

Comprehensive bioinformatic analysis of the docking of thirty-eight benzoazepine derivatives with topoisomerase I

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Submitted: August 03, 2026

DOI: 10.14295/bjs.v6i4.969

Accepted: October 01, 2026

URL: <https://doi.org/10.14295/bjs.v6i4.696>

Published: October 03, 2026

Abstract

Alterations in the topoisomerase I concentration have been associated with cancer development. It is important to mention that several drugs have been used as topoisomerase I inhibitors to treat cancer; however, some of these drugs can produce some secondary effects. Besides, their interaction is not clear; for this reason, the aim of this study was to determine the docking of thirty-eight benzazepine derivatives with topoisomerase I using the 1ej9 protein as a theoretical tool in the DockingServer program. In addition, compounds such as amantrone, belotecan, camptothecin, diflomotecan, exatecan, lurtetecan, and topotecan were used as controls. The results suggest that benzazepine derivatives 3, 5, 6, 8, 19, 20, and 24 have a good affinity for the 1ej9 protein surface, involving some amino acid residues such as Ala351, Phe361, and Tyr426. Besides, the *K_i* for compounds 3, 5, 6, 8, 19, 20, and 24 was different compared with the controls; this data suggests that benzazepine derivatives 3, 5, 6, 8, 19, 20, and 24 could act as 1ej9 protein inhibitors. This phenomenon could translate into changes in the biological activity of topoisomerase I. However, it is important to mention that alternative experiments are needed to evaluate the activity of these compounds in a biological model.

Keywords: topoisomerase I, cancer, benzazepine derivatives, docking server.

Análise bioinformática abrangente do docking de trinta e oito derivados de benzazepina com a topoisomerase I

Resumo

Alterações na concentração de topoisomerase I têm sido associadas ao desenvolvimento de câncer. É importante mencionar que diversos fármacos têm sido utilizados como inibidores da topoisomerase I no tratamento do câncer; no entanto, alguns desses fármacos podem causar efeitos secundários. Além disso, a natureza de sua interação não está totalmente esclarecida; por esse motivo, o objetivo deste estudo foi determinar o *docking* de trinta e oito derivados de benzazepina com a topoisomerase I, utilizando a proteína 1ej9 como ferramenta teórica no programa DockingServer. Adicionalmente, compostos como amantrona, belotecano, camptotecina, diflomotecano, exatecano, lurtetecano e topotecano foram utilizados como controles. Os resultados sugerem que os derivados de benzazepina 3, 5, 6, 8, 19, 20 e 24 apresentam boa afinidade pela superfície da proteína 1ej9, envolvendo resíduos de aminoácidos como Ala351, Phe361 e Tyr426. Além disso, o valor de *K_i* para os compostos 3, 5, 6, 8, 19, 20 e 24 diferiu do observado para os controles; esses dados sugerem que tais derivados de benzazepina poderiam atuar como inibidores da proteína 1ej9. Esse fenômeno poderia resultar em alterações na atividade biológica da topoisomerase I. Contudo, ressalta-se a necessidade de experimentos adicionais para avaliar a atividade desses

compostos em um modelo biológico.

Palavras-chave: topoisomerase I, câncer, derivados de benzazepina, servidor de docking.

1. Introduction

Epidemiological data indicate that cancer has increased worldwide in recent years, reducing the quality of life of the population (Raza, 2025; Roshandel et al., 2020; Diao et al., 2025; Nguyen et al., 2025; Vemula et al., 2025). There are several risk factors involved in cancer development, such as alcohol consumption (Green et al., 2025; Larson et al., 2025; Morford et al., 2025), smoking (Du et al., 2025; Lima et al., 2025; Zhang et al., 2025), obesity (Ingram et al., 2025; Nguyen et al., 2025; Solsona-Villarrasa; Vousden, 2025), genetics (Jia et al., 2025; Larcher et al., 2025; Manirakiza et al., 2025), and others.

Additionally, several studies indicate that overexpression of certain topoisomerases has been associated with cancer development; for example, one study found a relationship between high DNA topoisomerase I concentrations and colon cancer compared with normal colonic mucosa (Giovannella et al., 1989). Another report showed that topoisomerase I overexpression was inversely correlated with overall survival in patients with non-small cell lung cancer using the immunohistochemistry method (Lu et al., 2016). Furthermore, in another study, the topoisomerase I levels were quantified in crude extracts of colorectal and prostate tumors using the Northern blotting method; the results showed a 5- to 35-fold increase in topoisomerase I levels compared to normal colonic mucosa.

In the case of prostate tumors, the increase was 2- to 10-fold higher than in benign hyperplastic prostatic tissue from the same patients (Husain et al., 1994). It is important to mention that some drugs have been used as polymerase I inhibitors to treat some cancers, such as rebeccamsyn (Dezhenkova et al., 2014), camptothecin (Pommier et al., 1999), irinotecan and topotecan (Evasuedo et al., 1997), belotecan (Kumar et al., 2026), lurtetecan (Bai et al., 2022), exatecan, and others. However, some of these drugs can produce treatment resistance and secondary effects, resulting in a decrease in patients' quality of life (Gomez et al., 2024; Nitiss et al., 1996; Seiter, 2005; Paulik et al., 2020; Pilati et al., 2012).

This phenomenon may be due to several factors, such as i) the different doses employed; ii) differences in the chemical structure of each drug; and iii) differences in the drugs' interactions with the surface of topoisomerase I. In this way, some studies have been conducted to determine the interactions of several compounds with polymerase I using some methods; for example, a study showed the possible docking of compound CID-5271805 (Ginkgetina) with the amino acid residues Arg₃₆₄, Arg₄₈₈, Thr₅₀₁, Lys₄₉₃, and Lys₅₃₂ involved in the 1t8l protein surface (a human DNA topoisomerase I in complex with the camptothecin) using the Discovery Studio Software (Shinu, 2024).

Other data showed that narciclasine may interact with the amino acid residues Tyr₂₁₁, Arg₄₃₄, Glu₂₀₈, Asp₃₄₄, Asn₃₄₅, and His₃₄₆ involved in the chemical structure of the 1EJ9 protein (crystal structure of human DNA topoisomerase I) using Qvina-W and PyMOL programs (Wang et al., 2023). Besides, a study showed that an artemisin derivative interacts with the amino acid residues Arg₃₆₄ and Tyr₄₂₆ involved in the 1t8l protein using the AutoDock software (Madeddu et al., 2022). All these data indicate that some compounds can interact with different amino acid residues involved in the chemical structure of topoisomerase I. However, there is little information regarding the docking of benzazepine derivatives to topoisomerase I. For this reason, this study aimed to determine the molecular docking of thirty-eight benzazepine derivatives with topoisomerase I, using the 1ej9 protein as a theoretical tool in the DockingServer program.

2. Materials and Methods

This study was conducted to determine the molecular docking of some benzazepine derivatives (Figures 1 and 2; Table 1) with topoisomerase I as follows:

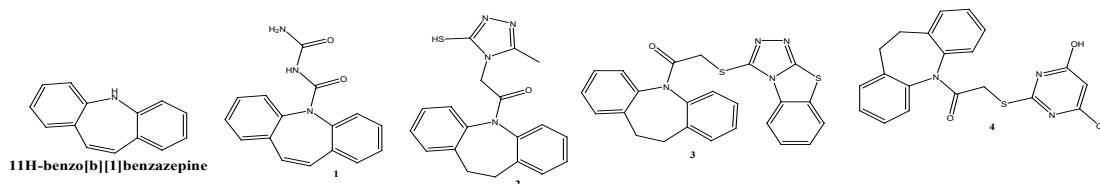


Figure 1. Chemical structure of benzazepine derivatives (1-4). Source: <https://pubchem.ncbi.nlm.nih.gov>

