Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1537989

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 7 - Issue 6: 1131-1138 / November 2024

# IN SILICO PREDICTION OF EGFR INHIBITORS FROM THIOPHENE DERIVATIVES

**Pınar SİYAH[1]***

[1]*Bahcesehir University, School of Pharmacy, Department of Biochemistry, 34330, Istanbul, Türkiye*

**Abstract:** Cancer is one of the biggest global health problems and is the second leading cause of death worldwide. Cancer also causes great damage to economy. Unfortunately, there is still no effective treatment method against this disease today, and the mortality rates in certain types are still very high. Medical research can now be done faster and safer with the aid of in silico studies. These studies save time for researchers and accelerate new drug discoveries. In our study, thiophene derivatives with important efficacy in cancer treatment were focused on and the affinity of the small molecule structures determined as candidates to the Epidermal Growth Factor Receptor (EGFR), known to be the key receptor in cancer, was examined. First, molecular docking studies were performed, and then long-term molecular dynamics (MD) simulations were carried out. Finally, anti-cancer activity predictions based on Quantitative Structure-Activity Relationship (QSAR) were performed. Co-crystallized ligand Erlotinib, taken from the Protein Data Bank (PDB), was used as a positive control and compared with candidate drugs using the same procedures. In light of the analysis of virtual screening, MD, MM/GBSA, and QSAR predictions, the top three molecules and their MM/GBSA scores were identified as follows: OSI 930 (-65.81 kcal/mol), Neltenexine (-49.53 kcal/mol), and Tenonitrozole (-41.95 kcal/mol). As a result, in this study, candidate molecules that inhibit EGFR and have the highest potential as anti-cancer drugs among thiophene-derived compounds were determined and detailed in silico analyzes were performed. This study holds importance as it may guide future anti-cancer drug discovery studies.

**Keywords:** EGFR, MD simulation, Molecular docking, Small molecules, Thiophene derivatives.

**Corresponding author:** Bahcesehir University, School of Pharmacy, Department of Biochemistry, 34330, İstanbul, Türkiye
**E mail:** pinar.siyah@med.bau.edu.tr (P. SİYAH)
Pınar Siyah https://orcid.org/0000-0003-1192-9416

**Received:** August 24, 2024
**Accepted:** September 30, 2024
**Published:** November 15, 2024

**Cite as:** Siyah P. 2024. In silico prediction of EGFR inhibitors from thiophene derivatives. BSJ Eng Sci, 7(6): 1131-1138.

## 1. Introduction

With the increasing number of cases, cancer is the second disease that causes the most deaths after cardiovascular diseases, and it is likely to come first in terms of both death and health expenditures in the coming years (Collaboration, 2019). In a developing country like Türkiye, currently, researchers are working hard on the treatment of this disease with low budget, and the number of articles on local cancer problems is increasing rapidly every year (Pramesh et al., 2022) . Considering the research budgets used and the number of researchers working, the process progresses very slowly with classical drug development methods. At the same time, it is very costly to pass a candidate drug molecule to phase stages. Discovery of a drug to prevent a disease is approximately $2-3 billion and takes 10 to 15 years but it is $300 million and 6.5 years for repurposing drugs (Weth et al., 2024). Moreover, there is no guarantee that a commercial product will be obtained as a result of these expenses. While normally only a few candidate drugs tested can be approved at the end of the process, this success rate can reach higher for drugs discovered in silico. Today, there is an urgent need to use computer-based drug discovery methods to overcome these limitations. Thanks to computer-based drug discovery, more effective, more target-specific and safer drug candidates can be found in a short time (Berdigaliyev and Aljofan, 2020).

In silico drug discovery process is a set of computer-based computational approaches, including high-throughput virtual screening, molecular docking, molecular dynamics simulations and artificial intelligence-based machine learning methods. In silico methods, which save on human resources, space and time, are increasing their effectiveness and usability day by day. With the aid of these methods, drug candidate molecules in the pool are evaluated for minimum toxicity (Stillman et al., 2020; Shaker et al., 2021).

It enables us to complete the processes including pre-clinical trials in a very short time, and to discover the best drug by enabling the selection of the most effective and target-specific candidate molecules (Gagic et al., 2020). For this purpose, drugs can be designed from scratch or screened from various databases containing many small drug molecules. Studies of repositioning existing, known, and also approved drugs against a different disease, such as the FDA-approved drug library, constitute the principle of drug repurposing studies. Drug repositioning studies appear to be the safest method because they enable the use of existing drugs with known side effects and pharmacodynamic potential

(Masuda et al., 2020; Turanli et al., 2021). At the same time, the first method used for emergency use in epidemic diseases such as COVID19 and extraordinary situations is again drug repurposing studies (Durdağı, 2020).

Thiophene derivatives are key structures consisting of a heterocyclic skeleton containing a ring of sulfur and four carbon atoms (Gramec et al., 2014). The higher number of the anticancer drugs used in clinics have a heterocyclic structure (Nehra et al., 2022). In this respect, many studies have shown that Thiophene derivatives, which are known to be a priority for drug discovery, have high anti-cancer potential. In addition, thiophenes are more reactive than their counterparts thanks to the sulfur atom in their structure. At the same time, they are more polar and exhibit a more biocompatible character thanks to their high solubility in water. The number of trials of thiophene derivatives, which stand out with these properties, in anti-cancer studies in the literature is increasing day by day, and they have high potential to become candidate drug molecules (Vallan et al., 2021; Kuchana et al., 2022).

