DISCOVERY OF POTENTIAL INHIBITORS AGAINST BLADDER CANCER USING IN SILICO APPROACH

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Article History: **Received:** 12.10.2023 **Revised:** 14.11.2023 **Accepted:** 13.12.2023

Abstract

Background: Bladder cancer remains a significant global health concern, necessitating the exploration of innovative therapeutic strategies. In silico approaches offer a promising avenue for the discovery of potential inhibitors targeting Fibroblast Growth Factor Receptor 3 (FGFR3), a key player in bladder cancer progression. This study focuses on the molecular docking analysis of various compounds, including mitomycin, ligand EVR, and 16 additional ligands, to identify novel inhibitors against FGFR3. **Methods:** A comprehensive in silico approach was employed, utilizing molecular docking simulations to assess the binding energies and interactions of compounds with the active site of FGFR3. The ligands, including laurifolin, were selected based on their diverse chemical properties and structural characteristics. Comparative analyses were conducted against established compounds to identify potential lead candidates. **Results:** The molecular docking results revealed substantial variations in binding energies among the tested compounds. Laurifolin exhibited the most favorable binding energy, suggesting a robust interaction with FGFR3. This compound demonstrated superior performance compared to mitomycin and ligand EVR, indicating its potential as a potent inhibitor of FGFR3. The specific structural features of laurifolin likely contribute to its enhanced binding energy, possibly involving hydrogen bonds and hydrophobic interactions within the FGFR3 binding pocket. **Conclusion:** The identification of laurifolin as a lead candidate underscores its potential as a novel therapeutic agent against FGFR3 in the context of bladder cancer. However, it is crucial to acknowledge that molecular docking results are computational predictions and necessitate validation through in vitro and in vivo studies.

Keywords: FGFR3 ; molecular docking ; laurifolin ; in silico approaches

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DOI: 10.53555/ecb/2023.12.12.269

INTRODUCTION

Bladder cancer is a relevant malignant disease with the 7th most common cancer in men and 17th in women, affects millions globally [1]. With an estimated 573,000 new cases diagnosed globally in 2020, its prevalence is particularly high in the United States, where it is the 13th most common cause of cancer death with an estimated more than 212,000 deaths worldwide [2]. The lifetime risk for individuals is significant, with 2.3% of men and women facing a potential diagnosis [3]. While age and gender play a role, smoking remains the leading risk factor, responsible for half of all cases. Early detection and understanding risk factors are key in combating this impactful disease. Fibroblast growth factor receptor 3 (FGFR3) genomic alterations are potent oncogenic drivers in bladder cancer. Development of novel selective FGFR3 inhibitors changed the therapeutic paradigm for patients with FGFR3-altered bladder cancer [4].

