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IN SILICO STUDY OF THE MECHANISM OF ANTICANCER ACTIVITY OF (*E*)-2-((5-(3-(2-FLUOROPHENYL)ACRYLOYL)-4-METHYLTHIAZOL-2-YL)AMINO)ISOINDOLINE-1,3-DIONE (LES-6489)

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INFORMATION

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thiazoles;
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anticancer activity;
molecular docking;
molecular dynamics simulations.

ABSTRACT

The aim of the work. To predict the mechanisms of anticancer activity using modern web tools for the compound Les-6489.
Materials and Methods. Molecular docking of EGFR and HER2 tyrosine kinases was performed for compound Les-6489. To evaluate the stability of complexes with Les-6489, molecular dynamics (MD) simulations were performed using GROMACS 13, which is accessed through the SiBioLead server.
Results and Discussion. As a result of in silico studies, a mechanism of antitumor activity was predicted for the studied compound Les-6489, which is implemented by inhibiting EGFR and HER2 tyrosine kinases.
Conclusions. The obtained results may become a platform for further structural optimization of the identified thiazole-isoindole hybrid molecule in the development of modern anticancer agents.

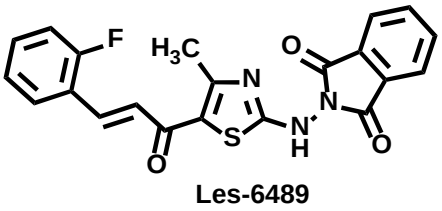
Introduction. Nitrogen- and sulfur-containing heterocycles, particularly thiazoles, have been extensively developed in medicinal and pharmaceutical chemistry due to their diverse biological activities and clinical applications [1]. Thiazole-based derivatives are a well-recognized class of biologically active compounds, serving as the key core for various drugs with antimicrobial, antiviral, antioxidant, anticancer, and anti-inflammatory properties [1]. Pharmacophore hybrid approach is an effective tool for the synthesis of multi targeted agents [2]. It is well known that isoindole fragment, therapeutically used in cancer diseases, and it possesses a wide spectrum of another activities, especially antimicrobial, and anti-inflammatory [3–5]. As a result, the purpose of our work was study of the *in silico* mechanism of biological activity of (*E*)-2-((5-(3-(2-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino) isoindoline-1,3-dione (Les-6489), which possess a high level of anticancer activity within the DTP NCI program [6].

To investigate the potential mode of action of (*E*)-2-((5-(3-(2-fluorophenyl)acryloyl)-4-methylthiazol-2-yl) amino)isoindoline-1,3-dione (Les-6489, Fig. 1), we employed the SwissTargetPrediction web server to predict its target enzyme classes [7]. The results demonstrated significant affinities for the kinase families (40 %) Additionally, the structure of Les-6489 is compatible with the general pharmacophore model of EGFR inhibitors [8].

Compound Les-6489 exhibits the characteristic pharmacophoric features of EGFR tyrosine kinase inhibitors. This class of anticancer drugs typically comprises four key components: (a) a terminal hydrophobic head that fits into a hydrophobic region, (b) a flat heteroaromatic ring that occupies the adenine binding pocket, (c) an imino linker, and (d) a hydrophobic

tail that interacts with another hydrophobic region of the EGFR tyrosine kinase enzymes (Fig. 2). To estimate the affinity of studied derivative for EGFR and HER2, we employed common *in silico* approaches, including molecular docking and molecular dynamics simulations. HER2 receptors were also included because EGFR and HER2 can form heterodimers [9] and some inhibitors that conform to the typical pharmacophore model of EGFR also exhibit affinities for HER2 receptors [10–12].

Materials and Methods. To investigate the hypothesis that antitumor activity is mediated through the inhibition of EGFR and HER2 tyrosine kinases, docking studies were conducted. The target enzymes selected for this study were EGFR (PDB ID: 3W32) [13] and HER2 (PDB ID: 3PP0) [14]. Due to the absence of certain amino acids in the selected PDB files, which could significantly influence docking and molecular dynamics results, the structures of EGFR and HER2 proteins were reconstructed using the Swiss-Model web server [15]. This reconstruction was based on their sequences and existing structures available on the PDB server. AutoDock Vina 1.2.5 was selected for its capability to accurately reproduce the positions of co-crystallized ligands within the selected enzymes with excellent RMSD values [16]. The energy-minimized 3D structure of Les-6489 was generated using Avogadro with the MMFF94 force field developed by Merck Research Laboratories (20,000 cycles) [17]. Protein structures were prepared for simulation using AutoDock Tools 1.5.6, which involved removing water molecules, adding



