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Research Article | Araştırma Makalesi

SUPPRESSING BREAST CANCER VIA 2KS1 PROTEIN USING BIOCHEMICAL *DE NOVO* DRUG DESIGN AND REPURPOSING OF VALPROIC ACID DERIVATIVES WITH BETTER INDICATIONS: COMPREHENSIVE MOLECULAR DOCKING, MOLECULAR DYNAMICS SIMULATIONS, ^{1}H NMR, AND ADMET ESTIMATIONS

2KS1 PROTEİNİ ARACILIĞIYLA MEME KANSERİNİN BASKILANMASI: GELİŞTİRİLMİŞ ENDİKASYONLARA SAHİP VALPROİK ASİT TÜREVLERİNİN BİYOKİMYASAL DE NOVO İLAÇ TASARIMI VE YENİDEN KONUMLANDIRILMASI; KAPSAMLI MOLEKÜLER KENETLENME, MOLEKÜLER DİNAMİK SİMÜLASYONLARI,^{1}H NMR VE ADMET TAHMİNLERİ

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ABSTRACT

Objective: This comprehensive *in silico* computational and theoretical biochemistry/organic chemistry research study covers the inhibition methodologies of 2KS1, which is an essential template for breast cancer-suppressing research.

Methods: To block the active site domain binding site of this protein, three *de novo* designed organic molecules by derivatizing Valproic Acid (VPA) were studied. Pharmacological organic chemistry effects of these analogues were enhanced to suppress 2KS1 better. The most recent molecular docking, molecular dynamics (MD), Proton Nuclear Magnetic Resonance (^{1}H NMR), and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) values showed that the derivatives of VPA exhibit better docking scores along with lower (better) inhibition constants and higher oral absorption percentage values compared to pristine VPA, meaning that the analogues possess better indications/pharmacological profiles.

Results: The new drug candidate derivatives showed approximately 50 to 200 times inhibition constant efficiency (1/50th to 1/200th of the dose usage) in the affinity and suppression capability compared to pristine VPA.

Conclusion: Being able to conduct such a crucial cancer study proves the fact that this study sheds light on the potential of new highly efficient drug molecules for future studies in terms of *in vitro* and *in vivo* for the suppression of breast cancer disease.

Keywords: Valproic acid, Breast cancer, In Silico drug design, Molecular docking, Molecular dynamics, ADMET

ÖZ

Amaç: Bu kapsamlı *in silico* hesaplamalı ve teorik biyokimya/organik kimya araştırma çalışması, meme kanseri baskılama araştırmaları için temel bir şablon olan 2KS1'in inhibisyon metodolojilerini kapsamaktadır.

Yöntem : Bu proteinin aktif bağlanma bölgesini bloklamak için, Valproik Asit (VPA) türevleri kullanılarak tasarlanan üç yeni organik molekül çalışılmıştır. Bu analogların, 2KS1'i daha iyi baskılamak için farmakolojik organik kimya etkileri güçlendirilmiştir. En son moleküler kenetlenme, moleküler dinamik (MD), Proton Nükleer Manyetik Rezonans (^{1}H NMR) ve Emilim, Dağılım, Metabolizma, Atılım ve Toksisite (ADMET) değerleri, VPA türevlerinin, orijinal VPA'ya kıyasla daha düşük (daha iyi) inhibisyon sabitleri ve daha yüksek oral emilim yüzdesi değerleri ile birlikte daha iyi kenetlenme puanları sergilediğini göstermiştir, bu da analogların daha iyi endikasyonlara/farmakolojik profillere sahip olduğu anlamına gelmektedir.

Bulgular: Yeni ilaç adayı türevleri, saf VPA'ya kıyasla afinite ve baskılama kabiliyetinde yaklaşık 50 ile 200 kat inhibisyon sabiti verimliliği (doz kullanımının 1/50 ile 1/200'ü) göstermiştir.

Sonuç: Böylesine önemli bir kanser çalışmasını yürütebilmek, bu çalışmanın meme kanseri hastalığının baskılanması için *in vitro* ve *in vivo* açısından gelecekteki çalışmalar için yeni çok etkin ilaç moleküllerinin potansiyeline ışık tuttuğunu kanıtlamaktadır.

