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SYNTHESIS AND CHARACTERIZATION OF PYRIMIDINE ANALOGUES FOR ANTICANCER AND ANTIHISTAMINIC PROPERTIES

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ABSTRACT

Spectroscopy, specifically NMR spectroscopy, was used to study the interaction of matter with electromagnetic radiation and characterize molecules like pyrimidine and its analogues. A Proline-complex-catalyzed multicomponent synthesis of pyrimidine was developed, optimizing reaction conditions through adjustments in temperature, time, molar ratios, and catalyst concentration. Molecular docking analysis demonstrated the synthesized compound's potential as an anti-cancer agent by exhibiting strong binding affinity and stable interactions with the Ras protein KRAS, commonly associated with cancer development. Additionally, the compound showed promising interactions with the HNMT protein, suggesting its potential for managing histaminic diseases. These findings emphasize the importance of spectroscopy, multicomponent synthesis, and molecular docking in drug discovery, offering insights into molecular interactions and aiding in the design of therapeutic agents.

Keywords: Spectroscopy, Pyrimidine, Docking, and Chemical Synthesis.

INTRODUCTION

Spectroscopy is a technique used to study the interaction of matter with electromagnetic radiation, providing insights into molecular structure and chemical properties(1). One of the most notable applications of pyrimidine in pharmaceutical chemistry is its role as a structural motif in nucleic acids (2). Pyrimidine bases, namely cytosine, thymine, and uracil, are fundamental building blocks of DNA and RNA (3). The mechanism of action of pyrimidine-based drugs varies depending on the specific compound and its target. However, there are several common mechanisms by which pyrimidine derivatives exert their therapeutic effects (4). Proline is an amino acid that plays a unique role in biochemistry and catalysis. The pharmacokinetics of pyrimidine and its derivatives depend on factors such as the route of administration, formulation, and specific compound characteristics (5). Pyrimidine derivatives can interact with nucleic acids, including DNA and RNA. They may inhibit DNA replication, transcription, or translation processes, leading to the suppression of viral replication or the inhibition of cancer cell growth (6). These drugs work by inhibiting the viral DNA polymerase enzyme, preventing viral replication and reducing the severity and duration of viral outbreaks(7). Pyrimidine analogues can be synthesized using classical or modern methods. Classical methods include the Biginelli, Gewald, Knorr, and Debus-Radziszewski reactions, which involve the condensation of aldehydes, ketones, amines, and carboxylic acids (8).

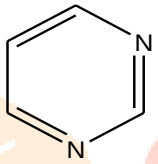


Fig. 1: Pyrimidine

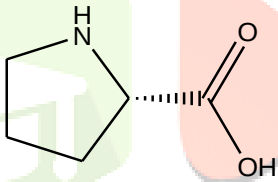


Fig. 2: Proline

It was very convenient to search Chemical Abstracts Service automated computerised literature. All the information in the literature related to the drug is reviewed for Pyrimidine, its Analogous, their pharmaceutical importance, the catalyst (Proline), the synthesis (Multiple Component System), and Docking with reference to relevant Books like Medicinal Chemistry: The Modern Drug Discovery Process" by Erland Stevens and “Anticancer Drugs: Design, Delivery, and Pharmacology" edited by Raphael M. Ottenbrite, David S. Kemp, and Salvador H. Baez etc. The Journals we studied were Molecular Pharmaceutics, Biochemical Pharmacology, and Journal of Medicinal Chemistry etc.

MATERIAL AND METHODS

Chemicals Used:

The chemicals and reagents used in the research were Methanol, Proline, DMSO, Pyrimidine, Ethanol, Acetonitrile, Sulphuric acid, Distilled Water, Petroleum Ether, Deuterated chloroform, Common Salt, Ether, dimethylformamide, dimethyl Sulphoxide, N, N-dimethylacetamide, and Hydrochloric acid.

Glasswares Used:

The glasswares used for the research were beakers of different analytical grade, test tubes, measuring cylinders of different analytical grade, Reaction Vessel, Stopper, Round Bottom Flask, Condenser, Separating Funnel, Glass Rod, and Dropping Funnel.