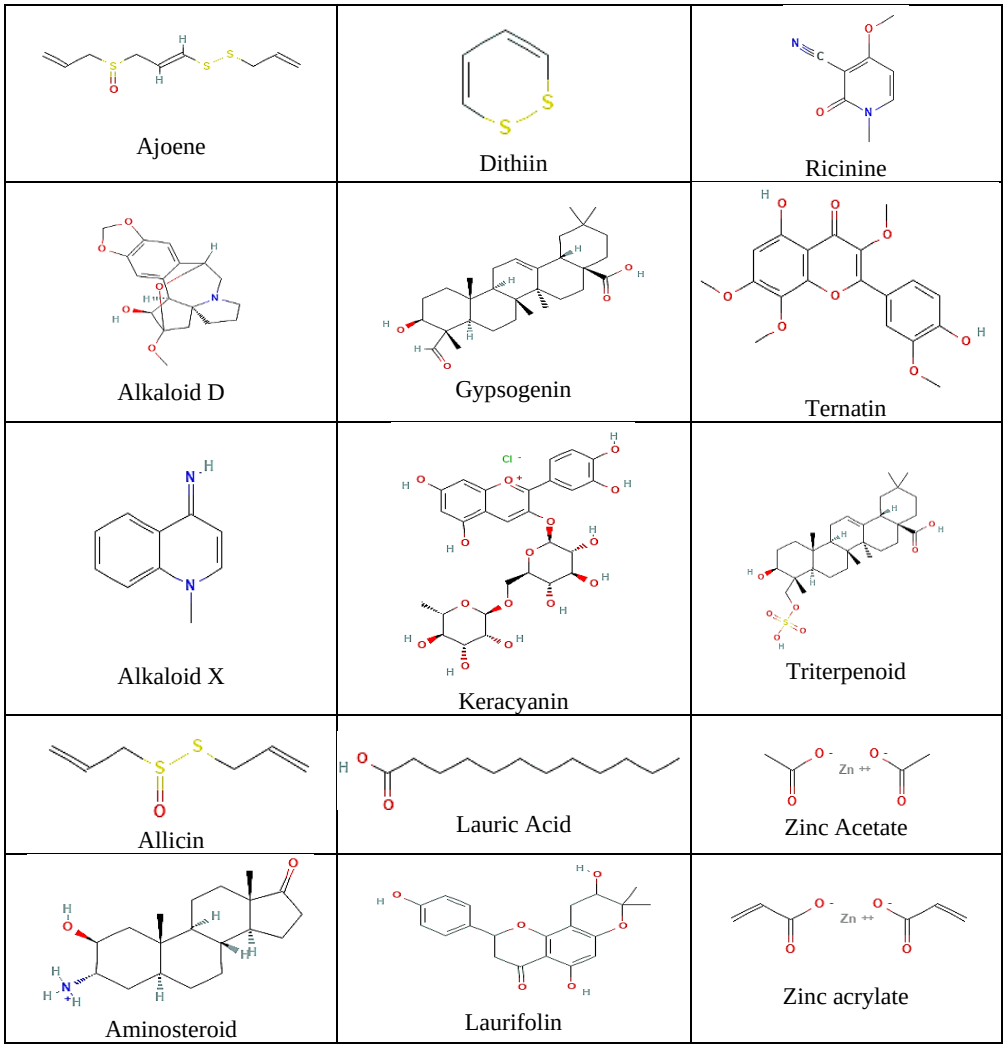
Erdafitinib, a pan-fibroblast growth factor receptor (FGFR) inhibitor, has been approved for treating patients with select FGFR2 and FGFR3 alterations and fusions since 2019. Since then, emerging data has demonstrated efficacy of combining erdafitinib with immunotherapy in treating FGFR-altered urothelial carcinoma, and with locally advanced or metastatic bladder cancer that has a type of susceptible genetic alteration known as FGFR3 or FGFR2 for adult patients' treatment [5]. However, erdafitinib has side effects such as nausea, vomiting, mouth sores/pain, change in how food tastes, abdominal pain, diarrhea, constipation, loss of appetite, weight loss, tiredness, dry mouth/skin, or muscle/joint pain may occur [6]. Other Intravesical treatments flush the bladder with drugs that kill cancer cells that remain after surgery. This lowers the chance of the cancer coming back. Mitomycin and gemcitabine are two chemotherapy drugs given as intravesical chemotherapy to treat bladder cancer. These drugs can also be given systemically. However, these drugs also have similar

side effects with erdafitinib [7].
Identifying potential bioactive compounds to combat bladder cancer through in silico approaches involves screening diverse chemical libraries. Several classes of compounds with known anticancer properties can be considered for virtual screening against the fibroblast growth factor receptor 3 (FGFR3). The aim of this study was to employ an in silico approach to identify and characterize bioactive compounds capable of inhibiting fibroblast growth factor receptor 3 (FGFR3) with the ultimate goal of combating bladder cancer. By utilizing computational tool such as molecular docking to evaluate and prioritize potential FGFR3 inhibitors. This research contributes to the advancement of precision medicine approaches in bladder cancer treatment, offering a more efficient and targeted strategy for developing FGFR3 inhibitors as potential anti-cancer agents.

