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Molecular docking study on biomolecules isolated from endophytic fungi

JANKO IGNJATOVIĆ[1], NEVENA ĐAJIĆ[1], JOVANA KRMAR[1], ANA PROTIĆ[1], BORUT ŠTRUKELJ[2] and BILJANA OTAŠEVIĆ[1]*

[1]*Department of Drug Analysis, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia and* [2]*Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia*

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Abstract: Recently, growing interest has been devoted to the investigation of compounds with antimicrobial activity due to rising cases of resistance of microbes to known therapies. A reliable and versatile source of novel drug discovery was recently found among endophytic fungi. Hitherto, the research usually enclosed the *in vitro* evaluation of antimicrobial activity and chemical structure elucidation of biomolecules extracted from fungal material. Therefore, this research was designed as an extension to previous investigations of endophytic fungi growing on conifer needles by means of conducting a molecular docking study. The *in silico* methods were used with the main goal to make a contribution to the understanding of the mechanisms underlying the interaction of biomolecules isolated from fungus *Phomopsis species* and eight different types of receptors that belong to usually multidrug resistant bacterial pathogens. The results revealed valuable interactions with receptors 3G7B (*Staphylococcus aureus*'s gyrase B), 1F0K (1.9 Å structure of *Escherichia coli*'s transferase) and 1SHV (*Klebsiella pneumoniae*'s SHV-1 β-lactamase) thus pointing out the receptors that trigger antibiotic response upon activation by the most potent compounds 325-3, 325-5, phomoenamide and phomol. These findings also recommended further discovery of novel potent and broad-spectrum antibiotics based on the structure of selected molecules.

Keywords: endophytes; antibacterial activity; *in silico* drug discovery.

INTRODUCTION

Antimicrobial drug resistance represents a global health problem. In order to effectively undertake the challenge of antimicrobial resistance, indispensable and sustainable use of antibiotics is necessary in order to control the disease, but also fostering innovation and development of new molecules with antibiotic potential. Development of novel antimicrobial therapies is necessary in order to enable

* Corresponding author. E-mail: biljana.otasevic@pharmacy.bg.ac.rs
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substitution for the declining effectiveness of already existing antibiotics.[1] Endophytic fungi were recently recognised as a naturally occurring repository of potent compounds for novel drug discovery regarding the fact that they represent microorganisms that usually inhabit medicinally potent plants and thus may inherit their medicinal abilities.[2–5] Endophytic fungi are defined as non-pathogenic microorganisms (bacteria or fungi) that are present in the inner tissues of plants and have a symbiotic relationship with their plant host by helping the host plant to overcome the invasion of pathogenic microorganisms by producing secondary metabolites.[6–11] A literature survey revealed that for these molecules, a variety of pharmacological activities, such as antifungal, antibacterial, antiviral, cytotoxic, anti-oxidant, *etc*. have already been reported.[12] In this regard, the authors have reported significant antibacterial activity coming from secondary metabolites of endophytic fungus *Phomopsis species* growing in Slovenian conifer forests.[13]

It is common knowledge that many antimicrobials express their effect through specific interaction with receptor targets that are present in microbes.[14,15] Nowadays, in line with experimental *in vitro* testing of the activity and interactions between potential ligands and receptors, achievements in the field of computer science allow a unique opportunity for computer aided drug design and simulation.[16–18] *In silico* methods enable high throughput screening for potential drug candidates and introduce scientifically more informative and rationalized pharmaceutical research. Bearing this in mind, as well as the already proven activity of biomolecules isolated from endophytic fungus *Phomopsis species* growing on conifer needles against *Staphylococcus aureus* and *Escherichia coli*,[13] the present study undertook consideration of common target receptors of known antibiotics related to these pathogens. It was decided that the antimicrobial activity against G^+ bacteria *S. aureus* should be investigated using receptors 3VSL (penicillin-binding protein 3 from methicilin-resistant *S. aureus*),[19] 3G7B (*S. aureus* gyrase B)[20] and 1JIJ (tyrosyl-tRNA synthetase of *S. aureus*).[20] In addition, receptor 3K3P from *Streptococcus mutans* (apo-form of D-alanine: D-alanine ligase),[21] was included as a valuable clue in further understanding of the resistance of G^+ type bacteria to known drugs. On the other hand, widely tested receptor targets for evaluation of antimicrobial activity on *E. coli*, as the most common representative of G^- bacteria, were 1F0K (1.9 Å structure of *E. coli*'s transferase),[22] 1KZN (24 kDa domain of *E. coli*'s isomerase)[21] and 4EMV (structure of *E. coli*'s topoisomerase ATP inhibitor).[22] Furthermore, the authors emphasized the importance of 1SHV receptor from *Klebsiella pneumoniae* (SHV-1 β-lactamase)[22] as an additional target for inhibition of G^- type bacteria.