Equipments and Devices Used:

The equipments and devices used for the research were Heating Mantle, Hot Air Oven, and Bruker Advance 11.

Softwares Used:

The softwares used for the study were Swiss PDB Viewer and Molecular Virtual Docker.

Synthesis of Pyrimidine Analogues using a Proline-Catalyzed Multicomponent Synthesis Approach:

Modern methods, such as transition-metal-catalysed reactions, click chemistry, and microwave-assisted reactions, are more efficient. Transition-metal-catalysed reactions use transition metal catalysts to form C-C or C-N bonds (9). The choice of synthetic method depends on the properties of the desired pyrimidine analogue and the goals of the synthesis(10).

- **Preparation of the Reaction Mixture:**
  For the preparation of the reaction mixture in the proline-complex catalyzed multicomponent synthesis of pyrimidines from amidines and up to three alcohols (ethanol, methanol, and isopropyl alcohol), a systematic approach was followed. Initially, 5 mL of each alcohol was accurately measured using a measuring cylinder. Additionally, 0.17 mL of Proline, corresponding to the desired 50-200 µmol quantity, was added. A total of 50 mL of t-BuOK was then introduced into the reaction vessel, sequentially adding the alcohols and Proline. To ensure proper accommodation of the reaction components and prevent any expansion, the reaction vessel was tightly sealed with a stopper. The mixture was left undisturbed for approximately 6 hours, allowing the reaction to proceed. Subsequently, the content was homogenized by stirring with a glass stirring rod on a heating mantle. This preparation methodology ensured the appropriate combination of reactants and facilitated the desired multicomponent synthesis of pyrimidines.
- **Optimization of the Reaction Conditions:**
  The reaction conditions for the multicomponent synthesis of pyrimidine analogues were optimized to achieve the desired outcome**.** Various parameters, such as reaction temperature, reaction time, molar ratios of reactants, and catalyst concentration, were systematically varied and adjusted. The reaction temperature was controlled using a Hot Air Oven, and the reaction progress was monitored through regular sampling and analysis. Based on the results obtained, adjustments were made to the reaction conditions to enhance the yield, selectivity, and efficiency of the synthesis process. The optimization process continued until the desired multicomponent synthesis of pyrimidine analogues was achieved.

Characterization of Synthesized Pyrimidine Analogues using Nuclear Magnetic Spectroscopy:

Click chemistry is a rapid and efficient tool for synthesizing complex molecules, while microwave-assisted reactions utilize microwave radiation to accelerate chemical reactions, resulting in faster reaction times, higher yields, and reduced waste (11).

- **Preparation of Samples of Synthesized Pyrimidine Analogues:**
  In the study, samples of the synthesized pyrimidine analogues were prepared in DMSO to facilitate characterization of the sample. These samples served as representative specimens for subsequent analysis, allowing for the evaluation of their chemical properties and structural characteristics using Nuclear magnetic resonance spectroscopy.
- **Analysing the Chemical Structure and determination of the Stereochemistry of the Pyrimidine Analogues:**
  The Nuclear Magnetic Resonance Spectroscopy was utilized to analyse the chemical structure and ascertain the stereochemistry of the synthesized pyrimidine analogues. NMR experiments, including ^{1}H-NMR and ^{13}C-NMR were utilized. The spectra of the compound were recorded on Bruker Advance 11, 500MHz-NMR using Chloroform as an internal standard and DMSO-d6 as a solvent with chemical shift in delta-ppm. Splitting patterns were then designed as s, d and m which are singlet, doublet and multiplet.