Anahtar Kelimeler: Valproik asit, Meme kanseri, *In Silico* ilaç tasarımı, Moleküler kenetlenme, Moleküler dinamik, ADMET

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Introduction

Regarding the chemical structure, historical background, and therapeutic potential of Valproic Acid (VPA), it is a branched short-chain fatty acid with the molecular formula $C_8H_{16}O_2$. It is structurally derived from valeric acid, the main naturally occurring component of Valeriana officinalis, commonly known as valerian. It was first synthesized by Burton in 1882. Its pharmacological potential as an antiepileptic agent was identified by Pierre Eymard in 1962, with clinical applications starting in France in 1967.[1,2]

Due to its generally pharmacologically versatile molecule, VPA is mainly applied in the management of epilepsy. Added to that, VPA has a wide range of pharmacological functions beyond anti-epileptic functions: an antioxidant function reducing oxidative stress and inflammation, neuroprotection against neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, anti-inflammatory action useful in arthritis, and atherosclerosis, among other disorders; cardioprotection through reduction of the level of homocysteine is the factor by which VPA reduces the risk of cardiovascular factor.[2,3]

The understanding of the mechanisms of its action in neurological and psychiatric disorders is very important. In fact, despite considerable efforts, the precise mechanisms of the therapeutic effect of VPA in epilepsy, migraine, mood disorders, anxiety, and bipolar disorder remain incompletely understood, but several molecular and cellular pathways have been suggested. Anticonvulsant properties have mainly been mediated by VPA with γ-aminobutyric acid or GABA. It promotes its synthesis and release, augmenting GABAergic transmission, acting essentially to depress hyperexcitability of the neuronal membranes.[4,5]

VPA has also been shown to reduce the release of excitatory amino acids, including β-hydroxybutyric acid, and prevent overactivation of the NMDA glutamate receptor subtype, thus stabilizing neuronal excitability and preventing seizure propagation. Furthermore, VPA blocks voltage-gated sodium channels, an important step in the prevention of recurrent neuronal firing in diseases such as epilepsy and other neurological disorders.[6,7]

Besides its neurological applications, VPA acts as an inhibitor of histone deacetylases, especially HDAC1, modulating HDAC9 and HDAC11 in cancer cell lines, regarding its epigenetic modulation and anticancer potential in the scientific literature. Through influencing histone acetylation, VPA can modulate cell cycle, differentiation, apoptosis, and DNA repair genes. Trans-inhibition of HDAC1 drives the expression of pro-apoptotic genes and tumor suppressors, whereas overexpression/activation of HDAC9 and HDAC11 has a role in modulating AML-related genes. These make VPA a putative ligand molecule in therapeutic approaches against cancers. For instance, VPA was found to synergize with other agents such as arsenic trioxide in lung cancer models and capecitabine in breast cancer cells through mechanisms including HDAC3 inhibition and gene modulation. Besides, it induces the expression of melatonin receptor MT1 in breast cancer cells and upregulates thymidine phosphorylase, suggesting a potential role for VPA in increasing the efficacy of existing chemotherapeutic agents. The role of VPA in leukemia, as well as the induction of autophagy, is not to be underestimated. Recent studies have documented its potential to increase the efficacy of ATRA in APL by inducing the induction of autophagy and differentiation in both ATRA-sensitive and ATRA-resistant cell lines. The mechanistic link between the induction of autophagy through VPA and its activity as an HDAC inhibitor underlines a multiple-agent role for VPA in hematological malignancies. Various biological activities and potentials involved in research about VPA derivatives are crucial in the context of drug repurposing and de novo drug discovery. VPA exerts various biological activities: antioxidant, anti-inflammatory, anti-carcinogenic, cardiovascular-protective, neuroprotective, and antimicrobial actions with wound-healing effects. These pharmacodynamic properties have established VPA as a flexible candidate for various diseases and pathological conditions. Due to the many therapeutic possibilities that it has, detailed investigations are needed concerning its mechanisms of action and the extension of its clinical use. With a wide array of molecular interactions and associated therapeutic features, VPA has remained a cornerstone in the treatment of epilepsy and psychiatric disorders and has emerged as a candidate adjuvant for therapies in cancers. The aptitude of VPA in modulating the epigenetic mechanisms, neurotransmitter pathways, and enhanced autophagy marks this molecule enough with pharmacological importance in multifarious domains.[8,9]

The HER family comprises four single-span transmembrane receptor tyrosine kinases: EGFR itself, often referred to as HER1 or ErbB1; HER2/c-erbB2/neu; HER3, also referred to as ErbB3; and finally, HER4 or ErbB4. These receptors signal through the process of ligand binding to form both homo- and heterodimeric complexes, examples of which are provided in Figure 1. This event triggers an intracellular cascade that controls such key cell features as proliferation, motility, and invasion. Dysregulation of HER family signaling is a common feature in human malignancies and underlines its crucial role in oncogenesis. Overexpression of HER2 is more frequently associated with ER-negative cancers and contributes to aggressive disease phenotypes, making it a vital therapeutic target. Thus, ErbB1 and ErbB2 play important roles in the development of breast cancer. For

that purpose, choosing the 2KS1 receptor protein to study its inhibition, where it has the dimeric structure of

