chemistry proceedings

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Proceeding Paper

High-Throughput Virtual Screening of Compounds with Electrophilic Fragments for New Potential Covalent Inhibitors of Bacterial Proteins †

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† Presented at the 26th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2022; Available online: https://ecsoc-26.sciforum.net/.

Abstract: Search for new antibacterial drugs has continued to be an urgent request. One of the approaches is development of covalent inhibitors using biochemoinformatics at initial stages. In this work, structures of few plant-derived substances with electrophilic unsaturated carbonyl & structures of small synthetic compounds suitable for fragment-based drug discovery (FBDD) with -CH_2-Br group were selected as ligands for sets of structures of bacterial proteins. Theoretical assessment was carried out using the Autodock Vina program for calculation and FYTdock for organization the process and analysis of results. Natural Ixerine D as well as synthetic 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan demonstrated the most promising results as potential Cys-targeted inhibitors.

Keywords: docking; antibacterial drugs; covalent inhibitors; bacterial proteins; fragment-based drug discovery

Citation: Yakovets, P.; Staravoitava, V.; Faletrov, Y.; Shkumatov, V. High-Throughput Virtual Screening of Compounds with Electrophilic Fragments for New Potential Covalent Inhibitors of Bacterial Proteins. *Chem. Proc.* **2022**, 4, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Published: 15 November 2022

1. Introduction

Natural antibiotics, their derivatives and synthetic antimicrobials are the primary tools to treat bacterial infections. A number of bacteria have developed resistance to many or even all such currently available drugs, hindering the treatment of these diseases. Therefore, the search for new antibacterial drugs does not cease to be an urgent scientific task. One of the approaches to create therapeutic agents is the development of covalent inhibitors. The initial stages of any modern drug design company include the use of modern methods of biochemoinformatics, in particular, molecular docking, i.e., computing ofligand-protein complexes with an assessment of their geometry and affinity [1–3].

In this work, ~20 structures of plant-derived electrophylic substances from the Pubchem database were selected as ligands as well as some structures suitable for the fragment-based ligand design approach (FBDD—Fragment-based drug discovery) containing the electrotrophic fragment -CH_2-Br. The electrophilic nature of the phytochemicals and the fragments provide a possibility of covalent medication of nucleophilic atoms of Cys and His residues in proteins. In this work the possibility was additionally evaluated in silico using the Autodock Vina program for docking simulations and FYTdock [4] to organize, run and analyze the docking results.

2. Materials and Methods

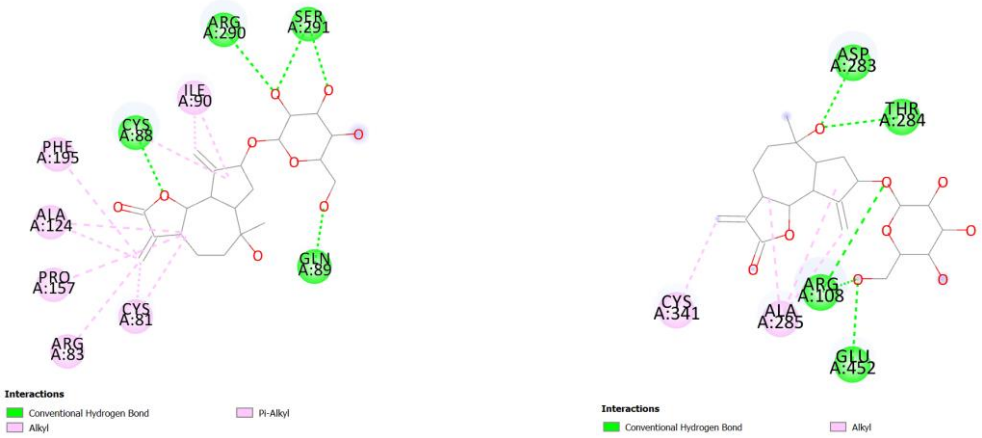
For molecular docking, AutoDock Vina 1.1.2 was used (docking area 4 × 4 × 4 nm in the center of the protein, step 0.1 nm, Exhaustiveness parameter 12, 5 models were calculated). Preparation of ligand and protein files and visualization of the results were performed using the MGL Tools software package (The Scripps research lab.). To automate the organization, run calculations using the Autodock Vina program, and analyze the results obtained, we used the original FYTdock assistant program [4]. As ligands, we chose 3 structures of compounds constructed by us using the FBDD approach (Fragment-based drug discovery): 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan, 2-bromo-1-(4-(nitrobenzofurazan-4-yl)piperazin-1-yl)ethanone, 2-bromo-N-(4-bromophenyl)acetamide, and a library of ~20 structures of plant-derived substances created using the Pubchem database taking into account their growth in the territory of the Republic of Belarus. Approximately 2900 protein structures of *Mycobacterium tuberculosis* and 500 random protein structures of some other bacterial species were selected to create a library of bacterial protein structures from the Protein Data Bank. Docking results were initially collected and processed using FYTdock software as an Excel spreadsheet showing binding energies, amino acid environment, protein ligand-amino acid interactions, protein-ligand complexes. The result was taken into account if the value of E_{bind} was no more than –6.0 kcal/mol and the distance from the electrophilic fragment of the ligand structure to the sulfur atom of the thiol group of cysteine residues in the protein-ligand complexes obtained in silico did not exceed each 0.45 nm (distance criterion). For graphical representation of the result, the Biovia program was used.

