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Structural insights into conformational stability of both wild-type and mutant Insulin Receptor Gene

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ABSTRACT

Type 2 diabetes (T2D) poses a health challenge. It can lead to complications such as heart disease, hypertension, heart failure, and stroke. Factors like obesity and lack of activity can contribute to insulin resistance. The insulin receptor gene (*INSR*) is responsible for producing insulin receptors. When this gene malfunctions, it can contribute to the development of T2D.

In this study, we investigated the stability of the structure of variants of *INSR* using an extended molecular dynamics simulation and the perturbation effect of compound CheBI_88339 on the protein structure. During the analysis, we observed that all three systems—the wild-type *INSR*, the R1191Q variant, and the R1191Q variant bound to compound CheBI_88339 (R1191Q-D) reached equilibrium in 30ns without any instability. Throughout the simulation process, it was generally observed that the wild-type *INSR* exhibited higher stability than the R1191Q variant and R1191Q-D. The root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of *INSR*, R1191Q and the variant bound to compound CheBI_88339 (R1191Q-D) are 9.28Å, 10.35Å, 8.65Å, 2.59Å, 2.98Å, and 2.89Å respectively.

These values indicate that the mutated *INSR* introduced levels of deviations and flexibility in the protein structure. However, considering the variant bound to compound CheBI_88339 suggests that this drug may contribute to stabilizing the dynamics of the mutant protein. Overall, our findings shed light on the effect of genetic variants and their impact on protein stability. This research provides further insight into the dynamics of *INSR* and the potential of CheBI_88339 in targeting *INSR*. However, this study is computational, and further experimental studies are required.

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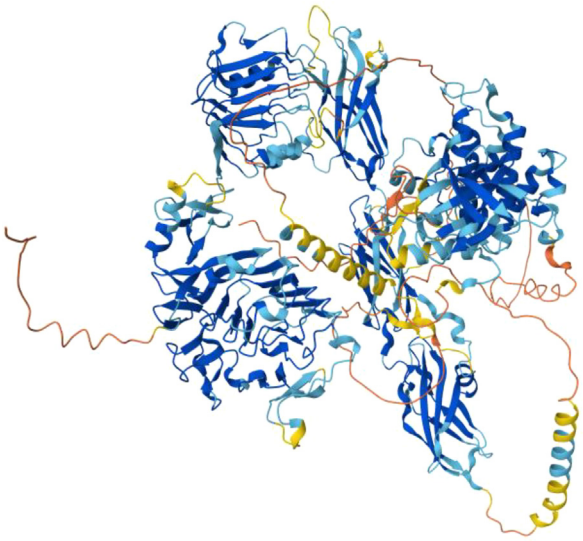

Fig. 1. 3-Dimensional structure of *INSR* [16]. This figure illustrates the three-dimensional structure of the *INSR* protein. The structure highlights the receptor's alpha and beta subunits, where the blue represents the membrane-spanning regions, the yellow denotes the insulin binding domains, and the orange indicates areas with regulatory importance.

Introduction

Diabetes mellitus is a major global health challenge. In 2021, approximately 537 million adults were living with this disease, and this figure is predicted to rise to 783 million by 2045. Over 95 % of diabetes patients have type 2 diabetes mellitus (T2D) [1]. T2D affects almost every organ in the human body, which can lead to severe complications such as coronary heart disease, hypertension, heart failure, and stroke [2]. Genetics and environment are the major risk factors for T2D. Lifestyle factors such as obesity and physical inactivity can lead to insulin resistance [3].

The insulin receptor gene (*INSR*) is the gene responsible for coding proteins called insulin receptors [4] (Fig. 1). These transmembrane protein receptors help to mediate insulin signalling, which in turn modulates cellular functions [5]. Elevated blood sugar level causes the pancreas to release insulin, which binds to the receptors on the target cells [6]. A cascade of intracellular events is initiated when this insulin binds to the receptors, leading to increased uptake of glucose, thereby ensuring that blood sugar level is regulated [7]. Insulin resistance, which is one of the hallmarks of T2D, is a result of the body cells' failure to respond effectively to insulin. This resistance means that the excess glucose in the bloodstream is barely taken up, thereby leading to elevated blood sugar level [8]. Mutations in the *INSR* gene can lead to the synthesis of dysfunctional insulin receptors, contributing to insulin resistance [9–11]. These genetic mutations can be inherited and might significantly increase an individual's susceptibility to T2D [12].