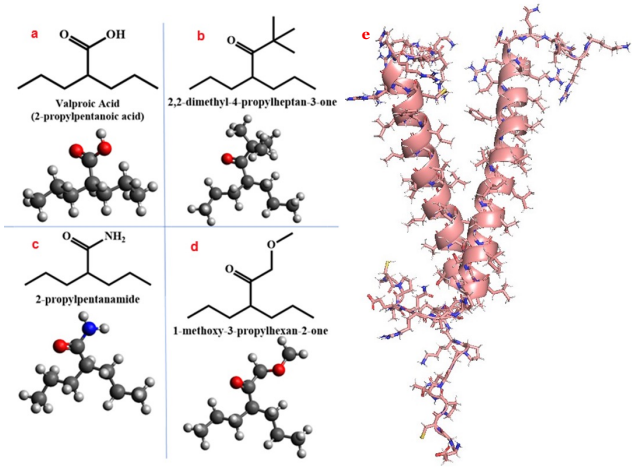

Figure 1. The organic chemical structures of pristine VPA and its three derivatized analogues that are drawn in both Chembiodraw and Avogadro software programs as well as the target molecule of 2KS1 on the right side: **a)** VPA Pristine, **b)** Isobutyl substituted/derivatized VPA (2,2-dimethyl-4-propylheptan-3-one), **c)** Amide substituted/derivatized VPA (2-propylpentanamide), **d)** Dimethylether substituted/derivatized VPA (1-methoxy-3-propylhexan-2-one) **e)** The illustrated heterodimeric ErbB2:ErbB1 protein structure of 2KS1 plays an important role in the development of breast cancer.

ErbB1:ErbB2 is one of the crucial steps as a target molecule suppression in breast cancer.[10]

To advance the understanding of VPA's therapeutic versatility, this study focuses on derivatized analogs of VPA, as illustrated in Figure 1. The investigation centers on three new structural derivatizations: isobutyl-, amide, and ether-substituted analogues. These functional groups were strategically selected based on both the authors' expertise in organic, biochemistry, and theoretical chemistry and insights from pharmaceutical chemistry databases. These databases have emphasized the immense contribution of certain organic substituents toward modulating anticancer activities, along with other pharmacological effects. In this context, some *in silico* theoretical studies were performed to predict and analyze the interactions and activities of these VPA derivatives. Functional groups are documented to influence electronic distribution, molecular reactivity, and bioavailability, all very critical parameters in drug design. The isobutyl substitution was included for its steric and hydrophobic effects, impacting molecular binding affinity and membrane permeability. With the inductive -I effect, the domain of the hydrogen bonds of VPA can be altered according to the study in pharmaceutical chemistry functional groups.[10] These would include etheric and amide alterations that serve the very same purpose. Thus, the increase in the hydrogen bond docking score while decreasing the inhibition constant occurs. *De novo* designed derivatives of VPA have the potential for rapid advancement via *in vitro* and *in vivo* studies, as well as clinical studies in human phases. Some of these analogues may stand a chance of becoming candidates with improved pharmacokinetic fine-tuning and an improved therapeutic index in the case of VPA.

Method

Ligand and receptor geometric optimization

Heterodimeric ErbB2:ErbB1 protein (PDB ID: 2KS1) was obtained from the Protein Data Bank to study with the VPA analogs were all already optimized utilizing the density functional theory (DFT)/B3LYP function along with the 6-31G(d,p) basis set principle[11] in the Gaussian 09 program.[12] The protocol successfully generated the most stable configurations of the molecular complexes, ensuring accurate electrostatic potential (ESP) charge assignments for each atom across the entire structures of 2KS1 and the druggable molecules. These refined configurations are critical for downstream computational and simulation-based analyses, providing a robust foundation for investigating molecular interactions and dynamics.[13] Post-processing of the output files was performed using a suite of computational tools, including Gauss View 6.0, Avogadro, and ChemBioDraw. Gauss View 6.0 facilitated visualization and analysis of Gaussian-derived data, Avogadro was employed for molecular editing and optimization, and ChemBioDraw enabled detailed structural representations. This integrated workflow ensured precise preparation and analysis of molecular models for subsequent simulation studies.[14] The ^{1}H NMR spectra estimation of the druggable molecules was gathered at this point.