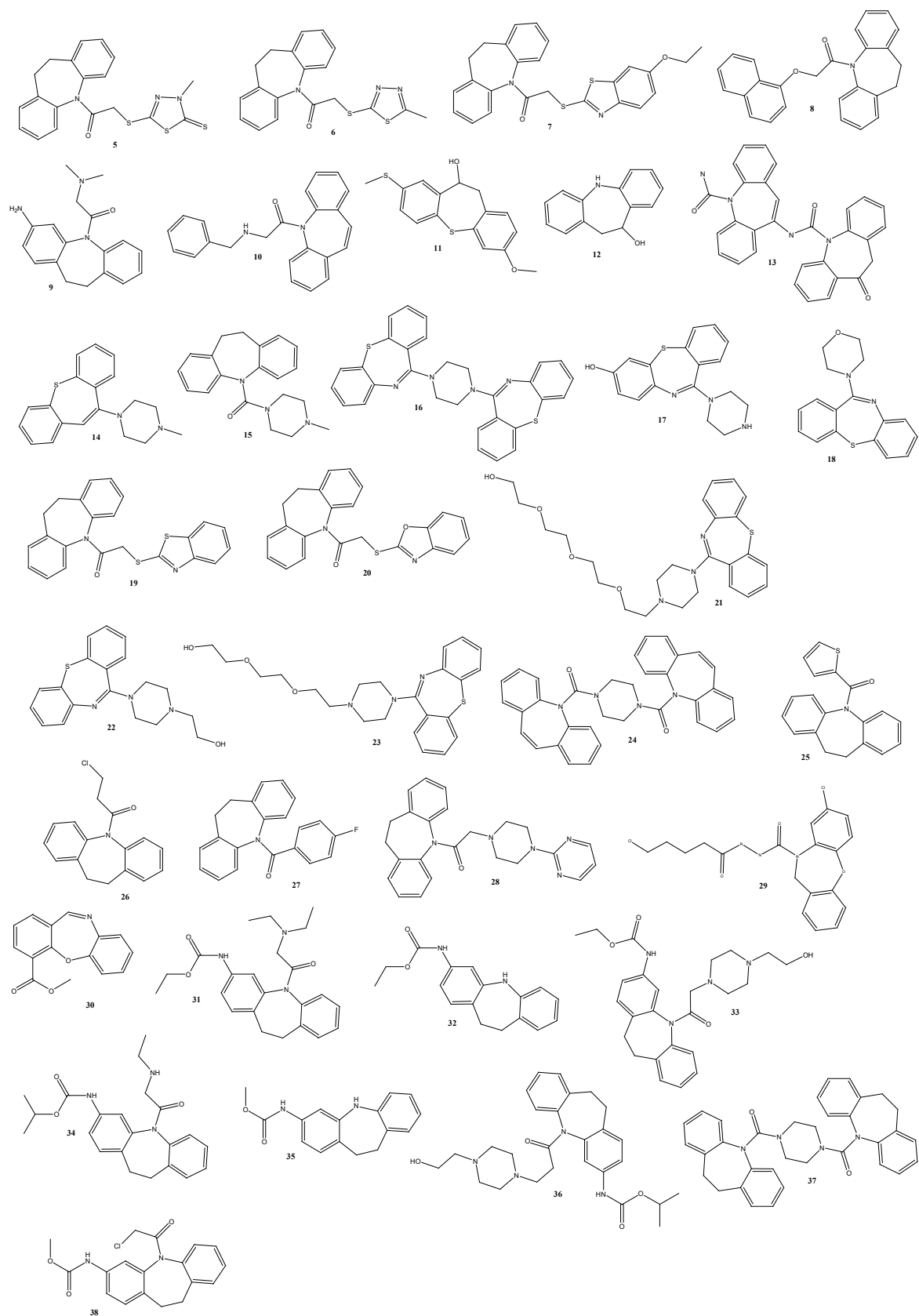


Figure 2. Chemical structure of benzodiazepine derivatives (5-38). Source: <https://pubchem.ncbi.nlm.nih.gov>

Table 1. IUPAC names of benzodiazepine derivatives.

Compound	Name
1	<i>N</i> -carbamoylbenzo[b][1]benzazepine-11-carboxamide
2	1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-(3-methyl-5-sulfanylidene-1 <i>H</i> -1,2,4-triazol-4-yl)ethanone
3	1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-([1,2,4] triazolo[3,4- <i>b</i>] [1,3]benzothiazol-1-ylsulfanyl)ethanone
4	2-[2-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-oxoethyl]sulfanyl-4-hydroxy-1 <i>H</i> -pyrimidin-6-one
5	1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-[(4-methyl-5-sulfanylidene-1,3,4-thiadiazol-2-yl)sulfanyl]ethanone
6	1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanyl]ethanone
7	1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-[(6-ethoxy-1,3-benzothiazol-2-yl)sulfanyl]ethanone
8	1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-naphthalen-1-yloxyethanone
9	1-(2-amino-5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-(dimethylamino)ethanone
10	1-benzo[b][1]benzazepin-11-yl-2-(benzylamino)ethanone
11	9-methoxy-3-methylsulfanyl-5,6-dihydrobenzo[b][1]benzothiepin-5-ol
12	6,11-dihydro-5 <i>H</i> -benzo[b][1]benzazepin-5-ol
13	5-[(5-oxo-6 <i>H</i> -benzo[b][1]benzazepine-11-carbonyl)amino]benzo[b][1]benzazepine-11-carboxamide
14	1-benzo[b][1]benzothiepin-5-yl-4-methylpiperazine
15	5,6-dihydrobenzo[b][1]benzazepin-11-yl-(4-methylpiperazin-1-yl)methanone
16	6-(4-benzo[b][1,4]benzothiazepin-6-yl)piperazin-1-ylbenzo[b][1,4]benzothiazepine
17	6-piperazin-1-ylbenzo[b][1,4]benzothiazepin-2-ol
18	4-benzo[b][1,4]benzothiazepin-6-ylmorpholin
19	2-(1,3-benzothiazol-2-ylsulfanyl)-1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)ethanone
20	2-(1,3-benzoxazol-2-ylsulfanyl)-1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)ethanone
21	2-[2-[2-(4-benzo[b][1,4]benzothiazepin-6-yl)piperazin-1-yl]ethoxy]ethoxy]ethoxy]ethanol
22	2-(4-benzo[b][1,4]benzothiazepin-6-yl)piperazin-1-yl)ethanol
23	2-[2-[2-(4-benzo[b][1,4]benzothiazepin-6-yl)piperazin-1-yl]ethoxy]ethoxy]ethanol
24	[4-(benzo[b][1]benzazepine-11-carbonyl)piperazin-1-yl]-benzo[b][1]benzazepin-11-ylmethanone
25	5,6-dihydrobenzo[b][1]benzazepin-11-yl(thiophen-2-yl)methanone
26	3-chloro-1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)propan-1-one
27	5,6-dihydrobenzo[b][1]benzazepin-11-yl-(4-fluorophenyl)methanone
28	1-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-2-(4-pyrimidin-2-yl)piperazin-1-yl)ethanone
29	3-chloro- <i>N'</i> -(5-chloropentanoyl)-6 <i>H</i> -benzo[b][1,4]benzoxazepine-5-carbohydrazide
30	methyl benzo[b][1,4]benzoxazepine-10-carboxylate
31	ethyl <i>N</i> -[11-[2-(diethylamino)acetyl]-5,6-dihydrobenzo[b][1]benzazepin-2-yl]carbamate
32	ethyl <i>N</i> -(6,11-dihydro-5 <i>H</i> -benzo[b][1]benzazepin-2-yl)carbamate

33	ethyl <i>N</i> -[11-[2-[4-(2-hydroxyethyl)piperazin-1-yl]acetyl]-5,6-dihydrobenzo[b][1] benzazepin-2-yl]carbamate chloride
34	propan-2-yl <i>N</i> -[11-[2-(ethylamino)acetyl]-5,6-dihydrobenzo[b][1]benzazepin-2-yl]carbamate
35	methyl <i>N</i> -(6,11-dihydro-5 <i>H</i> -benzo[b][1]benzazepin-2-yl)carbamate
36	propan-2-yl <i>N</i> -[11-[3-[4-(2-hydroxyethyl)piperazin-1-yl]propanoyl]-5,6-dihydrobenzo[b][1]benzazepin-2-yl]carbamate
37	[4-(5,6-dihydrobenzo[b][1]benzazepine-11-carbonyl)piperazin-1-yl]-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)methanone
38	methyl <i>N</i> -[11-(2-chloroacetyl)-5,6-dihydrobenzo[b][1]benzazepin-2-yl]carbamate

Source: <https://pubchem.ncbi.nlm.nih.gov>

2.1 Ligand-protein

The interaction of benzazepine derivatives (1-38) with human topoisomerase I was determined using the 1ej9 protein (crystal structure of the human topoisomerase I-DNA complex; <https://doi.org/10.2210/pdb1EJ9/pdb>) as a theoretical tool. It is noteworthy that the 1ej9 protein was previously characterized by X-ray diffraction at a resolution of 2.60 Å (Redinbo et al., 2000). Besides, its structure comprises several domains, such as the N-terminal domain, the central domain (or core), the linker domain, and the C-terminal domain (Alsner et al., 1992; Jain; Nagaraja, 2006; Stewart et al., 1996). It is important to mention that the docking of the benzazepine derivatives to the 1ej9 protein occurred at the A chains (Figure 3).

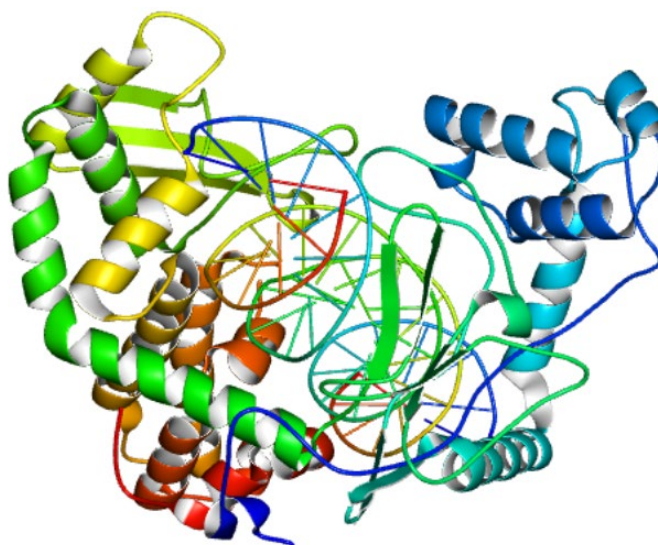


Figure 3. The scheme shows the 1ej9. Source: Protein Data Bank, <https://pdj.org/mine/summary/1ej9>

On the other hand, some topoisomerase I inhibitors such as ametantrone (Zagotto et al., 2014), belotecan (Kumar et al., 2026), camphotecin (Avemann et al., 1998), diflomotecan (Teicher, 2008), exatecan (Fuentes et al., 2026), lurtetecan (Bai et al., 2022), and topotecan (Mathijssen et al., 2002) were used as controls in the DockingServer program (<https://www.dockingserver.com/web>).

It is important to mention that DockingServer is a program that is used to determine the site, geometry, and energy of molecules interacting with proteins. Besides, DockingServer offers an easy way to compute ligand-protein interactions or virtual high-throughput screening calculations and is used to calculate other types of compounds (Lopez-Ramos et al., 2024). It is noteworthy that the MMFF94 force field (Halgren, 1996) was used for energy minimization of ligand molecules using DockingServer. Besides essential hydrogen atoms, Kollman united atom-type charges and solvation parameters were used, and the affinity (grid) maps were generated using the AutoGrid program (Morris et al., 1998).