The compound CheBI_88,339 is a reversible inhibitor of the insulin-like growth factor 1 receptor (IGF-1R) and insulin receptor (IR) family kinases. While this inhibitor has not been proven experimentally to be an inhibitor of *INSR*, it has some experimental backing for its activity as an inhibitor of insulin-like growth factor-1R/IR [13]. Currently, T2D treatment options involve strategies targeting insulin sensitivity or glucose absorption [14]. Channeling existing drugs and exploring other treatment options, including targeting IGF-1R and IR kinases, can lead to combination therapy, personalized medicine, and attractive lower medication costs. Molecular dynamic (MD) simulation is a computational technique utilised to study the behaviour of atoms, molecules, and the dynamics of protein-protein interaction [15]. This study aims to utilize MD simulation to explore how mutations in the *INSR* gene affect the stability of the structure and its binding affinity, thereby influencing insulin signaling pathways.

Materials and methods

INSR *variant retrieval*

We searched the open target platform for the genetic variants of the *INSR* gene reported to be associated with T2D. This platform provides information on the association between potential drug targets and different diseases [17]. We searched for genetic variation associated with *INSR* for patients affected by T2D. Only two variants, rs121913150 and rs1799816, were listed. Variant rs1799816 was reported to have uncertain clinical significance, while variant rs121913150 was reported to have a pathogenic clinical significance. Therefore, we selected variant rs121913150 (R1191Q) for downstream analysis [18–20].

Protein and ligand preparation

The 3D structure of *INSR* (*Homo sapiens*) was downloaded from AlphaFold, [16] with AFDB accession of AF-P06213-F1, while the 2D structure of CheBI_88,339 was downloaded from the ChEBI database [21]. This compound was then auto-optimized using the Avogadro Molecular editor [22]. UCSF Chimera [23] was deployed in the molecular docking of CheBI_88,339 into the *INSR* active site. The *INSR* variant structure was determined using the "swapaa" command in Chimera [23]. Docking validation was then accomplished using Autodock Tools, [24] by redocking the CheBI_88,339 and choosing docking scores and poses comparable to those produced by UCSF Chimaera.

Assessment of the drug likeliness of CheBI_88,339

The pharmacokinetic characteristics and drug-like nature of CheBI_88,339 were determined using SwissADME [25]. This software uses "Brain or Intestinal Estimated permeation method to compute the lipophilicity and polarity of small molecules.

Molecular dynamic simulation

The molecular dynamic simulation was done using AMBER software with the force field FF14SB [26]. The atomic charges of *INSR* were described using the restrained electrostatic potential (REP) and the general Amber force field (GAFF). Using the Leap variant in Amber 19, the system was neutralised, and hydrogen atoms were added. The system was solvated, and all the protein atoms were surrounded by an orthorhombic box of TIP3P water molecules at a distance of 9Å [27]. We deployed two different system minimization processes. The first was a system minimization in 2000 steps using a restriction potential of 500 kcal/mol. This partial minimization was necessary to reduce poor van der Waals contact in the surrounding solvent and keep the solute.

The second system minimization involves 10,000 steps, which are unrestricted. This was used to eliminate faulty contacts throughout the entire system. These were followed by gradually heating the system from 10 to 273 K at 50 ps at a collision frequency of 1.0 ps^{-1} using a Langevin thermostat. After that, 500 ps equilibration was then completed. Using a Berendsen barostat (isobaric-isothermal ensemble), a constant temperature and pressure at 300 k and 1 bar, respectively, were maintained. The simulation's steps were each run for 2 fs, and a single-precision floating-point precision model was used. The AMBER software's SNAKE algorithm was used to constrain (NTC = 2) all hydrogen-containing bonds [28]. This step is crucial because it eliminates the system's highest-frequency oscillation and the vibrations caused by hydrogen.