Molecular Docking Simulations

For the molecular docking simulations, the most updated AutoDock Vina 1.5.7 and PyRx were utilized.[15-18] Each simulation was run with 30 posed options with the exhaustiveness value of 16, which is a bio-statically required number, and each molecule was studied 5 times to verify the accuracy and precision of the results. Each molecule was studied three times with 100 poses for blind docking to see whether the ligands can find and bind to the active site of target molecule and to verify the accuracy and precision of the docking results. The grid box dimensions placed on the 2KS1 were 150 x 150 x 150 Å^3, wide enough to cover the whole protein, so that the results would be blind docking, meaning that the ligands themselves would choose the binding sites on 2KS1 freely. The center coordinates were 0, 0, 0 with 0.5 Å spacing since the study was blind docking where the grid was covering the whole target receptor. The docking scores, expressed in kcal/mol, represent the Gibbs free energy of binding Δ(ΔG), a critical parameter for assessing the affinity between ligands and target biomolecules. A detailed cluster analysis was conducted to ensure the reliability and accuracy of the docking results. This analysis facilitated the identification of the most favorable binding poses by evaluating accumulated clusters with optimal docking configurations. From these clusters, the docking poses demonstrating the lowest (most negative) Δ(ΔG) values, indicative of the most thermodynamically stable and favorable interactions, were selected as the initial configurations for subsequent

molecular dynamics (MD) simulations. This approach ensures that the selected ligand-receptor complexes are not only energetically favorable but also structurally representative, providing a robust starting point for in-depth dynamic and mechanistic investigations.

Molecular dynamics simulations

Using the latest edition of Schrödinger's Desmond program 2024-2,[19] MD simulations were run under the Optimized Potentials for Liquid Simulations (OPLS) 3.0 forcefield and a grid box of 150 x 150 x 150 $Å^3$. The initial structures for MD simulations were derived from the docking poses, illustrating optimal and favorable binding energy based on docking results.[20-22] Each MD run was repeated at least three times using different seed numbers to ensure the accuracy of simulation parameters and DNA-bound ligand complex structures, as stated and advised by the scientific literature.[23-26] Each run had 100 nanoseconds time. The goal of MD simulations was to assess the dynamic attributes of the ligand-receptor complex over time. Temperature and pressure parameters included NPT at 310 K with Nose-Hoover temperature coupling[27] and constant pressure of 1.01 bar via Martyna Tobias−Klein pressure coupling.[28] The systems had no constraints, and default initial velocity values were employed for forcefield calculations. As the post-MD application, utilizing the simulation event analysis tool of Schrodinger, Root Mean Square Deviations (RMSD) of all ligand-receptor studies were performed. After 20 – 22 ns, all pristine VPA and VPA derivatives reach equilibrium and they all stay under 1Å of oscillation distance among the molecules throughout the whole simulation time of 100 ns.

ADMET Estimations

Using the latest edition of Schrödinger's Desmond program and its QikProp ADMET Tool, ADMET simulations were run to determine the pharmacokinetic and physicochemical parameters to find out the drug-likeness of the four ligands in total.

Results

Docking Scores, Regioselectivity, and Molecular Dynamics Results

As can be seen from Table 1 and Figure 2a, the new substituted/derivatized druggable molecules have higher docking scores compared to the original VPA, and they also possess much greater inhibition constant values, meaning the fact that even much lower doses of those three *de novo* derivatives would be sufficient, if not better, therapeutic effect of VPA towards 2KS1 receptor. As a rough estimate, Isobutyl-derivatized VPA would exhibit at least the same affinity and suppression capability as the Pristine VPA, requiring only 1/189th of its dose while amid derivative requiring 1/208th of its dose. In the final pharmaceutical technological formulations, things can be arranged a bit differently since binding agents, etc., will also come into play. However, the issue is that even under much lower dosage utilization of the new three derivatives, they yield much greater hydrogen bonding docking scores.