Bioactive compounds labelled as 325-3 and 325-5, isolated from endophytic fungus *Phomopsis species* growing on conifer needles,[13] were used as a reference for a thorough literature search for secondary metabolites from other *Phomopsis species* strains, as well as structurally similar bioactive compounds.[23–32]

A set of molecules was selected that comprised phomoenamide and phomonitroester, secondary metabolites from endophyte *Phomopsis species* strain PSU-D15 with records on moderate *in vitro* antibacterial activity against *Mycobacterium tuberculosis*.[23,24] Five structurally similar compounds, hybrid peptide–polyketides named as curvularides A–E, obtained from the endophytic fungus *Curvularia geniculate* and isolated from the limbs of *Catunaregam tomentosa* were further selected because of their demonstrated antifungal activity against *Candida albicans*.[25] The set was complemented with compounds **6** and **7** isolated from endophytic fungus *Phomopsis species* from *Notobasis syriaca*, which also showed considerate antibacterial, anti-algal and antifungal activity.[26] Finally, phomol was recently promoted as a novel antibiotic isolated from *Phomopsis species* from the medicinal plant *Erythrina crista-galli*.[27]

EXPERIMENTAL

Molecular docking

Molecular docking is a practical *in silico* method employed in order to predict the orientation of a ligand in a receptor binding pocket.[33,34] Freely available software Autodock v4.2 (The Scripps Research Institute, La Jolla, CA, USA) was used to perform the docking studies, while analyses of the docking simulation was performed in AutoDockTools 1.5.6 (The Scripps Research Institute, La Jolla, CA, USA). Prior to docking simulation, the ligands and receptors were adequately prepared. The structures of the ligands were optimized to achieve the conformations with the minimum energy, while structures of the receptor were retrieved from Brookhaven protein data bank. Pre-calculation of a 3D grid of interaction energies was performed by AutoGrid based on a macromolecular target. Within this procedure, a cubic grid box and grid maps were created in order to represent the active region in which the native molecular structure is embedded.[35] A grid of 40 points in *x*-, *y*- and *z*-direction with grid spacing of 0.375 Å was built centred on a ligand. The maximum number of energy evaluations was 2.500.000. The Lamarckian genetic algorithm was used to identify the best conformers.[36,37] A maximum of 100 independent conformers for each compound were considered during the simulation. The docking exercise was executed between flexible ligands (tested molecular structures) and rigid protein receptors, allowing an evaluation of the free binding energy of the ligand and the macromolecule. Docked conformations with best *RMSD* (root mean square deviation) scoring function of all docked conformation were evaluated together with established key interactions.[38,39]

Preparation of receptors and ligand molecules

Crystal structures of G^+ and G^- bacterial type receptors were obtained from the Protein Data Bank (https://www.rcsb.org/).[20-22] A set of ligand molecules included compounds 325-3 and 325-5, curvularides A–E, compounds **6** and **7**, phomol, phomoenamide and phomonitroester (Fig. 1).[23-27] The antibiotic ampicillin served as the control ligand. All ligand molecules were set in their minimum energy conformations obtained by the MOPAC/AM1 method (job type: minimising the *RMS* gradient to 0.100; display: each iteration; AM1 theory; wave function: closed shell) in Chem 3D Ultra 7.0.0 (Surrey, UK).

