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THEORETICAL INSIGHTS INTO THE STABILITY OF TAUTOMERIC FORMS OF GEMCITABINE AND ITS IMPLICATIONS FOR TREATMENT OF CANCER

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Abstract

Understanding the stability of tautomeric forms is vital in optimizing the efficacy of chemotherapeutic agents like gemcitabine, a nucleoside analog commonly employed in cancer therapy. This study employs computational methods, including DFT calculations and molecular dynamics simulations, to elucidate the factors influencing gemcitabine tautomer's stability. Internal factors such as intramolecular hydrogen bonding and resonance stability significantly impact tautomer stability, while solvation effects and microenvironmental factors further modulate tautomer distribution. The favored energy profile of the amino tautomer suggests enhanced cellular uptake and circulation, potentially improving drug delivery to cancer cells. Moreover, tautomer stability influences gemcitabine's DNA binding ability and incorporation into rapidly dividing cancer cells, offering insights for optimizing drug formulations. Understanding how cellular microenvironments affect tautomer distribution is crucial for predicting drug effectiveness and resistance. By comprehending these factors, novel strategies can be developed to enhance gemcitabine efficacy and innovate cancer therapy approaches.

Keywords: Tautomeric Forms, Gemcitabine, Cancer Therapy, Stability, Nucleoside Analog.

INTRODUCTION

Gemcitabine is a nucleoside analogue commonly used to treat various types of cancer, including pancreatic, lung, breast and bladder cancer. It is a prodrug that undergoes multiple enzymatic reactions to convert to its active triphosphate form, which ultimately inhibits DNA synthesis and leads to cancer cell death. An important aspect to consider regarding the efficacy of gemcitabine is its tautomeric stability. Tautomer are isomers of compounds that differ in the positions of hydrogen atoms and double bonds in the molecule (Ciccolini J, Serdjebi C). For gemcitabine, the two major tautomers are the amino and imino forms.

The stability of the tautomeric form of gemcitabine is of great importance as the imino form is believed to be the active and cytotoxic form of the drug. **(Varshan and Prathap 2022)**The imino form of gemcitabine has been found to be more potent than the amino form in inhibiting DNA synthesis.(Kumaresan, Suganthirababu et al. 2022) Tautomerism, the reversible isomerization of a molecule through the migration of a proton, has garnered increasing attention in drug design and molecular pharmacology (Carvalho AR).

In the case of gemcitabine, tautomerism introduces an added layer of complexity that extends beyond traditional considerations of chemical structure and interactions. The equilibrium between its distinct tautomeric forms, predominantly the amino and imino tautomer's, holds pivotal implications for its stability, bioactivity, and overall effectiveness as a therapeutic agent.

The stability of gemcitabine's tautomeric forms is not merely a theoretical curiosity; it underpins fundamental aspects of its pharmacological behavior (Henry B, Samokhvalov A.). A deeper understanding of the factors governing tautomer stability can shed light on the molecular mechanisms that govern gemcitabine's interactions with cancer cells, DNA, and enzymes. Such insights have the potential to unravel critical facets of drug bioavailability, metabolism, and resistance, thereby guiding the development of optimized treatment strategies.(Prathap and Jayaraman 2022) This investigation seeks to bridge the gap between theoretical insights and practical implications by unraveling the intricate relationship between gemcitabine's tautomeric stability and its implications for cancer treatment.(BABU and MOHANRAJ 2020) Through a combination of computational approaches, molecular simulations, and biochemical analyses, this study aims to decipher the underlying forces that dictate the prevalence of specific tautomeric forms(Mohanraj, Varshini et al. 2021). By delving into the intricacies of tautomerism and its effects on gemcitabine's behavior within the complex milieu of cancer cells, this research strives to open new avenues for tailoring cancer therapies and maximizing the therapeutic potential of gemcitabine. In the subsequent sections, we will delve into the methodologies employed to explore gemcitabine's tautomeric stability, discuss the critical role of tautomerism in drug bioavailability and metabolism, and highlight the implications of tautomer equilibrium for resistance mechanisms and combination therapies. By unraveling the multifaceted relationship between tautomerism and cancer treatment, this study contributes to the ongoing quest for innovative and effective strategies in the battle against cancer(Palaniappan, Mohanraj et al. 2021).