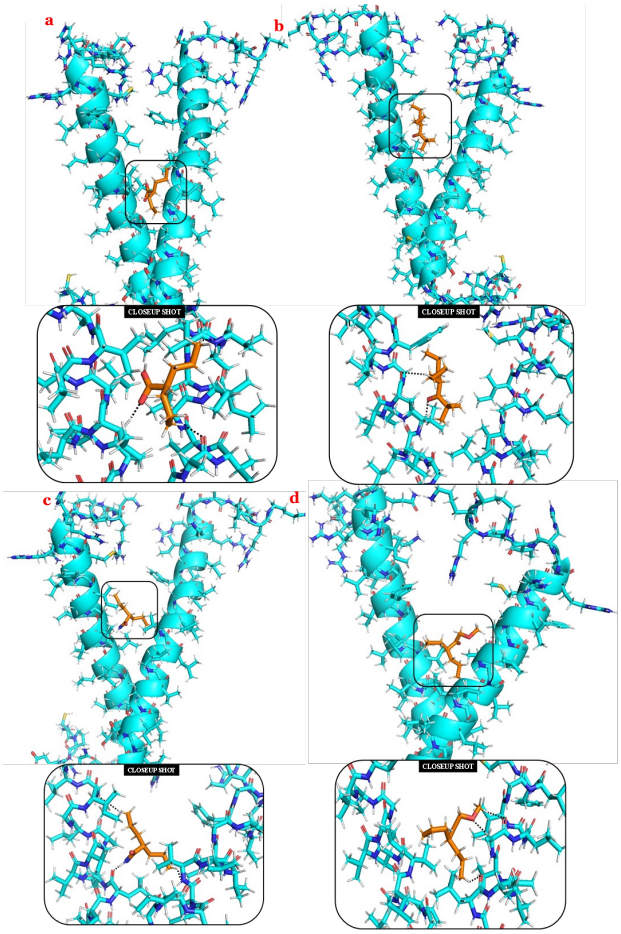

Figure 2. The best MD poses of **a)** VPA Pristine complexed with 2KS1; **b)** Isobutyl-substituted/derivatized VPA (2,2-dimethyl-4-propylheptan-3-one) complexed with 2KS1; **c)** Amide-substituted/derivatized VPA (2-propylpentanamide) complexed with 2KS1; **d)** Dimethyl ether-substituted/derivatized VPA (1-methoxy-3-propylhexan-2-one) complexed with 2KS1 within the most stabilized accumulated clusters under the OPLS 3.0 forcefield.

In Table 1b and Figure 2c, amide-derivatized VPA has the highest docking score of -10.47 kcal/mol. This is a great inhibition scoring value since, as it is well known from the scientific literature that, a good suppression/inhibition in proteins begins after -10, -11 kcal/mol values.[27] The amide's amine domain is pretty reactive in terms of creating hydrogen bonds. Following it, the isobutyl derivatized VPA yields us the data of -9.88 kcal/mol in Table 1c and Figure 2b. It is still a great number in terms of suppressing the active site of 2KS1 between the alpha helices. The β-carbon that is attached to the α-carbon of the carbonyl is at the center of three methyl groups. It is under the -I (minus inductive) effect of those three methyl groups surrounding it, making the β-carbon a bit more negatively charged, causing the α-carbon to be much more positively charged than it should be under the electronegative effect of the oxygen in the carbonyl bond. Since the carbonyl oxygen is more electronegative than that of carbonyl carbon, it attracts the π bonds towards itself, making itself negatively charged. Thus, the effect of β-carbon on α-carbon, making it more positively

Table 1. The comparison of docking energies, inhibition constants, and regioselectivities of Valproic Acid and its *de novo* designed derivatives on 2KS1 protein.

Drug Candidate	Docking Score (kcal/mol)	Inhibition Constant (μM)	Hydrogen Bond Distance (Å)	Ligand Functional Group(s)
a) Pristine VPA	-8.21	863.6	2.08, 2.11, 1.98 respectively	Carboxylic acid, Oxygen, and 2 Methyl Groups
b) Isobutyl-derivatized VPA (2,2-dimethyl-4-propylheptan-3-one)	-9.88	4.57	1.99, 2,05 respectively	Carboxylic acid, Oxygen, and a Methyl Group
c) Amide-derivatized VPA (2-propylpentanamide)	-10.47	4.14	1.92, 2.19, 2,07 respectively	Amide Nitrogen's Hydrogen and 2 Methyl Groups
d) Dimethyl ether-derivatized VPA (1-methoxy-3-propylhexan-2-one)	-9.67	17.09	2.01, 2.04, 1.97 respectively	Ether Oxygen, Methyl Hydrogen of Ether, and a Methyl group in the tail domain

charged, increases the carbonyl oxygen's nucleophilicity character and enhances its hydrogen bonding capability. This situation alteration within the organic mechanism causes the isobutyl substitution to have a higher docking energy compared to VPA. In the case of dimethyl ether derivatization in Table 1d and Figure 2d, it also yields a good docking score of -9.67 kcal/mol while maintaining an inhibition constant value of 17.09 μM. The inhibition constant is a bit much higher compared to that of isobutyl- and amide-functional groups, but that is not an issue at all, since compared to VPA's inhibition constant values of 863.6 μM, it is still much more effective in terms of dosage. The MD results of all three analogues are quite clear and accurate under the Forcefield OPLS 3.0 in terms of defining how the drug candidates bind to the 2KS1 receptor, as can be seen in Figures 2b, 2c, 2d.

