The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2023

Volume 9, Pages 14-20

ICGeHeS 2023: International Conference on General Health Sciences

Interaction of Wild Type and V804l and V804m-Mutated Ret Protein Kinase with Emodin: *In Silico* Approach

Emel Akbaba
Kirikkale University

Deniz Karatas
Manisa Celal Bayar University

Abstract: Medullary thyroid carcinoma (MTC) is a malignant endocrine tumor originating from parafollicular calcitonin-producing C cells. The proto-oncogene RET (REarranged during Transfection) is known as the main actor in the development and outbreak of MTC. Gain of function mutations of RET constitutively activate the receptor which are found to be responsible for the high percentage of MTC cases. The two FDA-approved drugs used for MTC, vandetanib and cabozantinib, are resistant to two mutant variants of RET which are V804L and V804M. In this study, the interactions of emodin, a natural molecule found in plants, with wild-type as well as V804L and V804M-mutated RET kinase were investigated via molecular docking. Pymol was used to create point mutations on wild type RET. Vandetanib and cabozantinib were used as the reference drugs. The binding free energy of vandetanib with wild-type RET, V804L and V804M variants were found to be -9.3, -9.1 and -8.6 kcal/mol. Similarly, the binding free energy of cabozantinib with wild-type RET, V804L and V804M variants were found to be -10.6, -10.4, and -9.5 kcal/mol, respectively. Clearly, the binding affinity of vandetanib and cabozantinib to RET kinase was found to be reduced in mutated variants as compared to wild type. In the meantime, the binding energy between emodin and wild-type RET was shown to be -9.3 kcal/mol. Interestingly, the binding affinity of emodin to V804L and V804M variants was determined to be increased (-9.9 and -9.8 kcal/mol, respectively) compared to wild type. Furthermore, many H-bonds and hydrophobic interactions between emodin and mutated RET variants were shown. Therefore, strong binding affinity of emodin to wild-type and the mutated variants of RET was suggested in this study. In conclusion, emodin was found to be a potential molecule to inhibit RET kinase activity and could be used as a therapeutical agent against medullary thyroid carcinoma.

Keywords: Medullary thyroid carcinoma, RET, V804L, V804M, Molecular docking

Introduction

Thyroid cancer is one of the most common cancers worldwide, affecting people in both developing and developed countries, with an incidence of 600,000 new cases diagnosed annually (Jayasinghe et al., 2022). Medullary thyroid cancer (MTC) is a rare cancer that arises from the neuroendocrine parafollicular C-cells of the thyroid gland comprising up to 3% of all thyroid cancers. However, MTC is associated with high mortality, with a disproportionate rate of 8.6% of thyroid cancer-related deaths (Wells et al. 2015). MTC can occur in a sporadic form and in a hereditary form, associated with multiple endocrine neoplasia (MEN) type 2, accounting for 75% and 25%, respectively (Kim & Kim, 2021). RET (REarranged during Transfection) proto-oncogene which is a protein tyrosine kinase, is the main oncogenic driver of MTC. As all hereditary MTC harbor a germline RET mutations and almost half of sporadic cases have a somatic RET mutation (Jaber, Dadu, and Hu 2021). RET mutations are associated with more aggressive disease in MTC. Furthermore, angiogenesis is more intense in RET-mutant MTC. The RET proto-oncogene is located on the long arm of chromosome 10 (10q11.2) and encodes the RET tyrosine kinase transmembrane receptor which is a 170-kDa protein monomer (Vodopivec

& Hu, 2022). RET is constitutively activated through two distinct mechanisms: mutations involving the cysteine-rich or kinase domains, and structural rearrangements leading to the fusion of RET to a 5′ upstream partner. Collectively, these alterations result in ligand-independent signaling and oncogenesis (Wirth et al., 2020). The physiologic signaling process of RET receptor starts with the binding of growth factors to a coreceptor, which in turn causes RET dimerization and phosphorylation of the intracellular kinase domain. This leads to the activation of RAS/ MAPK and PI3K/AKT pathways, involved in cell growth, proliferation, differentiation, survival, and migration (Ibáñez, 2013).