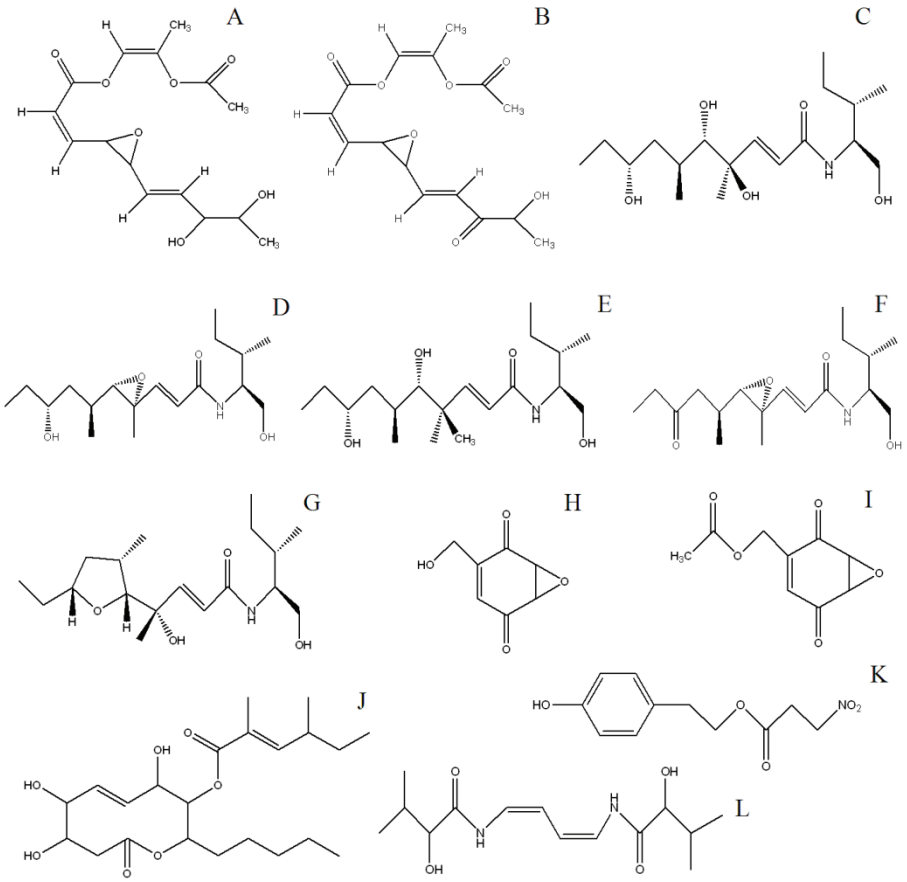

Fig. 1. Chemical structure of compound 325-3 (A), 325-5 (B), curvularide A (C), curvularide B (D), curvularide C (E), curvularide D (F), curvularide E (G), compound 6 (H), compound 7 (I), phomol (J), phomoenamide (K) and phomonitroester (L).

RESULTS AND DISCUSSION

As a result of the authors' previous research attempts, compounds 325-3 and 325-5 were isolated from endophytic fungus *Phomopsis* sp. and their activity against *Escherichia coli* and *Staphylococcus aureus* strains was experimentally evaluated. To understand the mechanism underlying the interaction between 325-3 or 325-5 and different type of receptors and to identify the receptor that triggers antibiotic response upon activation, a molecular docking study was further performed. Additionally, the pool of ligands was enriched with ten more endophytic biomolecules found through a literature surveillance. The most stable conformations proposed by docking study were selected based on the minimum binding energy contributing to more thermodynamically favoured pathway of the formation of the docked structure.

Each interaction was evaluated through certain parameters, *i.e.*, inhibition constant (K_i), *RMSD* value, free binding energy and potential presence of hydrogen bonds between the tested ligand and the receptor. In general, lower values of final and binding energies, lower values of inhibition constant, root mean square deviation (*RMSD*) with a threshold value of 2 Å and, in case of close interaction of the ligand and receptor, the potential for the formation of hydrogen bonds between them, may be considered as reliable indicators of potential binding of the tested ligand to a receptor.[40] The coordinates of the central grid point of the maps for all the tested ligands are provided in Table S-I of the Supplementary material to this paper.

