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Synthesis and Evaluation of Novel Thiosemicarbazone-1, 2, 3-Triazole Hybrids as Potential Anticancer Agents

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Abstract: The development of novel anticancer agents is crucial in addressing the growing challenge of cancer treatment. In this study, we report the synthesis and evaluation of novel thiosemicarbazone-1, 2, 3-triazole hybrids as potential anticancer agents. Thiosemicarbazones are well-known for their metal-chelating properties and ability to induce apoptosis in cancer cells, while 1, 2, 3-triazoles are stable pharmacophores that enhance molecular interactions with biological targets. By combining these two scaffolds, we designed hybrid molecules with the aim of improving anticancer efficacy and selectivity. The synthesis involved click chemistry to incorporate the triazole ring, followed by derivatization of the thiosemicarbazone moiety. The resulting hybrids were characterized using spectroscopic techniques such as NMR and mass spectrometry. In vitro cytotoxicity assays against various human cancer cell lines, including breast, lung, and colon cancers, demonstrated significant anticancer activity. Several compounds exhibited potent inhibition of cancer cell proliferation, with IC₅₀ values in the low micromolar range. Mechanistic studies suggested that these hybrids induce apoptosis through the generation of reactive oxygen species (ROS) and interference with metal ion homeostasis in cancer cells. These promising findings warrant further investigation of thiosemicarbazone-1, 2, 3-triazole hybrids as potential candidates for anticancer drug development.

Keywords: Thiosemicarbazone, 1, 2, 3-Triazole, Anticancer agents, Hybrid molecules, Cytotoxicity

1. Introduction

Colorectal cancer, often simply referred to as colon cancer, is a malignant growth that originates within the large intestine, specifically the colon or the rectum. It is a significant public health concern, ranking as the third most prevalent cancer and the fourth leading cause of cancer-related deaths globally. Colorectal cancer is the most common type of cancer of the gastrointestinal track worldwide and the second leading cause of cancer related death in the United States. In Crete, between 1992 and 2013 there were registered 25.1 new cases per 100,000 per year. Many genetic and environmental factors are associated with colorectal cancer, while surgery remains the gold standard of its treatment [1]. The incidence and mortality rates of colon cancer have traditionally been highest in affluent Western countries, but are now rapidly increasing in many other parts of the world as economic development progresses [2].

The risk factors associated with colon cancer are well established and include factors such as advanced age, male sex, smoking, excessive alcohol consumption, and physical inactivity, high consumption of red and processed meat, being overweight, and having a family history of the disease [3]. Dietary and lifestyle choices play a crucial role in the etiology of colon cancer, with a high intake of red

meat, animal fat, and refined carbohydrates being linked to a higher incidence, while a diet rich in fruits, vegetables, and whole grains has been shown to have a protective effect [4].

The development of colon cancer is a multistage process, progressing from normal mucosa to invasive carcinoma [5]. Epidemiological data suggest that diet is a major contributing factor, with high consumption of red meat, animal fat, alcohol, and refined cereals being associated with an increased risk, while the consumption of fruits, vegetables, and whole grains has been linked to a lower incidence of these cancers [6].

Phenolic compounds, which are found in a wide variety of fruits and vegetables, have been reported to possess important biological properties, including anticancer, antiviral, antioxidant, and anti-inflammatory activities [7]. These compounds may play a significant role in the prevention of colon cancer by modulating the various stages of carcinogenesis [8].

1, 2, 3-Triazoles are an important five-membered heterocyclic scaffold due to their extensive biological activities. These compounds exhibit high affinity for their biological targets, which is attributed to their improved solubility and ability to participate in various noncovalent interactions, such as hydrogen bonding, dipole-dipole interactions, and π -stacking [9]. The 1, 2, 3-triazole moiety can serve as a linker and exhibit bioisosteric effects on various pharmacophoric elements, including aromatic rings, peptide linkages, double bonds, and imidazole rings [9]. This scaffold is particularly noteworthy in the development of antifungal agents, with several marketed drugs such as itraconazole, fluconazole, ketoconazole, and voriconazole. Additionally, it has exhibited a broad spectrum of therapeutic activities, including anticancer, antitubercular, antioxidant, anti-inflammatory, acetylcholinesterase inhibition, and α -glucosidase inhibitor [10]. Thiosemicarbazones, on the other hand, have garnered significant pharmaceutical interest due to their diverse biological activities, including anti-inflammatory, anticancer, antiparasitic, antiviral, antitubercular, tyrosinase inhibition, anticonvulsant, antioxidant, and antimicrobial properties [11]. We have designed and synthesized a series of new thiosemicarbazone clubbed with 1, 4-disubstituted 1, 2, 3-triazoles. The synthesized compounds were evaluated for their *in vitro* anticancer activity.

2. MATERIALS AND METHODS

2.1. SYNTHETIC PROCEDURE

Synthetic Scheme:

Procedure.

Step: 1

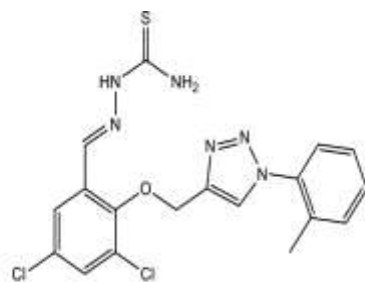
Compound (A) 1.0 gm was taken in 10 ml DMF, cooled at 0°C and NaOH was added to it. propargyl bromide was added dropwise to the reaction. Reaction was stirred at room temp for 3 hrs and monitored by tlc, starting consumed to give desired product (B).