MATERIALS AND METHODS

Materials

The receptor complex of fibroblast growth factor receptor 3 (FGFR3) was obtained from the Protein Data Bank Repository (PDB) with the identifier: 6LVM [8]. The corresponding files, in .pdb format, were downloaded. Additionally, a 3D file conformer of the commercial drug mitomycin, original ligand from the 3D structure of protein complex as pyrimidin derivative **(**2-[[5-[2-(3,5-dimethoxyphenyl)ethyl]-2-[[3-methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl]amino]pyrimidin-4-yl]amino]-N-ethyl-benzenesulfonamide) also known as ligand EVR, and 16 ligand files including: ajoene; alkaloid D; alkaloid X; allicin; aminosteroid; anisole; dithiin; gypsogenin; keracyanin; lauric acid; laurifolin; oleoyl chloride; ricinine; ternatin; triterpenoid; zinc acetate; zinc acrylate; zinc citrate were downloaded from PubChem [9]. These ligand files were in .sdf format. The 2D chemical structures of bioactive compounds were depicted in Figure 1.



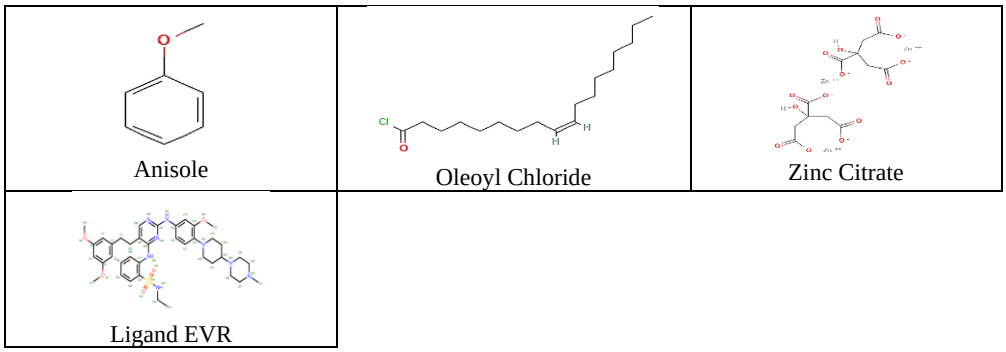


Figure 1: The 2D chemical structures of bioactive compounds.

Protein Preparation and Virtual Screening

After utilizing Discovery Studio Visualizer [10] to eliminate initial ligands and water molecules, the protein's .pdb files undergo a series of essential steps to facilitate molecular docking with PyRx [11]. These steps encompass acquiring the protein structure in a suitable format, importing it into PyRx, excluding water molecules, supplementing missing residues as needed, introducing hydrogen atoms, assigning atom types, optimizing the structure, and ultimately saving the meticulously prepared protein structure. Adhering to this procedural framework ensures that researchers adequately ready their protein structures for precise and dependable docking simulations.

Molecular Docking

The PyRx Tools software was utilized to prepare both the protein and ligand, converting them into .pdbqt format. To initiate molecular docking simulations, follow these steps: Commence by acquiring the protein structure from a database such as the Protein Data Bank (PDB) and import it into PyRx. Prepare the protein by eliminating water molecules, addressing missing residues, and introducing hydrogen atoms. Optionally, assign atom types and optimize the structure for heightened accuracy. Save the meticulously prepared protein structure in either PDB or PDBQT format. Subsequently, procure the ligand structure from a chemical database or generate it computationally, ensuring compatibility with PDB or SDF formats. Import the ligand into PyRx, add hydrogen atoms if necessary, assign atom types, and convert it to PDBQT format if required. Save the prepared ligand structure also in pdbqt format [11].

Protein and Ligand Interaction

The generation of docking data for both the protein and ligand was conducted in accordance with .pdb files. The PyRx program was utilized to seamlessly integrate the data, ensuring a uniform and cohesive representation for subsequent analyses. Additionally, PyMOL was employed for a systematic 3D visualization, facilitating a detailed examination of spatial arrangements, binding interfaces, and conformational changes [12]. Assessing the strength of interaction between the ligand and the target in molecular docking required using the binding energy (ΔG) value. To calculate inhibition constants (Ki), it was essential to determine the affinity with which a ligand binds to a target receptor, utilizing the formula: $Ki = e^{-RT/\Delta G}$.

