were progressed for IC_{50} determination. Analysis of compound activities by the endpoint or kinetic method revealed that the kinetic method generated more hits for follow-up in comparison to the endpoint method.

Based on IC_{50} screening, three compounds were identified as hits: two for TEM type beta-lactamases (Chembridge 6922126 and Chembridge 7030665) (Fig. 2) and one for CTX-M type beta-lactamases (ASN02750282). Their structures and IC_{50} values are shown on Fig. 3 and in Table 2.

A detailed study of the compound Chembridge 6922126 showed that it is prone to dimerization, and the dimer is characterized by lower solubility in aqueous solutions. The monomeric form of the ligand did not inhibit the activity of β-lactamases.

The ligand Chembridge 7030665, found as an analogue of the query ligand FTA, was characterized by the value of the equilibrium inhibition constant (K_i = 88 μM) determined in the hydrolysis of a CENTA substrate by recombinant β-lactamase TEM-171. This compound demonstrated a more effective inhibition of TEM type recombinant β-lactamase compared to FTA, for which K_i value is about five times worse (K_i = 490 μM [40]).

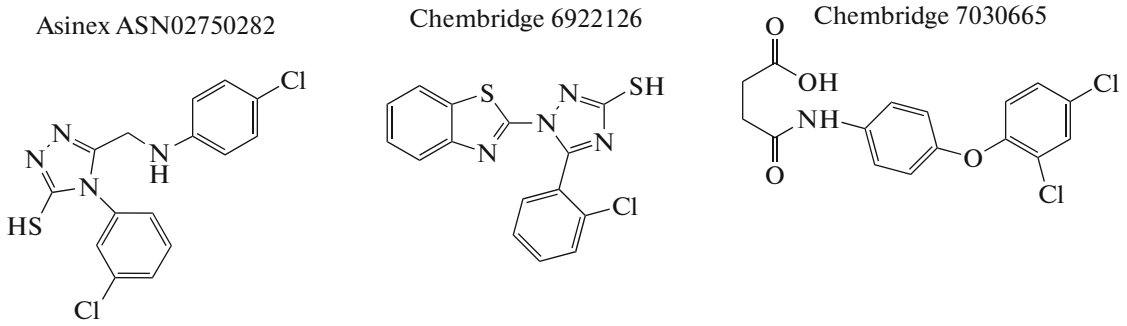


Fig. 3. Structures of three identified ligands-inhibitors of β-lactamases selected by ativity in the inhibition of β-lactam hydrolysis.

The ligand Chembridge 7030665 is a new type of non-covalent non-β-lactam inhibitor based on acylated phenoxyaniline. It was further characterized using infrared spectroscopy and fluorescence quenching [53]. Molecular docking analysis of the residues involved in the coordination of the inhibitor in the active site of the enzyme showed that some of them are conservative for β-lactamases of molecular class A. This suggests a broad specificity of this inhibitor with respect to clinically significant class A enzymes.

We also performed an additional chemical modification of the ligand Chembridge 7030665 in order to improve its ability to inhibit β-lactamases. The effect of various benzene ring substituents and the length of hydrocarbon chains on the efficiency of hydrolysis inhibition were investigated. As a result, a new thiourea-based non-β-lactam inhibitor was obtained, characterized by the inhibition constant K_i = 48 μM [55].

CONCLUSIONS

In this paper we introduced a new computational screening approach with scaffold hopping capabilities for ligand-based drug design. Taking query ligand it enables discovery of new compounds by shape and doesn't require structural information about protein target. In opposite to fingerprint-based methods, the presented method searches compounds with similar bioactivity to the query molecule, but with different scaffold. The compactness and simplicity in calculation of shape descriptors allow fast scanning of large molecular databases. The presented method for ligand-based drug design showed its applicability for scaffold hopping and allows fast scanning of large molecular databases. The method helped identify three non-β-lactam hit compounds showing higher inhibition of TEM type beta-lactamase compared to the used template compounds. The combination of the virtual screening method with the subsequent chemical design of the ligand molecule provides even more effective inhibitors.

Table 2. Summary of endpoint IC_{50} data for selected inhibitors of TEM β-lactamase

Supplier and compound ID	IC_{50}, μM
Asinex ASN02750282	36
Chembridge 6922126	58
Chembridge 7030665	88

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or using animals as experimental objects.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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