chemistry proceedings

MDPI

Proceeding Paper

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

Polina Yakovets [1,*], Viktoryia Staravoitava [2], Yaroslav Faletrov [1,2] and Vladimir Shkumatov [1,2,*]

[1] Department of Chemistry, Belarusian State University, 14 Leningradskaya St., 220006 Minsk, Belarus
[2] Research Institute for Physical Chemical Problems of the Belarusian State University, Minsk, Belarus 14 Leningradskaya St., 220030 Minsk, Belarus
* Correspondence: yakov.polina.42@gmail.com (P.Y.); vlad.shkumatov@tut.by (V.S.)
† Presented at the 26th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2022; Available online: https://ecsoc-26.sciforum.net/.

Abstract: The search for new antibacterial drugs has continued to be an urgent matter. One of the approaches is the development of covalent inhibitors using biochemoinformatics at the initial stages. In this work, structures of a few plant-derived substances with electrophilic unsaturated carbonyl and 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. The theoretical assessment was carried out using the Autodock Vina program for calculation and FYTdock for the organization of the process and the 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**, *12*, 87. https://doi.org/10.3390/ecsoc-26-13574

Academic Editor: Julio A. Seijas

Published: 14 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 creating 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 of ligand-protein complexes with an assessment of their geometry and affinity [1–3].

In this work, ~20 structures of plant-derived electrophilic 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 electrophilic 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, the exhaustiveness parameter was 12, and five models were calculated). Preparation of ligand and protein files and visualization of the results were

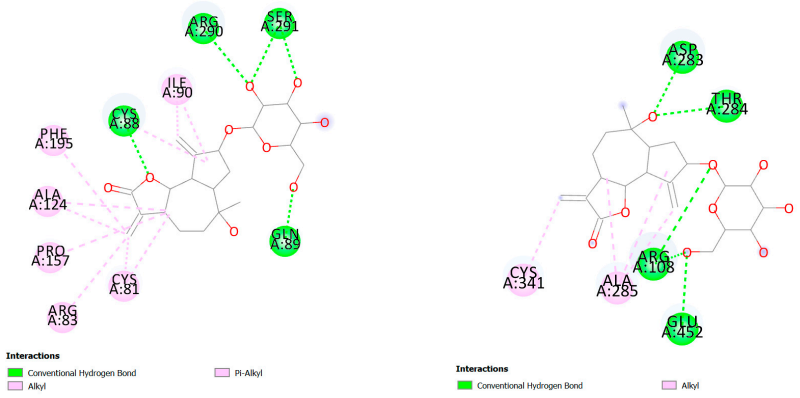
performed using the MGL Tools software package (The Scripps research lab.). To automate the organization, run calculations using the Autodock Vina program, and analyze the results obtained, we used the original FYTdock assistant program [4]. As ligands, we chose three structures of compounds constructed 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 a graphical representation of the result, the Biovia program was used.

3. Results

Fortunately, it was found that Ixerin D (Pubchem database number CID101553163) from dandelion, a common and widely grown plant, 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 were 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. The 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 a 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 the sulfur atom of a cysteine residue and their binding energies.