RESULTS

Protein and Ligand Interaction

This analysis employed Pyrx's gridbox tool to define the receptor docking region for molecular docking. Figure 2 and Figure 3 depicted the binding energy and 3D interactions between the fibroblast growth factor receptor 3 (FGFR3) and inhibition constant. Gypsogenin emerged as the compound with the most favorable binding affinity among the 18 tested, demonstrating promising potential for further investigation. Notably, the docking process was validated using the original ligand (ligand EVR) obtained from the protein-ligand complex 3D structure, confirming the accuracy of the simulations.

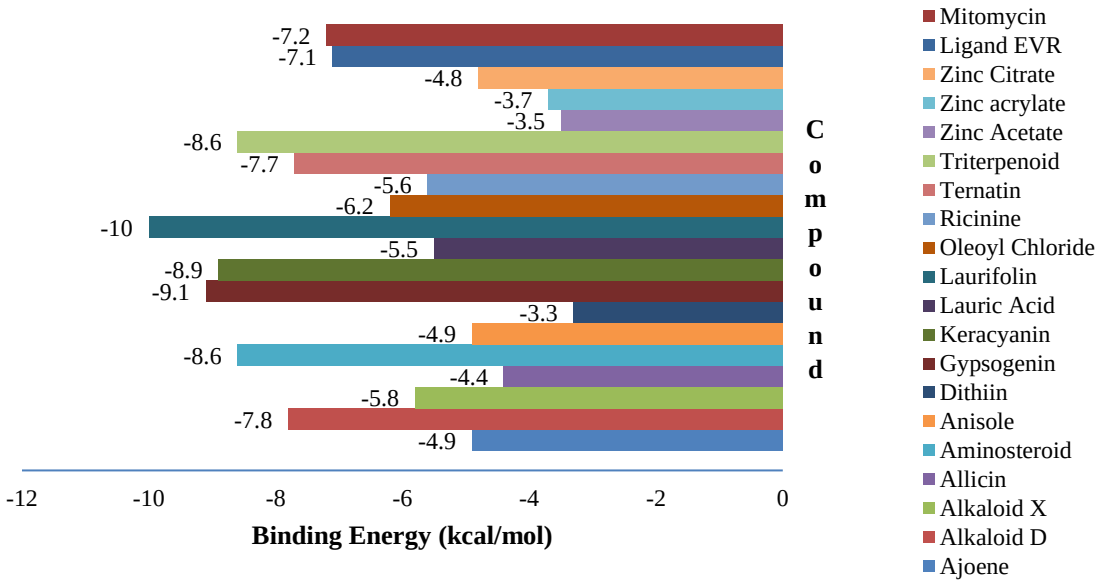


Figure 2: The energy of binding between bioactive compounds and fibroblast growth factor receptor 3 (FGFR3).

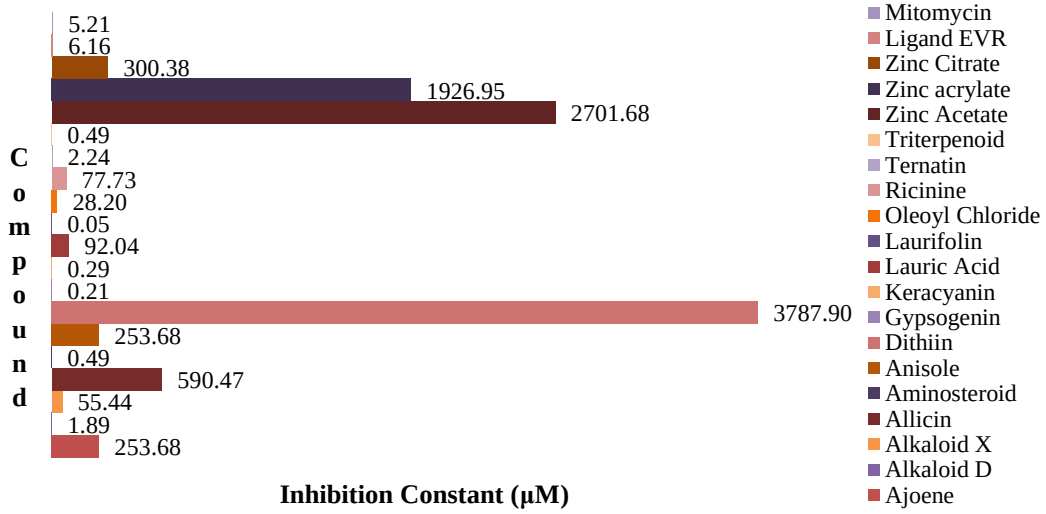


Figure 3: The inhibition constant of bioactive compounds and fibroblast growth factor receptor 3 (FGFR3) complex.