Les-6489

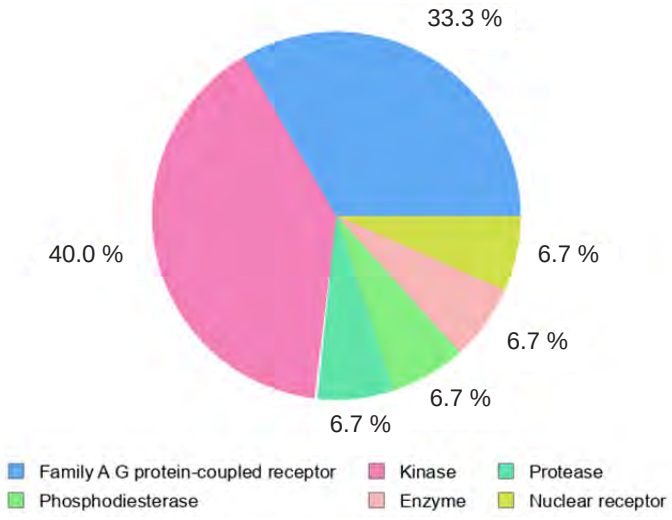


Fig. 1. The predicted target enzymes classes with probable affinity with Les-6489.

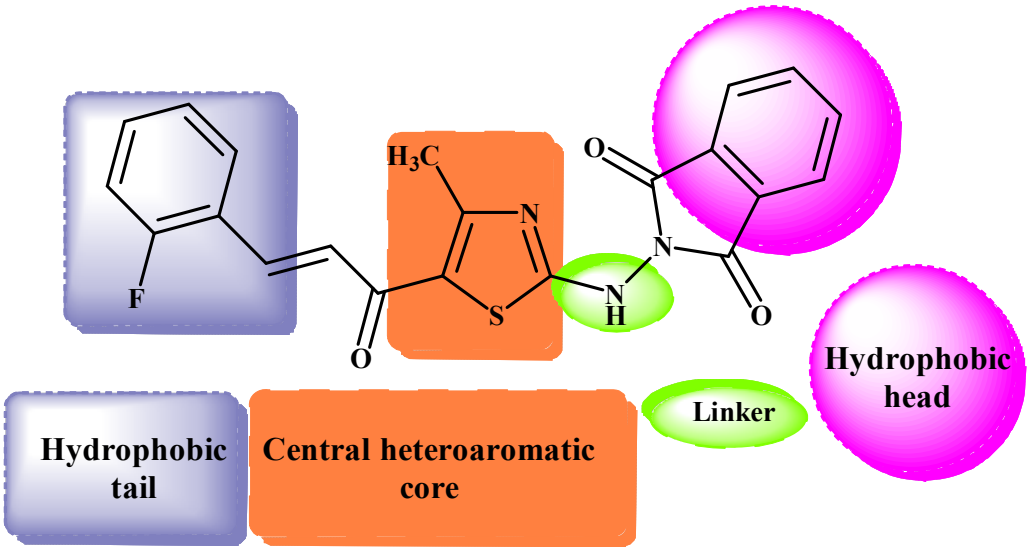


Fig. 2. Typical structural features of EGFR inhibitors compared with Les-6489.

polar hydrogen atoms, and assigning Kollman charges to residues. A cubic grid box of 55 Å was centered within the ATP binding site.

The binding energies of native ligands, which are experimentally confirmed potent tyrosine kinase inhibitors (BDBM50432376 for EGFR and BDBM50358456 for HER2), were obtained and used for comparison and inhibition activity assessment with Les-6489. To validate the obtained results through cross-docking, we included the structure of Tak-285, a potent dual EGFR/HER2 inhibitor, in the set of the used reference ligands [18].