Three systems were subsequently set up for molecular dynamics (MD) simulations. These systems include the wild-type *INSR*, the variant (R1191Q), and the variant bound to compound CheBI_88,339 (R1191Q-D). Further analyses, including root-mean-square deviation (RMSD),

Table 1
Pharmacokinetic profile of CheBI_88,339.

Properties	CheBI_88,339
Molecular Formula	$C_{23}H_{24}FN_9O$
Molecular Weight	461.49 g/mol
Lipophilicity (LogP)	2.49
Water Soluble	Moderately soluble
GIT absorption	High
BBB Permeability	No
Bioavailability	0.55
Synthetic accessibility	4.36
Druglikeness (Lipinski)	Yes

root-mean-square fluctuation (RMSF), principle component analysis (PCA), the radius of gyration (RoG), and solvent accessible surface area (SASA), were performed using the PTRAJ module of Amber 14 [29]. Using the analytical tool ORIGIN, we produced data plots and visualized them with UCSF Chimera [30].

Results

Drug-likeliness profile of CheBI_88,339

The table below provides a pharmacokinetic profile of a CheBI_88,339, detailing various chemical and pharmacological properties crucial for understanding its behaviour as a drug candidate (Table 1). These pharmacokinetic properties, potency, and safety are crucial and are greatly considered in the design of therapeutics. The ability of a compound to pass through the lipid bilayer is dependent on the value of its Lipophilicity (LogP). CheBI_88339 has a LogP value of 2.47, meaning it can achieve membrane permeability and first pass clearance. A LogP value between 2 and 3 is considered optimal [31]. The gastrointestinal (GIT) absorption value evaluates how easily a compound can be absorbed in the GIT. From the result, CheBI_88339 has a high GIT absorption potential. The blood-brain barrier value (BBB) ascertains the possibility of the compound passing through the blood-brain barrier. From our evaluation, CheBI_88339 has no chance of crossing the blood-brain barrier.

R1191Q elicited high structural Instability

Before undertaking molecular dynamic stimulation, we performed molecular docking by setting a grid box to a dimension of size x = 22.15, y = 23.78, and z = 25.65. The result showed that the docking score between CheBI_88339 and R1191Q is -8.321kj/mol.

Root mean square deviation (RMSD) is widely used in molecular dynamics simulations to assess how stable and consistent the structure of a simulated protein system is [32]. Lower RMSD values typically indicate stability, while higher values can suggest structural changes or increased flexibility [33,34]. In this analysis, we examined the RMSD values of the wild protein (*INSR*), its variant (R1191Q), and the variant when bound with a drug called CheBI_88339 throughout the simulation. The average RMSD for the wild-type protein during this simulation period was 9.28Å (Fig. 2). This indicates that there is some level of deviation or inherent flexibility in the wild-type protein. The variant protein displayed a RMSD value of 10.35Å, slightly higher than the wild type. This could suggest an increased level of deviations or flexibility in response to the introduced mutations or changes in structure that affect its behavior. However, when bound with the drug CheBI_88339, there was a decrease in RMSD for the variant protein, at 8.65Å compared to its standalone state without any drug interaction. This decrease suggests that the drug may contribute to stabilizing the structural dynamics of the variant protein, potentially through specific binding interactions or inducing conformational changes that restrict unwanted fluctuations.

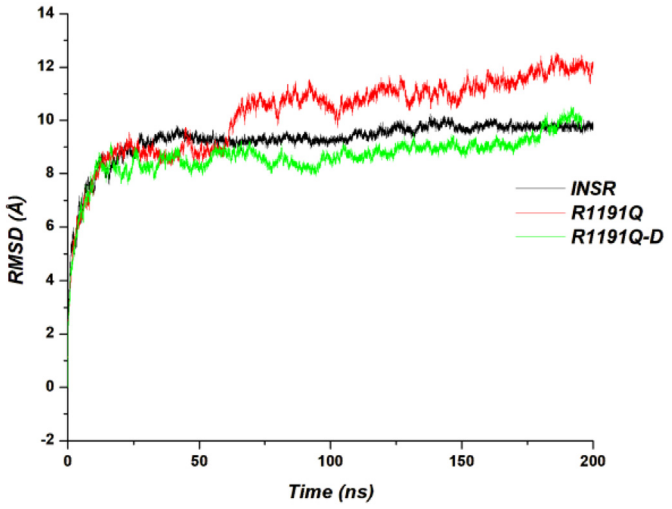

Fig. 2. C-α backbone RMSD of *INSR* (Black), R119Q (red), and R119Q-D (green). The graph shows the RMSD measured in Ångströms (Å) for the *INSR* protein and its mutants, R1191Q and R1191Q-D, as a function of time in nanoseconds (ns).