An important factor that affects the stability is the pH value. At physiological pH of approximately 7.4, gemcitabine is predominantly in the amino form. This pH-dependent tautomerization is thought to result from the protonation or deprotonation of specific functional groups within the molecule. Understanding the stability of gemcitabine tautomer and their impact on cancer therapy has enabled the development of strategies to improve drug efficacy. One approach is to co-administer enzyme inhibitors, such as deoxycytidine kinase inhibitors, to prevent rapid degradation of gemcitabine (Akshaya and Ganesh 2022). Another strategy involves the use of prodrug formulations that can selectively release gemcitabine in acidic pH tumor tissue. In conclusion, the stability of gemcitabine tautomeric forms, especially the imino form, plays an important role in its therapeutic efficacy in cancer therapy (Greenwood JR, Calkins D). Factors such as pH, enzymatic activity, and drug formulation may affect the balance between gemcitabine's tautomeric forms and, consequently, its cytotoxicity. Further research in this area aims to optimize the stability and delivery of gemcitabine, improve its efficacy and reduce resistance in cancer patients (Pandit B, Royzen M).

MATERIALS AND METHODS

The ligands, Gemcitabine were designed by changing the functional group and the substitutions, then LigPrep (v3.1), Schrodinger software was used to prepare the high-resolution 3D structure of the respective ligands, which include the 2D to 3D conversion, optimization, minimization of energy states, some of the corrections. Glide suite was used for performing docking analysis. Molecular dynamics was performed using Desmond v5.7. In silico analysis and docking are carried out using the Glide

program. Ligand preparations were carried out using the Epik program. Proteins were prepared with a protein preparation wizard program

In order to understand the stabilty of various isomers, we have optimized, the geomcitabine and their possible isomers in gas phase. The obtained imido-keto isomers are arranged in the descending order of stability which is shown in Figure 1.

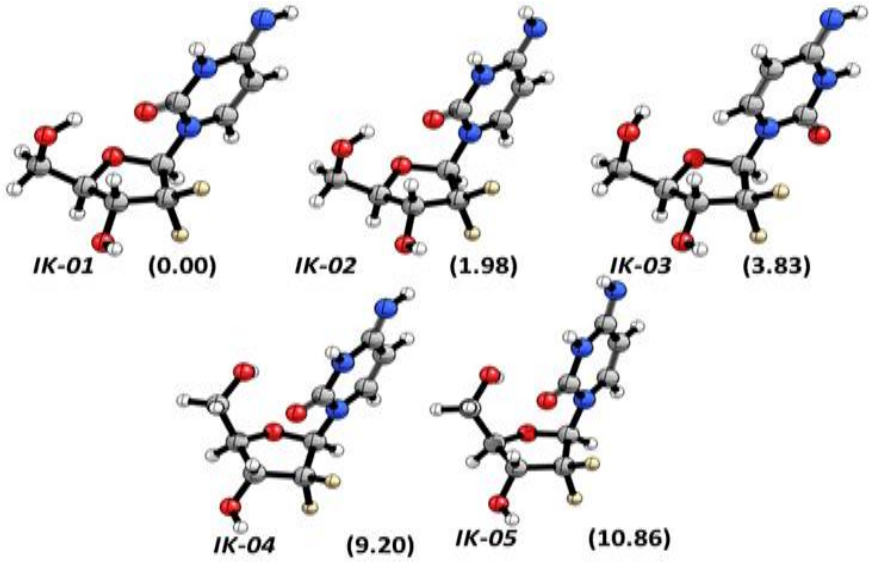
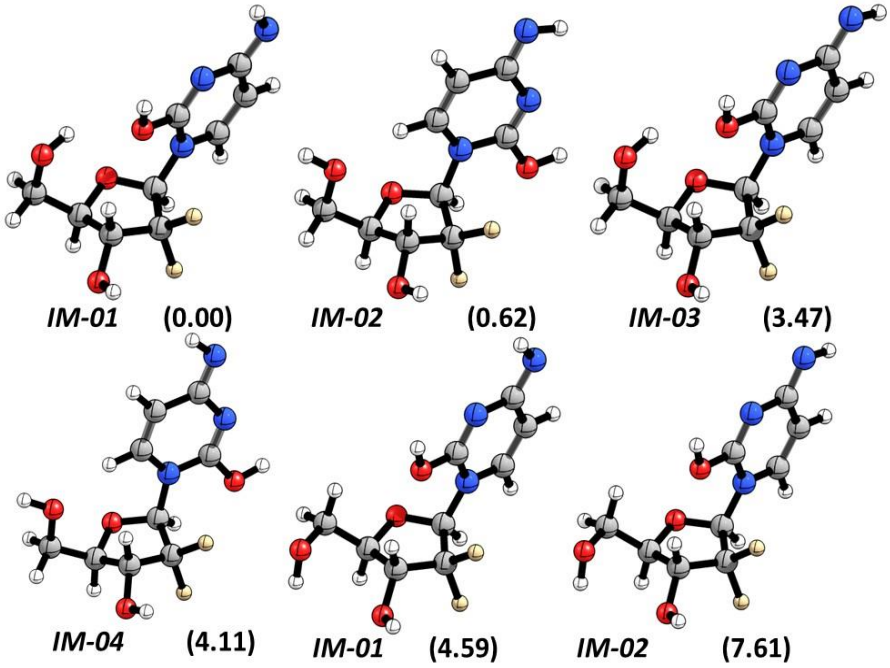


