In silico antibiofilm potency of phenolic *N*-acyl hydrazones against selected bacterial strains

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Abstract: In the present work, fourteen phenolic hydrazone derivatives were evaluated for their *in silico* inhibitory activity against selected *P. aeruginosa* and S. *maltophilia* proteins involved in drug resistance and biofilm formation. Molecular docking analysis revealed the highest binding affinity of analogs **n** (-8.4 kcal/mol) and **h** (-7.3 kcal/mol) towards *P. aeruginosa* **7m1m** and **7m1l** proteins, respectively. In the case of S. *maltophilia*, analog **k** (-8.4 kcal/mol) expressed the highest binding affinity to **6qw7**, whereas for **6uaf,** the lowest binding energy was calculated for derivative **d** (-8.1 kcal/mol). The obtained *in silico* results highlight the potential inhibition potency of the selected hydrazone analogs against investigated proteins and represent a good basis for future *in vitro* antibiofilm investigations.

Keywords: phenolics, hydrazone, antibiofilm activity, molecular docking

1. Introduction

The inhibition of biofilm formation has been recognized as a novel target for developing efficient broad-spectrum antibiotics and overcoming bacterial drug resistance [1]. Biofilm bacterial communities express higher resistance to drugs for multiple reasons, such as reduced antimicrobial penetration, slower metabolic state, easier resistance gene exchange between cells, etc. [2]. Among diverse bacteria species, *P. aeruginosa* and *S. maltophilia* are notorious for their ability to form biofilms, enabling their persistence and infectiousness, especially in hospitals and clinical settings [3], [4]. To overcome these concerns, targeting biofilm-related proteins is identified as a route for discovering effective antimicrobial agents [1]. Within numerous chemical entities, hydrazone-type derivatives are renowned for their antimicrobial virtues [5], as well as for their broad enzyme inhibition ability [6]. Bearing this in mind, fourteen phenolic hydrazone derivatives were subjected to molecular docking analysis with biofilm-related *P. aeruginosa* caseinolytic serine proteases (ClpPs) [7], as well as with *S. maltophilia* β-lactamases L1 and L2 involved in the antibiotic resistance [8].

2. Results and Discussion

A series of differently substituted phenolic *N*-acyl hydrazone derivatives (Figure 1) were subjected to molecular docking analysis using AutodockVina. The crystal structures of the selected proteins were acquired from the RSC protein data bank: *P. aeruginosa* ClpP1 (PDB: **7m1m**), *P. aeruginosa* ClpP2 (PDB: **7m1l**), *S. maltophilia* L2 complexed with relebactam (PDB: **6qw7**), and *S. maltophilia* metallo-beta-lactamase L1 in the complex with hydrolyzed imipenem (PDB: **6uaf**). The CASTp tool was used for the prediction of protein active sites. The cuboid grid box was set to embrace the whole protein in all cases.