There are known important protein targets in cancer such as BCL2 (Zhang et al., 2021), MCL2 (Harmanen et al., 2023), PI3K (Jones et al., 2022), PTEN (Turnham et al., 2020) and EGFR (Sun et al., 2021). Epidermal growth factor receptor (EGFR), which is involved in many pathways in the cell, such as cell signaling pathways that control cell division and survival, ensures homeostasis (Uribe et al., 2021). In recent years, research has focused on receptors expressed in cancerous cells, thus targeting drugs to a specific cell type has become possible. Personalized treatment has made significant progress, especially in diseases that can rapidly develop resistance to drugs, such as cancer. EGFR is one of the most targeted receptors and many researchers see it as a key target in the treatment of cancer (da Silva Santos et al., 2021; Tian et al., 2022). Within silico studies, various target molecules can be quickly tested against a receptor expressed in a known type of cancer (Sibuh et al., 2021). In this research, 10 different Thiophene derivatives were identified to be used in the treatment of cancer, which is a major global public health problem. Then, these thiophene derivatives were subjected to docking studies for the EGFR protein. Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) analysis was performed for the 5 derivatives with the best scores, and the results were evaluated by comparing them with the reference molecule.

## 2. Material and Methods

### 2.1. Preparation of the Thiophene-containing Compounds for Docking

In this study, ten compounds containing thiophene were obtained from the literature. Ligands were prepared using the LigPrep module of Schrödinger's Maestro molecular modeling package (Madhavi Sastry et al., 2013; Jamal et al., 2015). The physiological environment and ionization states of all ligands were adjusted at physiological pH 7.4 using Epik (Shelley et al., 2007).

### 2.2. Protein Preparation and Grid Box Generation

In this study, the tyrosine kinase domain of the Epidermal Growth Factor Receptor (EGFR) was utilized in a co-crystallized form with the 4-anilinoquinazoline inhibitor, erlotinib. The structure corresponding to this complex is identified by the Protein Data Bank (PDB) accession code 1M17. The Prime Module of the Schrödinger Molecular Modeling Package was used to complete structure with missing side chains and loops. Disulfide bonds were formed. Protonation states were set at a physiological pH of 7.4 using PROPKA (Bas et al., 2008). After the preparation of the ligand and protein, the grid box was created in the active region by taking the coordinates of the protein's co-crystallized ligand in the PDB as the center. For docking studies, the Glide docking program of the Maestro molecular modeling package was used with standard precision (SP) settings (Siyah et al., 2023). The conformation and the score of the docking poses obtained with the X-ray data of the co-crystallized structures were compared.

### 2.3. Molecular Dynamics Simulations

Atomic-level MD simulations contribute to the understanding of biological systems. In this study, classical MD simulations were applied for potentially effective compounds against the EF2K target. Through the Desmond MD simulation program, the compounds identified in the docking simulations along with the apo form of the target protein were added to the simulation (Bowers et al., 2006). The systems were surrounded with an orthorhombic box containing TIP3P water molecules, and interactions at the atomic level were determined using the OPLS3e force field (Roos et al., 2019). The systems were simulated with NPT assembly at 310K temperature and 1 bar pressure. The Nose-Hoover thermostat and the Martyna-Tobias-Klein barostat were used to keep temperature and pressure constant (Evans and Holian, 1985; Martyna et al., 1994). The systems were minimized and balanced with Desmond's default protocols, and MD simulation was performed for each system for 100 ns, 100 frames were recorded.

### 2.4. MM/GBSA Calculations

Interactions such as hydrogen bonds, hydrophobic interactions, ion pairs and water bridges established between the ligand and the active site are vital for the integration of small molecules into the catalytic part of the protein. The free energy values between protein and ligand of the molecules obtained from Maestro were calculated with the MM/GBSA methodology used in the Prime module of Schrödinger's molecular modeling package. Prime simulates these interactions in detail using the VSGB 2.0 resolution model (Jacobson et al., 2004).

### 2.5. ADME Analysis and Anticancer Activity Prediction with Binary QSAR Models

All molecules that underwent docking and 100ns MD were subjected to Metacore Metadrug analyzes from Clarivatives Analytics. Absorption, Distribution, Metabolism, and Excretion (ADME) properties were calculated. Therapeutic activity predictions for cancer were made with machine learning based binary QSAR models. The sensitivity, specificity, accuracy and model quality and accuracy of the models were validated with Matthews Correlation Coefficient (MCC). The cut off value was accepted as 0.5 (Ekins et al., 2006).

## 3. Results and Discussion

In this study, thiophene derivatives that we obtained from the literature and the reference molecule were docked to the EGFR target. Docking scores are the important metric that determines how strong a binding affinity a molecule can display with a target protein. Scores are expressed in negative energy units, with lower (more negative) scores meaning stronger binding affinity (Muegge and Rarey, 2001). In the light of the results of the docking study scores performed with the Glide module of the Maestro program, it was discovered that the Erlotinib molecule, which is stated as the reference molecule for EGFR and is co-crystallized in the PDB, binds to EGFR most strongly. The determination that it binds with the strongest score and it also binds through known interaction amino acids confirmed the accurate and reliable calculation results of the program we used (Türkmenoğlu, 2022).