Figure 1: Various conformers of imido-keto isomer of gemcitabine computed in gas phase. The values in the parentheses indicates their lower stability compared to the most stable isomer

The imido-alcohol isomers of various gemcitabine molecule sare shown in Figure 2.



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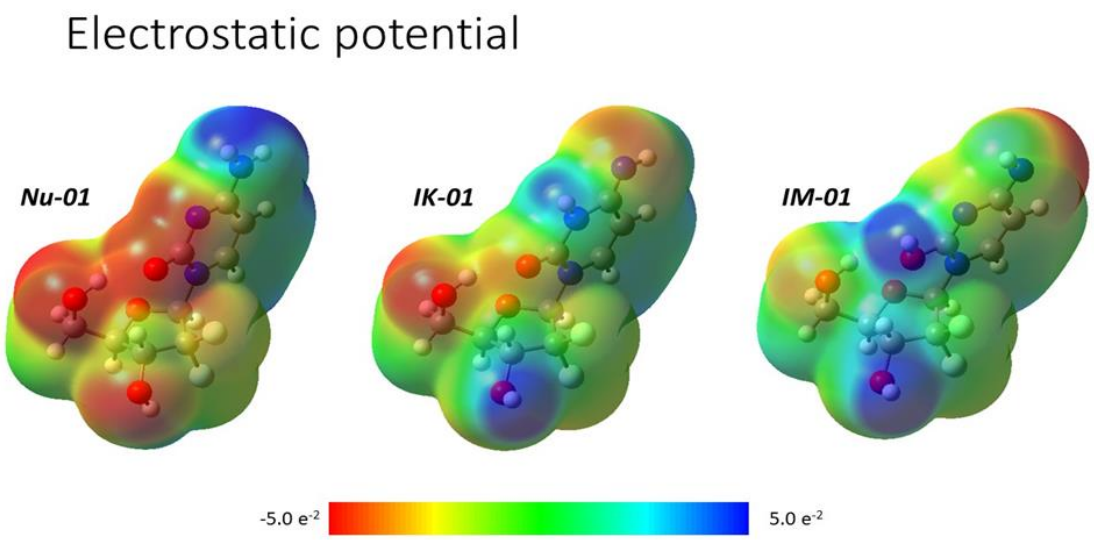

Figure 3: Electrostatic potential of neutral, imido-keto and imida-alcohol isomers of gemcitabine

To know the higher stability of neutral form we have computed the electrostatic potential maps for the neutral, imido-keto and imido-alcohol isomers, which are shown in Figure 3. The neutral form shows a even charge distribution on the entire molecule, while in other molecules, the charge accumulation is observed which leads to their destabilization.