Molecular dynamics (MD) simulations were performed using GROMACS [19], accessed via the SiBioLead server (https://sibiolead.com/), to assess the stability of the complexes with Les-6489. For comparative purposes, MD simulations were also performed with the native ligand. The complexes obtained from the simulations were checked and repaired using Swiss-PDB Viewer. The protein-ligand complexes were solvated in a triclinic box containing water, with 0.15 M NaCl added to neutralize the system's charge. The simulation system was parameterized with the CHARMM27 force field [20]. Before the production run, the system underwent equilibration using the NVT and NPT ensembles for 300 ps each. The MD simulation was conducted for 100 ns, utilizing the leap-frog integrator. Trajectory frames were recorded at 20 ps intervals throughout the 100 ns simulation, resulting in 5000 frames. The data obtained were analyzed using GROMACS' built-in trajectory analysis tools and Microsoft Excel.

Results and Discussion. The obtained models of EGFR and HER2 exhibited GMQE scores of 0.86 and 0.83, and QMEAN scores of 0.82±0.05 and 0.78±0.05, respectively. These values indicate that the quality of the predicted models is sufficient for docking and dynamic simulation [21]. Docking results demonstrated the potential affinity of Les-6489 for EGFR and HER2, with a slight preference for HER2 receptors. However, it should be noted that the binding energies are lower compared to the native ligands (Table).

In the EGFR compound, the NH linker forms a single hydrogen bond with ASN842, measuring 2.50 Å. The phthalimide core interacts with ARG841 through an alkyl interaction; however, this interaction is relatively weak due to the more hydrophilic nature of the interacting residue. The *o*-fluorophenyl substituent fits well into a pocket formed by Leu716, Val726, Lys745, and Leu788. The fluorine atom forms electrostatically-driven noncovalent interactions with Aal743 and Ile744, contributing significantly to the overall binding energy within the ATP binding site of EGFR (Fig. 3).

The Autodock docking score of Les-6489 is close to the reference ligand and Tak-285, compared to the results with EGFR. The molecule's tail and head fit well into the cavities formed by the lipophilic amino acids, interacting through π-alkyl non-covalent interactions. Additionally, the NH linker part of the molecule forms strong hydrogen bonds with ASP162 (2.25 Å), and the thiazole core interacts with Lys52 via a hydrogen bond (2.63 Å) (Fig. 4).

Molecular dynamics.

Dynamic simulation demonstrated the stability of both complexes, either Les-6489 or BDBM50358456, with HER2. Root Mean Square Deviation (RMSD) is a measure, used to quantify the average deviation of a ligand's positions from a reference start point over time. It provides insight into the overall structural stability and conformational changes of the molecule during the simulation. The RMSDs in both complexes are less than 0.3 nm. However, the native ligand demonstrated a more stable position during the 100 ns simulation, without any significant changes. In contrast, Les-6489, despite showing a better RMSD value, exhibited greater fluctuation during the 100 ns molecular dynamics simulation (Fig. 5).

Root Mean Square Fluctuation (RMSF) helps to quantify the flexibility of residues, over the time of the simulation. It indicates how much the position of each atom fluctuates around its average position during the simulation. The RMSF values for both complexes demonstrated similar stability, with a slight advantage observed for the native ligand compared to the complex with Les-6489 (Fig. 6).

The number of hydrogen bonds facilitates to estimate of the interactions and stability within and between HER2 and the tested ligands. Consistency in the number of hydrogen bonds over time may indicate the stabilityy of a molecular complex. Stable interactions are often

Table
Binding energies for Les-6489 and experimentally confirmed ligands of the EGRF and HER2

Compounds	EGFR (kcal/mol)	HER2 (kcal/mol)
Les-6489	-9.52	-10.32
BDBM50432376(PDB: W32)	-12.7	–
BDBM50358456 (PDB: 03Q)	–	-11.57
Tak-285	-11.08	-11.44