The kinase domain of RET receptor consists of an N-terminal lobe and a C-terminal lobe connected via a hinge. The N-terminal lobe consists of ß-sheets, whereas the C-terminal lobe contains α-helices. The catalytic cleft is located between the N-terminal lobe and the C-terminal lobe which is the focus of kinase inhibitor development. Val804 is the gatekeeper residue controlling access to the catalytic pocket (van Linden et al., 2014).

To date, only two multiple kinase inhibitors (MKIs) have been approved by the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) for the treatment of advanced MTC: Vandetanib and cabozantinib (Matrone et al. 2022). The IC50 values of vandetanib for the V804L and V804M variants are 3597 and 726 nM. However, the IC50 values of cabozantinib for V804L and V804M variants are 45 and 162 nM (Matrone et al. 2022). Unfortunately, vandetanib and cabozantinib are resistant to the V804L and V804M variants (La Pietra et al. 2018). MKIs generally are ineffective against RET V804 gatekeeper mutations (Subbiah et al., 2021).

Emodin, which is also known as 1,3,8 trihydroxy 6 methy anthraquinone, can be isolated from a number of medicinal herbs. Various studies have demonstrated that emodin inhibits growth in multiple cancer types, including lung and pancreatic cancer, and hepatocellular carcinoma. Emodin has been also reported to inhibit the proliferation of papillary thyroid cancer cells via activating AMPK pathway activity (Li et al., 2021). However, no studies reported the effect of emodin on RET kinase activity. Therefore, in this study we aimed to investigate the interaction of emodin with RET protein in its wild-type as well as V804L and V804M mutated variants via molecular docking analysis.

Method

In this study, RET protein kinase, the receptor molecule was taken from RCSB PDB databank (PDB ID: 2IVV) and ligand molecules, emodin (PubChem CID: 3220), vandetanib (PubChem CID: 3081361) and cabozantinib (PubChem CID: 25102847) were taken from PubChem databank. The 3D structures of the receptor and ligand molecules are displayed in Figure 1. The mutated RET variants were created via Pymol.

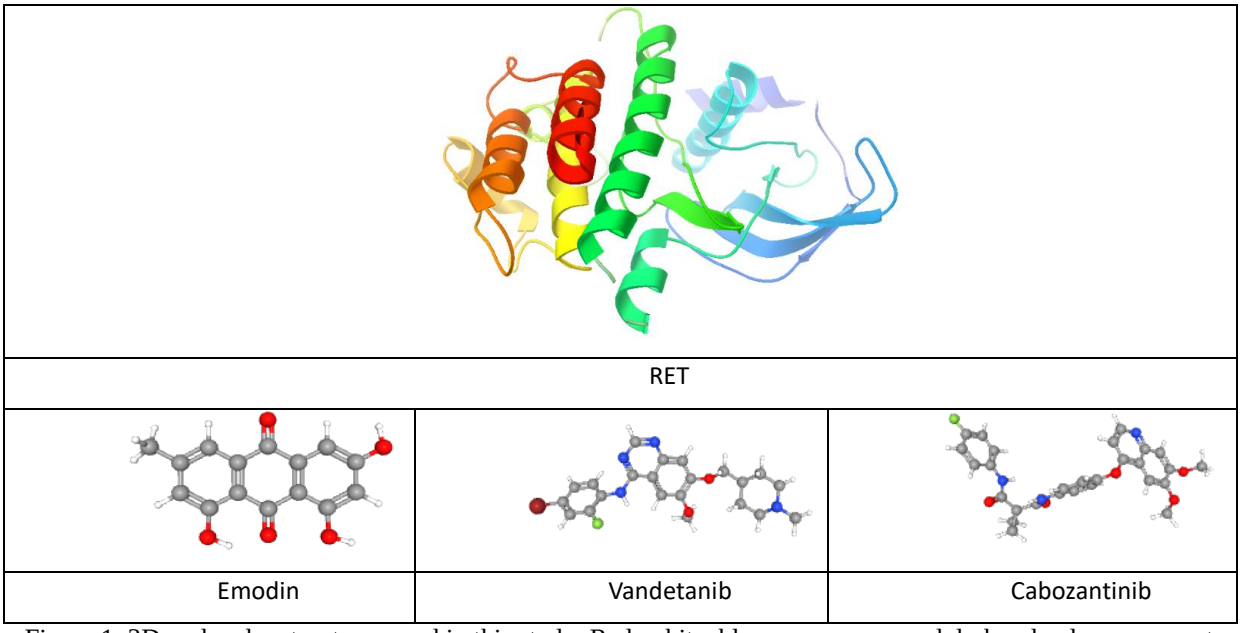

Figure 1. 3D molecular structures used in this study. Red, white, blue, green, gray and dark red colors represent oxygen (O), hydrogen (H), nitrogen (N), fluorine (F), carbon (C) and bromine (Br) atoms, respectively.