Figure 4 illustrated a detailed 2D representation of receptor-ligand interactions, presenting complex associations among various entities. In Figure 3A, the dynamic interplay between the fibroblast growth factor receptor 3 (FGFR3) and laurifolin was performed, emphasizing hydrogen bonds with Arg-564 and hydrophobic interactions such as Leu-478, Gly-479, Glu-480, Gly-481, Val-486, Ala-506, Lys-508, Ile-539, Val-555, Glu-556, Asn-562, Glu-565, Arg-621, Asn-622, Ala-634, Asp-635. In Figure 3B showed an enlarged perspective on the interaction between the receptor and the commercial drug mitomycin, highlighting a hydrogen interactions with Leu-478, Val-486, Leu-624 and Asp-635. Finally, Figure 3C captured the intricate interplay between the FGFR3 and the ligand EVR. This reveals nine hydrophobic interactions with Leu-522, Arg-621, Asn-622, Gly-481, Glu-480, Gly-479, Asn-562, Gly-561, Tyr-557, and fifteen hydrogen bonds with Asp-635, Glu-525, Phe-636, Lys-508, Ala-634, Ile-539, Val-553, Met-529, Val-486, Val-555, Leu-624, Glu-556, Ala-506, Ala-558, Leu-478.

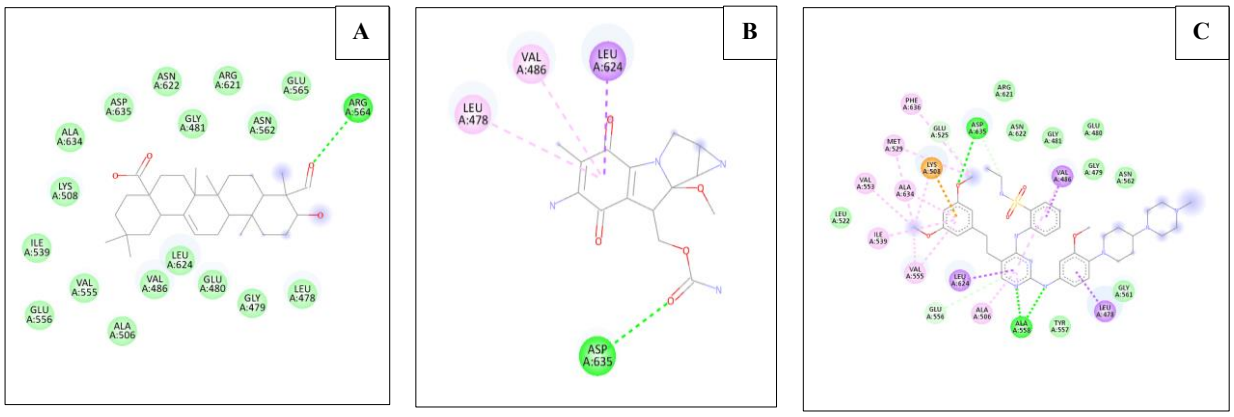


Figure 4: Visual representation in 2D of interactions between: A. fibroblast growth factor receptor 3 (FGFR3) and laurifolin; B. FGFR3 and mitomycin drug; C. FGFR3 and ligand EVR.

Figure 5 presented a three-dimensional (3D) visualization of interaction complexes involving the FGFR3 receptor and three distinct ligands: laurifolin, mitomycin drug, and ligand EVR.