The outcomes of the docking simulation for the 1F0K receptor with ligands (Table I and Table S-II of the Supplementary material) demonstrated that of the tested ligands, ampicillin exhibited the highest bonding potential by establishing 2 hydrogen bonds (Fig. 2), and having the lowest free binding energy of –6.47 kcal* mol^{-1}, an inhibition constant (K_i) of 18.08 μM and an *RMSD* value of 5.08. When comparing the affinity of the other ligands to ampicillin, 325-3, 325-5, phomoenamide and phomol stood out. Of the afore-mentioned compounds, lowest free binding energies were observed for compound 325-3 (–5.14 kcal mol^{-1}), phomoenamide (–4.97 kcal mol^{-1}) and 325-5 (–4.78 kcal mol^{-1}). Interestingly, the lowest K_i value, which implies high binding potential for the 1F0K receptor, was noted for phomol (K_i = 60.52 μM), followed by compounds 325-3 (K_i = = 172.04 μM) and phomoenamide (K_i = 227.74 μM). The lowest *RMSD* value was observed in case of phomol (*RMSD* = 3.26), followed compounds 325-3 and 325-5, which had the same *RMSD* value of 3.72 and phomoenamide with *RMSD* value of 4.46. Four hydrogen bonds were observed in case of compound 325-5 and curvularide A, though both compounds bind only to the GLN289 amino acid on the receptor, while additional hydrogen bonds were established with the other amino acids LEU265, SER192 and GLN193. This may imply that the 2 binding pockets of receptor 1F0K were in close vicinity. Compound 325-3, phomol and phomoenamide established 3 hydrogen bonds with the receptor. Since 325-5 and phomoenamide formed hydrogen bonds through THR266 amino acid and both compound 325-3 and 325-5 formed hydrogen bonds with amino acid GLN289 on the 1F0K receptor, it is suspected that compounds 325-3, 325-5 and phomoenamide bind to the same receptor pocket. On the other hand, phomol formed hydrogen bonds with amino acids GLY190 and GLN193, implying that it did not bond to the same place as the other compounds.

The analysis of docking results of ligands to 3G7B receptor showed, as expected, that among the investigated ligands, the lowest binding energy of –6.10 kcal mol^{-1} was observed for the ampicillin control (Table II and Table S-III of the Supplementary material). Of all tested ligands, compound 325-3 had the low-

* 1 kcal = 4184 J

TABLE I. Extracted crystal and experimental data for the molecular complexes with the 1F0K receptor

Tested ligand	Free binding energy, kcal mol^{-1}	Inhibition constant, μM	*RMSD*	Hydrogen bonds with receptor amino acids
Ampicillin	–6.47	18.08	5.08	THR266
325-3	–5.14	172.04	3.72	GLN289
325-5	–4.78	313.49	3.72	THR266 GLN289
Curvularide A	–4.76	324.52	6.17	LEU265, SER192, GLN193, GLN289
Curvularide B	–4.10	994.76	6.79	GLN289
Curvularide C	–4.70	361.64	4.74	VAL189
Curvularide D	–4.74	334.92	6.42	LEU265
Curvularide E	–4.73	343.80	4.90	GLU269
Phomoenamide	–4.97	227.74	4.46	THR266
Compound **6**	–3.88	1430	7.35	LEU265, THR266
Compound **7**	–4.56	454.39	5.83	ARG164, THR266
Phomol	–5.75	60.52	3.26	GLY190, GLN193
Phomonitroester	–4.81	296.63	5.89	GLU269, GLN289