Step 2:

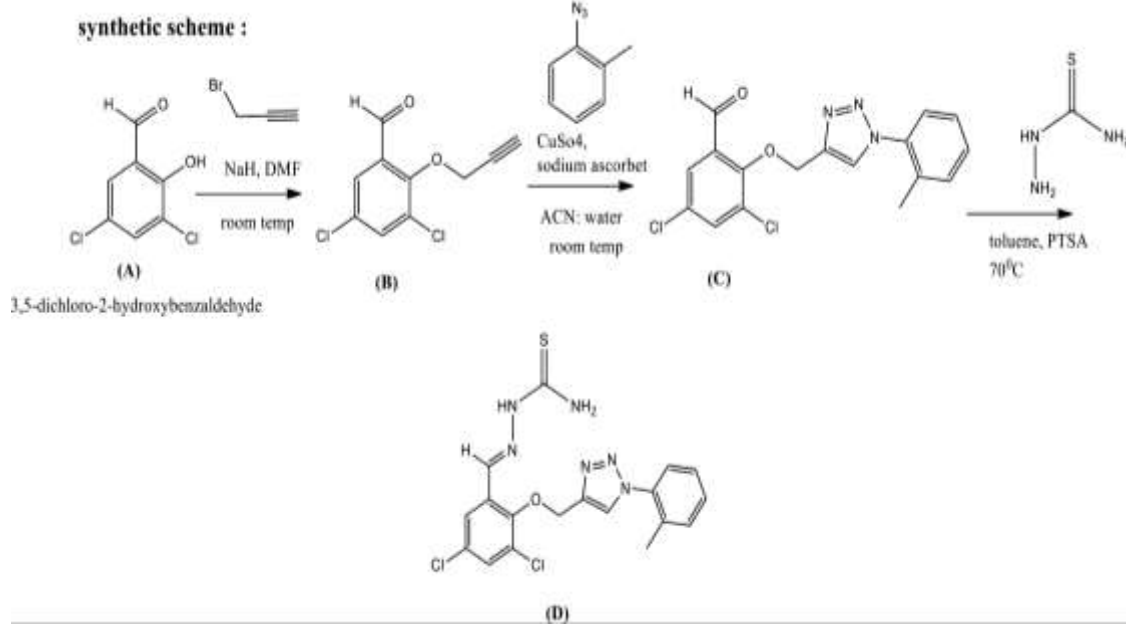
Compound (B) 1.0 gm was taken in 10 ml acetonitrile: water (8:2), CuSO₄, sodium ascorbate and substituted phenyl Azide was added to it. Reaction was stirred at room temp for 12 hrs and monitored by tlc, starting consumed to give desired product (C).

Step 3:

Compound (C) 1.0 gm was taken in 10 ml toluene, hydrazine thiocarbamide and PTSA catalytic was added to it. Reaction was stirred at 80°C for 12 hrs and monitored by tlc, starting consumed to give desired product (D).



(E)-2-(3,5-dichloro-2-((1-(o-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazinecarbothioamide



Molecular Formula	C ₁₈ H ₁₆ Cl ₂ N ₆ OS
Molecular Weight (g/mol)	435.0550
Melting Point (°C)	98-100°C
Yield (%w/w)	82%
Recrystallisation solvent	Ethanol
TLC	R _f = 0.73 Benzene:ethanol (1:1)
IR (ν, cm ⁻¹)	3358, 3237, 3120, 2926, 1608, 1590, 1512, 1024, 932, 849, 760, 732
¹ H NMR (DMSO)	2.19 (s, 3H), 5.26 (s, 2H), 6.46 (s, 1H), 7.29-7.34 (m, 2H), 7.36-7.39 (m, 2H), 7.42 (d, <i>J</i> = 4 Hz, 1H), 7.46 (d, <i>J</i> = 4 Hz, 1H), 7.70 (d, <i>J</i> = 4 Hz, 1H), 7.85 (s, 1H), 8.21 (s, 1H), 9.94 (s, 1H)

NMR Spectroscopy:**¹³C NMR:**

Thioamide Carbon: The carbon in the thioamide group is quite deshielded due to its attachment to the sulfur atom. Expect this around 180-210 ppm.

Alkyl and Aryl Carbons: Alkyl carbons will typically appear in the 10-50 ppm region. Aryl carbons will be found in the 100-160 ppm region. Carbons directly bonded to electronegative atoms or groups will appear downfield.

IR Spectroscopy:

For thioamides, which possess the general structure $R-C(S)-NR'R''$, the main IR peaks of interest arise from the C=S and C-N bonds, as well as any NH groups that might be present.

Here's what you might expect to see in the IR spectrum of a thioamide derivative:

C=S Stretch (Thioamide Group):

This is typically a strong and sharp peak.

Expect this in the range of $1050-1200\text{ cm}^{-1}$. However, the exact position can vary depending on the nature of the substituents.

C-N Stretch (Thioamide Group):

This peak is usually weaker than the C=S stretch.

Typically appears in the range of $1200-1350\text{ cm}^{-1}$

NH Stretch (If present in the thioamide):

For thioamides with a free NH or NH₂ group, there would be characteristic NH stretching peaks.

NH₂ Symmetrical and Asymmetrical Stretches: Usually in the range of $3300-3500\text{ cm}^{-1}$. They appear as two distinct, moderately strong peaks.