In addition, AutoDock parameter set- and distance-dependent dielectric functions were used to determine the van der Waals force and the electrostatic factors. Besides, docking simulations were performed using the Lamarckian genetic algorithm and the Solis & Wets method (Solis; Wets, 1981). It is noteworthy that the initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. Finally, the population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.

2.2 Root mean square deviation (RMSD)

The RMSD for benzazepine derivatives (1-38) was determined using the LigRMSD software. This program is used to determine the RMSD between two poses of the same ligand molecule docked on the same protein structure without the assumption of known atomic ordering between the two files (Velázquez-Libera et al., 2020).

2.3 Pharmacokinetic parameters

Some pharmacokinetic factors for benzazepine derivatives (3, 5, 6, 19, 20 and 24) were determined using the SwissADME and ADMETlab programs. These programs are used to determine different pharmacokinetic parameters for several drugs (Fu et al., 2024).

2.4 Toxicology analysis

Theoretical toxicity produced by benzazepine derivatives (3, 5, 6, 19, 20 and 24) was determined using the Gussar software (Askeroova, 2023). It is worth noting that the Gussar program was developed to create models based on the association between the chemical structure of various drugs and their toxicological activity (LD₅₀) through different routes (oral, intravenous, intraperitoneal, and subcutaneous). Furthermore, the toxicity level was calculated based on SYMYX MDL toxicity data, which includes information on approximately 10,000 chemical structures with acute toxicity data in rats represented as LD₅₀ values (log¹² [mmol kg⁻¹]).

3. Results

3.1 Ligand-protein complex

Molecular docking of benzazepine derivatives and the controls with the 1ej9 protein surface was determined using the DockingServer program (Table 2).

Table 2. Amino acid residues involved in the coupling of Dibenzo[b,f]azepine and their derivatives (1-38) with the 1ej9 protein surface.

Compounds	Aminoacid residues
Ametantrone	Asn ₃₅₂ ; Ser ₄₂₃ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈
Belotecan	Arg ₃₄₆ ; His ₃₆₇ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃
Camphotecin	Arg ₄₈₈ ; Lys ₅₃₂ ; Asp ₅₃₃ ; Ile ₅₃₅ ; Lys ₅₈₇ ; Arg ₅₉₀ ; His ₆₃₂ ; Thr ₇₁₈ ; Phe ₇₂₃
Diflomotecan	Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Ala ₄₈₉ ; Asn ₄₉₁ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Ser ₅₃₄
Exatecan	Arg ₄₈₈ ; Ile ₅₃₅ ; His ₆₃₂ ; Thr ₇₁₈ ; Asn ₇₂₂
Lurtetecan	Asn ₃₅₂ ; Phe ₃₆₁ ; Arg ₃₆₄ ; Gln ₄₂₁ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Asp ₅₃₃
Topotecan	Arg ₃₆₄ ; Lys ₄₉₃ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃
5H-Dibenzo[b,f]azepine	Arg ₃₆₄ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Ser ₅₃₄
1	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Thr ₄₉₈ ; Ala ₄₉₉ ; Asp ₅₃₃ ; Ser ₅₃₄
2	Arg ₄₈₈ ; Ala ₄₈₉ ; Asn ₄₉₁ ; Lys ₅₃₂ ; Lys ₅₈₇ ; Arg ₅₉₀
3	Ala ₃₅₁ ; Asn ₃₅₂ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈
4	Ala ₃₅₁ ; Asn ₃₅₂ ; Met ₄₂₈ ; Asn ₄₃₀ ; Pro ₄₃₁ ; Lys ₄₃₆
5	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Lys ₄₂₅ ; Thr ₄₉₈ ; Asp ₅₃₃

6	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Asp ₅₃₃
7	Ala ₃₅₁ ; Asn ₃₅₂ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Lys ₄₃₆
8	Ala ₃₅₁ ; Asn ₃₅₂ ; Glu ₃₅₆ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈
9	Ala ₄₈₉ ; Asn ₄₉₁ ; Lys ₄₉₃ ; Thr ₅₀₁ ; Ser ₅₀₆ ; Lys ₅₃₂ ; Asp ₅₃₃
10	Phe ₃₆₁ ; Arg ₃₆₄ ; Gln ₄₂₁ ; Ser ₄₂₃ ; Lys ₄₉₃ ; Thr ₅₀₁ ; Asp ₅₃₃ ; Ser ₅₃₄
11	Ala ₃₅₁ ; Asn ₃₅₂ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈
12	Ala ₃₅₁ ; Asn ₃₅₂ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈
13	Arg ₄₈₈ ; Lys ₅₃₂ ; Lys ₅₈₇ ; Arg ₅₉₀ ; Thr ₅₉₁ ; Thr ₇₁₈ ; Asn ₇₂₂ ; Phe ₇₂₃
14	Arg ₃₆₄ ; His ₃₆₇ ; Thr ₄₉₈ ; Asp ₅₃₃ ; Ser ₅₃₄
15	Phe ₃₆₁ ; Arg ₃₆₄ ; Gln ₄₂₁ ; Ser ₄₂₃ ; Lys ₄₂₅ ; Thr ₄₉₈ ; Asp ₅₃₃
16	Asn ₃₅₂ ; Phe ₃₆₁ ; Arg ₃₆₄ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Asp ₅₃₃
17	Asn ₃₅₂ ; Glu ₃₅₆ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Lys ₄₃₆
18	Arg ₃₆₄ ; His ₃₆₇ ; Thr ₄₉₈ ; Lys ₅₃₂ ; Asp ₅₃₃ ; Ser ₅₃₄
19	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃
20	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃ ; Ser ₅₃₄
21	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Ser ₄₂₃ ; Thr ₄₉₈ ; Lys ₅₃₂
22	Asn ₃₅₂ ; Glu ₃₅₆ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈
23	Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Thr ₄₉₈ ; Lys ₅₃₂ ; Asp ₅₃₃
24	Phe ₃₆₁ ; Arg ₃₆₄ ; Lys ₃₇₄ ; Glu ₄₁₈ ; Gln ₄₂₁ ; Ser ₄₂₃ ; Lys ₄₂₅ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Asp ₅₃₃
25	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃ ; Ser ₅₃₄
26	Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃
27	Arg ₃₆₄ ; Gln ₄₂₁ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Asp ₅₃₃ ; Ser ₅₃₄
28	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Glu ₄₁₈ ; Gln ₄₂₁ ; Ser ₄₂₃ ; Lys ₄₂₅ ; Thr ₄₉₈ ; Asp ₅₃₃
29	Ala ₃₅₁ ; Asn ₃₅₂ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Pro ₄₃₁ ; Lys ₄₃₆
30	Arg ₄₈₈ ; Ala ₄₈₉ ; Asn ₄₉₁ ; Lys ₄₉₃ ; Thr ₅₀₁ ; Lys ₅₃₂
31	Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Ser ₄₂₃ ; Lys ₄₉₃ ; Thr ₄₉₈ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃
32	Arg ₄₈₈ ; Ala ₄₈₉ ; Lys ₅₃₂ ; Lys ₅₈₇ ; Arg ₅₉₀ ; Phe ₇₂₃
33	Ala ₃₅₁ ; Asn ₃₅₂ ; Ser ₄₂₃ ; Ile ₄₂₄ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Lys ₄₃₆
34	Asn ₃₅₂ ; Glu ₃₅₆ ; Ser ₄₂₃ ; Ile ₄₂₄ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈
35	Ala ₃₅₁ ; Asn ₃₅₂ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Pro ₄₃₁ ; Lys ₄₃₆
36	Ala ₃₅₁ ; Asn ₃₅₂ ; Ser ₄₂₃ ; Lys ₄₂₅ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Lys ₄₃₆
37	Asn ₃₅₂ ; Glu ₃₅₆ ; Tyr ₄₂₆ ; Met ₄₂₈ ; Pro ₄₃₁ ; Lys ₄₃₆ ; Asn ₇₂₂
38	Phe ₃₆₁ ; Arg ₃₆₄ ; His ₃₆₇ ; Gln ₄₂₁ ; Ser ₄₂₃ ; Lys ₄₉₃ ; Thr ₅₀₁ ; Lys ₅₃₂ ; Asp ₅₃₃

Source: Authors, 2026.

3.2 Root mean square deviation (RMSD)

The RMSD values and percent of molecules matched for benzazepine derivatives (1-38) were determined using the program (Table 3).

Table 3. RMSD calculations among identical and similar benzazepine derivatives.

Compound	Type of conformation	RMSD (Å)	Percent of molecule match
1	Flexible	1.64	71.43
2	Flexible	1.77	24.00

3	Flexible	17.08	19.35
4	Flexible	1.68	22.22
5	Flexible	2.78	23.08
6	Flexible	1.77	24.00
7	Flexible	15.25	19.35
8	Flexible	2.76	20.69
9	Flexible	2.15	27.27
10	Flexible	2.56	57.69
11	Flexible	0.66	30.00
12	Flexible	2.02	37.50
13	Flexible	2.05	40.54
14	Flexible	0.98	27.27
15	Flexible	1.61	25.0
16	Flexible	2.47	16.67
17	Flexible	1.93	27.27
18	Flexible	1.41	28.57
19	Flexible	2.41	21.43
20	Flexible	3.18	21.43
21	Flexible	2.21	18.18
22	Flexible	1.96	25.00
23	Flexible	2.81	20.00
24	Flexible	4.46	37.50
25	Flexible	1.40	25.00
26	Flexible	0.40	30.00
27	Flexible	2.13	25.00
28	Flexible	3.64	20.00
29	Flexible	3.72	22.22
30	Flexible	1.87	31.58
31	Flexible	2.47	20.69
32	Flexible	1.09	28.57
33	Flexible	1.22	17.65
34	Flexible	2.07	21.43
35	Flexible	0.83	30.00
36	Flexible	3.71	17.14
37	Flexible	3.30	15.00
38	Flexible	1.01	25.00

Source: <https://ligrmsd.appsbio.utalcal/cl>

3.3 Thermodynamic parameters

The energy levels for the docking of benzazepine derivatives (1-38) and the controls with the 1ej9 protein surface were determined using DockingServer software (Table 4). Besides, the inhibition constant (K_i) for benzazepine derivatives 3 and 24 was lower compared with the controls.