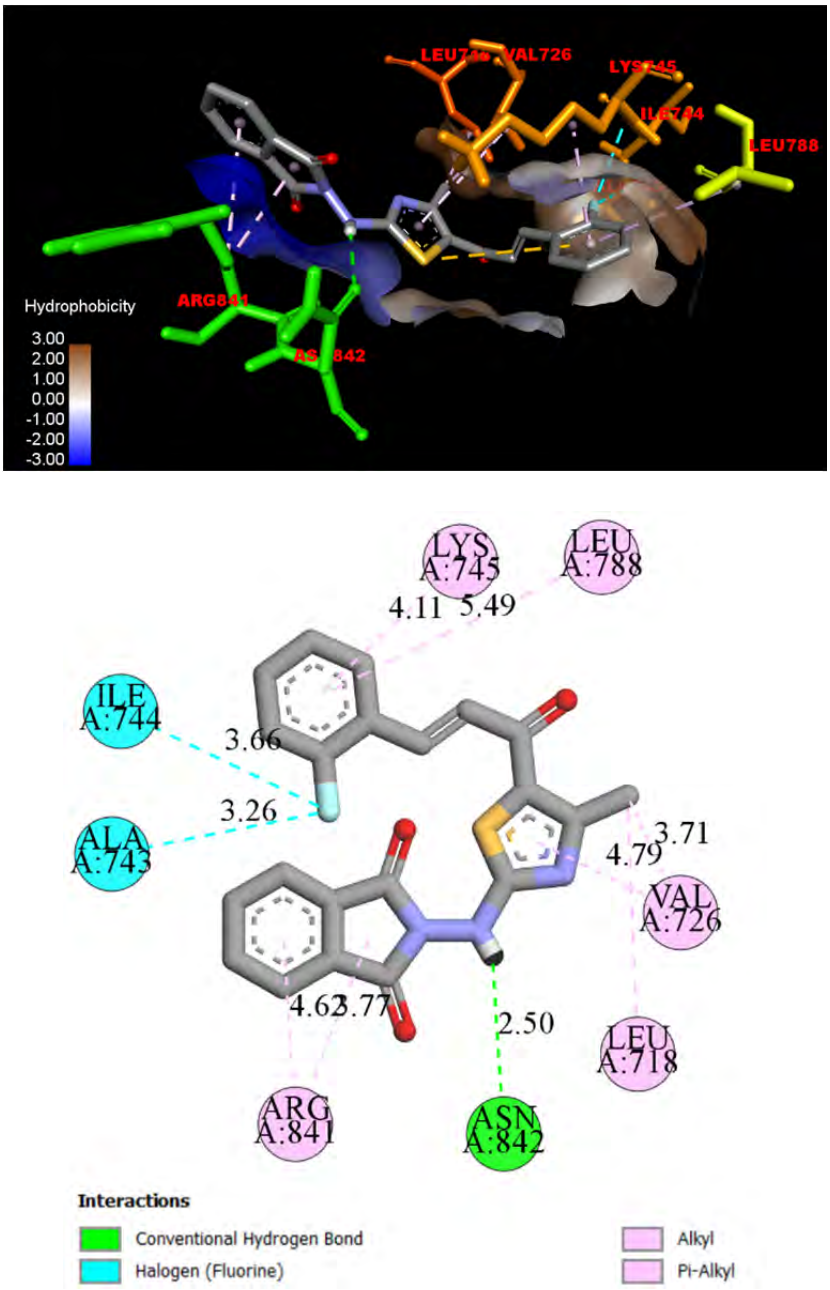


Fig. 3. 3D&2D schemes of the EGFR – Les-6489 interaction.

characterized by a consistent number of hydrogen bonds throughout the simulation. Both complexes exhibited a stable number of hydrogen bonds during the simulation. However, the native ligand maintained three hydrogen bonds for a longer duration compared to Les-6489, indicating better overall stability of the native ligand complex (Fig. 7).

The radius of gyration (***Rg***) quantifies the overall compactness and distribution of a molecule's mass around its center of mass, providing insights into the molecule's structural characteristics and changes during the simulation. A stable Rg value over time suggests that the molecule maintains a consistent overall structure, while fluctuations in ***Rg*** may indicate structural instability or transitions between different states. The ***Rg*** for HER2 – Les-6489 complexes indicates slight changes in the compactness of the complex during the simulation, whereas the complex with the native ligand exhibits more stable compactness. This stability suggests a more stable connection for the native ligand complex.

