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Research Article

Computational Analysis of Crizotinib Reactivity and Electronic Structure in the Presence of Neurotoxic β-Amyloid Aggregates

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ABSTRACT

Crizotinib, a tyrosine kinase inhibitor, is widely recognized for its role in oncology; however, its interactions with biomolecules implicated in neurodegenerative diseases remain underexplored. In this study, the structural optimization of Crizotinib was performed using Density Functional Theory (DFT) at the B3LYP/6-31G(d,p) level in Gaussian, ensuring a stable molecular geometry. To investigate its interaction with beta-amyloid (Aβ42) peptides, molecular dynamics (MD) simulations were conducted using the GROMACS package, applying the CHARMM force field for peptides and GAFF for Crizotinib. The system was solvated in a water box, neutralized, minimized, equilibrated, and subjected to a 100 ns production simulation under physiological conditions. Electronic structure analysis using DFT revealed that the HOMO and LUMO energies of Crizotinib shifted upon interaction with Aβ42. Specifically, the HOMO energy increased from –771.07 kJ·mol^{-1} (in the absence of Aβ42) to –684.12 kJ·mol^{-1} (in its presence), while the LUMO energy increased from –719.39 kJ·mol^{-1} to –673.19 kJ·mol^{-1}. This corresponds to a narrowing of the HOMO-LUMO gap from 51.68 kJ·mol^{-1} to 10.93 kJ·mol^{-1}, suggesting a substantial alteration in the molecule's electronic properties and potential reactivity. The HOMO and LUMO distributions further revealed an expanded electron cloud in the presence of Aβ42, indicating that non-covalent interactions, such as hydrogen bonding and π-π stacking, influence Crizotinib's electronic structure. These findings highlight the potential role of Crizotinib in modulating Aβ42-associated pathways, which are critical in Alzheimer's disease pathology. This study underscores the importance of computational approaches in elucidating drug–peptide interactions relevant to neurodegenerative disorders.

Keywords: Crizotinib, chemical reactivity, Alzheimer, Aggregation, Molecular dynamics

1. Introduction

Crizotinib is a small-molecule tyrosine kinase inhibitor (TKI) primarily developed for the treatment of non-small cell lung cancer (NSCLC) by targeting anaplastic lymphoma kinase (ALK), ROS1, and MET kinases[1]. By inhibiting these kinases, Crizotinib disrupts oncogenic signaling pathways, leading to reduced tumor growth and metastasis.

Beyond its established role in oncology, emerging studies suggest that Crizotinib may have potential neuroprotective properties, making it a candidate for repurposing in neurodegenerative disorders such as Alzheimer's disease (AD)[2].

Alzheimer's disease is a progressive neurodegenerative disorder characterized by memory loss, cognitive impairment, and neuronal dysfunction, with pathological hallmarks including beta-amyloid (Aβ) plaque deposition and tau protein hyperphosphorylation[3]. Among the various isoforms of Aβ peptides, Aβ42 is considered the most neurotoxic due to its higher aggregation propensity, leading to the formation of insoluble fibrils that contribute to synaptic dysfunction and neuronal death[4, 5]. The accumulation of Aβ42 oligomers triggers a cascade of molecular events, including oxidative stress, mitochondrial dysfunction, neuroinflammation, and kinase dysregulation, ultimately accelerating neurodegeneration[6, 7].

Recent investigations have suggested a link between Crizotinib and Alzheimer's disease pathology, particularly in modulating molecular pathways associated with Aβ aggregation and kinase activity[8]. Given that Crizotinib inhibits MET kinase, which has been implicated in synaptic plasticity and neuroprotection, its role in modulating Aβ-induced toxicity is of significant interest[9]. Additionally, molecular simulations and quantum chemical analyses have indicated that Crizotinib may interact with Aβ42 peptides, potentially influencing its aggregation dynamics and neurotoxic effects[10]. While the experimental evidence for direct

interaction remains limited, computational approaches provide a valuable theoretical framework for hypothesizing how small molecules like Crizotinib might influence Aβ42 structure, aggregation, and associated toxicity.Understanding these interactions at a molecular level can provide valuable insights into the therapeutic potential of Crizotinib in Alzheimer's disease and highlight its mechanistic influence on neurodegenerative processes. This study aims to explore the molecular interactions between Crizotinib and Aβ42 peptides, using computational modeling approaches to assess how this drug influences the electronic properties and binding behavior in the presence of amyloid aggregates.