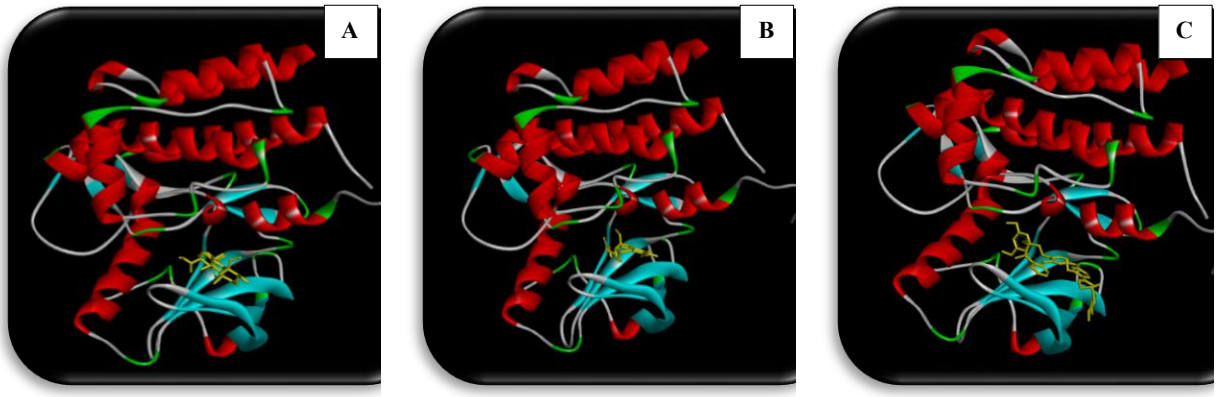


Figure 5: Visual representation in 3D of interactions between: A. fibroblast growth factor receptor 3 (FGFR3) and laurifolin; B. FGFR3 and mitomycin drug; C. FGFR3 and ligand EVR.

DISCUSSION

The results of the molecular docking analysis involving compounds mitomycin, ligand EVR, and 16 additional ligands revealed notable variations in their binding energies with the target protein. Among these ligands, laurifolin demonstrated the most favorable binding energy, suggesting a strong and stable interaction with the target protein, possibly Fibroblast Growth Factor Receptor 3 (FGFR3) in the context of bladder cancer therapy. This finding is of particular significance as it implies that laurifolin has the potential to serve as a potent inhibitor of FGFR3, making it a promising candidate for further exploration in the development of anti-bladder cancer therapeutics.

The superior binding energy of laurifolin may be attributed to its specific structural features that facilitate favorable interactions with the active site of FGFR3. It is crucial to consider the molecular docking results in conjunction with the chemical properties and structural characteristics of the ligands. Laurifolin's performance could be associated with its ability to form hydrogen bonds, hydrophobic interactions, or other favorable binding modes within the binding pocket of FGFR3.

Comparative analyses against established compounds like mitomycin and ligand EVR provide context to the significance of laurifolin's performance. The fact that laurifolin outperformed these compounds suggests its potential as a novel and effective inhibitor of FGFR3. However, it is essential to note that in vitro and in vivo validations are imperative to confirm the actual inhibitory activity and therapeutic efficacy of laurifolin against bladder cancer.

Moreover, the diverse set of ligands, including ajoene, alkaloid D, alkaloid X, allicin, aminosteroid, anisole, dithiin, gypsogenin, keracyanin, lauric acid, laurifolin, oleoyl chloride, ricinine, ternatin, triterpenoid, zinc acetate, zinc acrylate, and zinc citrate, provides a comprehensive overview of potential inhibitors. The identification of laurifolin as the lead candidate emphasizes the importance of employing a

broad screening approach to discover novel therapeutic agents for FGFR3 inhibition in bladder cancer. Laurifolin is a flavonoid derivative, has previously been shown to possess various biological activities, including anti-proliferative, anti-inflammatory, and antioxidant effects [13]. These properties may be relevant in the context of bladder cancer treatment. It is important to note that molecular docking is a computational method and does not guarantee actual biological activity. Further in vitro and in vivo studies are necessary to confirm the efficacy and safety of laurifolin as a potential bladder cancer therapeutic.

CONCLUSION

In conclusion, the molecular docking results highlight laurifolin as a promising compound with superior binding energy, laying the foundation for further experimental validation. The potential of laurifolin as an FGFR3 inhibitor underscores its significance in the development of targeted therapies for bladder cancer, though additional studies are needed to confirm its efficacy and safety

ACKNOWLEDGEMENT

We express our gratitude to the Institute for Research and Community Development at Unika Atma Jaya, the Directorate of Research and Development at UI, and the Institute for Research and Community Service at UNAS for their unwavering support.

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