The docking score of the reference molecule (Erlotinib), which is currently used as an anticancer drug (Zhou et al., 2011) and was included in our study as a positive control, was calculated as -8.21 (Figure 1). In the experimental group, the ligand that followed the reference drug with the closest score was the OSI930 molecule with -6.142 (Figure 2). This is followed by Tenosal with -6.06 kcal/mol and Neltenexine ligands with -6.04 kcal/mol. Suprofen molecule has average docking scores of -5.48, Cliprofen molecule -5.40, and Tenonitrozole molecule -5.27. The weakest binding ligands were VX-759, Midestein, VCH-916 and Taurostein, with docking scores of -4.994, -4.865, -4.442 and -4.340 kcal/mol, respectively. In this study, the cut off value was determined as -5.00 kcal/mol. While weaker binding ligands were eliminated, ligands with a stronger binding (more negative) score than -5.00 kcal/mol were selected for more detailed studies (Table 1).

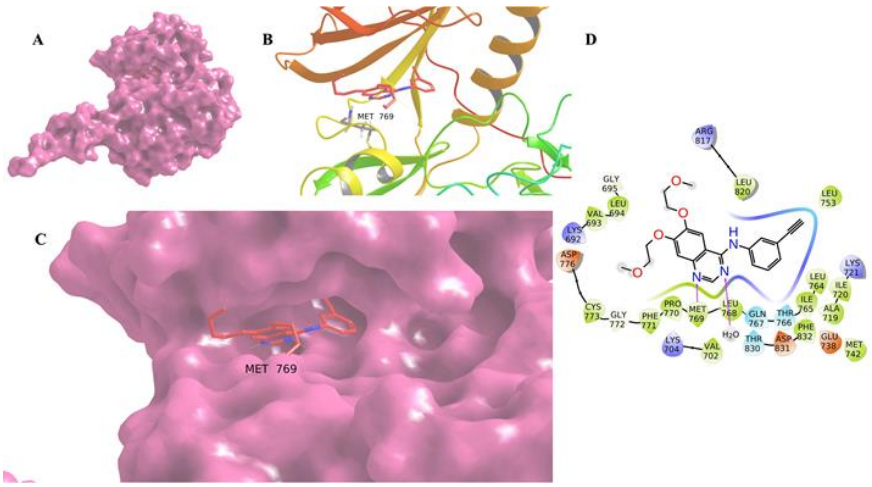


**Figure 1.** A) The best docking conformation of the Erlotinib ligand in the binding pocket of EGFR protein, B) Erlotinib-EGFR interaction in ribbons representation, C) Amino-acid contact of EGFR in EGFR- Erlotinib receptor-ligand interaction, D) 2D visualization of the interactions between EGFR and Erlotinib ligand.

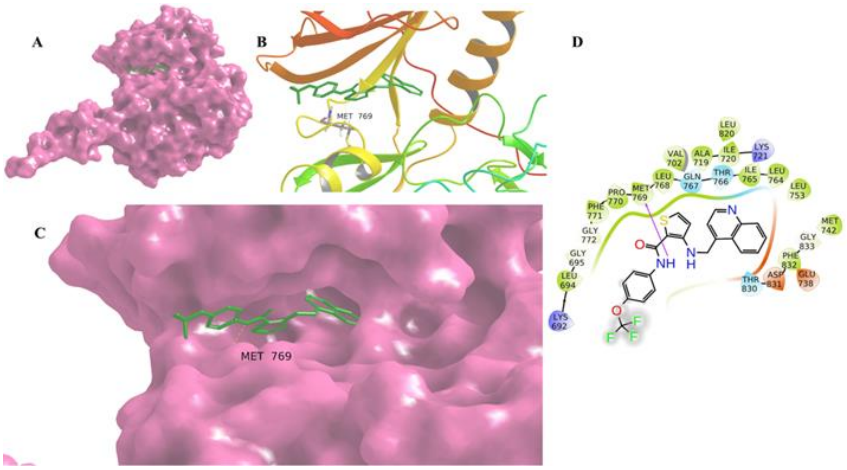

Figure 2. A) The best docking conformation of the OSI930 ligand in the binding pocket of EGFR protein, B) OSI930-EGFR interaction in ribbons representation, C) Amino-acid contact of EGFR in EGFR-OSI930 receptor-ligand interaction, D) 2D visualization of the interactions between EGFR and OSI930 ligand.

The complexes belonging to the best docking poses of the selected molecules were subjected to 100ns MD simulations to observe long-term protein-ligand interactions for detailed analyses (Agarwal et al., 2022). During the simulation, 100 frames were obtained, and the binding free energies of these frames were calculated with Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) analysis. MD-MM/GBSA analysis, an advanced method that uses molecular mechanics, is based on the calculation of the binding free energy change of the complex formed by small molecule drug candidates with the target biological macromolecule. This method evaluates the interactions of compounds with the target protein from a more detailed and dynamic perspective after molecular dynamics (MD) simulations. MM/GBSA analyzes were performed with the Prime module of the Maestro Molecular Modeling program (Palanivel et al., 2022).

**Table 1.** Docking (kcal/mol) and MM/GBSA (kcal/mol) scores of thiophene derivatives. Molecules are ranked from strongest MM/GBSA score to weakest

| Complex | Docking (kcal/mol) | 100 ns MD – MM/GBSA (kcal/mol) |
|---|---|---|
| OSI930 | -6.14 | -65.81 |
| Erlotinib (REF) | -8.21 | -64.72 |
| Neltenexine | -6.04 | -49.53 |
| Tenonitrozole | -5.27 | -41.95 |
| Suprofen | -5.48 | -34.79 |
| Cliprofen | -5.40 | -29.39 |
| Tenosal | -6.06 | -25.12 |

According to MM/GBSA scores, the binding free energy of Erlotinib is -64.72 kcal/mol. Remarkably, OSI930 emerged as the best candidate compound with a binding free energy of -65.81 kcal/mol, even outperforming the reference molecule.