Table 4. Thermodynamic parameters involved in the interaction of benzazepine derivatives with the 1ej9 protein surface.

Compound	A	B	C	D	E	F
Ametantrone	-4.43	0.56800	-2.62	1.37	-1.25	413.69
Belotecan	-5.62	0.07626	-6.35	-0.20	-6.56	770.54
Camphotecin	-6.07	0.03541	-5.90	-0.17	-6.08	744.03
Diflomotecan	-6.35	0.02215	-6.22	-0.05	-6.27	673.61
Exatecan	-4.29	0.71153	-5.16	0.56	-4.61	714.48
Lurtetecan	-3.42	3.13	-3.89	-0.00	-3.89	728.07
Topotecan	-4.12	0.95143	-5.59	0.42	-5.17	739.03
5H-Dibenzo[b,f]azepine	-4.79	0.30827	-4.74	-0.05	-4.79	461.57
1	-5.33	0.12478	-5.36	0.03	-5.33	512.86
2	-5.42	0.10629	-5.82	0.08	-5.74	638.43
3	-7.07	0.00654	-5.69	0.00	-5.70	501.34
4	-4.06	1.05	-4.34	-0.02	-4.36	464.81
5	-5.98	0.04169	-5.45	0.05	-5.40	658.20
6	-6.68	0.01265	-6.44	-0.13	-6.57	660.25
7	-5.75	0.06136	-5.15	0.03	-5.12	521.30
8	-6.34	0.02257	-5.47	-0.03	-5.50	518.97
9	-4.97	0.22871	-5.57	-0.05	-5.62	594.61
10	-5.76	0.05948	-6.26	-0.23	-6.49	663.43
11	-4.33	0.67370	-5.07	-0.03	-5.10	474.40
12	-4.37	0.63043	-4.56	0.00	-4.66	420.64
13	-5.88	0.04856	-6.25	0.07	-6.18	733.13
14	-4.31	0.69625	-5.27	0.67	-4.61	583.31
15	-4.35	0.64479	-4.44	0.09	-4.35	625.98
16	-5.88	0.04866	-6.46	0.01	-6.45	811.01
17	-2.70	10.53	-4.27	0.94	-3.33	521.67
18	-3.98	1.21	-4.94	0.67	-4.28	510.58
19	-6.71	0.01209	-6.09	0.01	-6.09	677.41
20	-6.58	0.01515	-6.55	-0.20	-6.75	694.22
21	-2.26	22.17	-5.13	1.52	-3.61	723.34
22	-3.40	3.23	-4.56	0.80	-3.75	397.99
23	-2.75	9.57	-4.16	0.93	-3.23	609.23
24	-7.11	0.00618	-7.09	-0.01	-7.11	823.22
25	-5.48	0.09553	-5.75	-0.03	-5.68	539.23
26	-5.21	0.15137	-5.46	-0.06	-5.53	530.76
27	-5.07	0.19271	-5.36	-0.01	-5.37	550.47
28	-5.29	0.13240	-5.23	0.01	-5.22	669.80
29	-6.18	0.02941	-6.43	-0.01	-6.45	612.83
30	-4.74	0.33735	-5.25	-0.13	-5.38	537.66
31	-4.98	0.22276	-5.73	0.05	-5.69	659.64
32	-4.25	0.76190	-5.13	0.00	-5.12	733.40

33	-4.37	0.62365	-6.32	0.46	-5.86	610.37
34	-3.20	4.50	-4.38	0.47	-3.91	471.42
35	-4.62	0.41144	-5.31	0.01	-5.30	478.97
36	-3.53	2.57	-5.49	0.73	-4.76	618.49
37	-5.70	0.06639	-5.72	0.02	-5.70	717.35
38	-5.63	0.07431	-5.98	-0.06	-6.03	616.75

Note: A = Est: Free Energy of Binding (kcal mol⁻¹); B = Inhibition Constant (*K_i*, mM); C = vdW + Hbond + desolvation Energy (kcal mol⁻¹); D = Electrostatic Energy (kcal mol⁻¹); E = Total Intermolecular Energy (kcal mol⁻¹); F = Interact. Surface.

3.4 Bond and distance

The types of bonds and distances involved in the docking of the benzazepine derivatives (3, 5, 6, 8, 19, 20, and 24) with the 1ej9 protein were determined using the DockingServer program (Table 5 and Figures 3-6).

Table 5. Bond type and distance levels (Å) involved in the interaction of benzazepine derivatives with the 1ej9 protein surface.

Com.	Hydrogen	Polar	Hydrophobic	π - π
3		Asn ₃₅₂ (N ₃ , 3.20)	Ala ₃₅₁ (C ₂₂ , 3.86) Ala ₃₅₁ (C ₂₃ , 3.43) Tyr ₄₂₆ (C ₁₃ , 3.32) Met ₄₂₈ (C ₂₂ , 3.86) Met ₄₂₈ (C ₂₃ , 3.21) Met ₄₂₈ (C ₂₄ , 3.37) Met ₄₂₈ (C ₁₂ , 3.26) Met ₄₂₈ (C ₁₃ , 3.29) Met ₄₂₈ (C ₁₇ , 3.35)	Tyr ₄₂₆ (C ₁₇ , 3.54)
5			Phe ₃₆₁ (C ₉ , 3.28) Phe ₃₆₁ (C ₈ , 3.68) His ₃₆₇ (C ₈ , 3.23)	Phe ₃₆₁ (C ₁₉ , 3.26) Phe ₃₆₁ (C ₁₀ , 3.90)
6	His ₃₆₇ (N ₁ , 3.04)	Arg ₃₆₄ (N ₂ , 3.23) His ₃₆₇ (N ₂ , 3.70)		Phe ₃₆₁ (C ₁₄ , 3.80) Phe ₃₆₁ (C ₁₅ , 3.56)
8			Ala ₃₅₁ (C ₁₉ , 3.83) Ala ₃₅₁ (C ₂₀ , 3.23) Met ₄₂₈ (C ₁₅ , 3.45) Met ₄₂₈ (C ₁₉ , 3.14)	Tyr ₄₂₆ (C ₆ , 3.29) Tyr ₄₂₆ (C ₇ , 3.72) Tyr ₄₂₆ (C ₈ , 3.25)
19				Phe ₃₆₁ (C ₁₅ , 3.10) Phe ₃₆₁ (C ₂₁ , 3.33) Phe ₃₆₁ (C ₂₂ , 3.54) Phe ₃₆₁ (C ₁₆ , 3.61) His ₃₆₇ (C ₁₅ , 3.62) His ₃₆₇ (C ₁₆ , 3.55)
20	Arg ₃₆₄ (O ₂ , 3.17) Lys ₄₉₃ (O ₁ , 3.11) Thr ₅₀₁ (N ₂ , 3.13)	His ₃₆₇ (O ₂ , 3.53) Lys ₄₉₃ (N ₂ , 3.75)		Phe ₃₆₁ (C ₁₀ , 3.72) Phe ₃₆₁ (C ₁₁ , 3.42)

24	Gln ₄₂₁ (N ₂ , 3.30)	Gln ₄₂₁ (N ₁ , 3.78)	Phe ₃₆₁ (C ₄ , 3.88)	Phe ₃₆₁ (C ₁₄ , 3.30)
		Lys ₄₂₅ (N ₃ , 3.65)		Phe ₃₆₁ (C ₁₅ , 3.63)
				Phe ₃₆₁ (C ₁₃ , 3.78)

Source: Authors, 2026.