Molecular docking was performed to explain the molecular interactions between the receptor and the ligands in Figure 1. Amino acids belonging to the catalytic region in the literature were taken as Leu730, Gly731, Val738, Ala756, Lys758, Ile788, Leu802, Ile803, Val804, Glu805, Tyr806, Ala807, Gly810, Leu881, Ser891, and Asp892. The geometric centers and xyz coordinates were calculated as 20,823, 6,794 and 10,789 Å, respectively, with the help of the AGFR program (Zhang et al., 2019). The grid box sizes were taken as 50 each, taking into account the volume occupied by these coordinates and ligands in the AutoDock tools visualizer (Morris et al. 2009). Based on these initial parameters, energy range 4 was selected and docking studies were carried out using the AutoDock Vina program (Trott & Olson 2010). These steps were performed exactly for each receptor and ligands and all parameters were taken as the same. Online Swissadme Pharmacokinetics Prediction Property was used [http://www.swissadme.ch/index.php] to predict ADME features of emodin.

Results and Discussion

Molecular Interactions between Vandetanib and RET Protein Kinase

The binding free energy between vandetanib and wild-type RET was found to be -9.3 kcal/mol. Figure 2 shows the best conformation of vandetanib/wild-type RET complex. Interestingly, no H-bond interactions were found between vandetanib and wild-type RET. However, many hydrophobic interactions were observed between vandetanib and Ala807, Leu730, Leu881, Ala756, Val804, Glu805, Ser891, Asp892, Lys758, Glu775, Val738, Gly731 and Gly810 residues of wild-type RET kinase.