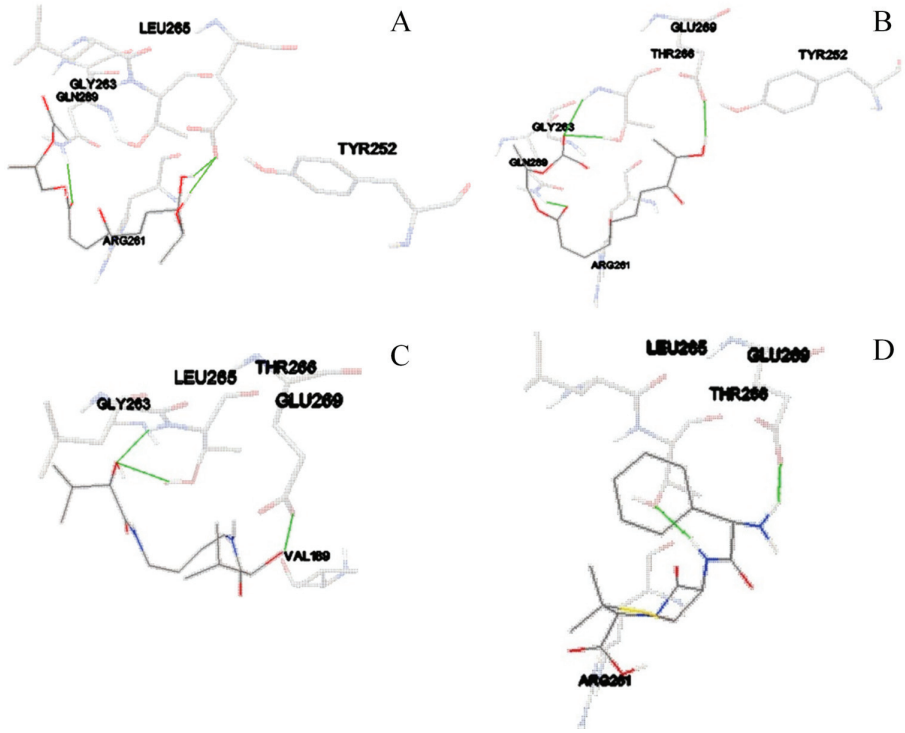

Fig. 2. Docking pose of ligands 325-3 (A), 325-5 (B), phomoenamide (C) and ampicillin (D) with binding pocket of 1F0K receptor. Only the portion of the receptor with interacting amino acid residues is displayed. Main hydrogenic bonds between ligand amino acid residues in receptor pocket are emphasized in green.

est binding energy of –5.27 kcal mol^{-1}. Moreover, a low binding energy with the 3G7B receptor was also observed for phomoenamide and the 325-5 ligand.

In addition, docking simulation revealed the establishment of 4 hydrogen bonds between the receptor 3G7B and both the ligand 325-3 and phomoenamide. Furthermore, 3 hydrogen bonds were observed between compound 325-3 and the 3G7B receptor. Similar binding spots on the 3G7B receptor (amino acids on positions ASP57, ASN54 and VAL131 of 3G7B receptor, Table S-III) were observed for both compounds 325-3 and 325-5 (Fig. 3), which may explain the antimicrobial activity against *S. aureus* experimentally observed in previous research. This also implied that compounds 325-3 and 325-5 interact with the same binding spot on the receptor, while phomoenamide or ampicillin bind to the other pockets of the 3G7B receptor. The lowest *RMSD* values were noted for phomoenamide (*RMSD* 3.27) and compound 325-5 (*RMSD* 3.92). Moreover, other tested ligands showed potential for interaction and binding with the 3G7B receptor. However, according to the free binding energy, the constant of inhibition and the *RMSD* value, compounds 325-3 (K_i = 135.56 μM, *RMSD* 5.04), 325-5 (K_i = 425.42 μM, *RMSD* 3.92) and phomoenamide *(*K_i = 397.98 μM, *RMSD* 3.27) showed the greatest potential.

TABLE II. Extracted crystal and experimental data for molecular complexes with the 3G7B receptor

Tested ligand	Free binding energy, kcal mol^{-1}	Inhibition constant, μM	*RMSD*	Hydrogen bonds with receptor amino acids
Ampicillin	–6.10	33.93	5.77	ASP53, GLU50
325-3	–5.27	135.56	5.04	ASP57, ASN54, VAL131
325-5	–4.60	425.42	3.92	ASP57VAL131, ASN54
CurvularideA	–2.58	12930	4.21	ASP53, GLU50, ASN54
CurvularideB	–4.36	632.03	3.98	GLU50, HIS46
CurvularideC	–3.21	4440	4.50	ASP57
CurvularideD	–4.51	495.24	4.19	ASP53, ASN54, VAL131
CurvularideE	–4.47	532.45	4.15	ASP52, ASN54
Phomoenamide	–4.64	397.98	3.27	ASN54, GLU50
Compound 6	–3.75	1790	6.72	VAL131, ASN54, GLU50
Compound 7	–4.20	838.08	4.80	ASN54, VAL131
Phomol	–3.74	1800	4.89	ASP53, ASN206
Phomonitroester	–3.88	1440	6.80	VAL130, GLU50, HIS46, VAL131