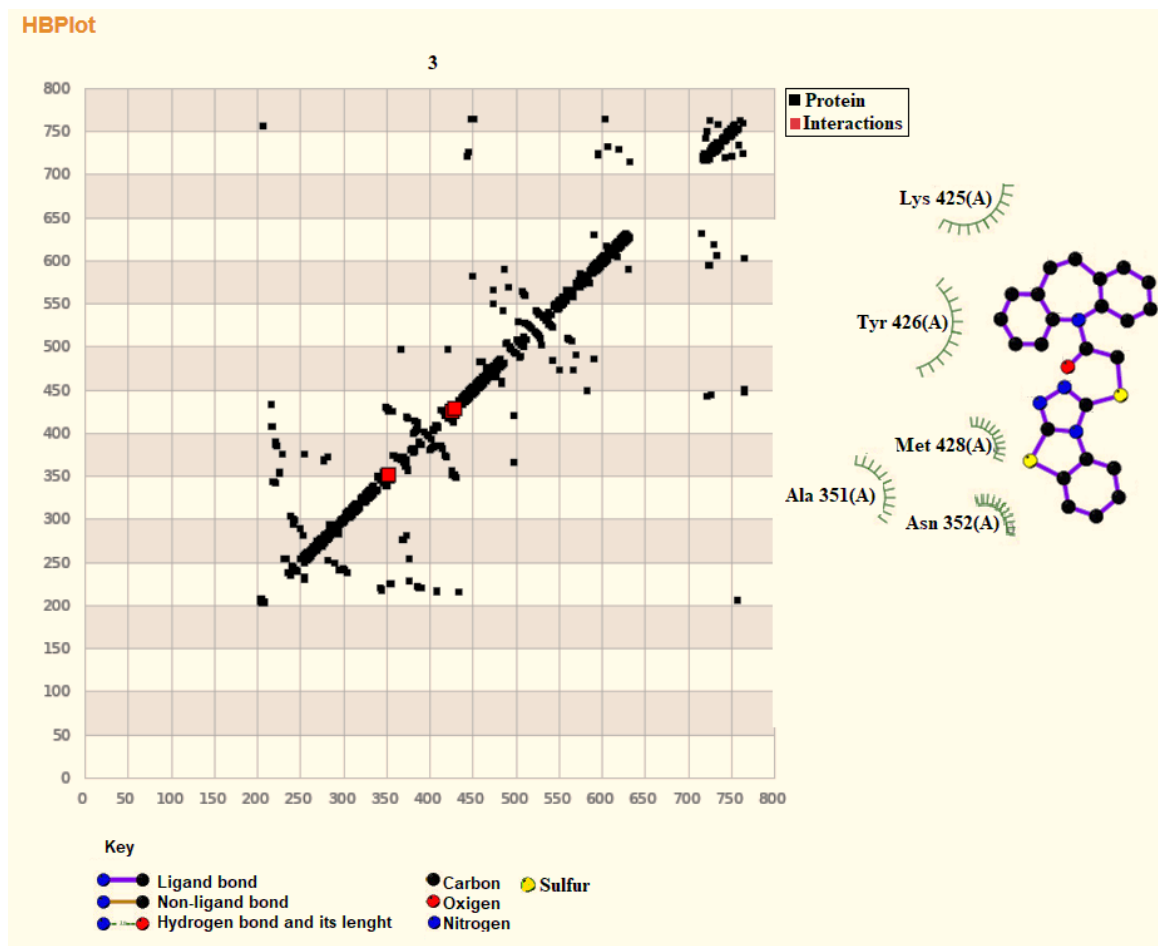


Figure 3. Docking of compound 3 with the 1ej9 protein surface. Visualized with the DockingServer program.
Source: Authors, 2026.

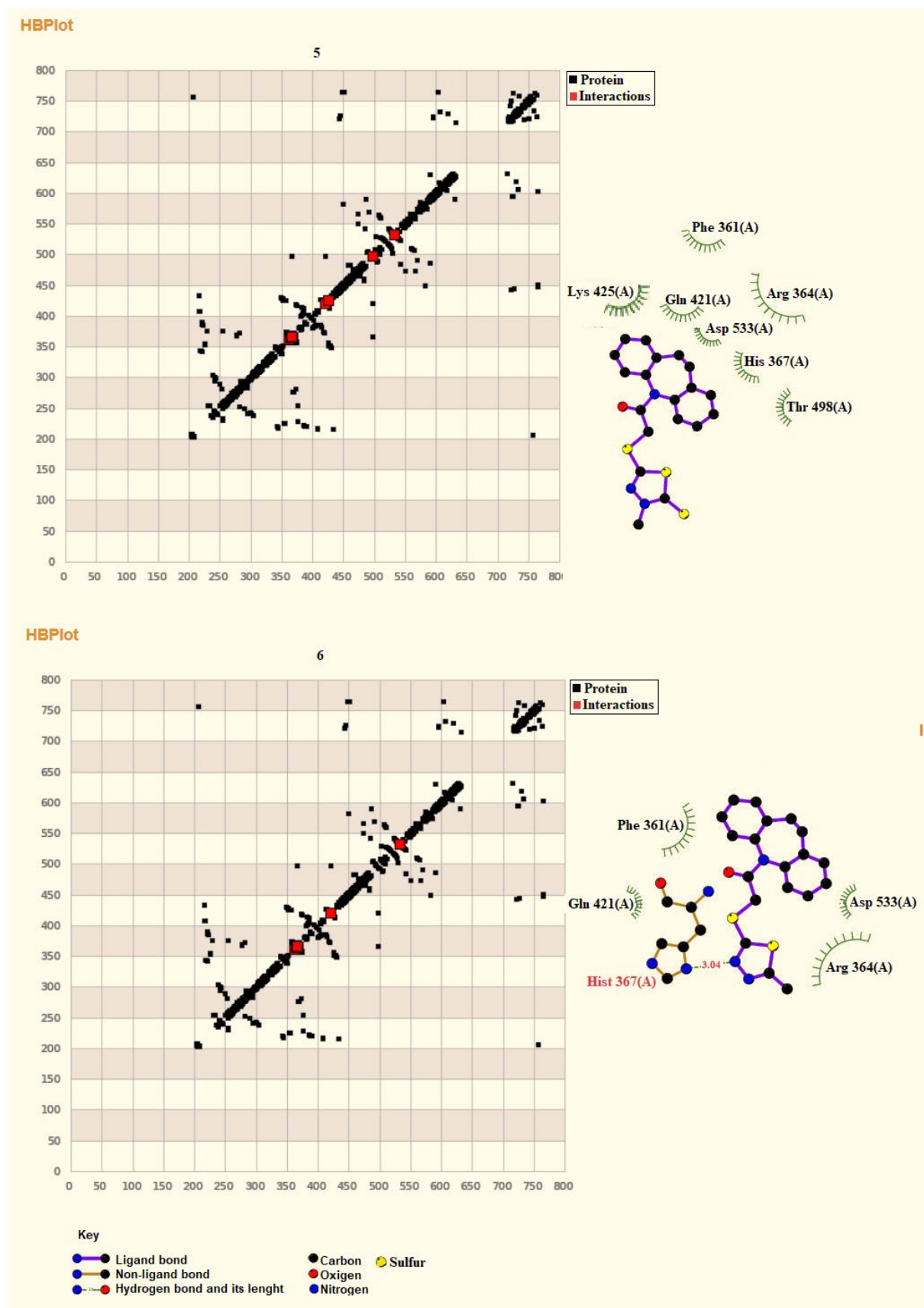


Figure 4. Interaction of compounds 5 and 6 with the 1ej9 protein surface. Visualized with the DockingServer program. Source: Authors, 2026.

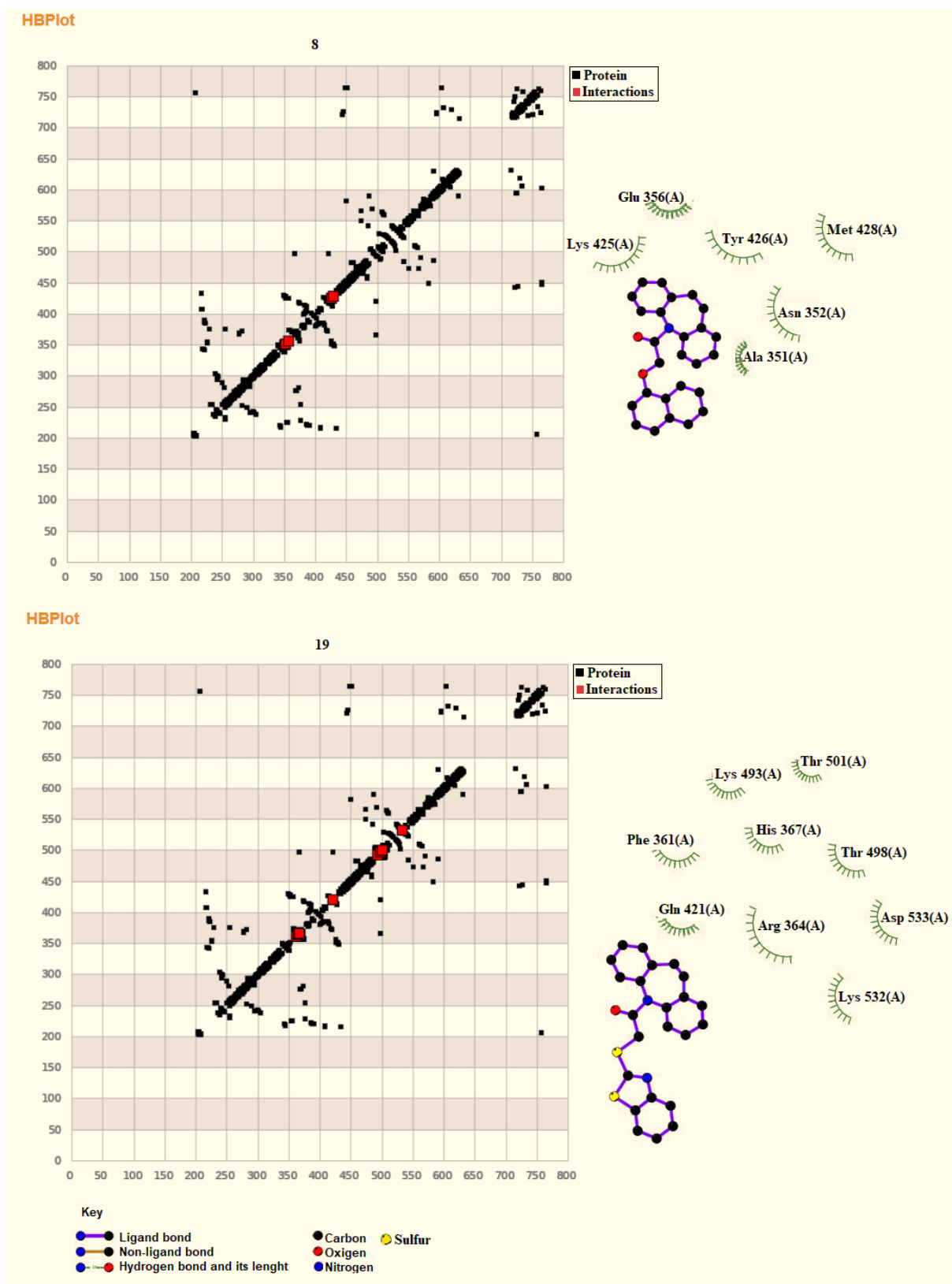


Figure 5. Docking of compounds 8 and 19 with the 1ej9 protein surface. Visualized with the DockingServer program. Source: Authors, 2026.