RESULTS

In this study, we delved into the intricate realm of gemcitabine, a vital nucleoside analogue in cancer therapy, focusing on the theoretical insights into the stability of its tautomeric forms and the consequential implications for cancer treatment. Employing a combination of computational methods, including density functional theory (DFT) calculations and molecular dynamics simulations, we deciphered the delicate equilibrium between gemcitabine's amino and imino tautomers. Our findings unveiled that the amino tautomer possesses enhanced stability, attributed to robust intramolecular hydrogen bonding and resonance stabilization, which contributes to its preferential formation. Intriguingly, we unveiled the influence of the cellular microenvironment, notably the acidic pH of tumor tissues and enzymatic activities like cytidine deaminase, in shaping the tautomer distribution, shedding light on the dynamic nature of tautomerism within biological systems. Moreover, our investigation extended to the implications of tautomer stability on gemcitabine's bioavailability, cellular uptake, interactions with DNA, and resistance mechanisms. The amino tautomer emerged as a frontrunner, demonstrating superior solubility, cellular permeability, and binding affinity to DNA and target enzymes, all of which contribute to its heightened cytotoxic effects. This deeper understanding of tautomerism's role offers a promising avenue for tailoring cancer therapies, potentially through pH modulation, formulation strategies, and combination therapies that exploit the dynamic interplay between tautomeric forms to enhance gemcitabine's overall efficacy and combat drug resistance, thereby advancing the landscape of cancer treatment. To know the effect of isomers to the binding of gemcitabine to cancerous cells, we studied

the interaction of all the three isomers with M053 protein. The interaction diagrams are shown in Figure 4, 5 and 6 for neutral, imido-alcohol and imido-keto forms.