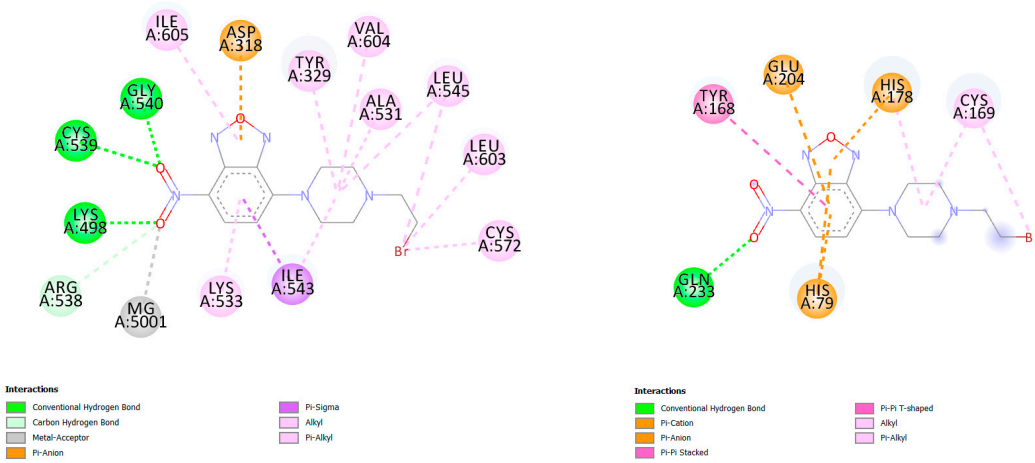
Protein PDB	Protein Name	Cysteine	E_{bind}, kcal/mol
6h27	S70C BlaC from *Mycobacterium tuberculosis*	CYS70	−7.3
3a2z	*E. coli* Gsp amidase Cys59 sulfenic acid	CYS59	−7.0
7ock	*E. coli* K-12 MAT	CYS96	−6.8
1qxa	Crystal structure of *Staphylococcus aureus* Sortase B	CYS223	−6.5

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. The 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

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).

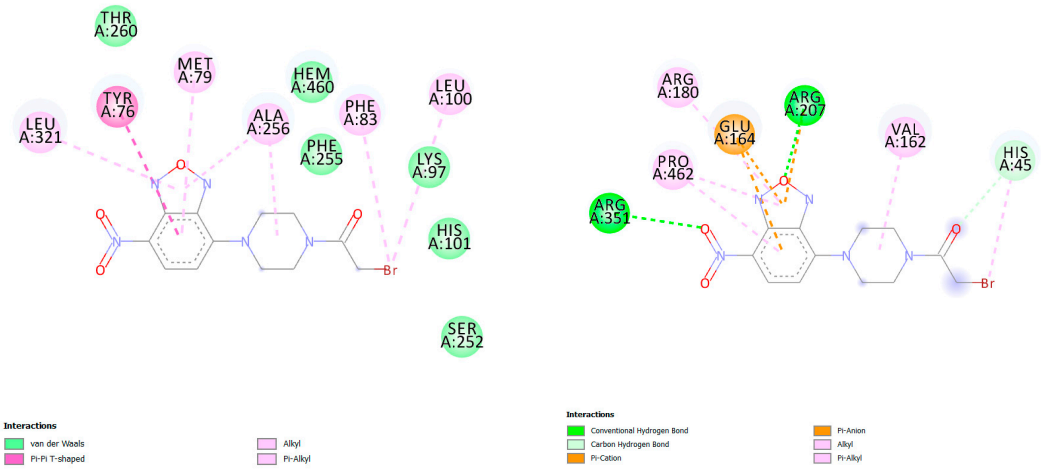


(**a**) (**b**)

Figure 2. The 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).

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).

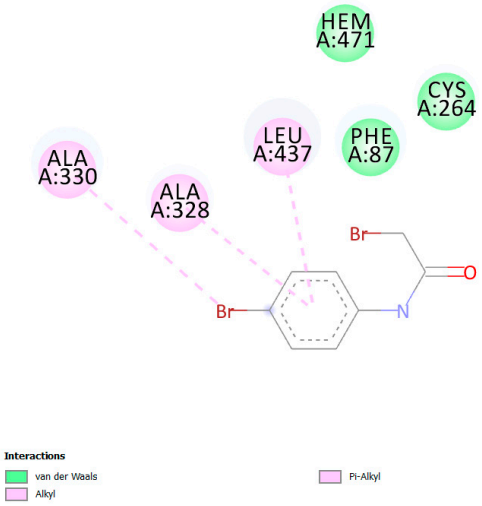


Figure 4. Calculated position of ligand 2-bromo-N-(4-bromophenyl)acetamide near A264C mutant heme domain of cytochrome P450 BM3 from *E. coli* (PDB code: 3EKB).