Neltenexine was the second-best drug candidate compound with a binding free energy of -49.53 kcal/mol MM/GBSA. Tenonitrozole was also one of the compounds that showed strong binding and high stability with -41.95 kcal/mol. Suprofen, Cliprofen and Tenosal MM/GBSA scores were determined as -34.79, -29.39 and -25.12 kcal/mol, respectively (Table 1). In the MM/GBSA analysis, the cut-off value was determined as -40.00 kcal/mol and compounds with better binding affinity were examined in further studies as potential EFGR inhibitor drug candidates.

The amino acids with which the reference drug Erlotinib interacts for 100 nanosecond (ns) and the type of these interactions were analyzed in detail. As the most important interaction, it was observed that Erlotinib continuously interacted with Met769 throughout the entire simulation period, thanks to H bonds. Leu694, Ala719, Leu820 were included as significant hydrophobic interactions. It formed salt bridges with Thr766, Cys773, Asp776 and Thr830. In the light of our results, it was determined that Leu694, Ala719, Thr766, Cys773, Asp776, Leu820, Leu830 residues, especially Met769, were the crucial amino acids that ensure the Erlotinib-EGFR interaction (Figure 3 and 4).

After determining the amino acids and bond structures that are important in EGFR binding via erlotinib, drug candidate target molecules were also subjected to the same analysis and how the proposed drug candidates interact with the EGFR receptor, with which bond type, over which amino acids and for how long they interact with this receptor, they were examined. It was determined that the compound OSI930, which had the best docking and MM/GBSA binding scores among the candidate compounds, interacted with Met769, the most important amino acid for EGFR interaction, similar to the known EGFR inhibitor Erlotinib. The crucial amino acids that provide OSI930-EGFR interaction were determined to be Leu694, Val702, Ala719, Lys721, Met769, Leu820. While it formed hydrogen bonds with Met769, its interactions with other amino acids were highly hydrophobic interactions. These hydrophobic interactions were also observed in the study of Chunaifah et al. (2024).

In addition, in our study, salt bridges with Cys773, Asp776 and Thr830 were also observed, similar to the reference drug, although not as strong as that of the reference drug used. The amino acids with which the prominent interactions occurred in our study are common with the key amino acids determined for Erlotinib and candidate drugs as a result of docking and simulation in the study of Eldehna et al (2022) (Eldehna et al., 2022). Saini et al. (2022) (Saini et al., 2022) also stated that EGFR interacts with Erlotinib via hydrogen bonding via Met769, which has a bond distance of 2.70 Å, and noted the importance of hydrophobic interactions with amino acids Leu694, Ala719, Lys721, Leu764 and Leu820. Studies by Yang et al. (2020) (Yang et al., 2020) have also indicated that Met769 has a key role in EGFR binding.

It is an important point that the binding patterns of different compounds may be slightly different from each other, which is responsible for the activity variations, but in general, inhibitors such as Erlotinib form a hydrogen bond with the NH backbone of Met769, especially in the hinge region. Moreover, often these compounds are deeply embedded in the EGFR through conserved hydrophobic interactions (Nasab et al., 2018). Our results support that the best compound, OSI930, which we claim has the potential to be an anti-cancer drug, is observed to be embedded in the EGFR, similar to Erlotinib, by forming a hydrogen bond with the NH backbone of Met769 in the hinge region and thanks to the hydrophobic interactions it carries out.

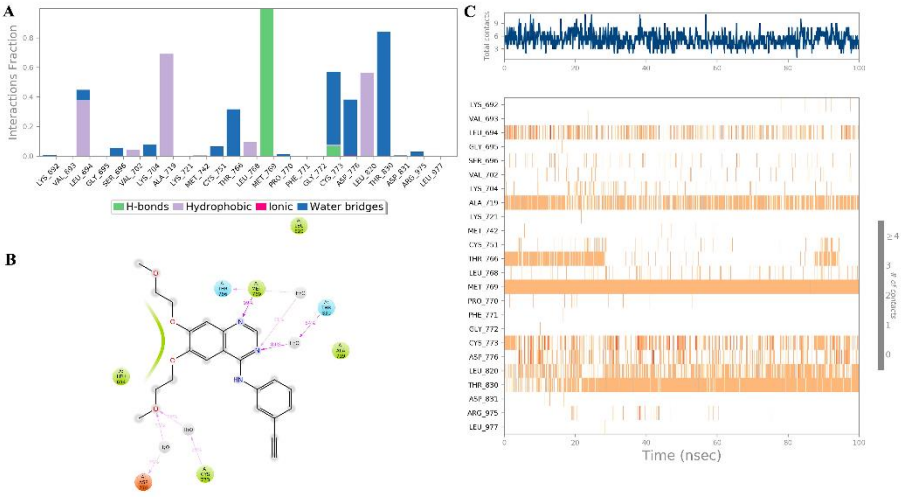


**Figure 3.** A) The type of interactions that occur between the Erlotinib ligand and the EGFR target during the MD simulation. B) 2D interaction analysis of the Erlotinib in the binding pocket of the protein. C) Time-dependent analysis of the Erlotinib-EGFR contact residues throughout 100 ns simulation.