The Advantages of Theoretical ^{1}H NMR Simulation Estimations

For these drug designs to work in wet-lab studies, the *in vitro* synthesis, purification, characterization, and theoretical simulations should be complete, and should be able to estimate that when things move on to organic/pharmaceutical chemistry synthesis, the products of *de novo* drug candidate designs should be easy to be characterized compared to the reactants. To achieve that purpose, the wet-lab synthesis and ^{1}H NMR characterizations, as well as the theoretical ^{1}H NMR characterizations, are all essential to understand how the spectrum of each analogue will vary.

As can be seen in Figure 3, VPA has a distinct 11.0 ppm peak that is helpful in its synthesis for drug production in pharmaceutical companies throughout the world. In the ^{1}H NMR of the isobutyl-derivative on the right side of VPA's NMR, the hydroxyl peak disappears immediately, which is a useful indicator for its characterization after the synthesis and purification steps that can be done in wet-lab studies. This obviously makes its characterization compared to VPA easy in any organic or pharmaceutical chemistry synthesis lab.

In the third ^{1}H NMR belonging to the amide analogue, the amine domain of amide has a pretty distinct ppm value of 7.16 that can make the compound distinct again in the characterization process. In the final derivative ^{1}H NMR spectrum of dimethyl ether-bound VPA derivative, additional peaks of 4.53 and 3.30 ppm values are added into the spectrum due to the introduction of new methyls around the oxygen of the ether. This also will make the chromatographic separations and the characterization of the new derivative easier when compared to VPA after a derivatization synthesis is done in a wet-lab.

Each derivatized analogue is unique in its own way for the chromatographic separation (column chromatography etc.) and ^{1}H NMR characterization. Thus, it should be noted that for future *in vitro* organic/pharmaceutical synthesis steps, these results of ^{1}H NMR will make things easier in the laboratory.

The ADMET Analysis

In Table 2, regarding the data yielded from the QikProp ADMET tool of Schrodinger's Desmond, the drug-likeness of the three *de novo* analogues of VPA falls into the range of a good drug candidate. Two of them, isobutyl and ether analogues have better oral absorption percentages, 100% absorption, compared to the VPA Pristine drug's percentage of 90%. In all other categories mentioned in the table and its explained legend, all derivatives comply with the ranges of druggable molecules.

The RMSD Data

In Figure 4a, the oscillations of the atoms of pristine 2KS1 are illustrated. It is under 0.4 Å and the equilibrium of MD reaches around 19-20 ns and keeps on going smooth for 100 ns. Most receptor RMSD oscillations stay under 1.0 Å.[13-23] In the case of Amide and Isobutyl complexes in Figures 4b, 4c, 4d it can be observed that they have lower deviations in their atoms since those structures as illustrated in Table 1 and Figure 2 have better biochemical affinity towards 2KS1 and therefore better results compared to pristine/parent VPA. Thus, this enhances the integrity of the complex in those three *de novo* analogues. All reach equilibrium around 20 ns. Finally, as of expected, pristine VPA complex with 2KS1 has the most oscillation in Figure 4e, confirming what the data states in Table 1. It has the worst docking energy and inhibition constant values compared to all new analogues. Hence, this situation causes the complex structure to have more RMSD oscillations compared to the rest.