NH Stretch: If there's just one hydrogen (NH), expect a broad peak in the $3200-3400\text{ cm}^{-1}$ range.

Amine C-N Stretch:

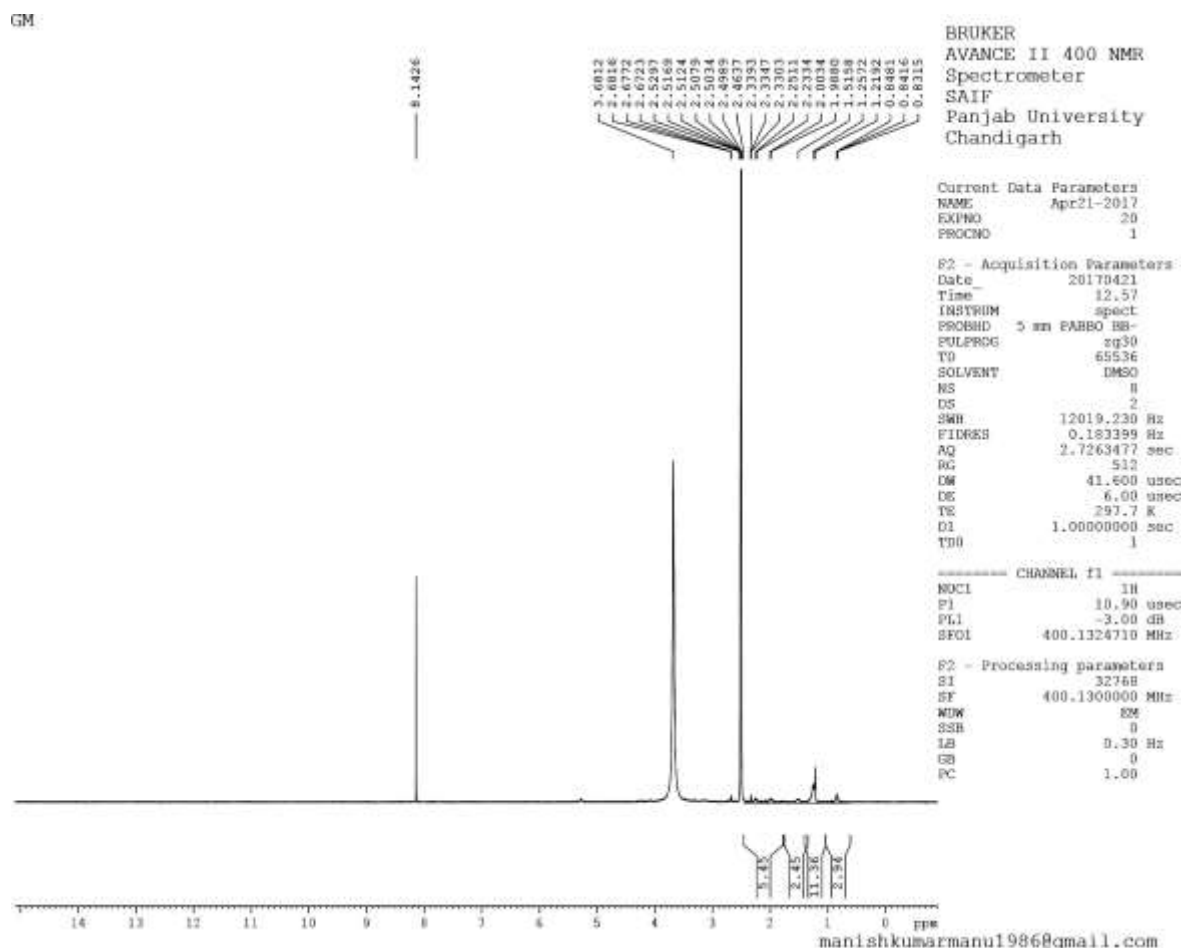


Fig 1. 13 C NMR

If the R' or R'' groups are alkyl groups, you might also observe a C-N stretch associated with the amine functionality.

This peak is usually in the $1000-1250\text{ cm}^{-1}$ range.

In addition to the above peaks, the IR spectrum will display bands associated with other functional groups or structural features present in the R, R', and R" substituents. For example, you might see alkane C-H stretches, aromatic ring vibrations, or other specific functional group vibrations.