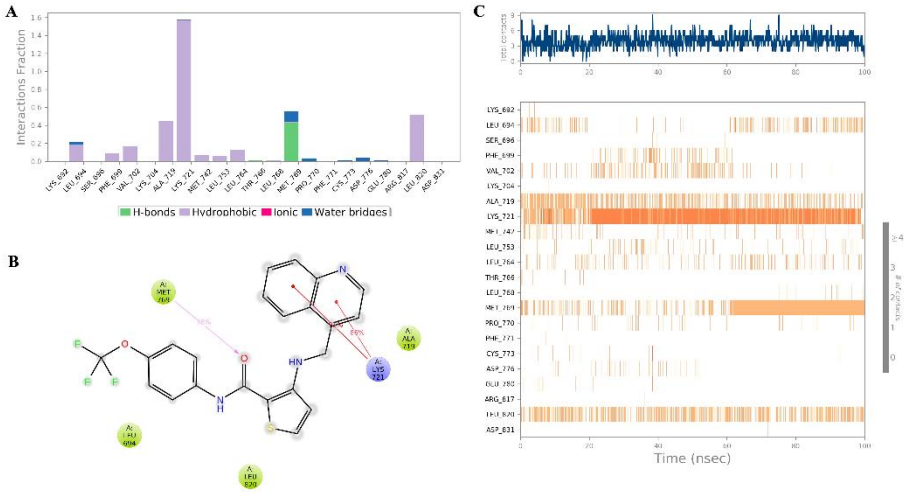


**Figure 4.** A) The type of interactions that occur between the OSI930 ligand and the EGFR target during the MD simulation. B) 2D interaction analysis of the OSI930 in the binding pocket of the protein. C) Time-dependent analysis of the OSI930-EGFR contact residues throughout 100 ns simulation.

After these studies, the ADME and anti-cancer properties of the reference molecule and selected drug candidate molecules were predicted in Clarivate analytics' MetaCore/MetaDrug platform for more detailed analysis (Celik et al., 2022). For this purpose, machine learning-based ADME and cancer therapeutic dual Quantitative structure–activity relationship (QSAR) models were used. Therapeutic activity values obtained from MetaCore Clarivate estimate the potential anti-cancer effects of the compounds. If the scores of compounds are higher than 0.5, it means that those compounds are predicted to show strong therapeutic potential and efficacy. Cancer therapeutic activity QSAR model predicted Erlotinib as a drug candidate that may have highly anti-cancer effects. This confirmed the accuracy and reliability of our studies and forecasting method. When the results are examined, the QSAR models we used expect Tenonitrozole to have a very high anti-cancer effect, and similarly, OSI930 and Neltenexine compounds were predicted by machine learning-based method to be good drug candidates in cancer. Cliprofen, Suprofen and Tenosal compounds, which were at the bottom of the MM/GBSA analysis, were found to be weak in terms of anti-cancer activity at this step (Table 2).

The novel small-molecule OSI-930 is a multi-kinase inhibitor which effectively targets vascular endothelial growth factor receptor 2 (VEGFR2)/kinase splicing domain receptor (KDR), platelet-derived growth factor receptor (PDGFR) and c-Kit (Garton et al., 2006). First-in-human phase I study was conducted to evaluate safety, maximum-tolerated dose (MTD), pharmacokinetics, pharmacodynamics, and antitumor activity of OSI-930 in

patients with advanced solid tumors and it was found that OSI-930 is well tolerated with clinically significant antitumor effects (Yap et al., 2013). However, there is no previous study showing that it may exert its effect by binding to the EGFR target. Although our study supports the literature and phase 1 studies, it is important in terms of showing the target it binds to, simulation of the interactions that occur, the type and duration of the interactions, and also the detection of important amino acids involved in the interaction.

It has been suggested that the co-inhibition of VEGF and EGFR, which are two key targets in cancers that have independent but highly interdependent interactions with each other, is important in cancer treatment and overcoming resistance to cancer (Tabernero, 2007). Macpherson et al. (2013) (Macpherson et al., 2013) study shows that the combined use of OSI-930 and erlotinib, a selective EGFR kinase inhibitor, has a synergistic effect. While finding the maximum tolerated dose of OSI-930 that could be combined with erlotinib. Discovering that OSI-930, which is primarily a VEGF inhibitor, also inhibits the EGFR protein underscores a crucial result for cancer therapy. In conclusion, our study emphasizes the importance of OSI-930 as a dual inhibitor that can target both EGFR and VEGF pathways and reveals its potential as a promising therapeutic option for cancer treatment (Falchook and Kurzrock, 2015; Wang et al., 2023).

Neltenexine, an elastase inhibitor, is a mucolytic agent that may be effective against pulmonary disease (Cattaneo, 2001). However, literature information about Neltenexine is limited. In-depth biochemical analyses are needed (Braga et al., 1995). Moreover, to date, no docking simulation studies have been conducted on Neltenexine. Therefore, its potential effects and mechanism of action need to be investigated in more detail. Our research provides an innovative perspective in that the Neltenexine molecule is docked for the first time and proposed as a repurposed EGFR inhibitor for the first time in this study.

Tenonitrozole, an antiprotozoal therapeutic agent approved by European regulatory authorities, prescribed primarily against urogenital trichomoniasis (Lynch et al., 2019; Guo et al., 2023). Tenonitrozole has not been previously investigated in cancer research. Our study pioneers the investigation to uncover its potential as an anti-cancer drug candidate by highlighting its EGFR inhibitory activity.