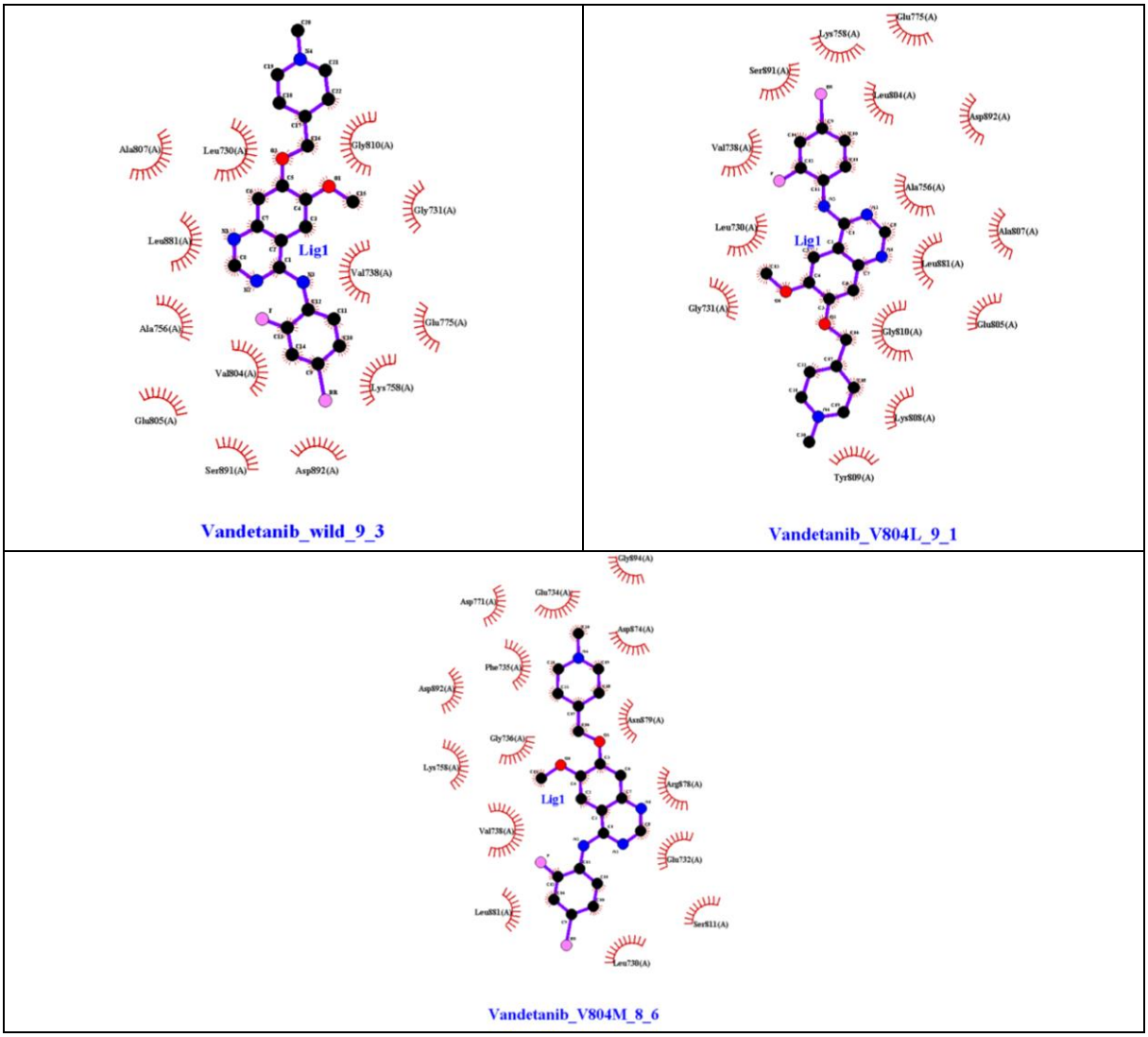

Figure 2. Molecular interactions between vandetanib and wild-type RET, V804L, and V804M.

The binding free energy between vandetanib and V804L was observed to be -9.1 kcal/mol. Like in vandetanib/wild-type RET complex, no-H bond interactions were determined between vandetanib and V804L variant. Nevertheless, hydrophobic interactions were shown between vandetanib and Glu775, Lys758, Ser891, Leu804, Asp892, Val738, Ala756, Ala807, Leu881, Leu730, Gly731, Gly810, Glu805, Lys808 and Tyr809 residues of V804L. The binding free energy between vandetanib and V804M was observed to be -8.6 kcal/mol. No H-bonds were observed between vandetanib and V804M; however, 15 hydrophobic interactions were shown in the vandetanib/V804M complex. The hydrophobic interactions were observed between vandetanib and Gly894, Glu734, Asp771, Asp874, Phe735, Asp892, Asn879, Gly736, Lys758, Val738, Arg878, Glu732, Leu881, Leu730, and Ser811 residues of V804M. These results show that no H-bond interactions were observed in the vandetanib/RET kinase complex with its wild-type or mutated forms. However, various hydrophobic interactions were determined in vandetanib/RET kinase complexes. The binding affinity of vandetanib to wild-type RET kinase was found to be stronger than to the mutated RET variants. These results are in accordance with the literature, indicating that vandetanib is resistant to V804L and V804M (La Pietra et al., 2018).

Molecular Interactions between Cabozantinib and RET Protein Kinase

The binding free energy between cabozantinib and wild-type RET was found to be -10.6 kcal/mol. Figure 3 shows the best conformation of cabozantinib/wild-type RET complex.