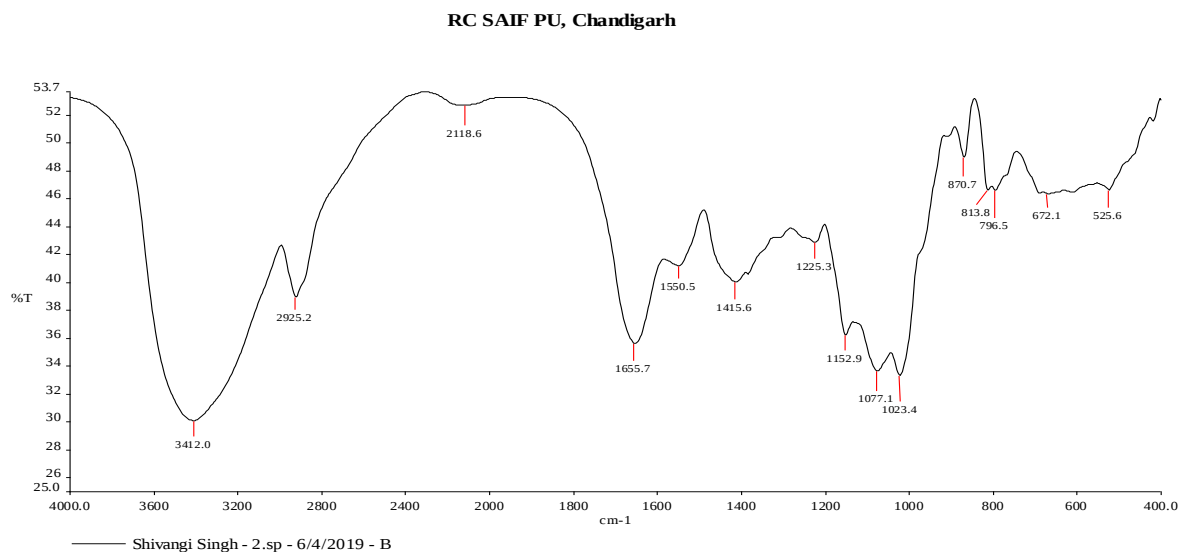


Fig 2: Ir spectroscopy

3. RESULTS AND DISCUSSIONS

3.1. Anticancer activity:

CTG Assay: Screening of Compounds: Protocol

The study conducted a cell viability assay using a 384-well culture plate, where cells were seeded at a density of 1,000 cells per well on day 1 in ATCC-formulated McCoy's 5a Medium Modified [12]. On day 2, the cells were treated with 5-Fluorouracil (5FU) as a standard control, as well as test compounds, both at varying concentrations [13]. The cells were then incubated at 37°C in a CO₂ incubator. After 72 hours of incubation, the CellTiter-Glo® 2.0 Cell Viability Assay Kit was used to measure the luminescence, which was used to calculate the percent cytotoxicity and determine the IC₅₀ values [14].

Discussions:

The present study investigated the cell viability of two test compounds (M4 and M5) and the standard control Fluorouracil (5-FU) in HCT-116 cells. The cell viability for the two test compounds was evaluated along with Fluorouracil (30 µg/mL, 3-fold dilution, 10-point dose-response curve) as a standard control [15]. Fluorouracil was tested at a concentration of 30 µg/mL with a 3-fold dilution and a 10-point dose-response curve [16]. The two test compounds were screened at 50 µM with a 3-fold dilution and a 10-point dose-response curve. The study observed an IC₅₀ of approximately 45 nM for Fluorouracil [17]. When comparing the potencies of the test compounds, the IC₅₀ for compound M4 was found to be more potent than compound M5, with IC₅₀ values of 1.5 µM and 6.7 µM, respectively. However, both test compounds were more potent compared to Fluorouracil [18].

The results suggest that the two test compounds have a higher potency compared to the standard control Fluorouracil in HCT-116 cells. Further investigation is needed to elucidate the underlying mechanisms and potential therapeutic applications of these compounds.

Docking study:

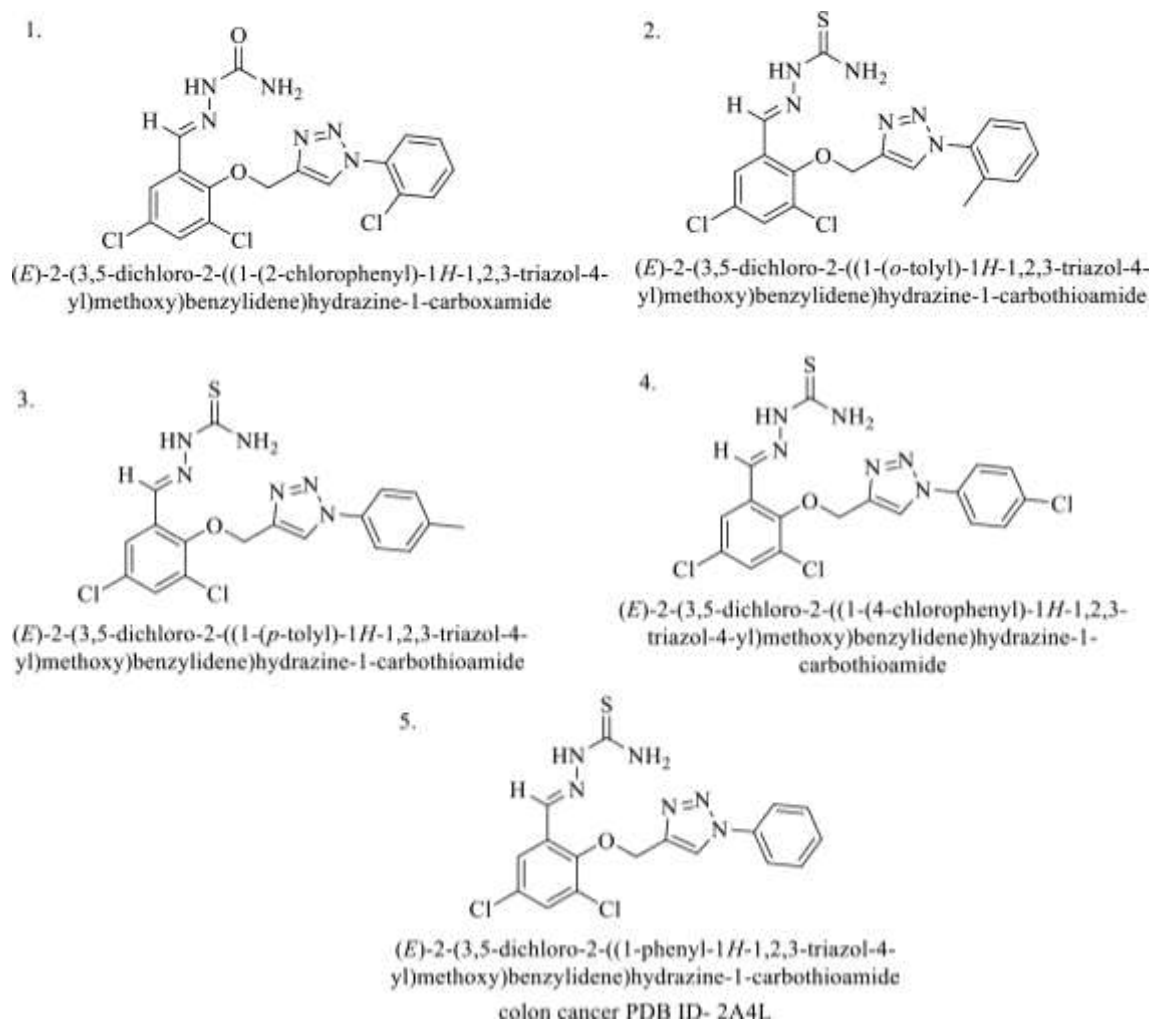
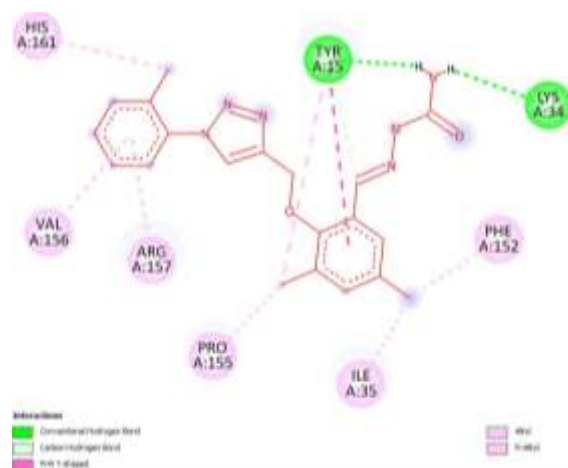
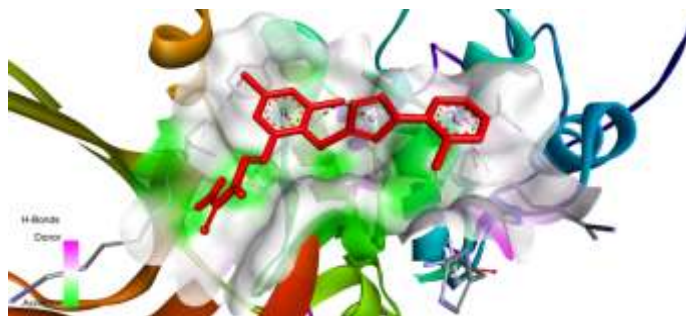


Fig 3: Molecular docking test information of Molecules (M1-M5) and colon cancer genes PDB ID: 2A4L

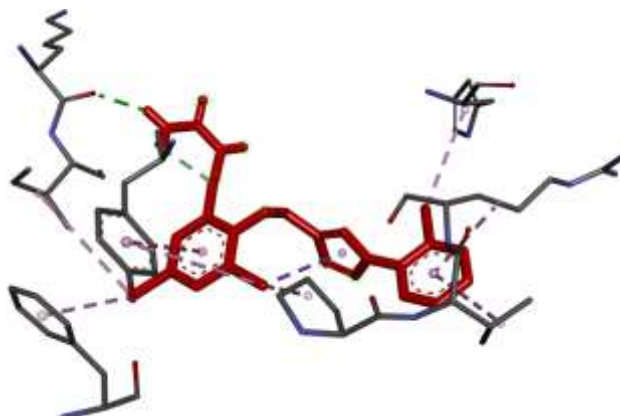
Molecular Structures:



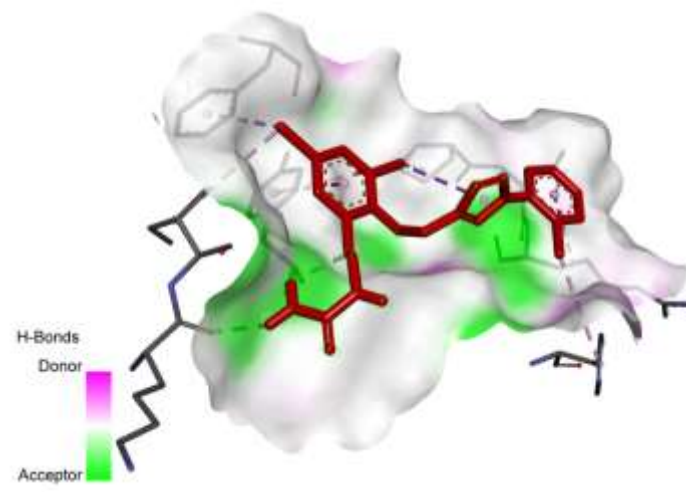
2D interaction image of M1 to CKD2



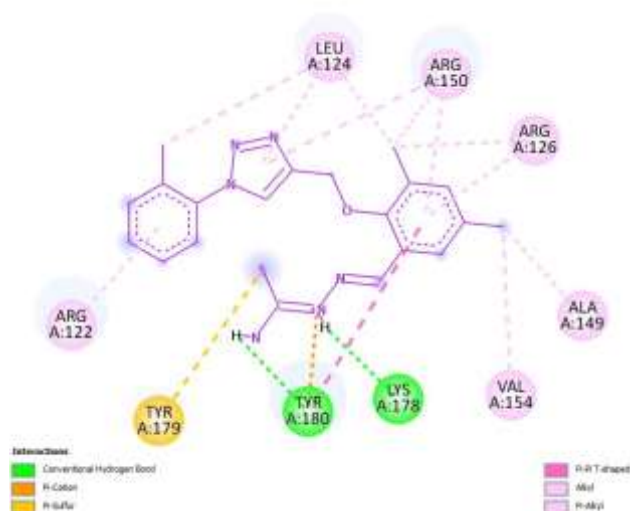
3D interaction image of M1 to CKD2



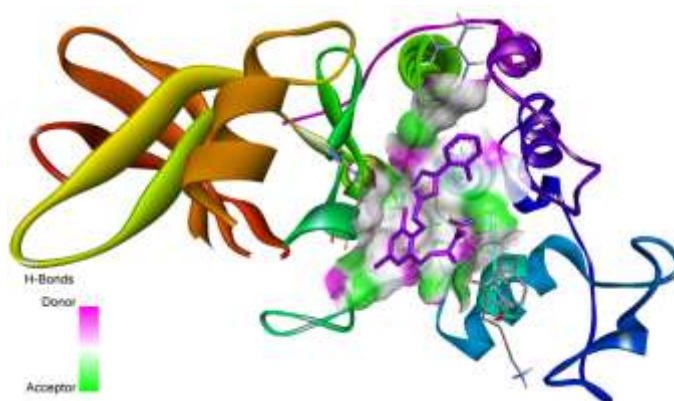
3D interaction image showing amino acids of M1 to CKD2



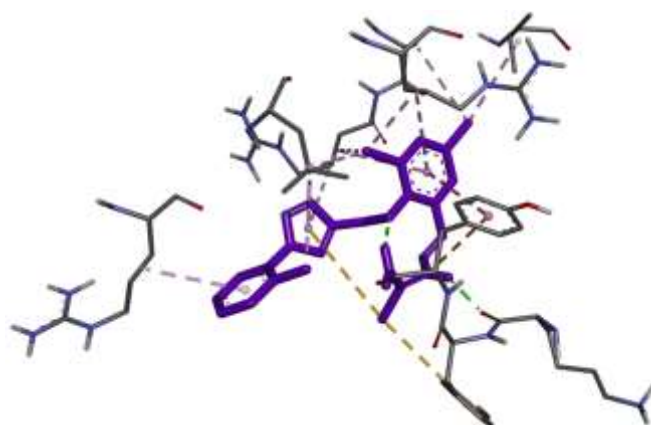
3D interaction image showing hydrogen bond of M1 to CKD2