**Table 2.** Prediction of cancer therapeutic activities of selected candidate drug molecules. Tanimato prioritization (TP) values are given in parentheses, indicating the similarity of the analyzed structure to the most similar compound in the training set.

| Name | MW | Rule Of 5 | RBN | HBA | HBD | Reactive | Cancer (TP) |
|---|---|---|---|---|---|---|---|
| Reference Drug (Erlotinib) | 393.443 | OK | 11 | 5 | 1 | OK | 0.86 (100.00) |
| Tenonitrozole | 255.27 | OK | 4 | 4 | 1 | R | 0.83 (34.05) |
| OSI930 | 443.44 | OK | 8 | 4 | 2 | OK | 0.79 (100.00) |
| Neltenexine | 489.25 | OK | 6 | 3 | 4 | R | 0.65 (52.11) |
| Cliprofen | 293.74 | OK | 4 | 3 | 0 | OK | 0.29 (46.34) |
| Suprofen | 259.3 | OK | 4 | 3 | 0 | OK | 0.28 (40.97) |
| Tenosal | 247.24 | OK | 4 | 4 | 0 | OK | 0.17 (41.95) |

1 Formula Molecular formula.
2 HBA Number of hydrogen bond acceptors.
3 HBD Number of hydrogen bond donors.
4 MW Molecular weight.
5 RBN Number of rotatable bonds.
6 Reactive Identify reactive groups in molecules. OK means that the metabolite does not contain spontaneously reactive groups, R means it does. MetaDrug currently includes 89 rules to predict likely reactive metabolites such as quinones, aromatic and hydroxyl amines, acyl glucuronides, acyl halides, nepoxides, thiophene-S-oxides, furans, phenoxyl radicals, phenols, and aniline radicals.
7 RuleOf5 Lipinski rule of five can be used to indicate whether a molecule is likely to be orally bioavailable. A molecule should not have more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, a molecular weight under 500, and a partition coefficient log P under 5. Rule of five-compliant molecules are marked as OK, non-compliant as Poor. Reference: Lipinski, et al., 2001 (PMID: 11259830).
8 Cancer Potential activity against cancer. Cutoff is 0.5. Values higher than 0.5 indicate potentially active compounds. Training set consists of approved drugs. Model description: Training set N=886, Test set N=167, Sensitivity= 0.89, Specificity=0.83, Accuracy=0.86, MCC=0.72. Reference: Clarivate Analytics.

## 4. Conclusion

In recent years, computer-based cancer candidate drug development research has gained momentum. In this study, molecules with the "thiophene scaffold", which is claimed to have a key role in cancer, were scanned from the literature and docked against the EGFR target, whose inhibition is known to be necessary in cancer in many publications. The duration, type and stability of the interactions between the drug candidates and the target protein, which were found to bind strongly with high affinity according to the docking score, and the important amino acid residues that play a role in these interactions were examined in detail with long-term MD simulations and mmgbsa analyses. Adme properties and anticancer activity of selected drugs were predicted with Machine Learning-based binary QSAR Models. In the light of the results, three candidate molecules were found to be

EGFR inhibitor drugs: OSI930, Tenonitrozole and Neltenexine. Upon evaluating MMGBSA calculations, concerning both binding free energy and binding stability, OSI930 emerges as the best candidate among these candidate molecules. Our research highlights the high potential of OSI930 as a dual inhibitor effectively targeting both EGFR and VEGF pathways, providing a promising strategy for cancer treatment. Furthermore, we expand the scope of potential therapeutics in cancer treatment by introducing Tenonitrozole and Neltenexine as novel EGFR inhibitor candidates. This research holds significance in providing novel inhibitor candidates against EGFR and providing guiding future studies. Future research should focus on performing extensive in vitro and in vivo experiments to validate the effectiveness and safety of these candidate molecules.

**Author Contributions**

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

| | P.S. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflicts of interest**

The author declare that there are no conflicts of interest regarding the publication of this research.

**Ethical approval**

No ethical approval was required for this research as no studies were conducted involving animals or humans

**References**


Agarwal G, Hajra A, Chakraborty S, Patel N, Biswas S, Adler MK, Lavie CJ. 2022. Predictors and mortality risk of venous thromboembolism in patients with COVID-19: systematic review and meta-analysis of observational studies. Ther Adv Cardiovasc Dis, 16: 17539447221105012.

Bas DC, Rogers DM, Jensen JH. 2008. Very fast prediction and rationalization of pKa values for protein–ligand complexes. Proteins, 73(3): 765-783.

Berdigaliyev N, Aljofan M. 2020. An overview of drug discovery and development. Future Med Chem, 12(10): 939-947.

Bowers KJ, Chow E, Xu H, Dror RO, Eastwood MP, Gregersen BA, Sacerdoti FD. 2006. Scalable algorithms for molecular dynamics simulations on commodity clusters. In: Proc. 2006 ACM/IEEE Conf. Supercomput, November 11-17, New York, NY, United States, pp: 84.

Braga PC, Piatti G, Limoli A, Dal Sasso M, Maci S. 1995. Neltenexine: morphological investigation of protection against elastase-induced emphysema in rats. Drugs Exp Clin Res, 21(2): 51-57.

Cattaneo C. 2001. Neltenexine tablets in smoking and non-smoking patients with COPD. A double-blind, randomised, controlled study versus placebo. Minerva Med, 92(4): 277-284.

Celik I, Ayhan-Kılcıgil G, Karayel A, Guven B, Onay-Besikci A. 2022. Synthesis, molecular docking, in silico ADME, and EGFR kinase inhibitor activity studies of some new benzimidazole derivatives bearing thiosemicarbazide, triazole, and thiadiazole. J Heterocycl Chem, 59(2): 371-387.