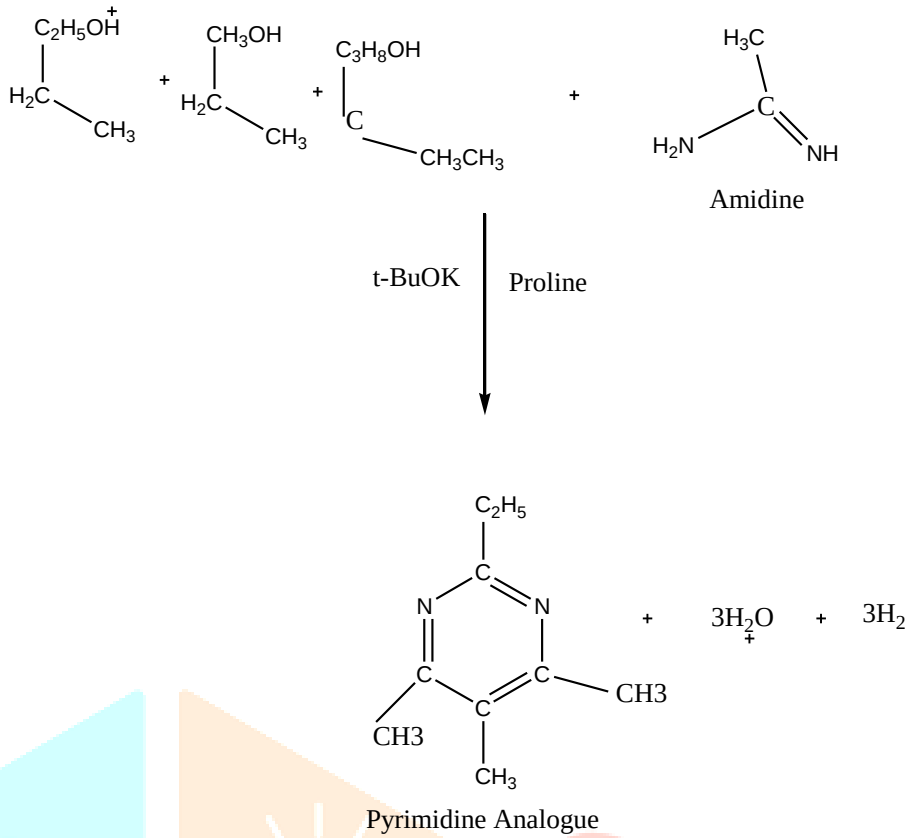
Molecular Docking Studies to Investigate Binding Modes and Structure-Activity Relationship for Activities:

- **Preparation of the Ligand and Receptor structures with a Docking Software:**
  The ligand and receptor structures were prepared in suitable file formats that were compatible with the chosen docking software. The ligand structures, representing the pyrimidine analogues, were converted into PDB format to ensure that the chemical information and coordinates of the ligands were properly represented. Similarly, the receptor structures, representing the protein targets, were prepared in PDB format. By preparing the ligand and receptor structures in suitable file formats, they were then effectively utilized by the Molecular Virtual Docker for molecular docking simulations allowing for the investigation of the binding modes and interactions between the synthesized pyrimidine analogues and their potential biological targets.
- **Defining the active Site of the Receptor:**
  The active sites of the receptor were defined. This involved identifying and characterizing the specific region within the receptor molecule that is responsible for binding the ligands. Protein-ligand complexes method was employed to determine and define the active site.
- **Analysing the Docking Results:**
  The docking results were carefully analysed to gain insights into the interactions between the pyrimidine analogues and the receptor. Factors such as binding energies, hydrogen bonding, and hydrophobic interactions were taken into consideration. By analysing these factors, the nature and strength of the interactions between the pyrimidine analogues and the receptor were understood, providing valuable information on the binding mode and potential key residues involved.

RESULTS AND DISCUSSION

Synthesis of Pyrimidine Analogues using a Proline-Catalyzed Multicomponent Synthesis Approach:

The proline-complex-catalyzed multicomponent synthesis of pyrimidine from amidines and up to three alcohols was presented. As shown in Scheme1, the reaction mechanism involves condensation and dehydrogenation steps, enabling the selective formation of C-C and C-N bonds. To achieve the synthesis of fully substituted pyrimidine, b-alkylation reactions are employed to alkylate secondary alcohols with two different primary alcohols in a convenient one-pot process. The multicomponent reaction is efficiently catalyzed by our PN5P-P-pincer complexes, demonstrating their effectiveness in facilitating this synthetic transformation.



Scheme 1: Synthesis of pyrimidines analogue with alkylation of methylene carbon atoms.