These results suggest that the highest affinity for the receptor 3G7B was observed for the ampicillin antibiotic control. Of the tested ligands, slightly lower affinity was noted for ligands 325-3, 325-5 and phomoenamide. Interestingly, binding places for ampicillin and phomoenamide included interactions in the receptor pocket in the vicinity of amino acid GLU50, while both compounds 325-3 and 325-5 included interactions with amino acids ASP57, ASP54 and

VAL131 on different receptor spot (Fig. 3). There was a difference in binding locations of the aforementioned compounds, which implied potential differences in mechanism of action of ampicillin and phomoenamide in comparison to 325-3 and 325-3.

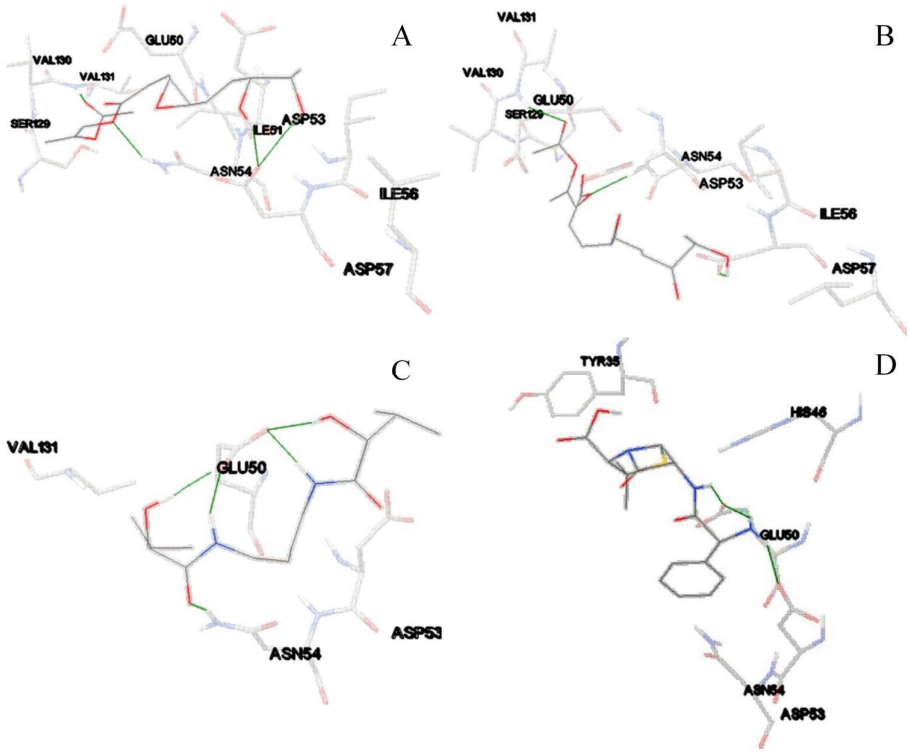

Fig. 3. Docking pose of ligands 325-3 (A), 325-5 (B), phomoenamide (C) and ampicillin (D) with 3G7B receptor binding pocket. Only the portion of the receptor with interacting amino acid residues is displayed. The main hydrogenic bonds between ligand amino acid residues in the receptor pocket are emphasized in green.