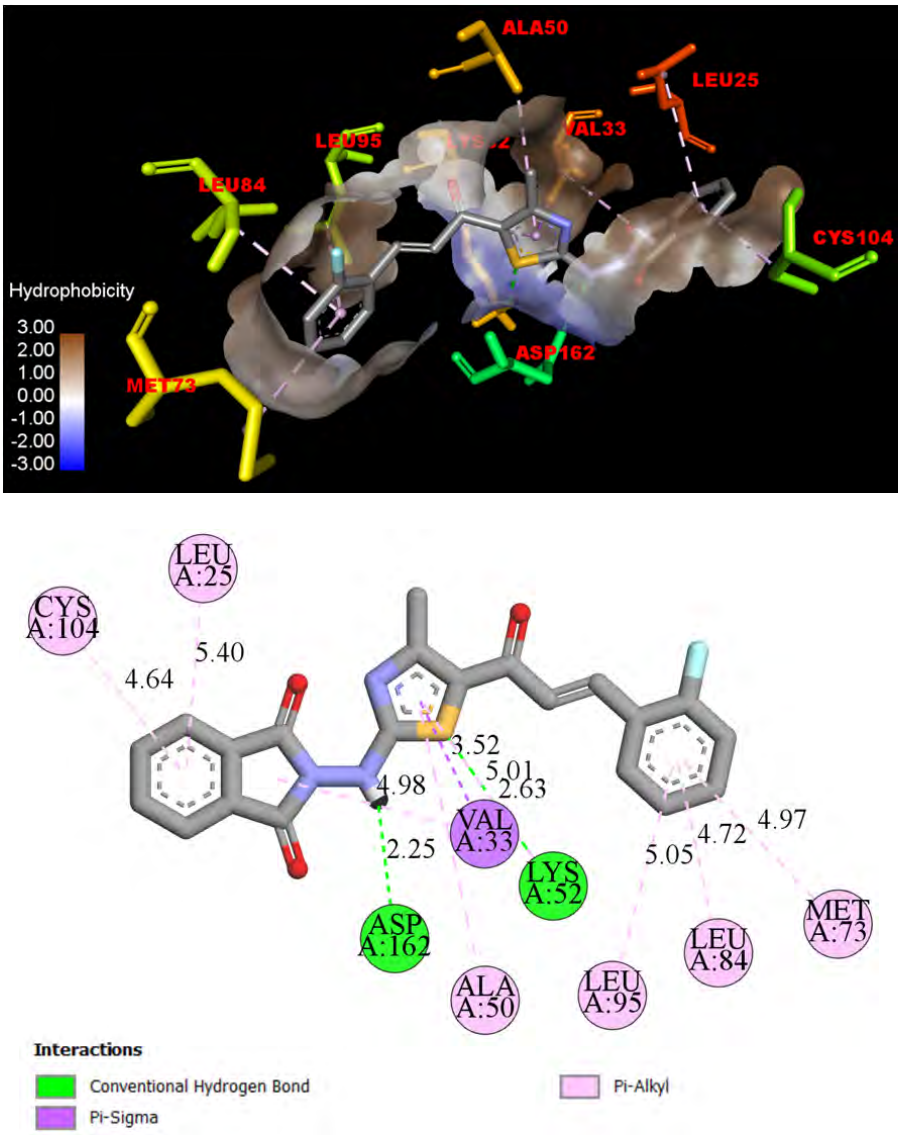


Fig. 4. 3D&2D schemes of the HER2 – Les-6489 interaction.

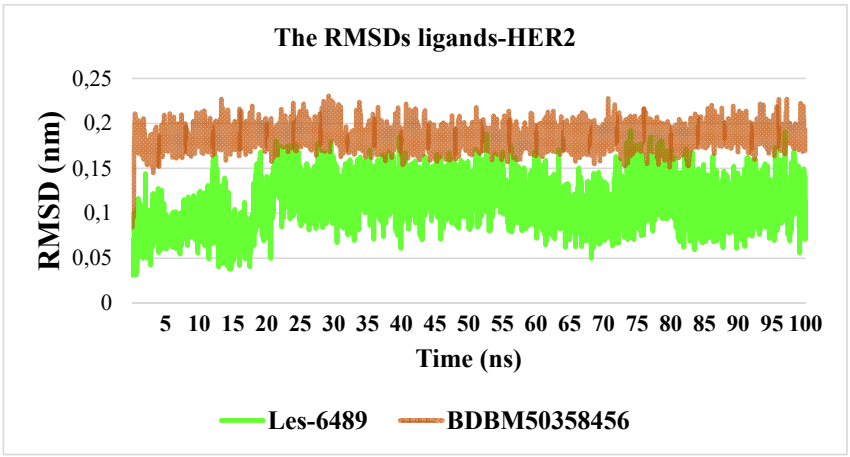


Fig. 5. The comparison of the RMSDs values for Les-6489 and BDBM50358456.