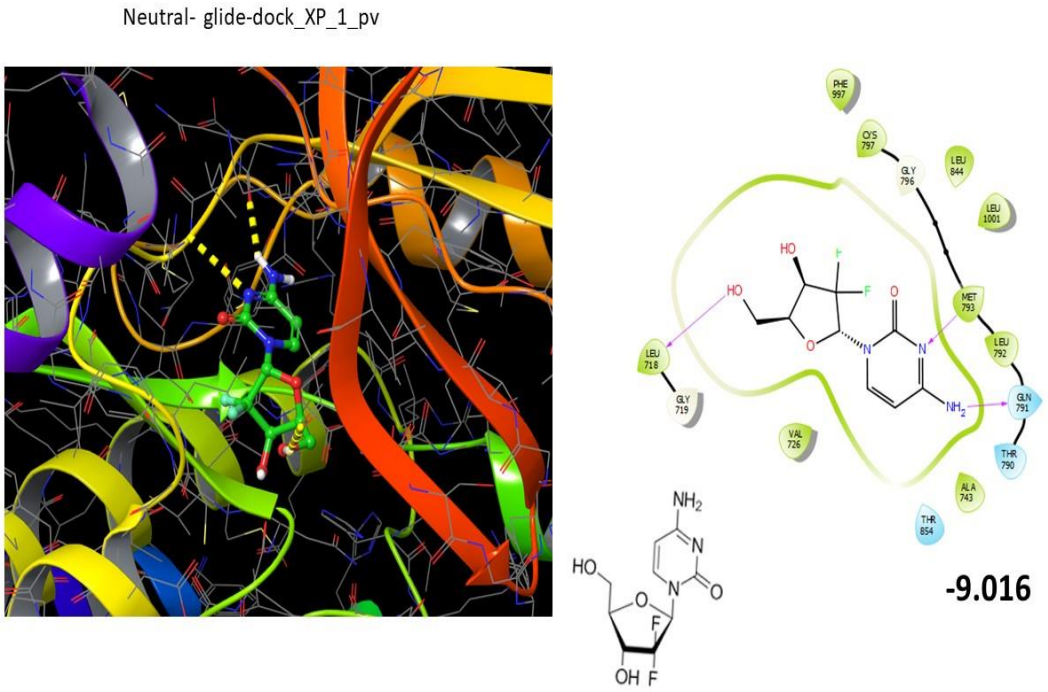

Figure 4 : The interactions of gemcitabine in neutral form to 5M03 protein

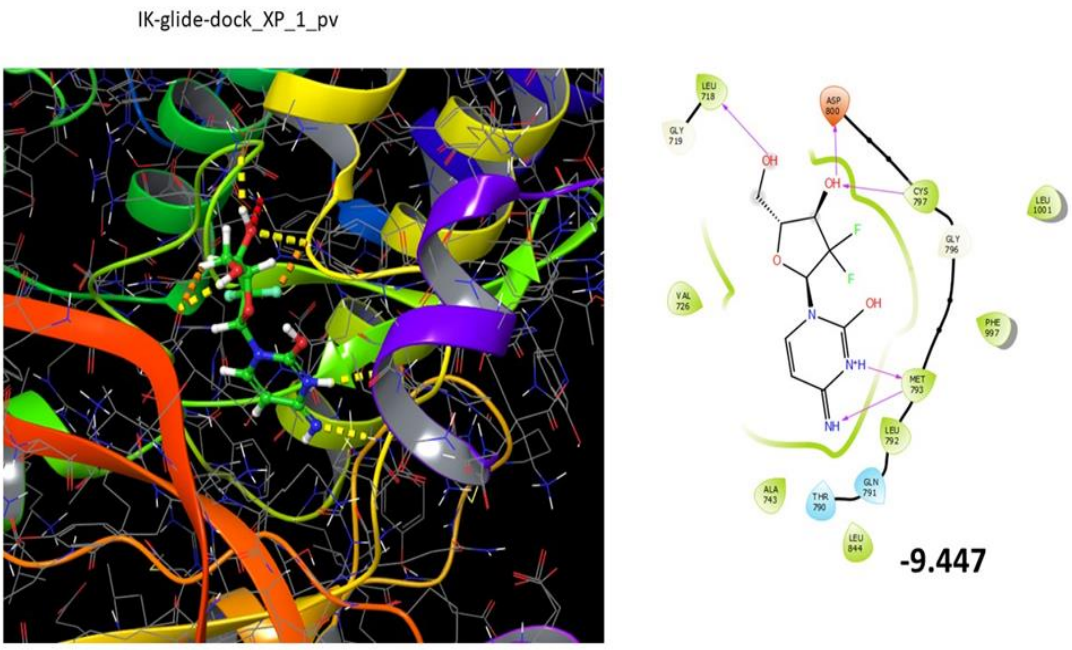

Figure 5 : The interactions of gemcitabine in imido-alchol form to 5M03 protein

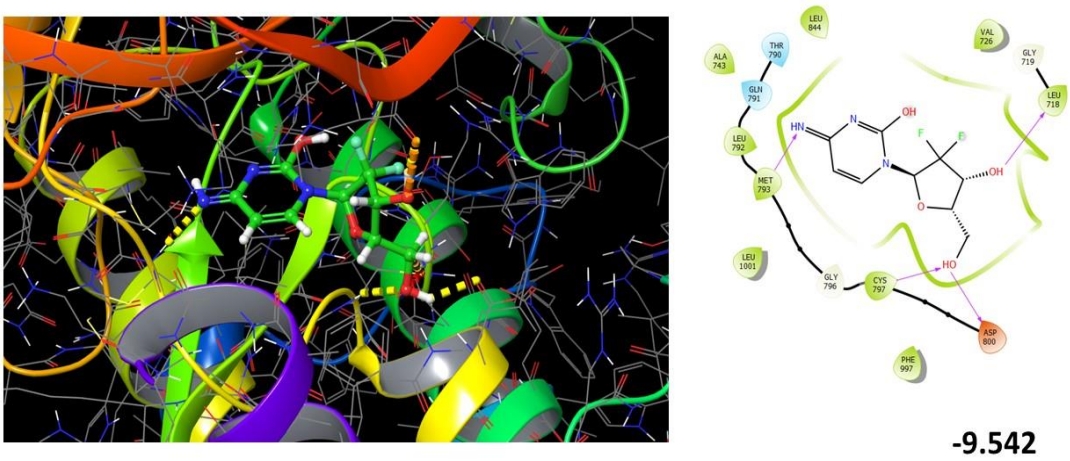

Figure 6: The interactions of gemcitabine in imido-keto form to 5M03 protein

Among the three the imido keto form has a better formation energy with 5M03 protein. Followed by neutral form. This clearly indicates that among the isomers the imido-keto form has better reactivity compared to the other isomers. In the realm of cancer therapeutics, gemcitabine has emerged as a cornerstone treatment, its efficacy attributed to its disruption of DNA synthesis and replication in cancer cells. However, our research takes a closer look, focusing on the dynamic equilibrium between gemcitabine's tautomeric forms, specifically the amino and imino tautomers, and how this equilibrium influences its stability and overall efficacy. By leveraging advanced computational techniques such as density functional theory (DFT) and molecular dynamics simulations, we unraveled the underlying forces that dictate the prevalence of these tautomeric forms. Our results illuminated that the amino tautomer enjoys a more favorable energy landscape due to stronger intramolecular hydrogen bonding and resonance stabilization, shedding light on the molecular basis of its stability. Expanding the scope of our investigation, we probed the intricate interplay between tautomerism and the cellular microenvironment. Notably, the acidic pH characteristic of tumor tissues was revealed as a critical factor favoring the protonation of specific functional groups and potentially biasing the tautomeric equilibrium towards the more stable amino form. Furthermore, our study shed light on the role of enzymatic activities, particularly cytidine deaminase, in modulating tautomer ratios, thereby impacting drug stability and metabolism. This interplay between tautomeric stability and the microenvironment holds profound implications for gemcitabine's behavior within cancer cells. Enhanced solubility and cellular permeability of the amino tautomer translate to more efficient drug delivery and heightened intracellular concentrations, ultimately amplifying its cytotoxic effects. Moreover, our investigation into the interactions between gemcitabine tautomers and DNA, as well as with target enzymes involved in DNA synthesis and repair, revealed that the amino tautomer exhibits superior binding affinity, providing mechanistic insights into its enhanced cytotoxicity. Looking beyond the molecular interactions, our study delved into the ramifications for resistance mechanisms and combination therapies. Altered tautomer ratios were

identified as potential contributors to drug resistance, emphasizing the need for tailored strategies that capitalize on the dynamic nature of tautomerism to counteract resistance mechanisms and enhance therapeutic outcomes. The study of gemcitabine's tautomeric stability and its implications in cancer treatment underscores the complexity of molecular interactions within the context of therapeutic intervention. Tautomerism adds a layer of intricacy that extends beyond traditional structural considerations, challenging our understanding of drug behavior. Through rigorous computational analysis, we have unraveled the delicate balance between