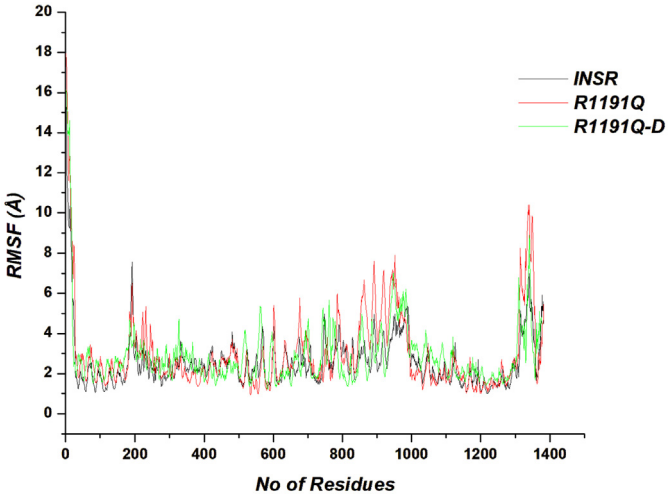

Fig. 3. Residual fluctuation of *INSR* (Black), R119Q (red), and R119Q-D (green). The graph represents the RMSF in Ångströms (Å) for each residue of the *INSR* and its mutants R1191Q and R1191Q-D.

Mutation increases INSR *flexibility*

Root mean square fluctuation (RMSF) measures how atoms, particularly the alpha carbons, in protein backbones deviate from their average positions during an MD simulation [35,36]. It provides insights into the flexibility of proteins and identifies regions that may play a role in transitions or functional mechanisms [35]. High RMSF indicates high deviation from its position and, hence, high structural mobility and vice versa. The wild-type protein displayed an RMSF of 2.59Å, indicating its flexibility across its residues, while the variant showed an average RMSF of 2.98Å (Fig. 3). This increase suggests that the mutations in the variant introduce additional flexibility or alter local dynamics in certain regions. Such enhanced mobility could result from changes in interactions within the molecule or modified structural constraints caused by the mutations. The drug-bound variant had a value of 2.89Å, lying between the wild-type protein and unbound variant. This indicates that drug binding moderates the variants' flexibility, bringing its dynamics closer to that of the wild-type but not completely reversing the effects of mutations. The RMSF data confirms that compared to the wild-type, the

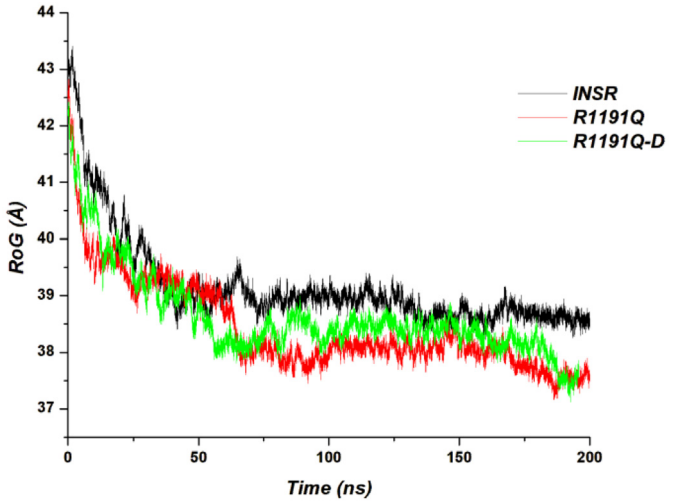

Fig. 4. C-α radius of gyration of *INSR* (Black), R119Q (red), and R119Q-D (green). This graph depicts the Radius of Gyration (Rg) measured in Ångströms (Å) for the *INSR* protein and its mutants R1191Q and R1191Q-D over a 200 nanosecond (ns) molecular dynamics simulation.