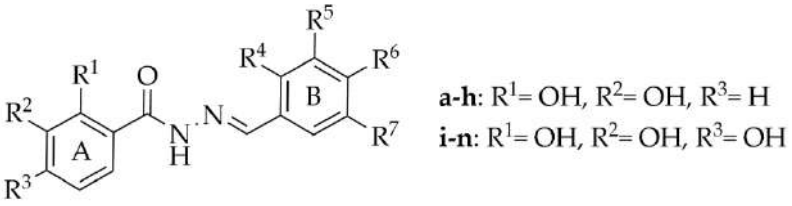


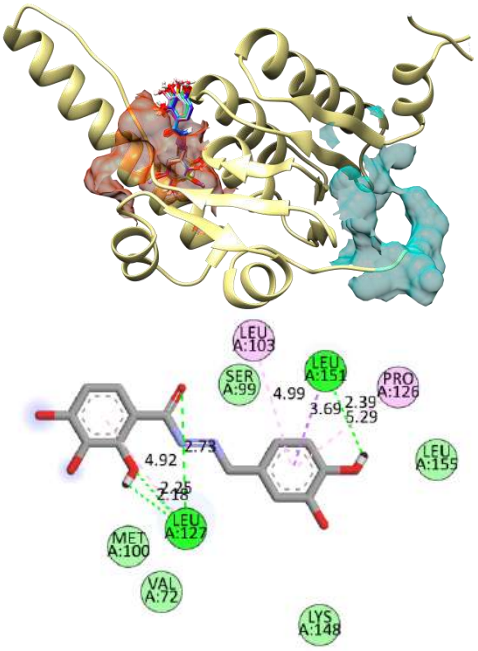
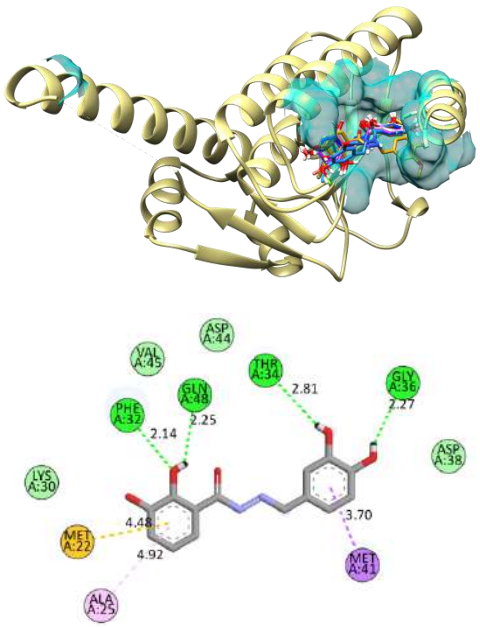
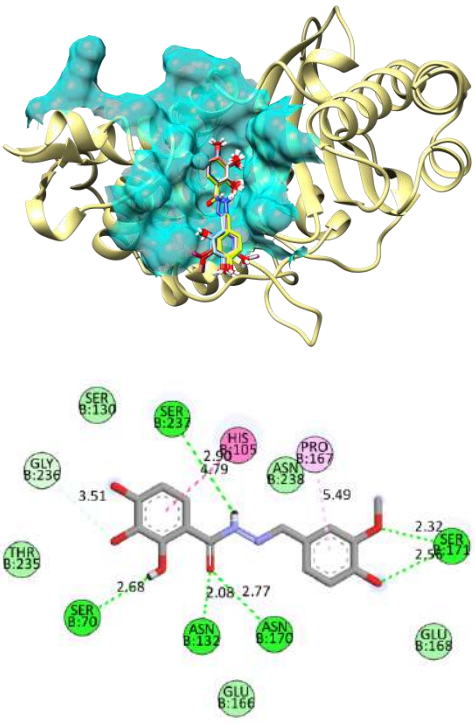
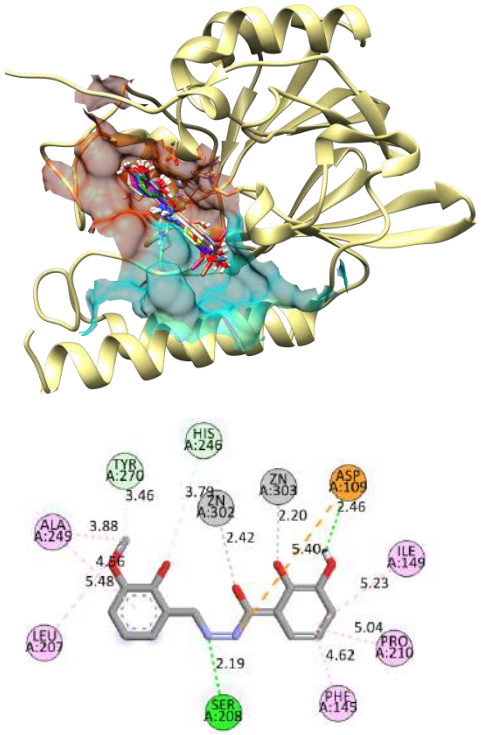
Figure 1. The general structure of the investigated compounds **a–n.**