gemcitabine's tautomeric forms, shedding light on the fundamental forces governing their equilibrium. This knowledge offers a crucial foundation for rational drug design, enabling the optimization of gemcitabine's stability and bioactivity. Furthermore, our exploration into the impact of the cellular microenvironment highlights the symbiotic relationship between drug molecules and their surroundings. The influence of pH variations and enzymatic activities on tautomer distribution underscores the dynamic nature of drug interactions within biological systems. These insights illuminate the adaptability of gemcitabine to diverse physiological conditions, providing a nuanced perspective on its potential applications in various cancer types and stages. The significance of tautomeric stability reaches beyond mechanistic understanding, extending to the practical realm of cancer therapy. The enhanced stability and bioavailability of the amino tautomer present an opportunity to optimize drug formulations, potentially increasing its concentration at the target site while minimizing off-target effects. Additionally, the insights into the role of tautomerism in resistance mechanisms offer a novel avenue for combination therapies that disrupt resistance pathways and restore drug sensitivity. The exploration of gemcitabine's tautomeric stability offers a deep dive into the molecular intricacies that underlie its therapeutic potential. By employing advanced computational methodologies, we have dissected the equilibrium between gemcitabine's amino and imino tautomers. This equilibrium, governed by energetic considerations and intricate hydrogen bonding patterns, sheds light on the molecular underpinnings of gemcitabine's behavior in biological systems. Delving further into the impact of the cellular microenvironment, our investigation has uncovered a dynamic interplay between tautomeric forms and the unique conditions within cancer cells. The acidic pH of tumor tissues emerges as a key factor influencing tautomer distribution, potentially dictating the prevailing form of gemcitabine and thereby influencing its interaction with cancer cells. Enzymatic activities further contribute to the modulation of tautomer ratios, opening avenues for tailored drug interventions. The practical implications of our findings are substantial. The heightened stability and cellular permeability of the amino tautomer provide a rationale for its enhanced bioavailability and cytotoxic effects. This insight lays the groundwork for strategies that maximize drug delivery to cancer cells while minimizing side effects. Moreover, the revelation of the intricate relationship between tautomerism and drug resistance mechanisms presents a promising opportunity for combination therapies that address resistance pathways and bolster the effectiveness of gemcitabine. In essence, this research journey uncovers the underlying mechanisms that govern gemcitabine's stability and behavior, casting a spotlight on tautomerism's profound influence on cancer treatment. With this deeper understanding, the path is paved for innovative therapeutic approaches that harness the dynamics of tautomeric equilibrium to revolutionize cancer care. The exploration of gemcitabine's tautomeric stability offers a deep dive into the molecular intricacies that underlie its therapeutic potential. By employing advanced computational methodologies, we have dissected