It is known that antibiotics can exhibit their effect through inhibition of *β*-lactamase. Therefore, docking simulations between SHV-1 *β*-lactamase as receptor and all the investigated ligands were performed and evaluated (Tables III and S-IV (Supplementary material)). Ampicillin exhibited the lowest free binding energy of –1.90 kcal mol^{-1} and an *RMSD* of 5.08. However, compounds **6** and phomonitroester had the lowest free binding energy (–2.85 and –2.67 kcal mol^{-1}, respectively), followed by compound **6** (–2.48 kcal mol^{-1}) and compound 325-5 (–1.79 kcal mol^{-1}). This might imply that they potentially have greater affinity for the receptor in comparison to the ampicillin control. For all compounds, higher values for the inhibition constants were observed, with the lowest value of

8.10 mM for compound **7**. Moreover, 4 hydrogen bonds were obtained in the case of compound **7** and phomonitroester, while 3 potential hydrogen bonds were formed between compound 325-5 and amino acids ARG202 and ARG205 on the 1SHV receptor.

TABLE III. Crystal and experimental data for molecular complexes with the 1SHV receptor

Tested ligand	Free binding energy, kcal mol^{-1}	Inhibition constant, μM	*RMSD*	Hydrogen bonds with receptor amino acids
Ampicillin	–1.90	40320	5.08	ARG205
325-3	0.33	N.A.	7.26	ARG205
325-5	–1.79	48870	5.65	ARG202, ARG205
CurvularideA	–0.41	497240	5.07	ARG202
CurvularideB	–0.92	210410	7.41	ARG202ARG205
CurvularideC	0.75	N.A.	4.74	None
CurvularideD	–1.57	70350	6.97	ARG202, ARG205
CurvularideE	–1.27	116590	5.05	ARG202, ARG205
Phomoenamide	–1.78	49500	6.51	GLU92
Compound **6**	–2.48	15230	7.48	ARG202, ARG205
Compound **7**	–2.85	8100	5.97	ARG202, ARG205
Phomol	–0.55	395820	5.28	ARG202, ARG205
Phomonitroester	–2.67	11060	7.46	ARG202, ARG205

Overall, a lower binding potential was observed in case of docking of all the tested ligands with 1SHV receptor in comparison to both the 1F0K and 3G7B receptors. Moreover, the highest affinity for the 1SHV receptor was observed for compound **7** followed by phomonitroester, the ampicillin control and compound 325-5. For all ligands, the binding pocket within the receptor seemed to be the same, since all hydrogen bonds were formed with amino acids on position ARG202 and ARG205 (Fig. 4).

In docking simulations with receptor 3VSL, all compounds showed a weak potential for interaction (Table S-V). None of the compounds had the potential to form hydrogen bonds, also the values of the free binding energy were high, implying a weak potential for any interaction, and hence the inhibition constant could not be calculated. A similar lack of interaction potential for all tested compounds with 4EMV, 1JIJ, 1KZN and 3K3P receptor was also evident (Tables S-VI–IX of the supplementary material). According to the obtained data, it seemed that none of the investigated compounds interacted with these receptors.

CONCLUSIONS

As an extension of practical *in vitro* experiments for antimicrobial evaluation, separation and characterization of the biomolecules of endophytic fungi, *in silico* molecular docking was proposed with the aim to introduce additional effort and to reliably recognize which active structures could serve as leading molecules for further *in silico* antibiotic drug discovery. Within this study, light was shed on