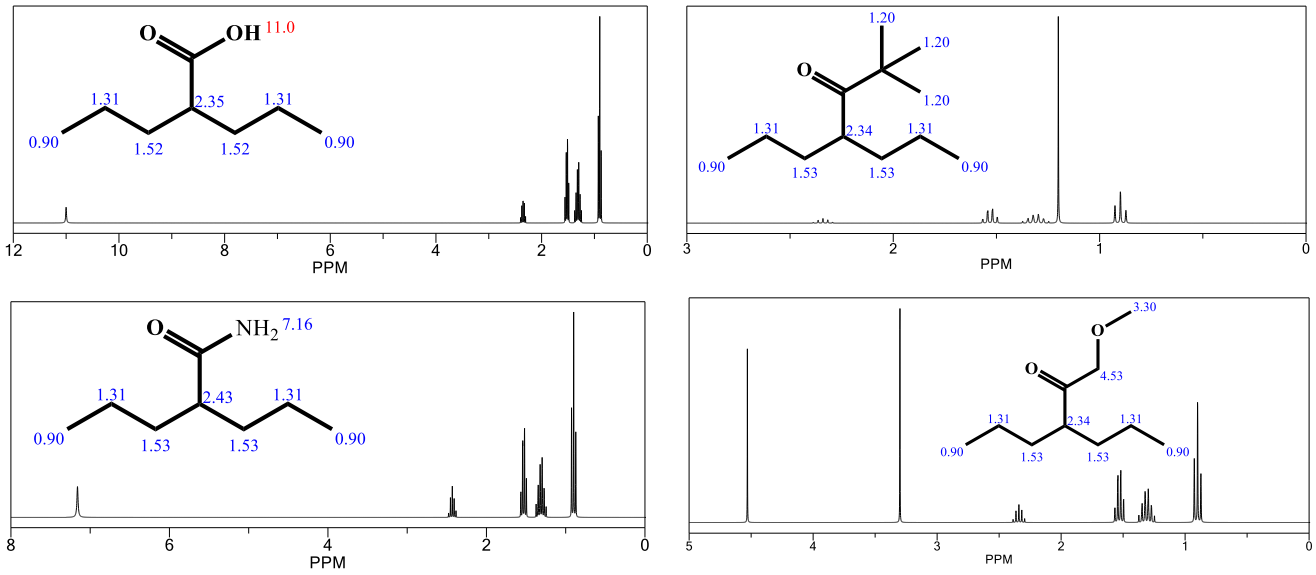

Figure 3. Theoretical simulation ^{1}H NMR estimations of Pristine VPA, Isobutyl-substituted/derivatized VPA (2,2-dimethyl-4-propylheptan-3-one), Amide-substituted/derivatized VPA (2-propylpentanamide), and Dimethyl ether-substituted/derivatized VPA (1-methoxy-3-propylhexan-2-one).

Table 2. ADMET and drug-likeness properties of VPA and its derivatized analogues, as determined by QikProp of Schrodinger Desmond Maestro 2024-2.

Compound name	MW[a]	Acceptor HB[b]	SASA[c]	QPlogPo/w[d]	QPlogBB[e]	QPlogS[f]	QPlogHERG[g]	% Human Oral Absorption[h]
a) Pristine VPA	144.213	2.000	396.716	2.743	-0.372	-1.874	-1.374	90.903
b) Isobutyl derivatized VPA (2,2-dimethyl-4-propylheptan-3-one)	184.321	2.000	471.548	3.416	0.038	-3.129	-3.395	100.000
c) Amide-derivatized VPA (2-propylpentanamide)	143.228	2.500	397.302	0.728	-0.507	-0.672	-1.834	82.526
d) Dimethyl ether derivatized VPA (1-methoxy-3-propylhexan-2-one)	172.267	3.700	462.981	2.157	-0.171	-1.914	-3.735	100.000

[a]Molecular weight (acceptable range: <500 g/mol). [b]Hydrogen bond acceptor (acceptable range: ≤10). [c]Total solvent accessible surface area using a probe with a 1.4 radius (acceptable range: 300 – 1000). [d]Predicted octanol/water partition coefficient (acceptable range: 2.0 – 6.5). [e]Predicted blood-brain partition coefficient (acceptable range: 3.0 – 1.2). [f]Predicted aqueous solubility, S in mol/dm^{-3} (acceptable range: 6.5 – 0.5). [g]Predicted IC_{50} value for blockage of HERG K^{+}channels: <−5=concern. [h]Predicted human oral absorption on a 0 to 100% scale (<25% is poor and >80% is high).