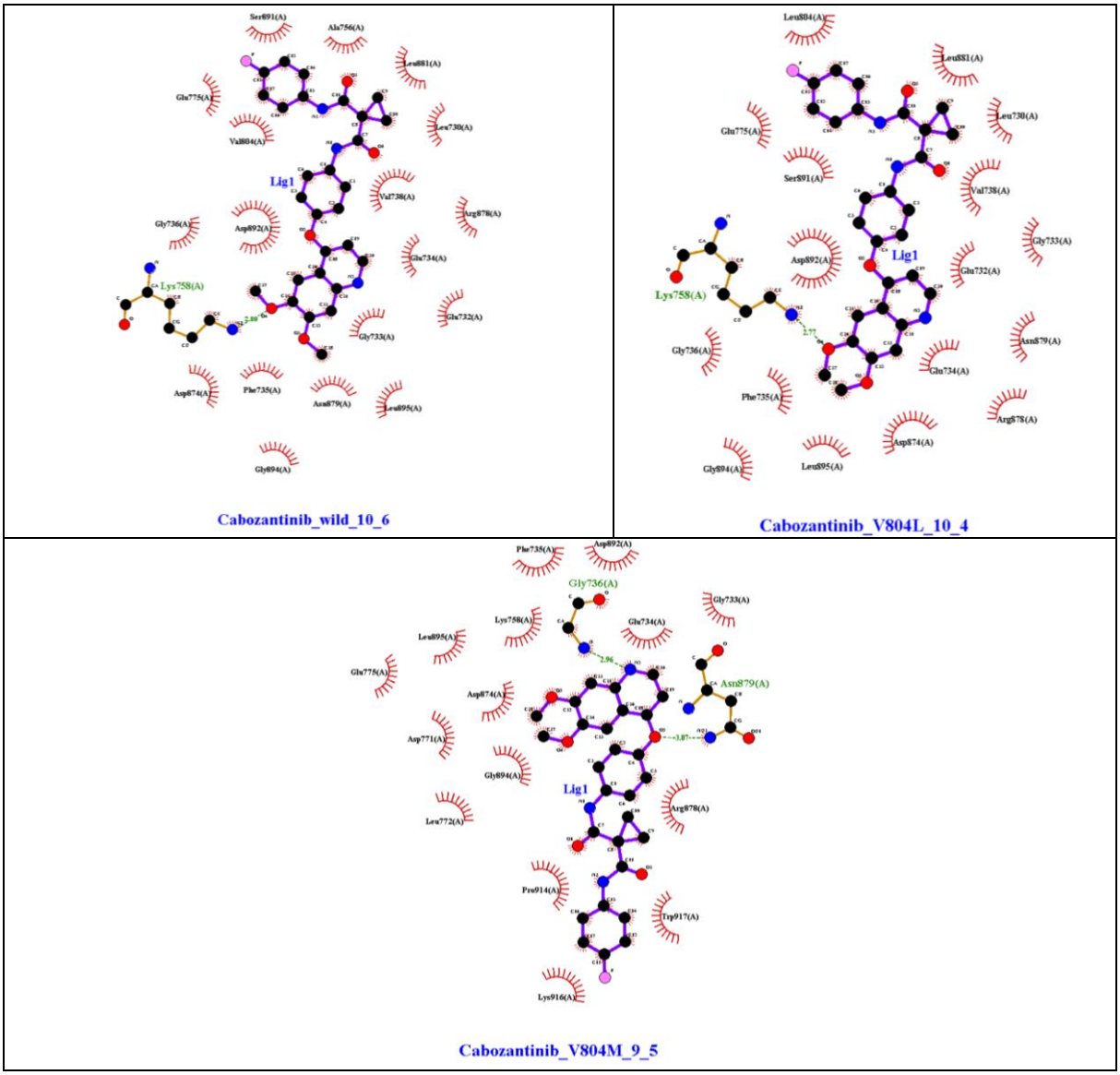

Figure 3. Molecular interactions between cabozantinib and wild-type RET, V804L, and V804M.

In Figure 3, only one H-bond interaction was shown between cabozantinib and Lys758 (2.80 A°) of wild-type RET. However, various hydrophobic interactions were determined between cabozantinib and Ser891, Ala756, Leu881, Leu730, Val738, Arg878, Glu734, Glu775, Val804, Asp892, Gly736, Glu732, Gly733, Asp874, Phe735, Asn879, Leu895, and Gly894 residues of wild-type RET. The free energy of binding in cabozantinib/V804L complex was found to be -10.4 kcal/mol. As in the cabozantinib/wild-type RET complex, only one H-bond (2.74 A°) was observed between cabozantinib and V804L. However, 17 hydrophobic interactions were determined with Gly736, Phe735, Gly894, Leu895, Asp874, Arg878, Glu734, Asn879, Glu732, Gly733, Val738, Leu730, Asp892, Ser891, Glu775, Leu881, Leu804 of V804L. The binding free energy between cabozantinib and V804M was found to be -9.5 kcal/mol. 2 H-bonds were shown in cabozantinib/V804M complex with Gly736 (2.94 A°) and Asn879 (3.07 A°). Hydrophobic interactions, on the other hand, were observed with Glu734, Gly733, Lys916, Asp892, Phe735, Trp917, Lys758, Leu895, Glu775, Asp874, Asp771, Pro914, Gly894, Leu772, and Arg878 residues of V804M. Clearly, the binding affinity of cabozantinib to wild-type RET kinase was determined to be stronger than to V804L and V804M.