Chunaifah I, Venilita RE, Tjitda PJP, Astuti E, Wahyuningsih TD. 2024. Thiophene-based N-phenyl pyrazolines: Synthesis, anticancer activity, molecular docking and ADME study. J Appl Pharm Sci, 14(4): 63-71.

Collaboration GB of DC. 2019. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. JAMA Oncol, 5(12): 1749-1768. doi:10.1001/jamaoncol.2019.2996

da Silva Santos E, Nogueira KAB, Fernandes LCC, Martins JRP, Reis AVF, Neto JdeBV, Eloy JO. 2021. EGFR targeting for cancer therapy: Pharmacology and immunoconjugates with drugs and nanoparticles. Int J Pharm, 592: 120082.

Durdagi S. 2020. Virtual drug repurposing study against SARS-CoV-2 TMPRSS2 target. Turk. J. Biol., 44(7): 185-191.

Ekins S, Bugrim A, Brovold L, Kirillov E, Nikolsky Y, Rakhmatulin E, Melnikov A. 2006. Algorithms for network analysis in systems-ADME/Tox using the MetaCore and MetaDrug platforms. Xenobiotica, 36(10-11): 877-901.

Eldehna WM, El Hassab MA, Elsayed ZM, Al-Warhi T, Elkady H, Abo-Ashour MF, Abdel-Aziz HA. 2022. Design, synthesis, in vitro biological assessment and molecular modeling insights for novel 3-(naphthalen-1-yl)-4, 5-dihydropyrazoles as anticancer agents with potential EGFR inhibitory activity. Sci Rep, 12(1): 12821.

Evans DJ, Holian BL. 1985. The nose–hoover thermostat. J Chem Phys, 83(8): 4069-4074.

Falchook GS, Kurzrock R. 2015. VEGF and dual-EGFR inhibition in colorectal cancer. Cell Cycle, 14(8): 1129-1130.

Gagic Z, Ruzic D, Djokovic N, Djikic T, Nikolic K. 2020. In silico methods for design of kinase inhibitors as anticancer drugs. Front Chem, 7: 873.

Garton AJ, Crew APA, Franklin M, Cooke AR, Wynne GM, Castaldo L, Brown EN. 2006. OSI-930: a novel selective inhibitor of Kit and kinase insert domain receptor tyrosine kinases with antitumor activity in mouse xenograft models. Cancer Res, 66(2): 1015-1024.

Gramec D, Peterlin Mašič L, Sollner Dolenc M. 2014. Bioactivation potential of thiophene-containing drugs. Chem Res Toxicol, 27(8): 1344-1358.

Guo M, Yu X, Zhu YZ, Yu Y. 2023. From bench to bedside: What do we know about imidazothiazole derivatives so far? Molecules, 28(13): 5052.

Harmanen M, Klaavuniemi T, Sorigue M, Khan M, Prusila R, Kari

E, Jukkola A. 2023. Real-world Data: MCL2 protocol demonstrates excellent treatment results among patients with mantle cell lymphoma not fulfilling the original trial inclusion criteria. HemaSphere, 7(10): e954.

Jacobson MP, Pincus DL, Rapp CS, Day TJF, Honig B, Shaw DE, Friesner RA. 2004. A hierarchical approach to all-atom protein loop prediction. Proteins, 55(2): 351-367.

Jamal S, Goyal S, Shanker A, Grover A. 2015. Checking the STEP-associated trafficking and internalization of glutamate receptors for reduced cognitive deficits: a machine learning approach-based cheminformatics study and its application for drug repurposing. PLoS One, 10(6): e0129370.

Jones W, Tait D, Livasy C, Ganapathi M, Ganapathi R. 2022. PLK3 amplification and tumor immune microenvironment of metastatic tumors are linked to adjuvant treatment outcomes in uterine serous cancer. NAR Cancer, 4(3): zcac026.

Kuchana V, Kashetti V, Tangeda SJ, Manga V. 2022. Design, synthesis and molecular docking study of thiophenyl hydrazone derivatives as tubulin polymerization inhibitors. Synth Commun, 52(21): 2029-2047.

Lynch C, Mackowiak B, Huang R, Li L, Heyward S, Sakamuru S, Xia M. 2019. Identification of modulators that activate the constitutive androstane receptor from the Tox21 10K compound library. Toxicol Sci, 167(1): 282-292.

Macpherson IR, Poondru S, Simon GR, Gedrich R, Brock K, Hopkins CA, Evans TRJ. 2013. A phase 1 study of OSI-930 in combination with erlotinib in patients with advanced solid tumours. Eur J Cancer, 49(4): 782-789.

Madhavi Sastry G, Adzhigirey M, Day T, Annabhimoju R, Sherman W. 2013. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aided Mol Des, 27: 221-234.

Martyna GJ, Tobias DJ, Klein ML. 1994. Constant pressure molecular dynamics algorithms. J Chem Phys, 101(5): 4177-4189.

Masuda T, Tsuruda Y, Matsumoto Y, Uchida H, Nakayama KI, Mimori K. 2020. Drug repositioning in cancer: The current situation in Japan. Cancer Sci, 111(4): 1039-1046.

Muegge I, Rarey M. 2001. Small molecule docking and scoring. Rev Comput Chem, 17: 1-60.

Nasab RR, Mansourian M, Hassanzadeh F, Shahlaei M. 2018. Exploring the interaction between epidermal growth factor receptor tyrosine kinase and some of the synthesized inhibitors using combination of in-silico and in-vitro cytotoxicity methods. Res Pharm Sci, 13(6): 509-522.