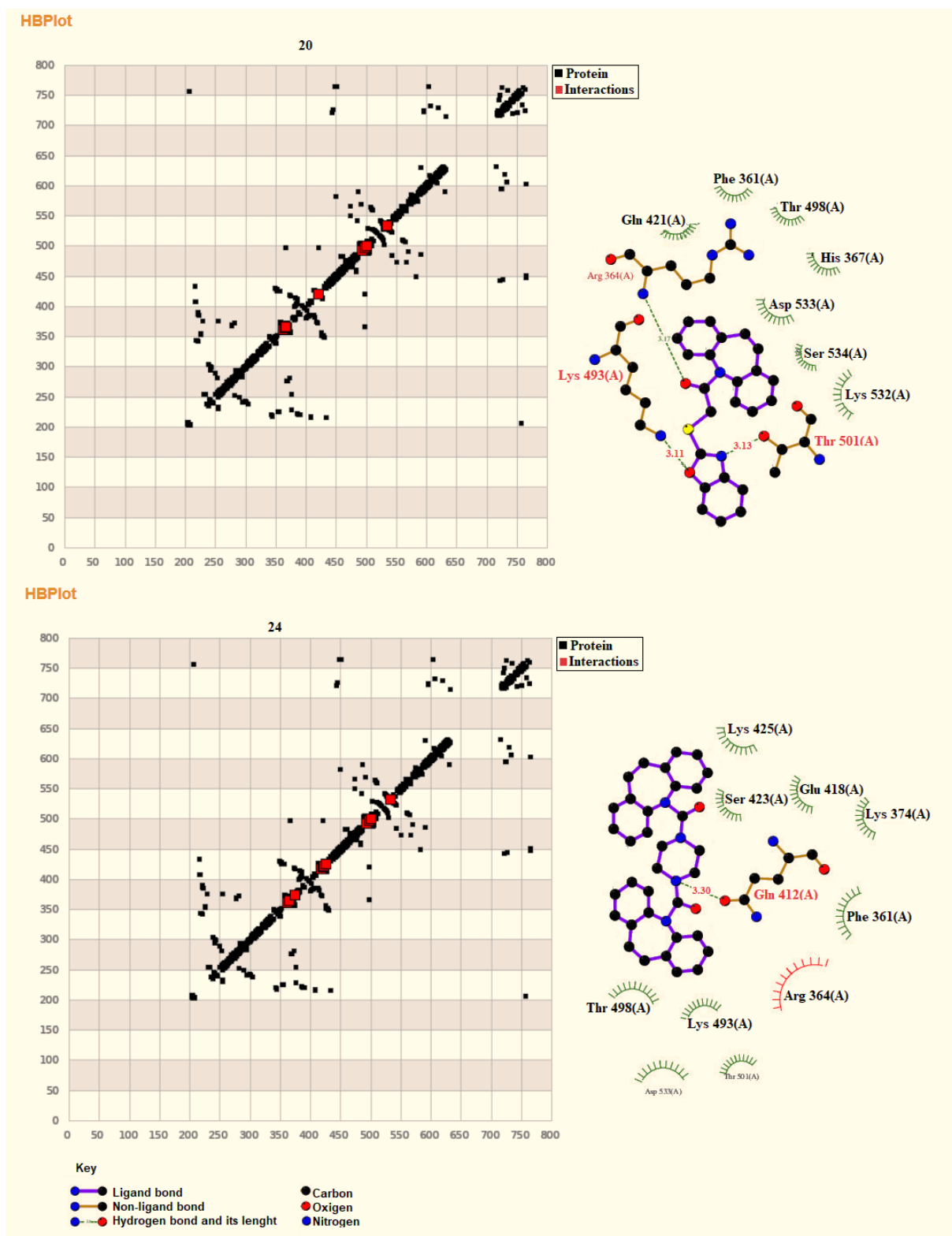
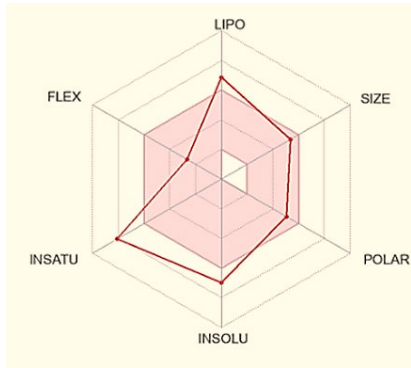
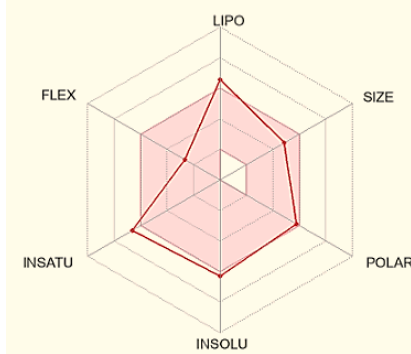
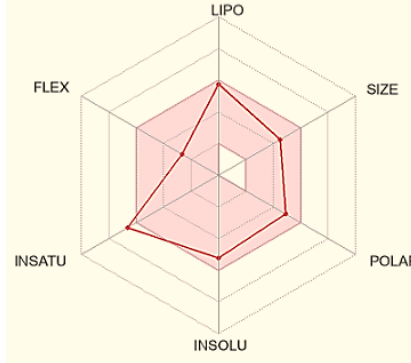
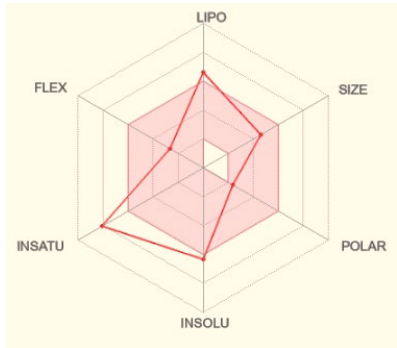


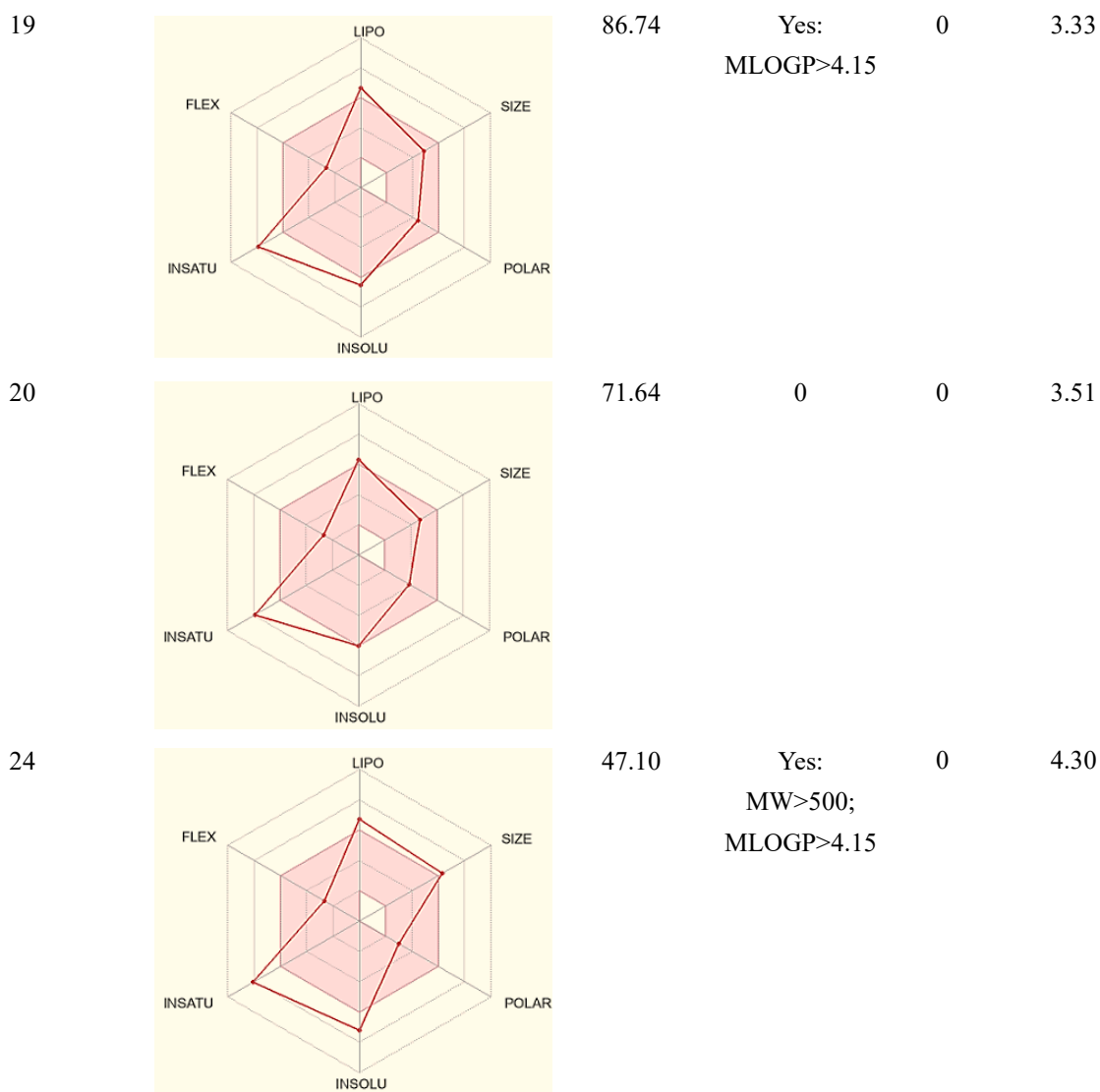
Figure 6. Interaction of compounds 20 and 24 with the 1ej9 protein surface. Visualized with the DockingServer program. Source: Authors, 2026.