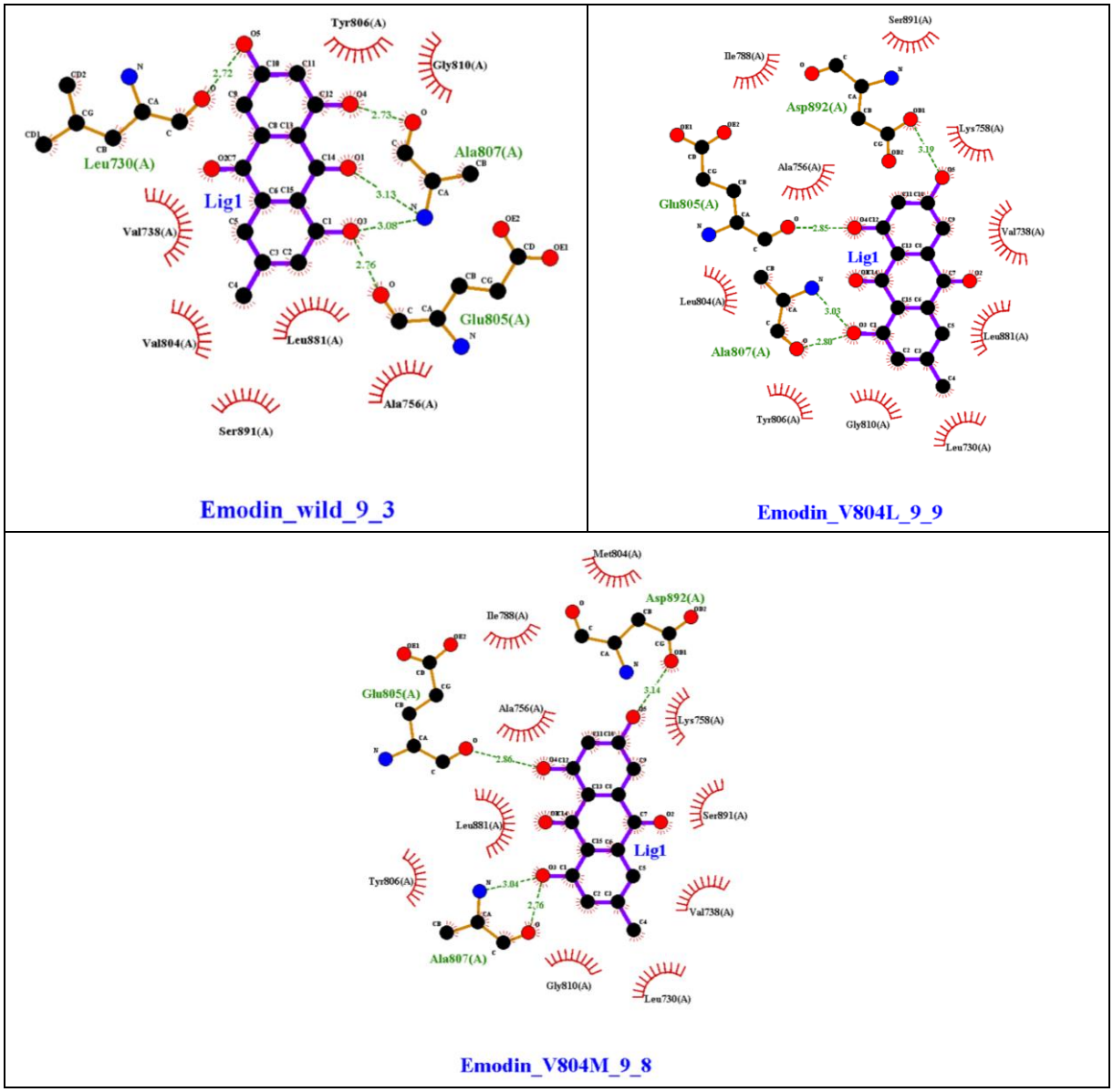

Figure 4. Molecular interactions between emodin and wild-type RET, V804L, and V804M