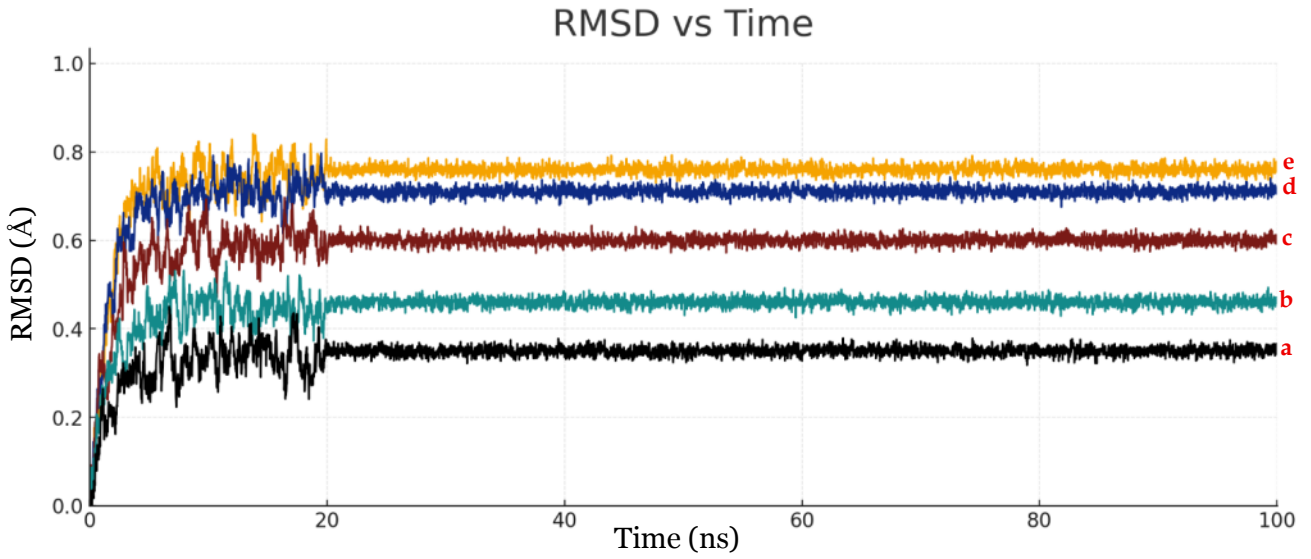

Figure 4. The RMSD Data of 2KS1 and the complexes bound to it. a) Black colored: solely Pristine 2KS1 receptor protein; b) Teal/Green colored: The complex of Amide-derivatized VPA with 2KS1; c) Red colored: The complex of Isobutyl-derivatized VPA with 2KS1; d) Blue/Magenta colored: The complex of Dimethylether-derivatized VPA with 2KS1; and e) Orange/Yellow colored: Pristine VPA with 2KS1. The forcefield is OPLS 3.0.

Discussion

Comparing the results of Table 2, Figures 2 and 3 to see whether the findings vindicate each other or not, it can be clearly expressed that all fit to each other. In the MD studies, along with its ADMET estimation results of all the analogues of VPA have better docking score results as well as much better inhibition constant values, meaning that using much fewer doses of the drug candidates would be enough to give the same indications of VPA.

The structurally *de novo* derivatized analogues of VPA demonstrated remarkable enhanced molecular interactions with the 2KS1 receptor, as reflected by their markedly elevated docking scores relative to the parent compound. Thus, these derivatives exhibited significantly improved inhibition constant, a strong pharmacological affinity 200-fold to the target receptor. This strongly means that a superior binding affinity and pharmacodynamic potency occurred with the new analogues. In practical pharmacological terms, such an improvement indicates that even considerably lower concentrations of the three *de novo* designed derivatives would suffice to elicit a therapeutic response (oncological toxicity towards the breast cancer cells) comparable to, or potentially exceeding, that of unmodified pristine/parent VPA. Among them, the amide, isobutyl and dimethylether-substituted VPA analog are predicted to retain receptor affinities comparable to the pristine molecule, with calculated inhibition constants that are theoretically lower by approximately $1/208^{th}$, $1/189^{th}$, $1/50^{th}$ respectively.

In terms of QPlogHERG IC_{50} toxicity evaluations, the new druggable molecules are also in the fine range value of being safe, so this means that for further *in vitro* and *in vivo* evaluations, they are suitable for a research study. This research highlights the significant potential of Valproic Acid to serve as an anticancer agent through *de novo* drug design and repurposing, despite its established role as a widely used antiepileptic drug. Modifications involving key functional groups, such as isobutyl, amide, and dimethyl ether, markedly enhance its pharmacological profile. These alterations improve docking scores, lower inhibition constants, and increase the affinity of hydrogen bond interactions with the 2KS1 receptor protein, demonstrating superior binding characteristics and indications towards the target molecule. The study employs a multidisciplinary approach, integrating theoretical chemistry, biochemistry, organic chemistry, and pharmaceutical chemistry to optimize and characterize the enhanced VPA analogues. Comprehensive *in silico* analyses, including molecular docking and molecular dynamics simulations, were supported by additional experimental insights from ^{1}H NMR spectroscopy and ADMET profiling. These results collectively validate the potential of VPA derivatives to be advanced as oncological therapeutics. The findings pave the way for future experimental validations, encompassing *in vitro*, *in vivo* studies, and human clinical trials. By transitioning these VPA-derived molecules into *de novo* synthetic frameworks, the research opens new avenues for their application as targeted anticancer agents, reinforcing their value in oncological drug development.

Limitations

This study is based entirely on *in silico* analyses, which provide predictive insights but cannot fully represent complex biological systems. The calculated binding affinities, inhibition constants, and ADMET properties are theoretical and do not directly indicate biological or clinical efficacy. Experimental validation through *in vitro* and *in vivo* studies is therefore required.

Ethical Approval

None. The *in silico* nature of all of these studies comply with laws of National Ethics Committee, so there is no need for ethical application.

Conflict of Interest

There is a single author. There is no conflict of interest.

Author Contributions

There is a single author.

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