3.5 Pharmacokinetic parameters

The pharmacokinetic values for benzazepine derivatives 3, 5, 6, 8, 19, 20 and 24 were determined using SwissADME and ADMETlab programs (Tables 6 and 7).

Table 6. Physicochemical parameters for benzazepine derivatives 3, 5, 6, 8, 19, 20 and 24.

Compound	Area*	TPSA ($\text{\AA}^2$)	Lipinski (violations)	Pains (alerts)	Synthetic accessibility
3		104.04	Yes; 1 violation: MLOGP>4.15	0	3.71
5		123.76	0	0	3.43
6		99.63	0	0	3.28
8		29.54	0	0	3.06



Note: *The colored zone is the suitable physicochemical space for oral bioavailability: LIPO (Lipophilicity): $-0.7 < \text{XLOGP3} < 5.0$; SIZE: $150 \text{ g mol}^{-1} < \text{MW} < 500 \text{ g mol}^{-1}$; POLAR (Polarity): $20 \text{ \AA}^2 < \text{TPSA} < 130 \text{ \AA}^2$; INSOLU (Insolubility): $-6 < \log S (\text{ESOL}) < 0$; INSATU (Insaturation): $0.25 < \text{Fraction Csp}^3 < 1$; FLEX (Flexibility): $0 < \text{Num. rotatable bonds} < 9$. ®Probability of F > 10% in rat implemented from Martin et al. (2005).

Table 7. Pharmacokinetic parameters for benzazepine derivatives 3, 5, 6, 8, 19, 20 and 24.

Parameter	3	5	6	8	19	20	24
Caco-2 Permeability	-4.94	-5.162	-4.680	-4.792	-4.663	-4.720	-4.642
Pgp inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pgp substrate	No	No	No	No	No	No	No
Human intestinal Absorption	No	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	Yes	No	Yes	Yes	Yes	Yes
CYP1A2 substrate	Yes	No	No	Yes	Yes	Yes	Yes
CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C19 substrate	Yes	No	Yes	Yes	Yes	No	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C9 substrate	Yes	No	No	Yes	No	No	Yes

CYP2D6 inhibitor	No	No	No	No	No	No	No
CYP2D6 substrate	Yes	No	No	No	No	No	Yes
CYP3A4 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes	Yes	No
CYP2B6 inhibitor	No	Yes	No	Yes	No	No	No
CYP2B6 substrate	Yes	No	No	No	No	No	Yes
CYP2C8 inhibitor	Yes	Yes	Yes	No	Yes	Yes	Yes
Human Liver Microsomal Stability	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Plasma protein binding (%)	97.9	98.0	95.7	98.4	98.4	98.3	97.6
Volume distribution (L kg ⁻¹)	0.166	0.151	-0.003	0.89	0.06	0.009	0.085
Blood-Brain Barrier	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CL _{plasma} (mL min kg ⁻¹)	3.15	2.272	2.193	7.267	2.997	2.713	4.633
T _{1/2} (L Kg ⁻¹)	0.484	0.727	0.682	0.338	0.447	0.519	0.414

Source: Authors, 2026.

3.6 Toxicity analysis

The Gussar program was used to calculate the toxicity values (Table 8) for benzazepine derivatives (3, 5, 6, 8, 19, 20, and 24). The results showed variations in dose concentration across various administration routes.

Table 8. Analysis of toxicity produced by benzazepine derivatives (3, 5, 6, 8, 19, 20, and 24) using the Gussar program.

Compound	IP LD ₅₀ (mg kg ⁻¹)	IV LD ₅₀ (mg kg ⁻¹)	Oral LD ₅₀ (mg kg ⁻¹)	SC LD ₅₀ (mg kg ⁻¹)
3	558.70	118.80	375.50	728.60
5	347.70	95.14	555.40	528.80
6	333.80	84.82	677.30	683.10
8	919.30	99.71	1228.00	1773.00
19	506.80	118.70	2344.00	1075.00
20	579.30	83.54	924.40	769.40
24	424.90	45.68	1990.00	1138.00

Note: IP - Intraperitoneal route of administration; IV - Intravenous route of administration; Oral - Oral route of administration; SC - Subcutaneous route of administration. Source: Authors, 2026.

4. Discussion

Several studies indicate that overexpression of topoisomerase I has been associated with several types of cancers (Fry et al., 1991; Mazzoleni et al., 2025; McLeod et al., 1994; Sandri et al., 1996). It is noteworthy that several drugs have been developed as topoisomerase I inhibitors to treat cancer cell growth (Anjaneyulu et al., 2026; Martin-Encinas et al., 2025; Sahu et al., 2025; Turini et al., 2025).

However, some of these drugs can cause toxicity and resistance (Boos; Stopper, 2000; Nagao et al., 1999; Pilati et al., 2012; Seiter, 2005; Tan et al., 1989). Therefore, it is necessary to develop a series of compounds with biological activity against topoisomerase I to treat cancer cell growth. In this way, this study aimed to determine the possible docking of some benzazepine derivatives with topoisomerase I using several strategies as follows:

4.1 Ligand-protein complex

For several years, some theoretical methods have been used as tools for the development of anticancer drugs (Alaviet; Sharma, 2023; Solanki et al., 2023; Uppathi et al., 2025). For example, a study showed the synthesis of the compound cyclohexyl(2-phenylquinazolin-4-yl)amine, which exhibited biological activity against some cancer cell strains, and its molecular docking with topoisomerase I using a theoretical model (Le et al., 2011).

Another study displayed the docking of 3-(5-((methoxymethoxy)methyl)benzo[d][1,3]dioxol-6-yl)isoquinolin-1(2*H*)-one with topoisomerase I using the 1sc7 protein [a human DNA-topoisomerase I in complex with the indenoisoquinoline] as a theoretical tool in Surflex-Dock in the Sybyl version 8.1.1 program (Van et al., 2012). Finally, a study displays the molecular docking of compound 2-((5-(2-(4-Ethoxyphenyl)-1*H*-benzo[d]imidazol-6-yl)-1,3,4-oxadiazol-2-yl)thio-1-(4-(pyridin-2-yl)-piperazin-1-yl)-ethane-1-one with human topoisomerase I using the Glide 7.1 software (Acar Çevik et al., 2020).

These data indicate that several methods have been used to determine the interaction of different compounds with topoisomerase I; however, there is little data on the docking of benzazepine derivatives with this biomolecule. For this reason, in this study, the molecular docking of some benzazepine derivatives with topoisomerase I was determined using the 1ej9 protein as a theoretical tool in the DockingServer program. It is important to mention that some studies indicate that topoisomerase I has a catalytic center that involves some amino acid residues such as Tyr⁷²³, Arg⁵⁹⁰, Arg⁴⁸⁸, His⁶³², and Lys⁵³², and a catalytic water molecule stands out (Redinbo et al., 2000; Wang, 1996).

On the other hand, the compounds ametantrone, belotecan, camptothecin, diflotecan, exatecan, lurtotecan, topotecan, and 5*H*-dibenzo[b,f]azepine were used as controls. The results showed differences in the number of amino acid residues involved in the docking of benzoazepine derivatives with the 1ej9 protein compared with the controls (Table 3). For example, camptothecin may interact in the catalytic center with Arg⁵⁹⁰ and His⁶³²; exatecan with His⁶³²; 5*H*-Dibenzo[b,f]azepine and compounds 2, 9, 13, 1-20, 23, 25, 26, 30, and 31 with Lys⁵³². This phenomenon may be due to i) different chemical characteristics of each compound; ii) the different types of conformations adopted by each molecule. For this reason, the RMSD was determined as follows:

4.2 RMSD

Several studies have been used to determine the different conformational changes in the ligand-protein complex formation (Celej et al., 2003; Nicklaus et al., 1995; Sedov; Zuev, 2025; Sim et al., 2025). In this way, the ligand-protein complex formation may produce different conformational rearrangements, from local movements of the side chains involved in the ligand's chemical structure to changes in the protein's conformation. It is noteworthy that different conformations adopted by identical or similar chemical compounds have been determined using some programs, such as pyRMSD (Gil; Guallar, 2013), DockRMSD (Zhang, 2020), Dock6 (Sangande et al., 2020), LibDock (Rao et al., 2007), and LigRMSD (Valdés-Albuernes et al., 2025; Velázquez-Libera et al., 2020).

It is worth noting that the LigRMSD program is based on two methods: the first involves strict correspondence, which considers identical atom and bond types, and the second involves a flexible correspondence, which allows for the comparison of atom or bond pairs of different types. For this reason, in this study the LigRMSD program was used to determine the different RMSD values for the benzazepine derivatives. The results displayed differences in the percentage of molecules matching and RMSD values; these data suggest that the different chemical structures of each compound could condition the type of conformation adopted in the formation of the ligand-protein complex.