2. Experimental

Method

The structural optimization of Crizotinib was performed using Density Functional Theory (DFT) within the Gaussian software package, employing the B3LYP functional and the 6-31G(d,p) basis set to achieve an optimized molecular geometry[11]. Convergence criteria were set to ensure a stable minimum energy structure, and vibrational frequency calculations were conducted to confirm the absence of imaginary frequencies.

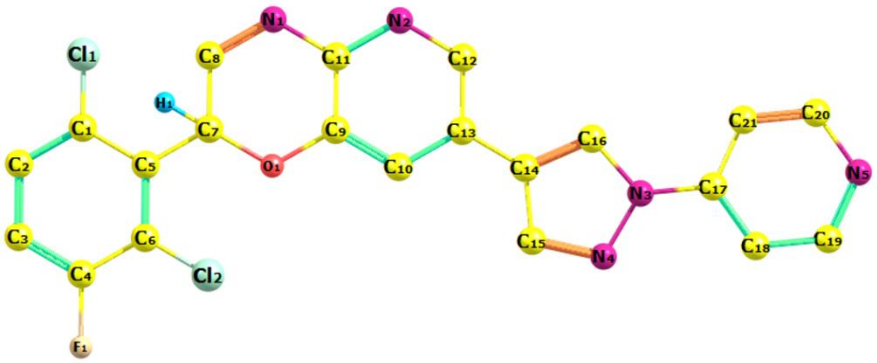

Fig. 1. optimized structure of crizotinib

To investigate the interaction between Crizotinib and beta-amyloid (Aβ42) peptide, molecular dynamics (MD) simulations were conducted using the GROMACS 2021 package[12]. The CHARMM force field was applied for the peptide and Crizotinib[13]. The initial coordinates of Aβ42 were taken from the Protein Data Bank (1Z0Q), and the ligand topology was generated using the SwissParam tool[14]. The Crizotinib-Aβ42 system was solvated in a cubic simulation box with SPC water molecules, ensuring a 10 Å padding distance from the solute, and counterions (Na+ and Cl−) were added to neutralize the system.The simulation employed a cut-off distance of 1.4 Å for both electrostatic and van der Waals interactions. Periodic boundary conditions (PBC) were applied in all directions to mimic an infinite system, and sodium and chlorine ions were added to neutralize the system.

A consistent random seed was used to ensure reproducibility of the simulation results.Energy minimization was performed using the steepest descent algorithm for 50,000 steps to remove steric clashes[15]. The system was equilibrated in two steps: (1) NVT equilibration at 310 K for 100 ps using the velocity-rescaling (V-rescale) thermostat and (2) NPT equilibration at 1 atm for 100 ps using a Berendsen barostat to stabilize the density[16].

A 100 ns production simulation was performed with a 2-fs time step under periodic boundary conditions (PBC), employing the Particle Mesh Ewald (PME) method for long-range electrostatic interactions and LINCS constraints for hydrogen bonds[17]. To examine the effect of beta-amyloid on Crizotinib’s electronic properties, the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) were calculated in both the absence and presence of beta-amyloid. Single-point energy calculations were conducted using DFT at the B3LYP/6-31G(d,p) level in Gaussian, and the molecular orbital distributions were analyzed to assess changes in electronic properties due to peptide interaction.