3. Results

Fortunately, it was found that Ixerin D (Pubchem database number CID101553163) from common widely grown plant dandelion demonstrated a number of interactions with high affinity and the location of its electrophilic fragment within 0.4 nm from the sulfur atom of the cysteine of Mtb proteins, lipoyl synthase, inosine monophosphate dehydrogenase, and beta-ketoacyl-acyl carrier protein synthase III from *Mycobacterium tuberculosis* (Table 1 and Figure 1).

Table 1. The proteins from protein-ligand complexes where an electrophilic carbon of the Ixerin D located within 0.4 nm from the sulfur atom of a cysteine residue and their binding energies.

Protein PDB	Protein Name	Cysteine	E_{bind}, kcal/mol
5EXI	Lipoyl synthase	CYS81	−10.7
4ZQR	The catalytic domain of the inosine monophosphate dehydrogenase	CYS341	−9.4
1M1M	Beta-ketoacyl-acyl carrier protein synthase III	CYS123	−9.2
2AJ9	Beta-ketoacyl-acyl carrier protein synthase III	CYS122	−9.1
2AHB	Beta-ketoacyl-acyl carrier protein synthase III	CYS122	−9.0



(**a**) (**b**)

Figure 1. Calculated position of ligand Ixerin D inside of *Mycobacterium tuberculosis* proteins: (**a**) Lipoyl synthase (PDB code: 5EXI); (**b**) The catalytic domain of the inosine monophosphate dehydrogenase (PDB code: 4ZQR).

This compound is metabolite of the common dandelion (*Taraxacum officinale*) and is probably of low toxicity to humans due to the use of parts of this plant as food or medicine by humans and some animals. The beta-ketoacyl-acyl carrier protein synthase III is very important for fatty acid biosynthesis and for the normal life cycle of Mtb [5]. Such calculated and theoretical data indicate the possibility of a favorable outcome of the biological testing of Ixerin D, and it can be obtained from a natural source, which does not make it necessary to develop a scheme for its chemical synthesis.

For the synthetic ligand 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan, compiled using the FBDD approach, the -CH_2-Br fragment was found to be located close to the cysteine sulfur atom in Sortase B from *Staphylococcus aureus*, a human pathogen [6], *E. Coli* Gsp amidase, which regulates the redox state of *E. coli* cells [7], β-lactamase S70C BlaC from *Mycobacterium tuberculosis*, which contributes to the development of the bacteria natural resistance to β-lactam antibiotics [8] (Table 2).

Table 2. The proteins from protein-ligand complexes where an electrophilic fragment of the 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan located near from the sulfur atom of a cysteine residue and their binding energies.

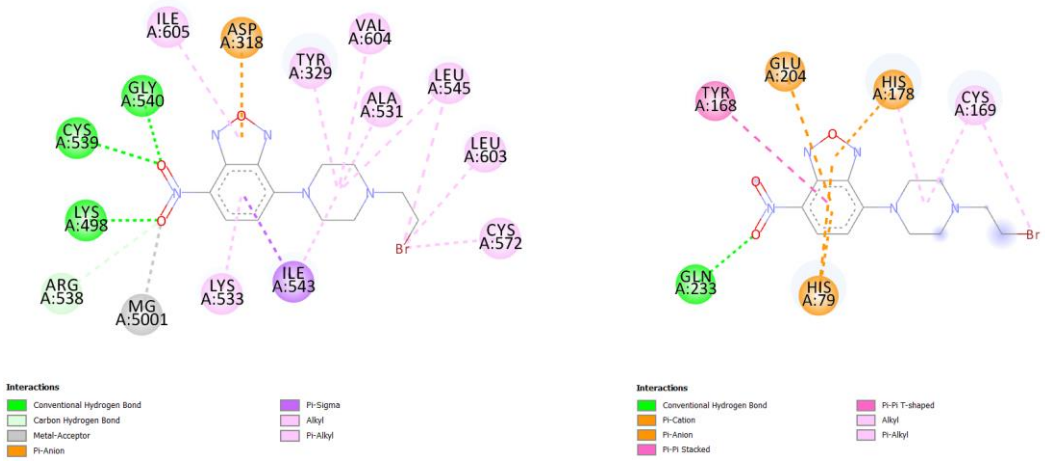
Protein PDB	Protein Name	Cysteine	E_{bind}, kcal/mol
6h27	S70C BlaC from *Mycobacterium tuberculosis*	CYS70	−7.3
3a2z	*E. Coli* Gsp amidase Cys59 sulfenic acid	CYS59	−7.0
7ock	*E. Coli K-12* MAT	CYS96	−6.8
1qxa	Crystal structure of *Staphylococcus aureus* Sortase B	CYS223	−6.5