Nehra B, Mathew B, Chawla PA. 2022. A medicinal chemist's perspective towards structure activity relationship of heterocycle based anticancer agents. Curr Top Med Chem, 22(6): 493-528.

Palanivel S, Yli-Harja O, Kandhavelu M. 2022. Molecular interaction study of novel indoline derivatives with EGFR-kinase domain using multiple computational analysis. J Biomol Struct Dyn, 40(16): 7545-7554.

Pramesh CS, Badwe RA, Bhoo-Pathy N, Booth CM, Chinnaswamy G, Dare AJ, Gospodarowicz M. 2022. Priorities for cancer research in low-and middle-income countries: a global perspective. Nat Med, 28(4): 649-657.

Roos K, Wu C, Damm W, Reboul M, Stevenson JM, Lu C, Wang L. 2019. OPLS3e: Extending force field coverage for drug-like small molecules. J Chem Theory Comput, 15(3): 1863-1874.

Saini N, Grewal AS, Lather V, Gahlawat SK. 2022. Natural alkaloids targeting EGFR in non-small cell lung cancer: Molecular docking and ADMET predictions. Chem Biol Interact, 358: 109901.

Shaker B, Ahmad S, Lee J, Jung C, Na D. 2021. In silico methods and tools for drug discovery. Comput Biol Med, 137: 104851.

Shelley JC, Cholleti A, Frye LL, Greenwood JR, Timlin MR, Uchimaya M. 2007. Epik: a software program for pKa prediction and protonation state generation for drug-like molecules. J Comput Aided Mol Des, 21: 681-691.

Sibuh BZ, Gupta PK, Taneja P, Khanna S, Sarkar P, Pachisia S, Singh SK. 2021. Synthesis, in silico study, and anti-cancer activity of thiosemicarbazone derivatives. Biomedicines, 9(10): 1375.

Siyah P, Durdagi S, Aksoydan B. 2023. Discovery of potential PD-L1 small molecule inhibitors as novel cancer therapeutics using machine learning-based QSAR models: A virtual drug repurposing study. Biophys J, 122(3): 144.

Stillman NR, Kovacevic M, Balaz I, Hauert S. 2020. In silico modelling of cancer nanomedicine, across scales and transport barriers. NPJ Comput Mater, 6(1): 92.

Sun M, Wang T, Li L, Li X, Zhai Y, Zhang J, Li W. 2021. The application of inorganic nanoparticles in molecular targeted cancer therapy: EGFR targeting. Front Pharmacol, 12: 702445.

Tabernero J. 2007. The role of VEGF and EGFR inhibition: implications for combining anti–VEGF and anti–EGFR agents. Mol Cancer Res, 5(3): 203-220.

Tian X, Gu T, Lee M-H, Dong Z. 2022. Challenge and countermeasures for EGFR targeted therapy in non-small cell lung cancer. Biochim Biophys Acta Rev Cancer, 1877(1): 188645.

Turanli B, Altay O, Borén J, Turkez H, Nielsen J, Uhlen M, Mardinoglu A. 2021. Systems biology based drug repositioning for development of cancer therapy. Semin Cancer Biol, 68: 47-58.

Türkmenoğlu B. 2022. Investigation of novel compounds via in silico approaches of EGFR inhibitors as anticancer agents. J Indian Chem Soc, 99(8): 100601.

Turnham DJ, Bullock N, Dass MS, Staffurth JN, Pearson HB. 2020. The PTEN conundrum: how to target PTEN-deficient prostate cancer. Cells, 9(11): 2342.

Uribe ML, Marrocco I, Yarden Y. 2021. EGFR in cancer: Signaling mechanisms, drugs, and acquired resistance. Cancers (Basel), 13(11): 2748.

Vallan L, Istif E, Gómez IJ, Alegret N, Mantione D. 2021. Thiophene-based trimers and their bioapplications: an overview. Polymers (Basel), 13(12): 1977.

Wang Q, Zeng A, Zhu M, Song L. 2023. Dual inhibition of EGFR VEGF: An effective approach to the treatment of advanced non small cell lung cancer with EGFR mutation. Int J Oncol, 62(2): 1-10.

Weth FR, Hoggarth GB, Weth AF, Paterson E, White MPJ, Tan ST, Gray C. 2024. Unlocking hidden potential: advancements, approaches, and obstacles in repurposing drugs for cancer therapy. Br J Cancer, 130(5): 703-715.

Yang X, Hou Z, Wang D, Mou Y, Guo C. 2020. Design, synthesis and biological evaluation of novel heptamethine cyanine dye-erlotinib conjugates as antitumor agents. Bioorg Med Chem Lett, 30(23): 127557.

Yap TA, Arkenau HT, Camidge DR, George S, Serkova NJ, Gwyther SJ, Desouza NM. 2013. First-in-human phase I trial of two schedules of OSI-930, a novel multikinase inhibitor, incorporating translational proof-of-mechanism studies. Clin Cancer Res, 19(4): 909-919.

Zhang L, Lu Z, Zhao X. 2021. Targeting Bcl-2 for cancer therapy. Biochim Biophys Acta Rev Cancer, 1876(1): 188569.

Zhou C, Wu Y-L, Chen G, Feng J, Liu X-Q, Wang C, Ren S. 2011. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-

label, randomised, phase 3 study. Lancet Oncol, 12(8): 735-742.