3. Result and Discussion:

The last 10 nanoseconds of the simulation were sampled. The sampled structure of the beta-amyloid peptide alongsidecrizotinib is shown in the following figure:

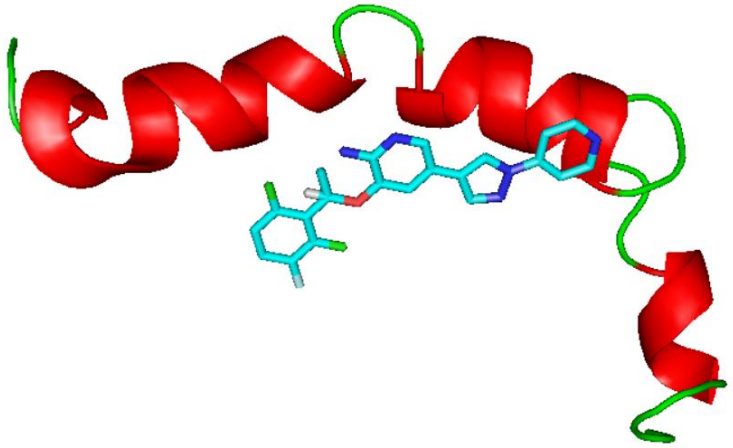

Fig. 2. The structure of crizotinib in the beta-amyloid peptide binding site, sampled from performed molecular dynamics simulation.

In this figure, the peptide structure is shown in cartoon form and the drug structure is shown in stick form. The red areas in the peptide structure correspond to the helix and the green areas correspond to the random coil structure. It can be seen that the drug approaches the peptide from its six- and five-membered rings and interacts with it.In order to investigate the effect of beta-amyloid peptide on crizotinib reactivity, the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) orbitals of the drug were obtained in the absence and presence of the peptide. The results are shown in the figures below.

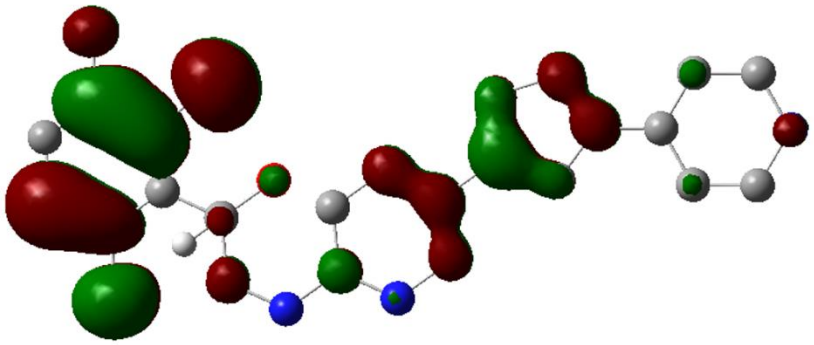

Fig. 3.HOMO orbital of Crizotinib in absence of beta amyloid

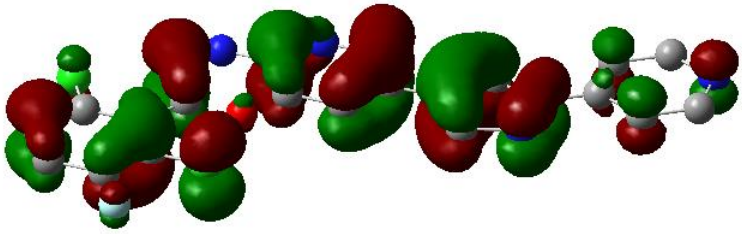

Fig. 4. HOMO orbital of Crizotinib in presence of beta amyloid

The image presents two molecular orbital visualizations of Crizotinib, a known kinase inhibitor, showing its Highest Occupied Molecular Orbital (HOMO) in two different environments: the Fig. 4 represents the HOMO orbital of Crizotinib in the absence of beta-amyloid, while the Fig. 5 shows the HOMO orbital in the presence of beta-amyloid. The HOMO represents the highest energy molecular orbital occupied by electrons and is critical in understanding the molecule's chemical reactivity, interaction with biomolecules, and electronic properties. In the absence of beta-amyloid, the electronic density (depicted as green and red lobes) is more localized, particularly over key functional groups, indicating a constrained electronic structure. This suggests that in isolation, Crizotinib maintains its standard electronic and binding properties. In contrast, in the presence of beta-amyloid, the electron cloud distribution becomes more spread out, extending over a wider molecular region, suggesting that molecular interactions (such as hydrogen bonding, van der Waals forces, or π-π stacking) alter the electronic properties of Crizotinib. This change in electronic

structure could influence Crizotinib's chemical reactivity, binding affinity, and potentially its biological activity. The implications of this include altered reactivity, potential changes in drug-target interactions, and possible effects on Crizotinib's role in neurological conditions, especially in relation to beta-amyloid aggregation, which is linked to Alzheimer's disease.