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)ethenone, 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 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: Conceptualization, Y.F., P.Y. and V.S. (Viktoryia Staravoitava); writing—original draft preparation, P.Y. and V.S. (Viktoryia Staravoitava); writing—review and editing, Y.F., P.Y. and V.S. (Viktoryia Staravoitava); supervision, V.S. (Vladimir Shkumatov). All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by governmental grants of republic of Belarus No. of registration 20210560.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Kibou, Z.; Aissaoui, N.; Daoud, I.; Seijas, J.A.; Vázquez-Tato, M.P.; Khelil, N.K.; Choukchou-Braham, N. Efficient Synthesis of 2-Aminopyridine Derivatives: Antibacterial Activity Assessment and Molecular Docking Studies. *Molecules* **2022**, *27*, 3439. [CrossRef] [PubMed]
2. Faletrov, Y.; Brzostek, A.; Plocinska, R.; Dziadek, J.; Rudaya, E.; Edimecheva, I.; Shkumatov, V. Uptake and Metabolism of Fluorescent Steroids by Mycobacterial Cells. *Steroids* **2017**, *117*, 29–37. [CrossRef]
3. Faletrov, Y.V.; Karpushenkova, V.S.; Zavalinich, V.A.; Yakovets, P.S.; Shkredava, A.D.; Shkumatov, V.M. Interaction of Nitrobenzoxadiazole Derivatives of Piperazine and Aniline with Bovine Serum Albumine in Silico and in Vitro. *J. Belarusian State Univ. Chem.* **2021**, *2*, 25–35. [CrossRef]
4. Faletrov, Y.V.; Staravoitava, V.A.; Dudko, A.R.; Shkumatov, V.M. Application of Docking-Based Inverse High Throughput Virtual Screening to Found Phytochemical Covalent Inhibitors of SARS-CoV-2 Main Protease, NSP12 and NSP16. 2022, *preprint*. [CrossRef]
5. Sachdeva, S.; Reynolds, K.A. Mycobacterium tuberculosis β-Ketoacyl Acyl Carrier Protein Synthase III (mtFabH) Assay: Principles and Method. In *New Antibiotic Targets. Methods In Molecular Medicine™*; Champney, W.S., Ed.; Humana Press: Totowa, NJ, USA, 2008; Volume 142, pp. 205–213. [CrossRef]
6. Zong, Y.; Mazmanian, S.K.; Schneewind, O.; Narayana, S.V.L. The Structure of Sortase B, a Cysteine Transpeptidase That Tethers Surface Protein to the *Staphylococcus Aureus* Cell Wall. *Structure* **2004**, *12*, 105–112. [CrossRef] [PubMed]
7. Chiang, B.-Y.; Chen, T.-C.; Pai, C.-H.; Chou, C.-C.; Chen, H.-H.; Ko, T.-P.; Hsu, W.-H.; Chang, C.-Y.; Wu, W.-F.; Wang, A.H.-J.; et al. Protein S-Thiolation by Glutathionylspermidine (Gsp). *J. Biol. Chem.* **2010**, *285*, 25345–25353. [CrossRef]
8. Tassoni, R.; Blok, A.; Pannu, N.S.; Ubbink, M. New Conformations of Acylation Adducts of Inhibitors of β-Lactamase from *Mycobacterium tuberculosis*. *Biochemistry* **2019**, *58*, 997–1009. [CrossRef]
9. Pai, C.-H.; Chiang, B.-Y.; Ko, T.-P.; Chou, C.-C.; Chong, C.-M.; Yen, F.-J.; Chen, S.; Coward, J.K.; Wang, A.H.-J.; Lin, C.-H. Dual Binding Sites for Translocation Catalysis by *Escherichia coli* Glutathionylspermidine Synthetase. *EMBO J.* **2006**, *25*, 5970–5982. [CrossRef] [PubMed]
10. Evdokimov, A.G.; Pokross, M.; Walter, R.L.; Mekel, M.; Barnett, B.L.; Amburgey, J.; Seibel, W.L.; Soper, S.J.; Djung, J.F.; Fairweather, N.; et al. Serendipitous Discovery of Novel Bacterial Methionine Aminopeptidase Inhibitors. *Proteins* **2006**, *66*, 538–546. [CrossRef]