It is important to note that, despite the localization of the electrophilic fragment of the ligand near cysteine, definitely these ligand-receptor interactions can be hindered due to geometrical features and energetically favorable location of the ligand in the protein. 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan showed interactions with high affinity and favorable location of the -CH_2-Br fragment for covalent binding with the following bacterial proteins: *E. Coli* bifunctional glutathionylspermidine synthetase/amidase and *E. Coli* K-12 methionine aminopeptidase with binding energies of −8.3 kcal/mol and −7.0 kcal/mol, respectively (Table 3 and Figure 2). These enzymes are

potential new drug targets, and inhibitors of these enzymes may be useful as prototypes of new antibacterial agents [9,10].

Table 3. The proteins from protein-ligand complexes where an electrophilic fragment of 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan located within 0.4 nm from the sulfur atom of a cysteine residue and their binding energies.

Protein PDB	Protein Name	Cysteine	E_{bind}, kcal/mol
2ioa	*E. Coli* Bifunctional glutathionylspermidine synthetase/amidase	CYS572	−8.3
2gg7	*E. Coli K-12* methionine aminopeptidase	CYS169	−7.0



(**a**) (**b**)

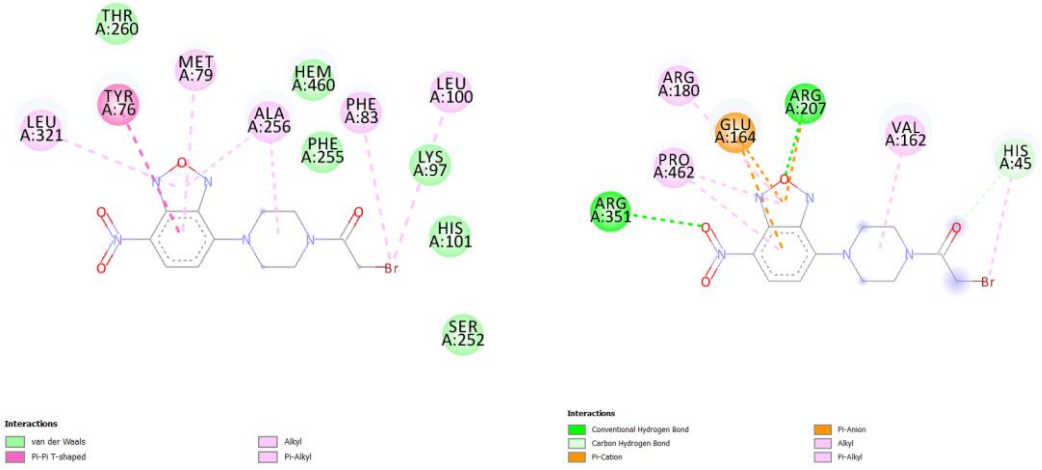
Figure 2. Calculated position of ligand 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan inside of bacterial proteins: (**a**) *E. Coli* Bifunctional glutathionylspermidine synthetase/amidase (PDB code: 2ioa); (**b**) *E. Coli K-12* methionine aminopeptidase (PDB code: 2gg7).

The compound 2-bromo-1-(4-(nitrobenzofurazan-4-yl)piperazin-1-yl)ethanone had the orientation of the -CH_2-Br fragment near the C37L/C151T/C442A histidine of the CYP51 triple mutant from *Mycobacterium tuberculosis*, Microcin-processing metalloprotease TldD/E from *E. Coli*, CYP134A1 with a closed-loop substrate binding from *Bacillus subtilis* and other bacterial proteins with binding energies from −6.7 to −9.0 kcal/mol (Table 4 and Figure 3).