The redistribution of HOMO orbitals suggests that Crizotinib might exhibit different pharmacological effects in the presence of beta-amyloid, impacting its bioavailability, stability, or metabolic pathways. These findings highlight the importance of studying quantum chemical properties and molecular interactions to better understand drug behavior in complex biological environments.

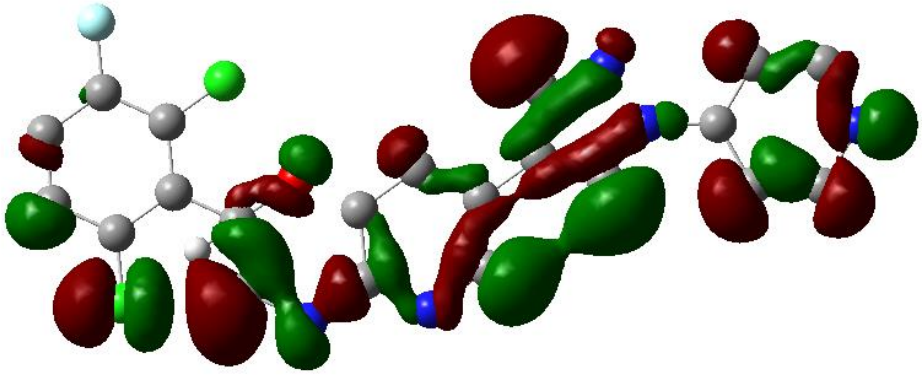

Fig. 5. LUMO orbital of Crizotinib in absence of beta amyloid

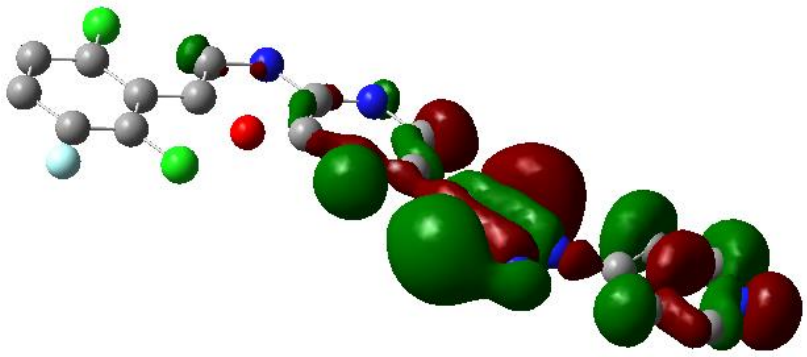

Fig. 6. LUMO orbital of Crizotinib in presence of beta amyloid

The image presents two molecular orbital visualizations ofCrizotinib, illustrating itsLowest Unoccupied Molecular Orbital (LUMO)in two different conditions: thetop imagerepresents the LUMO orbital of Crizotinib in the absence of beta-amyloid (Fig. 5), while thebottom imageshows the LUMO orbital in the presence of beta-amyloid (Fig. 6). The LUMO represents the lowest energy molecular orbital available for electron acceptance and is crucial for understanding a molecule'selectrophilic nature, reactivity, and potential interactions with biological molecules. In theabsence of beta-amyloid, the electronic density (depicted as green and red lobes) islocalized in distinct molecular regions, suggesting that Crizotinib'selectronic acceptor sites are well-definedand concentrated over specific functional groups, maintaining its standard electronic structure. However, in thepresence of beta-amyloid, the electron cloud distribution becomes more dispersed, with the LUMO extending across a broader molecular framework. Thisshift in electron density suggests an alteration in Crizotinib's electronic properties, likely due tomolecular interactions with beta-amyloid, such as hydrogen bonding, electrostatic interactions, or π-π stacking. These changes in the LUMO distribution could impactCrizotinib's chemical reactivity, charge transfer dynamics, and its ability to engage in electron-exchange interactions with biological molecules. Theimplicationsof these findings include apotential alteration in Crizotinib's drug-target interactions, which might influence itsbinding affinity, pharmacological effectiveness, and possibly its role in neurodegenerative conditionslike Alzheimer's disease, where beta-amyloid aggregation plays a critical role. The shift in orbital distribution may also indicatemodified bioavailability, metabolism, or stability in the presence of beta-amyloid. To assess the impact of Aβ42 interaction on the electronic properties of Crizotinib, HOMO and LUMO energies were calculated using DFT at the B3LYP/6-31G(d,p) level. In the absence of Aβ42, the HOMO and LUMO energies of Crizotinib were found to be –771.07 $kJ \cdot mol^{-1}$ and –719.39 $kJ \cdot mol^{-1}$, respectively. Upon interaction with Aβ42, these values shifted to –684.12 $kJ \cdot mol^{-1}$ (HOMO) and –673.19