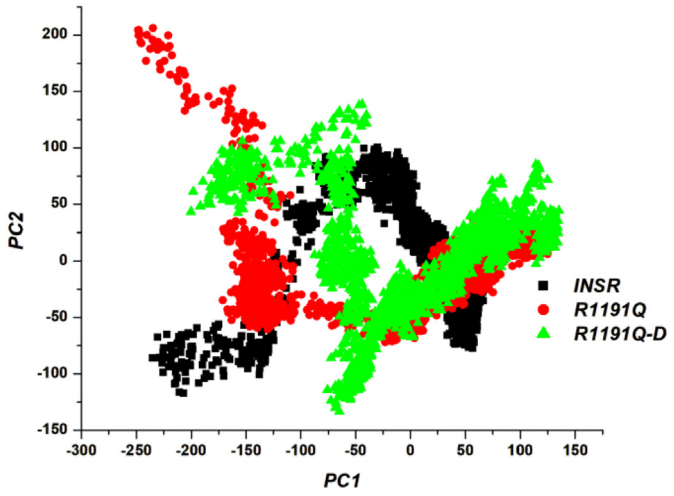

Fig. 5. Principal component of *INSR* (Black), R119Q (red), and R119Q-D (green). This plot shows the principal component analysis (PCA) results on the *INSR* protein and its mutants, R1191Q and R1191Q-D, across simulated conditions.

variant exhibits structural deviation from its initial configuration suggesting global instability or structural changes. The RMSF data, which shows enhanced localised flexibility in the variant, supports this. When bound to the variant, the compound CheBI_88339 appears to stabilize both the local dynamics (as demonstrated by the moderated RMSF) and the overall structure (as shown by the lowered RMSF), highlighting its potential as a therapeutic option to restore some stability to the mutated protein.

Alpha carbon atom compactness and structural folding

The radius of gyration (Rg) provides insights into the compactness of a protein structure during dynamics simulations [37]. It measures how spread out the atoms are from the center of mass in a protein. Our study observed that the wildtype protein had an Rg value of 39.14Å, indicating its compactness or spatial arrangement throughout the simulation. The variant exhibited a reduced average Rg value of 38.39Å (Fig. 4), suggesting that it may have a more compact or tightly folded conformation than the wild type. This can result from the mutations causing specific regions to fold inward or form interactions within the molecule. Interestingly, when we introduced CheBI_88339 and bound it to the variant, we noticed an increase in its Rg value to 38.63 Å However, this was still lower than observed for the wild-type protein. This indicates that while drug binding partially restores some of the distribution in the variant, it does not fully revert it to its conformational state, which resembles that of the wild type. The Rg adds another dimension; despite its increased flexibility and deviations, the variant remains more compact than its wild-type counterpart. This compartment could potentially be a mechanism or a consequence of disrupted interactions leading to a denser structure.

Principal component analysis

The wild-type *INSR* appears relatively compact, mainly located in the quadrant of PC1. This implies that the natural shape of *INSR* has some flexibility, which seems to play a role in insulin binding and receptor activation. The R1191Q variant is widely distributed across both PC1 and PC2 axes. This suggests that the R1191Q mutation introduces changes that may result in a range of states potentially interfering with insulin binding or signal transduction by the receptor. The R1191Q-D complex (represented by triangles) occupies a position between the *INSR* and the mutant R1191Q (Fig. 5). Interestingly, when the drug is present, it appears to limit the range of motion of the receptor and bring it closer to the wild-type *INSR*. This suggests that the drug might restore some stability or functionality lost due to the R1191Q mutation.