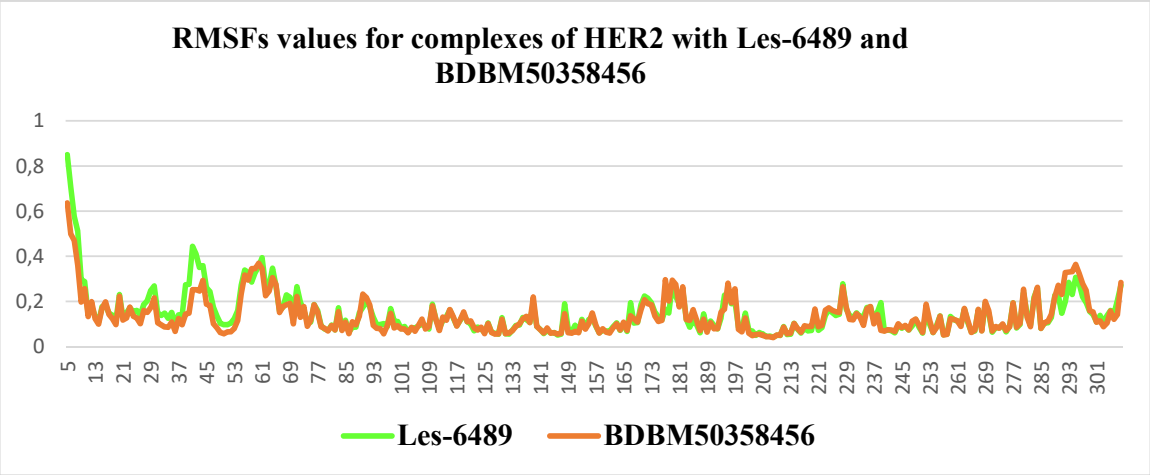


Fig. 6. The calculated average RMSF for HER2 complexes with Les-6489 and BDBM50358456.

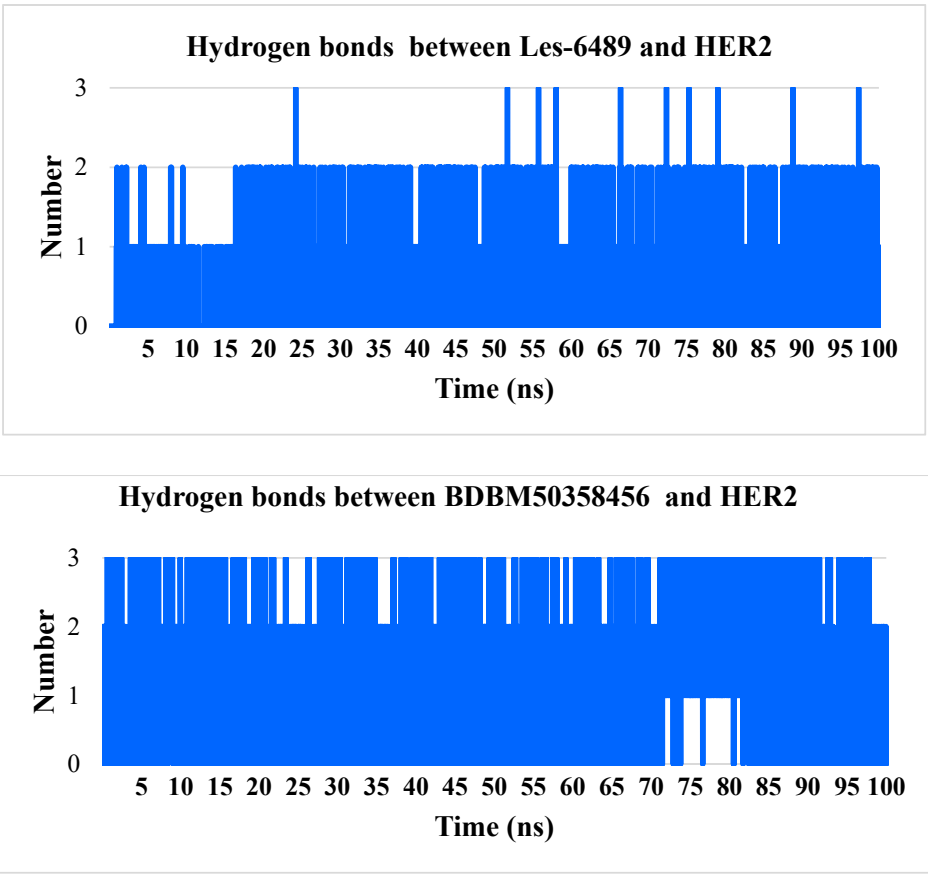


Fig. 7. The number of hydrogen bonds during the simulation for complexes of HER2 with Les-6489 and BDBM50358456.