kJ·mol^{-1} (LUMO). This indicates a narrowing of the HOMO–LUMO energy gap from 51.68 kJ·mol^{-1} to 10.93 kJ·mol^{-1}, suggesting increased chemical reactivity.

Conclusion:

This study investigated the interaction between Crizotinib, a kinase inhibitor, and beta-amyloid peptide, focusing on the drug's electronic properties and reactivity in different environments. By analyzing the Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) of Crizotinib in the presence and absence of beta-amyloid, we observed significant changes in its electronic structure. Molecular dynamics simulations revealed that Crizotinib interacts with beta-amyloid via its six- and five-membered rings, forming interactions that alter its electronic properties.The HOMO analysis showed that in the absence of beta-amyloid, the electronic density was well-localized over specific functional groups, indicating a constrained electronic structure with stable reactivity and binding properties. However, in the presence of beta-amyloid, the electron cloud distribution became more delocalized, spreading across a wider molecular region. This shift suggests that interactions such as hydrogen bonding, van der Waals forces, and π-π stacking modify the electronic landscape of Crizotinib, potentially influencing its reactivity, stability, and pharmacological activity. A similar pattern was observed in the LUMO analysis, where the electron density was originally concentrated over distinct molecular regions but became more dispersed in the presence of beta-amyloid. This suggests that beta-amyloid binding affects Crizotinib’s electrophilic nature and charge transfer dynamics, which may alter its behavior in biological environments.These findings have several important implications.

The observed modifications in HOMO and LUMO distribution indicate that Crizotinib may exhibit altered drug-target interactions, bioavailability, and metabolism when in contact

with beta-amyloid. Given that beta-amyloid aggregation is a hallmark of Alzheimer's disease, Crizotinib's altered reactivity in this environment suggests a potential role in neurological conditions. The study highlights the necessity of quantum chemical analyses and molecular simulations in understanding how small molecules interact with biological macromolecules in complex environments. Further investigations, including density functional theory (DFT) calculations, molecular docking studies, and experimental validations, are required to fully elucidate the pharmacological consequences of Crizotinib's interactions with beta-amyloid.

Understanding these effects could provide insights into drug repurposing strategies for neurodegenerative diseases and aid in the design of new therapeutic agents with optimized properties for treating conditions associated with beta-amyloid aggregation.To build upon the computational findings presented here, future experimental studies are encouraged to validate the predicted interaction between Crizotinib and Aβ42 peptides, for example through spectroscopic techniques (e.g., NMR, fluorescence quenching) or surfaceplasmon resonance to detect binding events. Additionally, cell-based assays could assess whether Crizotinib modulates Aβ42-induced cytotoxicity, providing functional insights. In the long term, preclinical studies in Alzheimer's disease models may help evaluate the therapeutic relevance of Crizotinib repurposing in a biological context.While this study provides valuable insights into the electronic structure alterations of Crizotinib in the presence of Aβ42 using DFT and molecular dynamics simulations, certain methodological limitations should be acknowledged.

The absence of experimental validation, limited sampling of Aβ42 conformational diversity, and the lack of advanced quantum descriptors (e.g., Fukui functions, ESP maps) may constrain the depth of mechanistic interpretation. Additionally, specific non-covalent interactions were inferred from electronic features rather than directly quantified. These limitations highlight opportunities for future studies combining extended simulation timescales, experimental data, and more comprehensive quantum chemical analyses.

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