The obtained binding affinity values exposed derivatives **n** (R^4, R^7=H; R^5, R^6= OH; -8.4 kcal/mol) and **h** (R^4, R^7=H; R^5, R^6= OH; -7.3 kcal/mol) as most prominent binders of *P. aeruginosa* **7m1m** and **7m1l**, respectively (Table 1). Excellent agreement with the CASTp was achieved in all cases since all ligands were docked within the predicted active sites (Table 2). In the case of beta-lactamases **6qw7** and **6uaf**, almost all investigated compounds expressed higher binding affinities in comparison to standard inhibitors (SI). Molecular docking exposed derivative **k** (R^4, R^7=H; R^5=OCH_3; R^6=OH) with the lowest binding energy to **6qw7** of -8.4 kcal/mol. Significantly lower binding energies were obtained for **a–n** with **6uaf** in comparison to SI imipenem, revealing analog **d** (R^4=OH; R^5=OCH_3; R^6, R^7=H) as the most prominent binder of metallo-beta-lactamase L1 with the binding energy of -8.1 kcal/mol. Potential bioactive conformations of compounds **a–n** and 2D diagrams for the highest affinity compounds (Table 2) provided insight into the binding modes and multiple established interactions with both A and B moieties (hydrogen bonds (green); Van der Waals interactions (light green); alkyl, π-alkyl, π-π, and π-sigma interactions (purple); attractive charges and π-sulfur interactions (orange); as well as metal-acceptor (gray)). Particularly, the presence of catechol, vanillin, *o*-vanillin, and syringaldehyde moiety on the B side of the molecule was identified as most favorable for interaction establishment in all cases.

Table 1. Calculated binding affinities (kcal/mol) of compounds **a–n** against selected proteins.

	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**m**	**n**	**SI**
7m1m	-8.2	-8.2	-8.2	-8.3	-8.0	-8.2	-8.2	-8.4	-8.1	-8.2	-8.0	-7.4	-8.1	-8.4	/
7m1l	-6.9	-6.9	-7.1	-7.0	-7.1	-6.0	-7.3	-7.3	-6.4	-6.4	-6.6	-5.8	-6.7	-6.9	/
6qw7	-7.8	-7.4	-7.9	-7.5	-8.3	-8.2	-7.8	-8.1	-8.0	-7.8	-8.4	-8.3	-7.9	-8.2	-7.7
6uaf	-7.6	-7.9	-7.3	-8.1	-7.4	-7.4	-7.9	-8.1	-7.4	-7.2	-6.7	-6.7	-7.1	-7.6	-5.2

Table 2. Selected *P. aeruginosa and S. maltophilia* proteins presented with potential bioactive conformations of compounds **a-n** and 2D interaction diagrams for the highest binding affinity compounds.

PDB: **7m1m**	PDB: **7m1l**
	
analog **n**	analog **h**
PDB: **6qw7**	PDB: **6uaf**
	
analog **k**	analog **d**

3. Conclusions

In this research, a series of phenolic *N*-acyl hydrazones were subjected to molecular docking analysis with *P. aeruginosa and S. maltophilia* biofilm-related and drug-resistance proteins. The obtained results revealed analogs **n** (-8.4 kcal/mol) and **h** (-7.3 kcal/mol) with the highest binding affinity towards *P. aeruginosa* caseinolytic serine proteases **7m1m** and **7m1l,** respectively**.** Molecular docking performed on *S. maltophilia* beta-lactamases exposed significantly lower values of binding energies for compounds **a–n** in comparison to standard inhibitors. Here, derivative **k** (-8.4 kcal/mol) was identified as the most effective binder of **6qw7**, whereas in the case of **6auf**, the lowest binding energy was calculated for derivative **d** (-8.1 kcal/mol). Insight into the binding modes exposed multiple favorable interactions responsible for the enhanced binding affinity towards selected proteins. The presence of catechol, vanillin, *o*-vanillin, and syringaldehyde moiety on the B ring was recognized as most beneficial for interaction establishment in all cases.

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