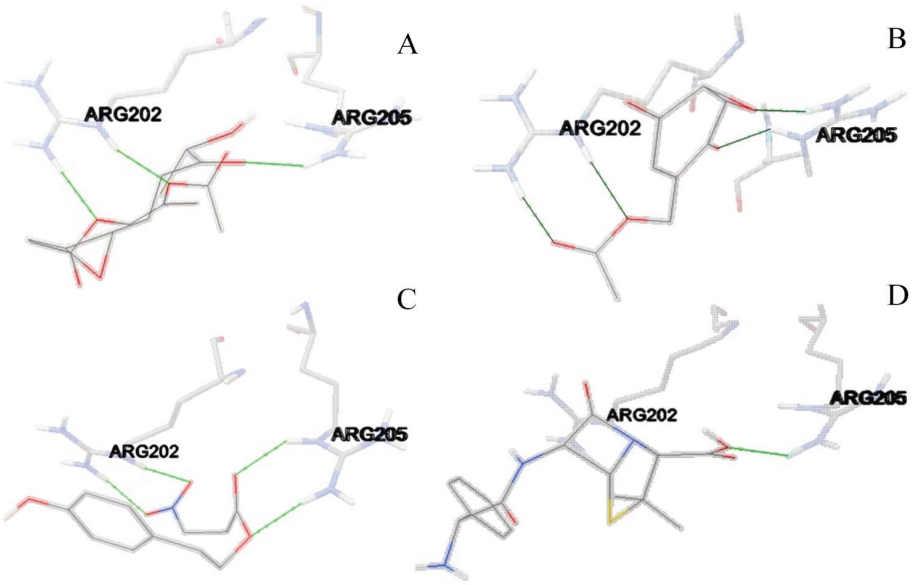

Fig. 4. Docking pose of ligands 325-5 (A), compound **7** (B), phomonitroester (C) and ampicillin (D) with the 1SHV receptor binding pocket. Only the portion of the receptor with interacting amino acid residues is displayed. The main hydrogenic bonds between the ligand amino acid residues in receptor pocket are emphasized in green.

the interactions between a series of twelve compounds and their potential targeted receptors. According to the overall criteria for docking evaluations, which included the value of the free binding energy, constant of inhibition, *RMSD* value and potential for establishment of hydrogen bonds with the receptor, it was concluded that the highest potential for docking interaction was observed in case of 3G7B, 1F0K and 1SHV receptors located in pathogens *Staphylococcus aureus*, *Escherichia Coli* and *Klebsiella pneumonia*. The results from this docking study suggest that structural similarities as well as some specific properties of compounds 325-3, 325-5, phomoenamide and phomol may hopefully be used as directions for the further development of their derivatives as novel antibiotics with potent, broad-spectrum activity. In addition, these findings may also indicate how to perform further optimization of biomolecule production by endophytic fungi and/or more effective processing of gathered biomaterial. Moreover, the investigated compounds might also interact with other targets involving different mechanisms of action.

SUPPLEMENTARY MATERIAL

Tables S-I–S-IX, containing coordinates of central grid point of maps for all tested ligands and overall docking results of twelve antimicrobial structures and control ligand against eight receptors, are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/index, or from the corresponding author on request.

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ИЗВОД

СТУДИЈА МОЛЕКУЛСКОГ ДОКИНГА СА БИОМОЛЕКУЛИМА ИЗОЛОВАНИМ ИЗ ЕНДОФИТНИХ ГЉИВА

ЈАНКО ИГЊАТОВИЋ[1], НЕВЕНА ЂАЈИЋ[1], ЈОВАНА КРМАР[1], АНА ПРОТИЋ[1], БОРУТ ШТРУКЕЉ[2] и БИЉАНА ОТАШЕВИЋ[1]

[1]Катедра за аналитику лекова, Универзитет у Београду – Фармацеутски факултет, Војводе Степе 450, 11221 Београд и [2]Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

У последње време, као одговор на повећање резистенције микроорганизама на познату терапију, све већа пажња се поклања истраживању једињења са антимикробном активношћу. Ендофитне гљиве су недавно представљене као поуздан и богат извор за развој нових лекова. До сада, истраживања су се углавном ограничавала на *in vitro* процену антимикробне активности и разоткривање хемијске структуре биомолекула изолованих из материјала гљива. Из тог разлога, ово истраживање је осмишљено као проширење претходно спроведених испитивања ендофита које расту на иглицама четинара путем *in silico* студије молекулског докинга. Главни циљ употребе *in silico* метода је био да се направи прилог разумевању механизама који стоје иза интеракције биомолекула изолованих из гљиве *Phomopsis species* са осам различитих типова рецептора који припадају патогеним бактеријама уобичајено мултирезистентних на лекове. Резултати су указали на важне интеракције са рецепторима 3G7B (*Staphylococcus aureus* гираза Б), 1F0K (структура *Escherichia Coli* трансферазе величине 1,9 Å) и 1SHV (SHV-1 β-лактамаза *Klebsiella pneumoniae*) указујући на тај начин на рецепторе путем којих се започиње антибиотски одговор након активације најпотентнијим једињењима, 325-3, 325-5, фомоенамидом и фомолом. Овим открићем се такође препоручује будући развој нових моћних антибиотика са широким спектром деловања базиран на структури изабраних молекула.

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