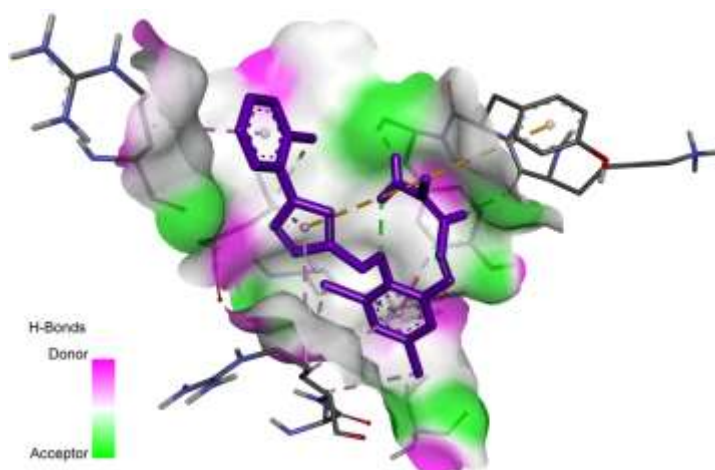
2D interaction image of M2 to CKD2



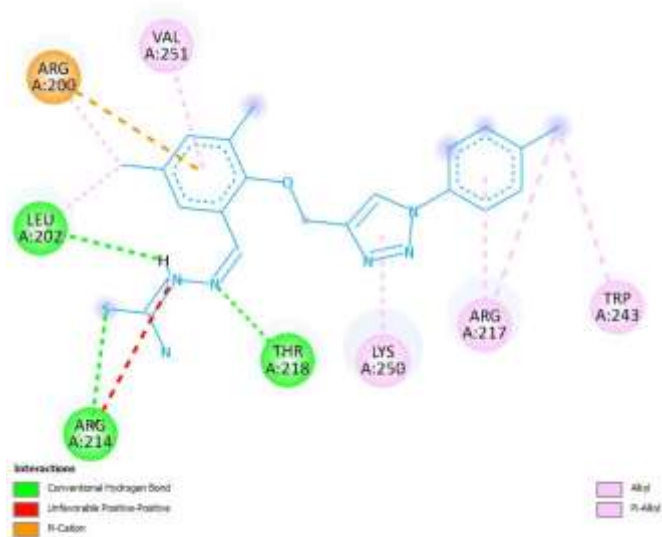
3D interaction image of M2 to CKD2



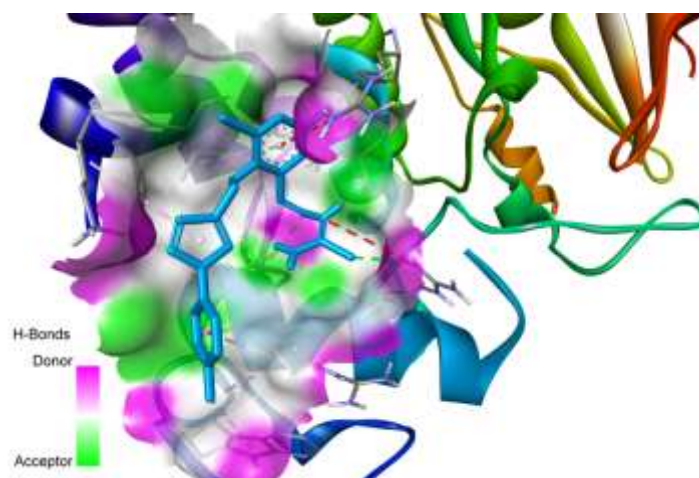
3D interaction image showing amino acids of M2 to CKD2



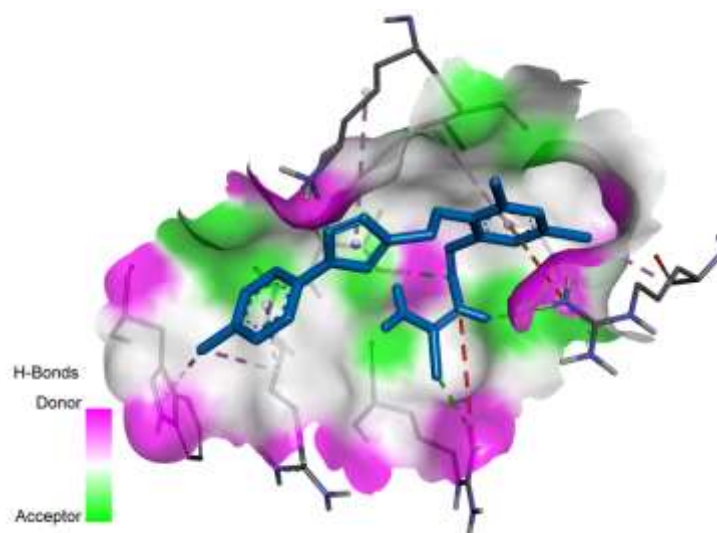
3D interaction image showing hydrogen bond of M2 to CKD2



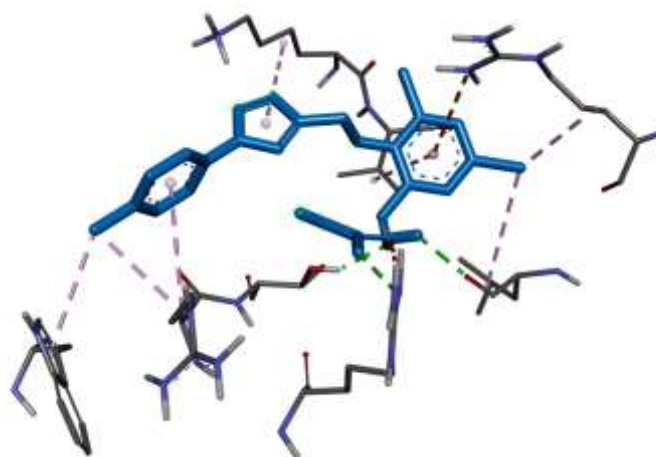
2D interaction image of M3 to CKD2



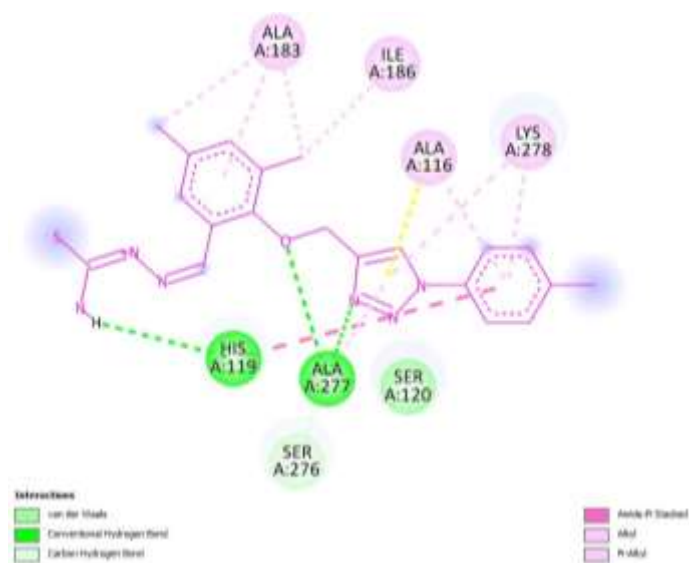
3D interaction image of M3 to CKD2



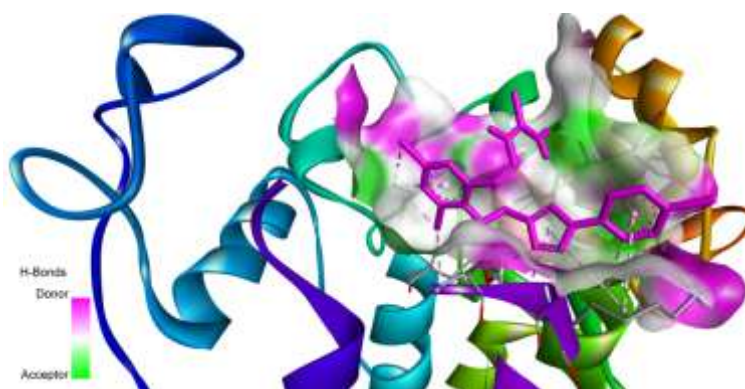
3D interaction image showing hydrogen bond of M3 to CKD2