Molecular Interactions between Emodin and RET Protein Kinase

The binding free energy between emodin and wild-type RET was found to be -9.3 kcal/mol. Figure 4 shows the best conformation of emodin with wild-type RET. Many H-bonds between oxygen atoms of emodin and wild-

type RET were determined with Leu730 (2.72 A°), Ala807 (2.73 A°), Ala807 (3.13 A°), Ala807 (3.08 A°) and Glu805 (2.76 A°) residues, as illustrated in Figure 2. Furthermore, hydrophobic interactions were observed between emodin and Val738, Val804, Leu881, Ser891, Ala756, Tyr806, and Gly810 residues of wild-type RET. The binding free energy between emodin and V804L variant of RET was determined to be -9.9 kcal/mol. H-bond interactions between emodin and Glu805 (2.85 A°), Ala807 (3.03 A°), Ala807 (2.80 A°) and Asp892 (3.19 A°) residues of V804L variant. Hydrophobic interactions, on the other hand, were observed between emodin and Ile788, Ser891, Lys758, Gly810, Val738, Leu881, Leu730, Tyr806, Leu804, and Ala756 residues of V804L variant. The binding free energy between emodin and V804M was found to be -9.8 kcal/mol. H-bonds between emodin and Asp892 (3.14 A°), Glu805 (2.86 A°), Ala807 (3.04 A°) and Ala807 (2.76 A°) of V804M were determined. In addition, hydrophobic interactions in emodin with Met804, Ile788, Ala756, Lys758, Ser891, Leu881, Tyr806, Val738, Gly810 and Leu730 of V804M were observed. The strong binding affinity of emodin to both wild type and the mutated variants of RET protein kinase were suggested in this study. Interestingly, the binding free energy between emodin and the mutated variants were much lower than the one with wild-type RET.

Therefore, the molecular interactions between emodin and the mutated variants (V804L and V804M) were suggested to be stronger than the one with wild-type RET kinase. To be effective as a drug, a potent molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Lipinski's rule-of-five examined orally active compounds to define physicochemical ranges for high probability to be an oral drug (Daina, Michielin & Zoete, 2017). ADME (Absorption, Distribution, Metabolism, and Excretion) features of emodin was identified in this study. Table 1 shows ADME properties of emodin, predicting high lipophilicity and water solubility. No Cytochrome P inhibitory activity of emodin suggests high clearence of emodin with no drug-drug interactions. Emodin was shown to have high bioavailability.

Table 1. ADME Properties of Emodin

Property	Emodin	Property	Emodin
Consensus Log P o/w	0.12	CYP2C9 inhibitor	No
Log S (ESOL)	-1.43	CYP2D6 inhibitor	No
Class	Very soluble	CYP3A4 inhibitor	No
GI absorption	High	Lipinski	Yes
BBB permeant	No	Egan	Yes
P-gp substrate	Yes	Veber	Yes
CYP1A2 inhibitor	No	Muegge	Yes
CYP2C19 inhibitor	No	Bioavailability score	0.55

Conclusion

This study shows the molecular interaction of emodin with RET kinase via molecular docking for the first time. The strong binding affinity of emodin, especially for the mutated variants suggests that emodin could be a good candidate as a RET kinase inhibitor. Furthermore, high bioavailability score of emodin suggests a potential candidate as a drug. Therefore, emodin could be used for therapeutical purposes against medullary thyroid cancer.

Recommendations

This study shows strong binding affinity of emodin to both wild-type and the mutated RET variants, namely V804L and V804M via molecular docking. Further bioinformatics tools such as molecular modelling are suggested to evaluate the interaction of emodin with RET kinase. In-vitro and in-vivo studies are also highly necessary to verify the RET kinase inhibitory activity of emodin.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the author.