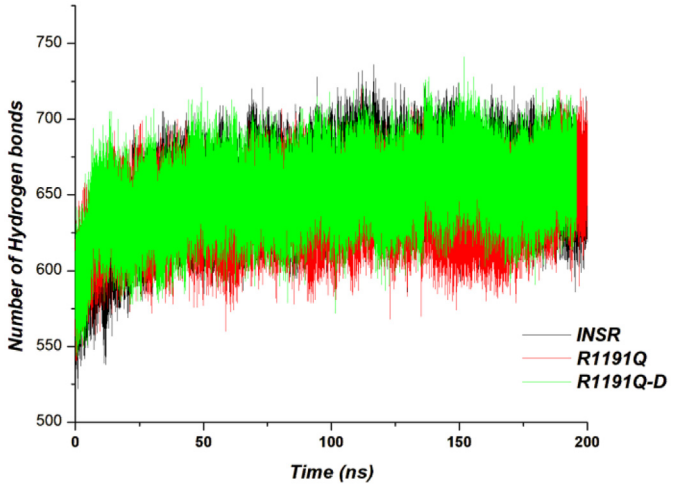

Fig. 6. Hydrogen bond of *INSR* (Black), R119Q (red), and R119Q-D (green). This graph presents the number of hydrogen bonds measured for the *INSR* protein and its variants, R1191Q and R1191Q-D, over 200 nanoseconds (ns) simulation time.

Evaluation of hydrogen bond during the simulation period

Hydrogen bonding plays a role in the stability and functioning of proteins [37]. They are essential in insulin receptor (*INSR*) for maintaining its structure and enabling interaction with insulin. During most of the simulation (Fig. 6), the number of hydrogen bonds fluctuates, with the baseline above 650. This indicates that the receptor's native form maintains a network of hydrogen bonds, which is expected for a folded and functional protein. There is a decrease in the number of hydrogen bonds of the R1191Q variant compared to the wild-type *INSR* (Fig. 6). This suggests that the R1191Q mutation impacts the receptor's stability by disrupting hydrogen bonds essential for maintaining its structure and function. R1191Q-D complex had increased hydrogen bonds compared to the R1191Q variant alone. This suggests that some of those

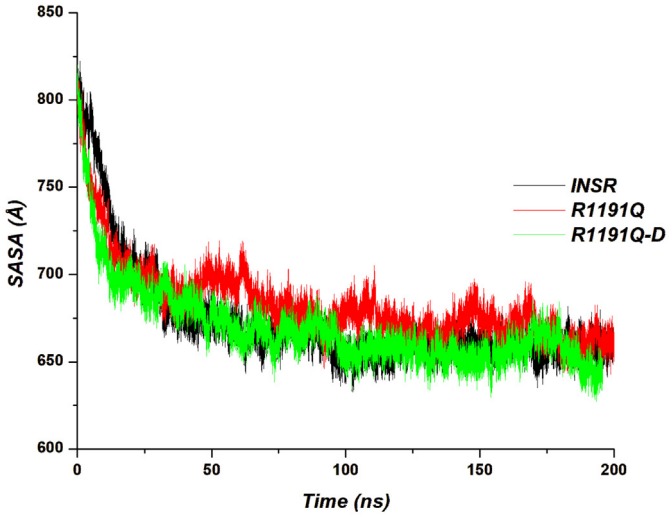

Fig. 7. Solvent Accessible Surface Area (**SASA) plot of *INSR* (Black), R119Q (red), and R119Q-D (green).** This graph tracks the SASA in Ångströms squared (Å^2) for the *INSR* protein and its variants R1191Q and R1191Q-D over a 200 nanosecond (ns) period.

disrupted hydrogen bonds caused by mutation may be restored when bound with this drug. However, the R1191Q-D complex shows fewer hydrogen bonds compared to the *INSR*. This suggests that while the drug can improve some of the destabilizing effects caused by the mutation, it does not completely restore the original hydrogen bond network. The decrease in hydrogen bonds observed in the R1191Q variant may be linked to a loss of integrity, potentially affecting the receptor's ability to function correctly. The hydrogen bonds are partially restored in the presence of the drug. This indicates a stabilizing effect that could lead to regained functionality or increased stability in this receptor variation.