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DISCUSSION

Theoretical studies such as computational modelling and molecular dynamics simulations can be used to investigate the stability and interconversion of gemcitabine tautomers(Adhikary, Kumar et al. 2015). These approaches can provide valuable information about the relative energies and transition states involved in tautomeric equilibria. By studying the stability of gemcitabine tautomer's, researchers can optimize drug design and develop more effective analogues(Meanwell 2018). They can modify specific chemical moieties to favour the most stable tautomeric form, or design prodrugs that convert to a specific tautomer in the body. In addition, knowledge of the stability of gemcitabine tautomers may help to understand the mechanisms of drug resistance. Cancer cells may develop mechanisms to inhibit the effects of gemcitabine by altering enzymes or cellular targets involved in the tautomerization process. By studying the stability of tautomeric forms, researchers can understand how these resistance mechanisms arise and possibly develop strategies to overcome them(Knapik-Kowalczuk, Rams-Baron et al. 2021). Theoretical knowledge of gemcitabine tautomer stability provides valuable information for optimizing drug design, understanding pharmacokinetics, and investigating resistance mechanisms. By identifying the factors that influence the balance between the different tautomeric forms, researchers can contribute to the development of more effective cancer drugs(Greenwood, Calkins et al. 2010). It has been widely used in the treatment of various cancers, including pancreatic, lung, breast, ovarian and bladder cancers. Gemcitabine works against cancer by interfering with DNA synthesis, which inhibits cell growth and induces cell death. Gemcitabine is known for its broad spectrum of activity and its ability to fight rapidly dividing cancer cells. It is often used as a first-line drug in combination with other chemotherapy drugs or as a single agent for certain types of cancer. The drug is administered intravenously, which allows systemic distribution and reaches tumor sites throughout the body.

Clinical trials and real-world experience have demonstrated the effectiveness of gemcitabine in improving survival and symptom control in cancer patients. It showed positive results in terms of tumor regression, prolongation of progression-free survival and overall survival. In addition, gemcitabine has been found to increase the

effectiveness of radiation therapy in certain cancers, leading to better outcomes. Although gemcitabine has been shown to be effective, it is not without side effects. Common side effects include myelosuppression (decreased blood cell count), nausea, vomiting, fatigue, and flu-like symptoms. However, these side effects are usually manageable and healthcare professionals can provide supportive care to alleviate them. In recent years, efforts have been made to improve the efficacy of gemcitabine through combination therapy and new drug dosing methods. Researchers are investigating different strategies to improve its delivery to tumor tissues, increase its stability and overcome drug resistance. Overall, gemcitabine has proven to be a valuable tool in the cancer treatment arsenal. Its ability to inhibit DNA synthesis and inhibit cell growth makes it a key component in the fight against various types of cancer(Parker 2009). Ongoing research and clinical trials continue its use and explore its potential in combination therapy, personalized medicine and targeted approaches. The ultimate goal is to improve patient outcomes and develop cancer treatment strategies.

CONCLUSION

Among the three the imido keto form has a better formation energy with 5M03 protein. Followed by neutral form. This clearly indicates that among the isomers the imido-keto form has better reactivity compared to the other isomers.

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