4.3 Thermodynamic parameters

The interaction of several drugs with some biomolecules to form the protein-ligand complex involves different factors, including binding free energy, total intermolecular energy, and van der Waals (vdW) interactions, among others (Limongelli, 2020; Nguyen et al., 2025; Perozzo et al., 2004; Woo; Roux, 2005). For example, a study showed the docking of a benzazepine derivative ((1*S*,2*S*)-2-methyl-3-(4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-benzazepine-1,7-diol) with the protein 3qel (crystal structure of the N-terminal domains of the GluN1 and GluN2B subunits of the NMDA receptor in complex with ifenprodil) yields a binding energy of -61.12 kcal mol⁻¹ using the MM/GBSA method (Tewes et al., 2015).

Another report indicates that the docking of a benzazepine derivative (9,10-dimethoxy-1-phenyl-6,7-dihydro-5*H*-pyrrolo[2,1-*a*][2] benzazepine-2-carbaldehyde) with the 3qx3 protein (human beta topoisomerase II together with

DNA and etoposide) can generate a binding energy of -8.1 kcal/mol (Nevskaya et al., 2026). All these data suggest that binding energy may depend on the chemical characteristics of each compound; for this reason, in this investigation, the binding free energy and other thermodynamic parameters involved in the docking of some benzazepine derivatives with the 1ej9 protein were determined using the DockingServer software. Besides, ametantrone, belotecan, camphotecin, diflomotecan, exatecan, lutetecan, topotecan, and 5H-dibenzoazepine were used as controls. The results showed that the binding free energy was lower for compounds 3 (-7.07 kcal mol⁻¹) and 24 (-7.11 kcal mol⁻¹) compared with compounds 1, 2, 4-23, 25-38, and the controls. These results suggest that differences in binding free energy values could influence the affinity of each compound for the 1ej9 protein surface.

4.4 Inhibition constant (*K_i*)

For several years, there has been a growing interest in the pharmaceutical field in characterizing the affinity that some drugs have for different biomolecules. For this purpose, several methods have been used to measure the strength and specificity of protein-ligand interactions. For example, a study conducted molecular docking of camptothecin-lysinate hydrochloride with the 1t8i protein (human DNA topoisomerase I in complex with the toxic agent camptothecin and forming a covalent complex with a 22-base-pair DNA duplex) using a theoretical model. The results showed a binding energy of -24.066 kcal/mol and an affinity (*K_i*) of 0.9602 (Arthur et al., 2018).

In addition, molecular docking studies were conducted on other compounds, such as pyrostrubin and doxorubicin, with the 1t8i protein using AutoDock v. 4.2 software. The results showed binding energies of -7.78 kcal mol⁻¹ and a *K_i* of 1.98 for pyrostrubin; finally, the binding energy value for doxorubicin was -9.65 kcal mol⁻¹, with a *K_i* of 0.083 (Jadaun et al., 2017). All these data indicate that the affinity of various drugs for topoisomerase I could depend on the differences in the chemical structure of each compound.

Analyzing these data in this study, the *K_i* values of the benzazepine derivatives were determined using the DockingServer program in order to compare them with the *K_i* values obtained for the controls. The results showed that *K_i* for benzazepine derivatives 3 and 24 was lower compared with compounds 1, 2, 4-23, 25-38 and the controls. Other data indicate that *K_i* for benzazepine analogs 6 and 19 was lower in comparison with compounds 1, 2, 4-18, 20-23, 25-38 and the controls. Besides, the *K_i* for compound 5 was lower compared with ametantrone, elotecan, lurtetecan, exatecan, and 5H-dibenzoazepine. Finally, the *K_i* for compound 8 was lower in comparison with camptothecin, ametantrone, belotecan, lurtetecan, exatecan, topotecan, and 5H-dibenzoazepine.

4.5 Bond type

Some studies indicate that the type of bond can influence ligand-protein formation (Bitencourt-Ferreira et al., 2019; Brylinski, 2018; Ferreira; Shapira, 2017; Patil et al., 2010). For this reason, in this study, the type of bond and the distance involved in the docking between benzazepine derivatives (3, 5, 6, 8, 19, 20 and 24) and the 1ej9 protein surface were determined using the DockingServer program. The results showed that the amino acid residue His₃₆₇ can interact with compounds 6 and 20 through polar bonds. Furthermore, Ala₃₅₁ can couple to benzoazepine derivatives 3 and 8 via hydrophobic bonding. Other data indicate that Phe₃₆₁ might be specific for compounds 5 and 24. In addition, compounds 3 and 8 can interact with the amino acid residue Tyr₄₂₆ via a π - π bond. Finally, 5, 6, 19, 20, and 24 may interact with Phe₃₆₁ through a π - π bond.

4.6 Physicochemical and pharmacokinetic parameters

For several years, several compounds have been developed to treat different types of cancer; however, their biological activity depends on several pharmacokinetic and pharmacodynamic factors. It is noteworthy that there are currently some theoretical methods available to predict the pharmacokinetic activity of different compounds, such as PKSolver (Zhan et al., 2010), PharmCalc (Borsi et al., 1988), PHARM (Gomeni, 1984), PKCALC (Shumaker, 1986), SwissADME (Yusuf, 2025), ADMETab (Cheohen et al., 2024), and others.

In this study, some physicochemical and pharmacokinetic parameters were determined using the SwissADME program; the results display the appropriate physicochemical area for the oral bioavailability of the benzodiazepine derivatives 3, 5, 6, 8, 19, 20, and 24. The results indicate that compounds 5, 6, 8, and 20 may have favorable oral bioavailability because they meet the criteria of Lipinski's rule of five [an empirical rule used to predict the biological activity of a compound in relation to other established drugs] (Lohit et al., 2024; Walters, 2012). Other data indicate that topological polar surface area (TPSA) values were less than 130 Å² for compounds 3, 5, 6, 8, 19,

20, and 24. It is noteworthy that TPSA has been used as a molecular descriptor to determine some biological properties in the transport of drugs, such as intestinal absorption and blood-brain barrier penetration (Daina; Zoete, 2016; Shityakov et al., 2013). The results suggest that this physicochemical property of benzodiazepine derivatives could influence intestinal absorption and their passage through the blood-brain barrier.

On the other hand, other pharmacokinetic parameters were determined using the ADMETab 3.0 program; the results showed that compounds 3, 5, 6, 8, 19, 20, and 24 could act as P-glycoprotein (P-gp) inhibitors. Other results indicate that compounds 3, 5, 8, 19, 20, and 24 could inhibit the biological activity of CYP1A2. Furthermore, the benzodiazepine derivatives 3, 5, 6, 8, 19, 20, and 24 may act as CYP2C19, CYP2C9, and CYP3B4 inhibitors. Additionally, other data suggest that compounds 5 and 8 could interact with CYP2B6. Finally, other data suggest that compounds 3, 5, 6, 19, 20, and 24 may act as CYP2C8 inhibitors. All these data suggest that benzodiazepine derivatives could be metabolized differently, which may be of interest for future research.

4.6 Toxicity analysis

In the literature, there are several methods to predict the toxicity degree of different drugs, such as Tox II (Klopman and Rosenkranz, 1995), VirtualToxLab (Vedani et al., 2012), ToxCast (Dix et al., 2007), Gussar (Askerova et al., 2023), and others. In this research, the toxicity degree produced by benzodiazepine derivatives 3, 5, 6, 8, 19, 20, and 24 was determined using the Gussar program. The data indicate that compound 6 requires a lower dose administered intraperitoneally compared with 3, 5, 8, 19, 20, and 24. Other data suggest that benzodiazepine derivative 24 requires a lower dose through the intravenous route to produce toxicity compared to compounds 3, 5, 6, 8, 19, and 20. Additionally, other results showed that compound 3 requires a lower dose administered orally to produce toxicity. Finally, compound 5 could produce some degree of toxicity through the subcutaneous route of administration. All these data suggest that the degree of toxicity could depend on different chemical characteristics of each compound and the route of administration.

5. Conclusions

This study conducted the docking of thirty-eight benzodiazepine derivatives with topoisomerase I, using the 1ej9 protein as a theoretical tool in the DockingServer program. The results suggest that benzodiazepine derivatives 3, 5, 6, 8, 19, 20, and 24 have a good affinity for the 1ej9 protein surface, involving some amino acid residues such as Ala₃₅₁, Phe₃₆₁, and Tyr₄₂₆. Besides, the Ki values for compounds 3, 5, 6, 8, 19, 20, and 24 were different compared with the controls. All this data suggest that benzodiazepine derivatives 3, 5, 6, 8, 19, 20, and 24 could act as 1ej9 protein inhibitors. This phenomenon could translate into changes in the biological activity of topoisomerase I. However, it is important to mention that alternative experiments are needed to evaluate the activity of these compounds in a biological model.

6. Authors' Contributions

Bonilla-Zavaleta: data curation, funding acquisition, investigation, methodology, visualization, writing—original draft, writing—review & editing. *Rosas-Nexticapa Marcela*: data curation, formal analysis, funding acquisition, investigation, methodology, visualization, writing—original draft, writing—review & editing. *Álvarez-Ramírez Magdalena*: formal analysis, funding acquisition, investigation, methodology, visualization., methodology, visualization, writing—original draft, writing—review & editing. *Ramírez-Higuera Abril*: formal analysis, funding acquisition, investigation, visualization, writing—review & editing. *Rascon-Diaz Martha Paola*: formal analysis, funding acquisition, visualization. *Bonilla-Zavaleta Enrique*: funding acquisition, visualization, writing—review & editing.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. Artificial Intelligence Statement: Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that generative AI and AI-assisted tools were used exclusively for English and Portuguese grammar and language editing during the preparation of this manuscript. No AI tools were used to generate scientific content, analyze data, interpret results, or draw conclusions. All revisions suggested by AI were carefully reviewed, verified, and approved by the authors, who assume full responsibility for the accuracy, integrity, and originality of the manuscript. AI tools are not authors and do not assume any responsibility for the content.

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Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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