Solvent accessible surface area (SASA)

SASA plays a role in understanding protein folding, function, and interaction, as it gives us insights into the parts of a protein that are exposed and could be significant for interactions [38]. The wildtype protein showed a sharp decrease in SASA at the beginning of the simulation, which then stabilized quickly. This drop might indicate that the protein relaxes from a conformation to one more energetically favorable and possibly more compact after starting the simulation. Likewise, the R1191Q also starts with a decreasing trend but consistently maintains a higher SASA than the wild type. This suggests that there might be some disruption in protein folding due to this mutation, resulting in exposed areas to solvent and a less compact structure. The R1191Q-D exhibited SASA values that fall between those of mutant types, indicating that drug binding induces some changes resulting in a slightly more compact structure (Fig. 7) than when it's unbound but still not as compact as seen in typical receptors. The observed higher SASA for the R1191Q variant might indicate that the protein structure has been altered or destabilized due to the mutation. It seems that the drug partially counteracts this effect, which could have implications for how the drug works. The increased SASA in the R1191Q variant suggests that its interaction profile with ligands or other molecular partners may have changed, potentially leading to consequences. The binding of the drug to R1191Q appears to affect how exposed the protein is to its surroundings, which could impact how it interacts with insulin or other molecules.

Discussion

In this study, we examined the dynamics of *INSR* and its variant R1191Q using molecular docking and molecular dynamic simulation. We also used the same method to assess the effect of CheBI_88,339 on R1191Q. We observed a decrease in the molecular instability of R1191Q when bound with CheBI_88,339.

As shown in previous research, mutation within a protein influences protein stability and flexibility, which sometimes increases the pathogenicity of such proteins (39–41). The decreased RMSD observed upon binding CheBI_88339 with R1191Q relative to the wild type aligns with previous findings that small molecular weight inhibitors could potentially increase the stability and restore instability of a protein caused by mutation (42–44). Similarly, our study shows that protein flexibility increases with mutation (45). However, this reduces with binding with an inhibitor, which may help restore the protein function (45). The ability of CheBI_88339 to moderate these changes presents an avenue for intervention (46,47), which could be further explored in drug repurposing and personalized medicine, where drug molecules are tailored to address specific mutational effects.

One strength of this study is the use of extended molecular dynamic stimulation, which provided detailed and time-wise insight into how the wild-type and mutant *INSR* behave at the atomic level. This allowed for proper comparisons of the stability and flexibility between the wild, mutant, and drug-bound systems.

This study's limitations primarily stem from the nature of simulations. While MD simulations are a tool for predicting protein dynamics, they may not always encompass the range of natural molecular interactions. Furthermore, the accuracy of force fields utilized in these simulations plays a role, and any limitations in their accuracy can impact the results obtained. Additionally, due to the scope of this simulation, it may not be possible to observe changes that could occur under physiological conditions. Also, this research is computational, relying on the inherent limitations of the algorithms and tools. Hence, experimental studies are needed to support the results.

This research contributes to understanding how structural deviations and mutations can influence protein stability and function. Additionally, it is worth noting that the binding of CheBI_88339 appears to mitigate these effects, stabilizing the protein structure and potentially restoring functionality. These insights enhance our understanding of the *INSR* dynamics and offer a promising outlook for developing targeted therapies for conditions stemming from similar mutations. The study underscores the importance of integrating computational simulations with experimental validation to accelerate the path toward personalized medicine.

In conclusion, the observation that CheBI_88339 stabilized the R1191Q mutant suggests that it could be a promising therapeutic option that could be repurposed for this mutation in T2D patients when repurposed. However, it is important to undertake experimental research to validate and provide a deep dive into the biological mechanism.

Consent for publication

All authors give consent for publication.

Declaration of competing interest

The authors declare that there are no competing interest.

CRediT authorship contribution statement

Chisom Soremekun: Conceptualization, Formal analysis, Writing – original draft. **Daudi Jjingo:** Supervision, Writing – review & editing. **David Kateete:** Writing – review & editing. **Oyekanmi Nash:** Writing – review & editing. **Harald Grallert:** Supervision, Writing – review & editing. **Annette Peters:** Supervision. **Tinashe Chikowore:** Writing – review & editing. **Chiara Batini:** Supervision, Writing – review & editing. **Opeyemi Soremekun:** Conceptualization, Validation, Writing – original draft. **Segun Fatumo:** Conceptualization, Supervision, Writing – review & editing.

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Data Availability

This study only used publicly available data. No original data were collected.

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