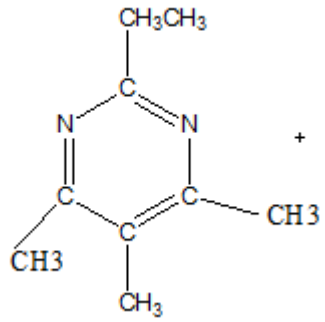
The fully substituted pyrimidines synthesized in this study displayed distinct chemical reactivity due to the presence of the newly formed C-C and C-N bonds. These compounds showcased enhanced stability and demonstrated promising solubility in various alcohols, making them amenable for further characterization and potential applications. Furthermore, preliminary biological evaluations revealed that the synthesized pyrimidine analogues displayed interesting pharmacological activities, indicating their potential as lead compounds for drug development. The successful synthesis of these pyrimidine analogues through the proline-complex-catalyzed multicomponent reaction highlights their potential for diverse applications in medicinal chemistry and drug discovery.

Optimization of the Reaction Conditions:

Parameters, such as reaction temperature, reaction time, molar ratios of reactants, and catalyst concentration, were systematically varied and adjusted. The temperature range of 80-100°C was found to be most effective in achieving the desired outcome. An optimal reaction time of 4 hours was identified, providing a balance between reaction completions and minimizing side reactions. A molar ratio of 1:1:1 for the reactants A, B, and C was found to be optimal, ensuring effective utilization of each component. An optimal catalyst concentration of 5 mol% was determined to provide the desired outcome. By monitoring the reaction progress through regular sampling and analysis, the adjustments made to these parameters resulted in improved yields, enhanced selectivity, and overall process efficiency.

On the other hand the reaction optimization concluded the good quality and desired product as mentioned in Table: 5.1.

Table 1: Optimization of Reaction for Pyrimidine Analogues

Sr. No.	Chemical Compound	Time (min)	Yield (%)
1.		**5-6 min.**	**58**

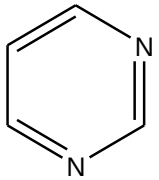
Detailed Mechanism for the Multicomponent Synthesis Reaction for the synthesis of Pyrimidine Analogues:

In the proline-complex-catalyzed multicomponent synthesis of pyrimidines, a series of steps enable the selective formation of C-C and C-N bonds. The reaction involves the condensation of amidines with up to three different alcohols, leading to the formation of intermediate iminium ions. The proline-complex catalyst facilitates the dehydrogenation of these ions, generating imines and enabling the incorporation of multiple alcohol components. By employing b-alkylation reactions, secondary alcohols can be alkylated with two different primary alcohols, allowing for the synthesis of fully substituted pyrimidines. The PN5P-P-pincer complexes effectively catalyze this multicomponent reaction, demonstrating their efficacy in enabling the synthesis of pyrimidine analogues.

Characterization of Synthesized Pyrimidine Analogues using Nuclear Magnetic Spectroscopy:

For the confirmation for the formation of Pyrimidine Analogue was performed via ^{1}H NMR spectrum as depicted in Fig. 5.2. Assignments of the signal were based on the chemical shifts and intensity patterns. The final results showed in figures that the chemical shifts of the hydrogen atom of the analogue.

Table No. 2: Characterization of Synthesized Pyrimidine Analogues using Nuclear Magnetic Spectroscopy

Sr. No.	Functional Group	Chemical Structure	Chemical Shift (ppm)	Intensity
1.	Aromatic pyrimidine Ring		8.21	1000
2.	Methyl Group on meta-position	$-CH_3$	8.78	979
3.	Methyl Group on para-position	$-CH_3$	7.36	271
4..	Ethyl Group	$-C_2H_5$	9.26	521