Table 4. The proteins from protein-ligand complexes where an electrophilic fragment of 2-bromo-1-(4-(nitrobenzofurazan-4-yl)piperazin-1-yl)ethanone located within 0.4 nm from the sulfur atom of a cysteine residue and their binding energies.

Protein PDB	Protein Name	Cysteine	E_{bind}, kcal/mol
1u13	Crystal structure of C37L/C151T/C442A-triple mutant of CYP51 from *Mycobacterium tuberculosis*	HIS101	-9.0
5nj5	*E. Coli* Microcin-processing metalloprotease TldD/E	HIS45	-8.4
3NC3	*Bacillus subtilis* CYP134A1 structure with a closed substrate binding loop	HIS351	-8.3

5njf	*E. Coli* Microcin-processing metalloprotease TldD/E (TldD H262A mutant)	HIS45	-8.0
5njc	*E. Coli* Microcin-processing metalloprotease TldD/E (TldD E263A mutant)	HIS45	-7.7
2VZM	Crystal structure of *E. Coli* PikC D50N mutant	HIS238	-7.7
3LXI	Crystal Structure of *E. Coli* CYP101D1	HIS400	-7.4
4BF4	PikC D50N mutant from *Streptomyces venezuelae*	HIS238	-7.4
2irv	Crystal structure of *E. Coli* K-12 GlpG, a rhomboid intramembrane serine protease	HIS150	-7.3
3zeb	*E. Coli* BL21(DE3) GlpG	HIS141	-7.3
5hdi	*Mycobacterium tuberculosis* cytochrome P450 CYP144A1	HIS325	-7.3
1T2B	Crystal Structure of *Citrobacter braakii* cytochrome P450cin	HIS391	-7.1
4EGM	The X-ray crystal structure of *Rhodopseudomonas palustris* HaA2 CYP199A4	HIS202	-6.9
3ZC3	*Nostoc* sp. PCC 7119 Ferredoxin-NADP reductase (mutation S80A)	HIS42	-6.8
1P7R	Crystal structure of *Pseudomonas putida* cytochrome P450CAM	HIS391	-6.7



(**a**) (**b**)

Figure 3. Calculated position of ligand 2-bromo-1-(4-(nitrobenzofurazan-4-yl)piperazin-1-yl)ethanone near bacterial proteins: (**a**) Crystal structure analysis of the C37L/C151T/C442A-triple mutant of CYP51 from *Mycobacterium tuberculosis* (PDB code: 1u13); (**b**) *E. Coli* Microcin-processing metalloprotease TldD/E (PDB code: 5nj5).

The ligand of 2-bromo-N-(4-bromophenyl)acetamide bound only to the mutant heme domain A264C of cytochrome P450 BM3 from *E. Coli* (PDB code: 3EKB) with localization of the bromine atom of the compound close to cysteine (CYS264) and binding energy of -6.0 kcal/mol (Figure 4).

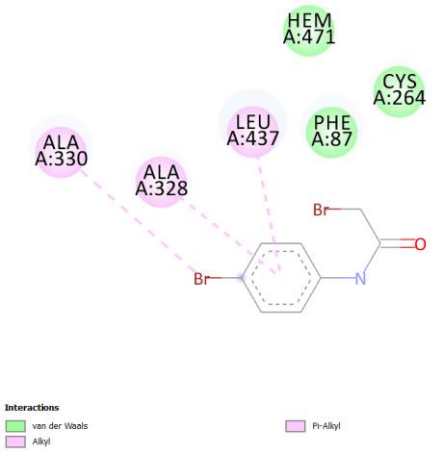


Figure 4. Calculated position of ligand 2-bromo-N-(4-bromophenyl)acetamide near A264C mutant heme domain of cytochrome P450 BM3 from *E. Coli* (PDB code: 3EKB).

4. Conclusions

Based on in silico molecular docking, natural compound Ixerin D from common dandelion, as well as synthetic ligands 4-(4-(2-bromoethyl)piperazin-1-yl)-7-nitrobenzofurazan, 2-bromo-1-(4-(nitrobenzofurazan-4-yl)piperazin-1-yl)ethanone and 2-bromo-N-(4-bromophenyl)acetamide, fragments for structures of new covalent molecular tools or drugs, were identified to be able to covalently modified inhibitors of various bacterial proteins with localization of their electrophilic fragments within 0.4 nm from functional amino acid fragments. residues and a binding energy in the range from -6.0 to −10.7 kcal/mol. Thus, the results substantiate perspectives of experimental studies of these ligands as potential antibacterial agents or molecular tools with covalent modifier properties.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: This study was supported by GSPSR (Belarus) № 20210560.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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