Acknowledgements or Notes

* This article was presented as oral presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Marmaris/Turkey on April 27-30, 2023.

References

Daina., Antoine, Michielin, O., & Zoete, V. (2017). Swissadme: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7*: 42717.

Ibáñez., & Carlos, F. (2013). Structure and physiology of the ret receptor tyrosine kinase. *Cold Spring Harbor Perspectives in Biology, 5*(2), a009134.

Jaber., Tania., Dadu, R., & Hu, M. I. (2021). Medullary thyroid carcinoma. *Current Opinion in Endocrinology, Diabetes, and Obesity, 28*(5), 540–46.

Jayasinghe., Ravindri., Basnayake, O., Jayarajah, U., & Seneviratne, S. (2022). Management of medullary carcinoma of the thyroid: a review. *The Journal of International Medical Research, 50*(7), 3000605221110698.

Kim., Mijin., & Kim, B. H. (2021). Current guidelines for management of medullary thyroid carcinoma. *Endocrinology and Metabolism, 36*(3), 514–24.

Li., Weilong., Wang, D., Li, M., & Li, B. (2021). Emodin inhibits the proliferation of papillary thyroid carcinoma by activating ampk. *Experimental and Therapeutic Medicine, 22*(4), 1075.

Linden., P J van, O., Kooistra, A. J., Leurs, R., J P de Esch, I., & de Graaf, C. (2014). Klıfs: a knowledge-based structural database to navigate kinase–ligand ınteraction space. *Journal of Medicinal Chemistry, 57*(2), 249–77.

Matrone., Antonio., Gambale, C., Prete, A., & Elisei, R. (2022). Sporadic Medullary Thyroid Carcinoma: Towards a Precision Medicine. *Frontiers in Endocrinology,* 13: 864253.

Morris., Garrett M, Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). Autodock4 and autodocktools4: automated docking with selective receptor flexibility. *Journal of Computational Chemistry, 30* (16), 2785–91.

Pietra., La, V., Sartini, S., Botta, L., Antonelli, A., Ferrari, S. M., Fallahi, P., Moriconi, A. (2018). Challenging clinically unresponsive medullary thyroid cancer: discovery and pharmacological activity of novel ret ınhibitors. *European Journal of Medicinal Chemistry, 150*, 491–505.

Subbiah, Shen V. T., Terzyan, S. S., Liu, X., Hu, X., Patel, K. P., Hu, M. (2021). Structural basis of acquired resistance to selpercatinib and pralsetinib mediated by non-gatekeeper ret mutations. *Annals of Oncology, 32* (2), 261–68.

Trott., Oleg., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry, 31*(2), 455–61.

Vodopivec., Danica, M, & Hu, M. I. (2022). Ret kinase ınhibitors for ret-altered thyroid cancers. *Therapeutic Advances in Medical Oncology, 14*: 17588359221101692.

Wells., Jr, S. A., Asa, S. J., Dralle, H., Elisei, R., Evans, D. B., Gagel, R. F., & Lee, N. (2015). Revised American thyroid association guidelines for the management of medullary thyroid carcinoma. *Thyroid : Official Journal of the American Thyroid Association, 25*(6), 567–610.

Wirth., Lori J, Sherman, E., Robinson, B., Solomon, B., Kang, H., Lorch, J., & Worden, F. (2020). Efficacy of selpercatinib in ret-altered thyroid cancers. *The New England Journal of Medicine, 383*(9), 825–35.

Zhang., Yuqi. Forli, S., Omelchenko, A., & Sanner, M. F. (2019). Autogridfr: ımprovements on autodock affinity maps and associated software tools. *Journal of Computational Chemistry, 40*(32), 2882–86.

Author Information

Emel Akbaba
Faculty of Medicine, Kirikkale University, 71450, Kirikkale, Turkey
Contact e-mail: *eakbaba@kku.edu.tr*

Deniz Karataş
Bioengineering Department, Yunusemre, Manisa, 45140, Turkey

To cite this article:

Akbaba, E. & Karataş, D. (2023). Interaction of wild type and V804L and V804M-mutated RET protein kinase with emodin: *In silico* approach. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 9*, 14-20.