Nevertheless, both complexes are quite comparable in terms of their overall structural characteristics (Fig. 8).

Dynamic simulation demonstrated the stability of both Les-6489 and BDBM50358456 complexes with HER2. Overall, the native ligand complex demonstrated superior stability across multiple metrics compared to the Les-6489 complex. Nevertheless, both complexes were quite comparable

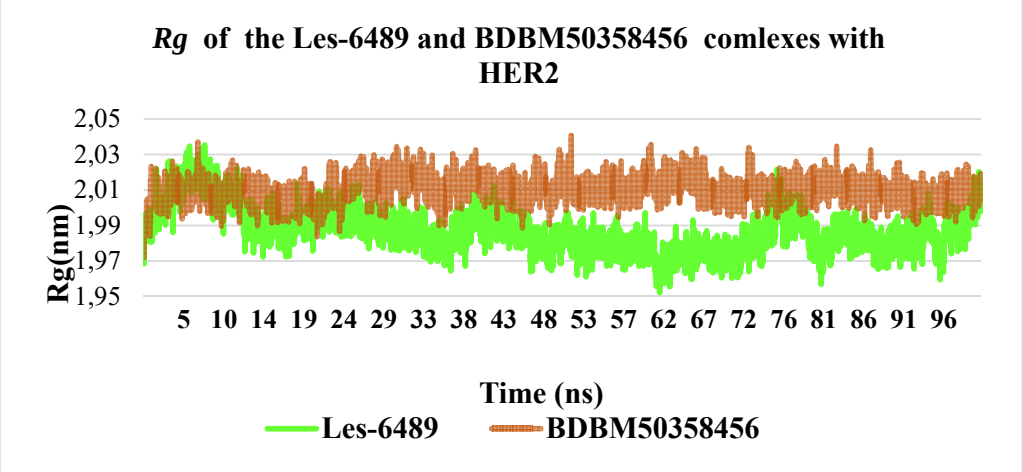


Fig. 8. The ***Rg*** values for HER2 with compound 3 and BDBM50358456.

in their overall structural characteristics, with each showing distinct advantages in specific areas of stability assessment.

Conclusion. Complex studies based on molecular docking and molecular dynamics (MD) simulations have established a significant potential of the compound Les-6489, which can be considered as a potential direction for further in-depth studies of anticancer activity of thiazole derivatives with isoindole fragments in the structure.

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Conflicts of interest: authors have no conflict of interest to declare.

Конфлікт інтересів: відсутній.

IN SILICO ДОСЛІДЖЕННЯ МЕХАНІЗМУ ПРОТИПУХЛИННОЇ АКТИВНОСТІ (*E*)-2-((5-(3-(2-ФЛУОРОФЕНІЛ)АКРИЛОЇЛ)-4-МЕТИЛТІАЗОЛ-2-ІЛ)АМІНО)ІЗОІНДОЛІН-1,3-ДІОНУ (LES-6489)

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Мета роботи. Здійснити прогнозування механізмів реалізації протипухлинної активності з використанням сучасних веб-інструментів для сполуки Les-6489.

Матеріали і методи. Для сполуки Les-6489 проведено молекулярний докінг щодо EGFR та HER2 тирозинкіназ. Для оцінки стабільності комплексів з Les-6489 було проведено моделювання молекулярної динаміки (МД) за допомогою GROMACS-13, доступ до якого здійснюється через сервер SiBioLead.

Результати й обговорення. У результаті проведених *in silico* досліджень для сполуки Les-6489, яку вивчали, було спрогнозовано механізм протипухлинної активності, що реалізується шляхом інгібування EGFR та HER2 тирозинкіназ.

Висновки. Результати, отримані в процесі досліджень, можуть стати платформою для подальшої структурної оптимізації ідентифікованої сполуки на основі тіазол-ізоіндольних гібридних молекул у розробці сучасних протипухлинних агентів.

Ключові слова: тіазоли; ізоіндол; протиракова активність; молекулярний докінг; моделювання молекулярної динаміки.

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