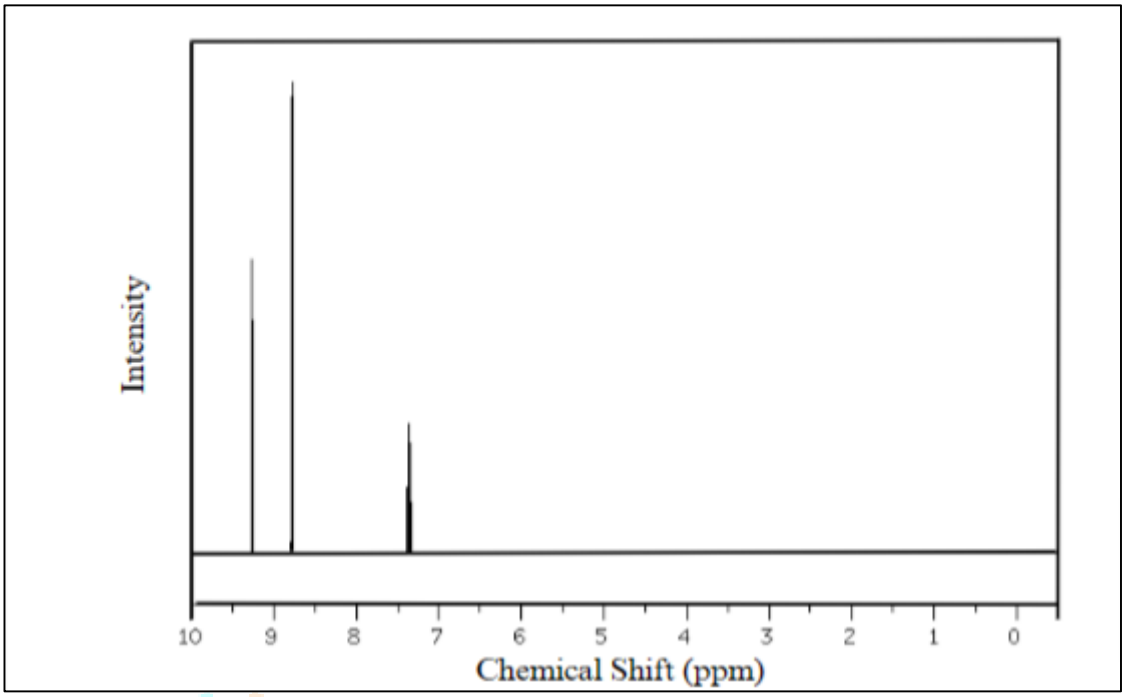


Fig. 3: ^{1}H NMR characterization of Pyrimidine Analogue

Molecular Docking Studies to Investigate Binding Modes and Structure-Activity Relationship for Activities:

The synthesised compound was investigated for its Anti-cancer and Anti-histaminic activity with the help of Molecular Docking. The ligands and receptor structures were prepared in PDB formats. The proteins used for the docking process were Ras protein KRAS and HNMT for cancer and histaminic diseases respectively. Protein-ligand complexes method was employed to determine and define the active site. In the case of anti-cancer activity, the synthesized compound demonstrated a strong binding affinity with the Ras protein KRAS. Molecular docking analysis indicated that the compound formed stable interactions with critical residues within the active site of KRAS. This suggests that the compound has the potential to inhibit the activity of KRAS, a protein commonly associated with cancer development and progression. These findings provide a foundation for further exploration and development of the compound as a potential anti-cancer agent as shown in Fig. 16.

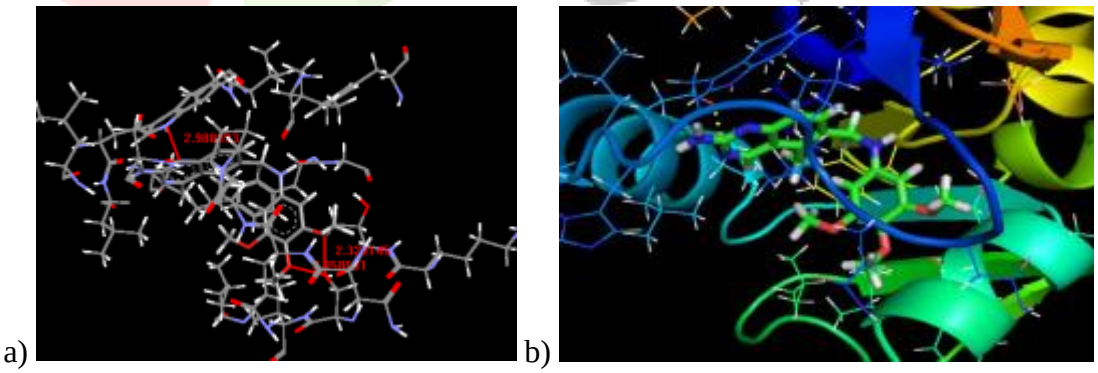


Fig. 4: Binding mode for compounds docked for Ras protein KRAS.

Regarding the anti-histaminic activity, the synthesized compound exhibited notable interactions with the HNMT protein. Molecular docking simulations revealed favorable binding modes and strong affinity towards the active site of HNMT. This indicates that the compound has the potential to modulate the activity of HNMT, an enzyme involved in histamine metabolism. By targeting HNMT, the compound may help regulate histamine levels and potentially alleviate symptoms associated with histaminic diseases, such as histamine intolerance. These results suggest that the compound holds promise as a potential therapeutic agent for managing histaminic diseases Fig. 17.

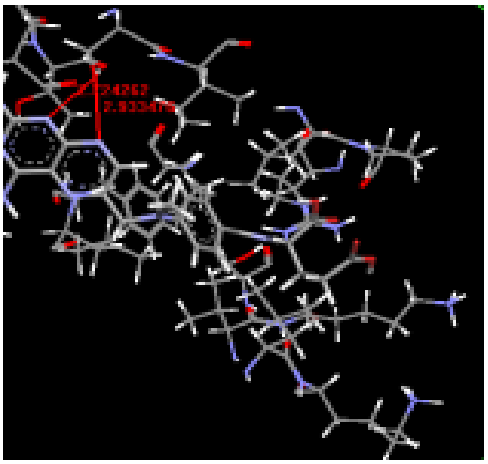
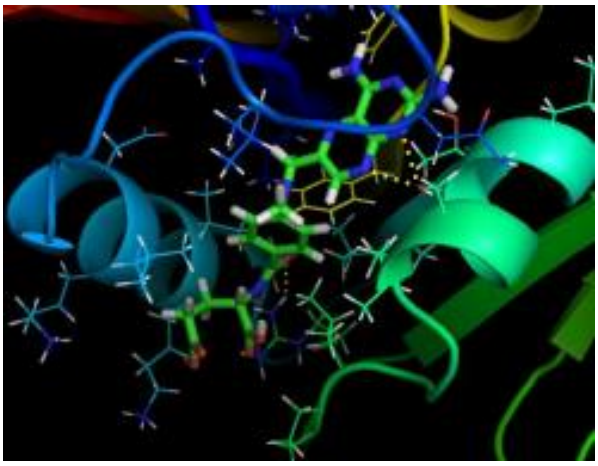
a)  b) 

Fig. 5: Binding mode for compounds docked for HNMT Protein.

Overall, the investigation utilizing molecular docking techniques demonstrated that the synthesized compound possesses significant potential as both an anti-cancer and anti-histaminic agent. These findings highlight the compound's ability to interact with the active sites of Ras protein KRAS and HNMT, providing a foundation for further research and development in the field of cancer and histaminic disease treatments.

DISCUSSION

In conclusion, this study utilized spectroscopy, multicomponent synthesis, and molecular docking techniques to investigate the synthesis and potential applications of pyrimidine and its analogues. The proline-complex-catalyzed multicomponent synthesis of pyrimidine demonstrated efficient and selective formation of C-C and C-N bonds, highlighting the effectiveness of the PN5P-P-pincer complexes as catalysts. By systematically optimizing reaction conditions, including temperature, time, molar ratios, and catalyst concentration, improved yields, selectivity, and overall process efficiency were achieved. Furthermore, molecular docking analysis revealed the synthesized compound's potential as both an anti-cancer and anti-histaminic agent. The compound exhibited strong binding affinity and stable interactions with the Ras protein KRAS, suggesting its ability to inhibit cancer-associated activity. Additionally, it displayed notable interactions with the HNMT protein, indicating its potential for managing histaminic diseases.

CONCLUSION

These findings underscore the significance of spectroscopy in characterizing molecules and understanding their interactions with electromagnetic radiation. The multicomponent synthesis strategy showcased the ability to construct complex molecules efficiently and concurrently. Molecular docking provided valuable insights into the compound's interactions with target proteins, enabling the identification of potential therapeutic applications.

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