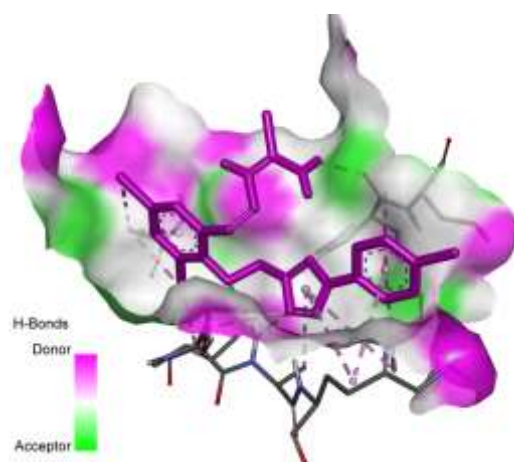
3D interaction image showing amino acids of M3 to CKD2



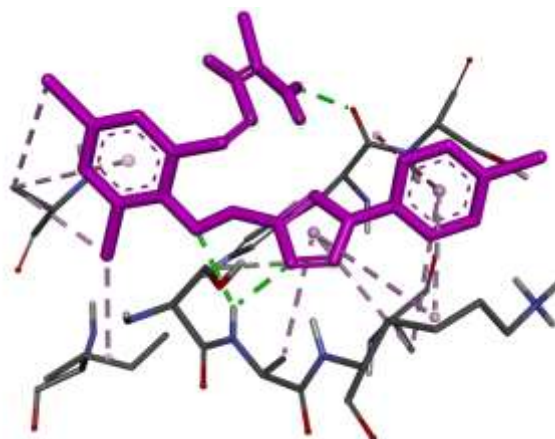
2D interaction image of M4 to CKD2



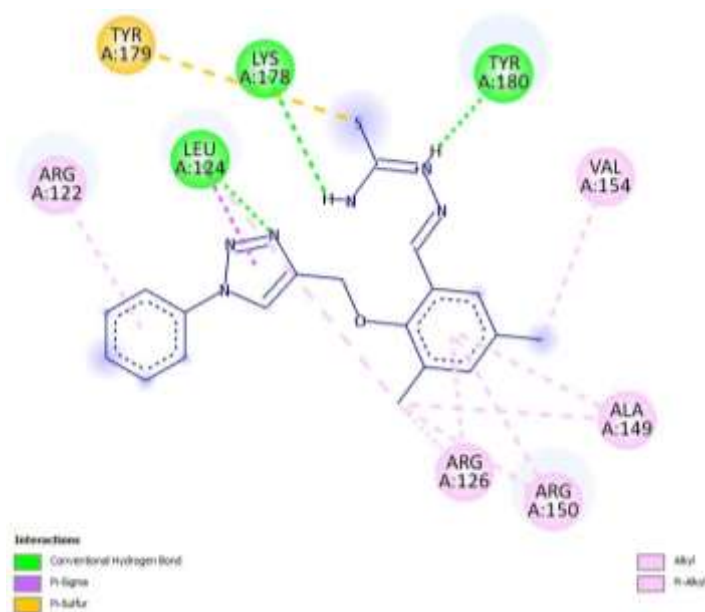
3D interaction image of M4 to CKD2



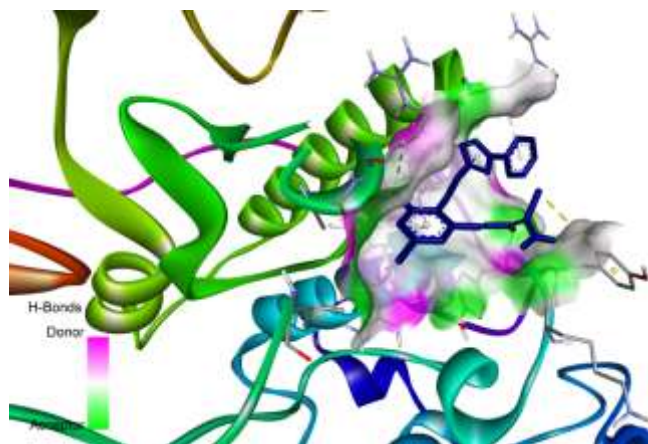
3D interaction image showing hydrogen bond of M4 to CKD2



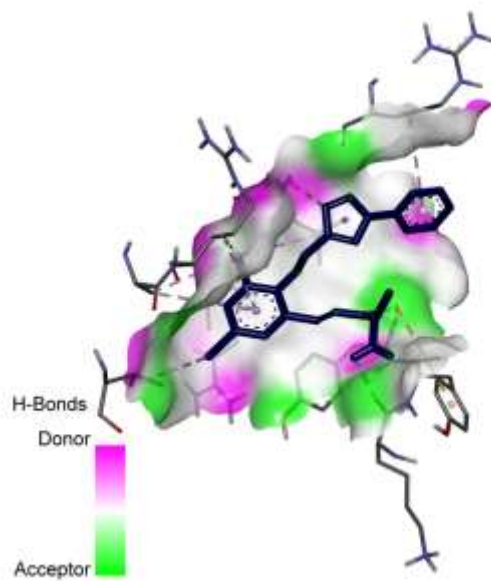
3D interaction image showing amino acids of M4 to CKD2



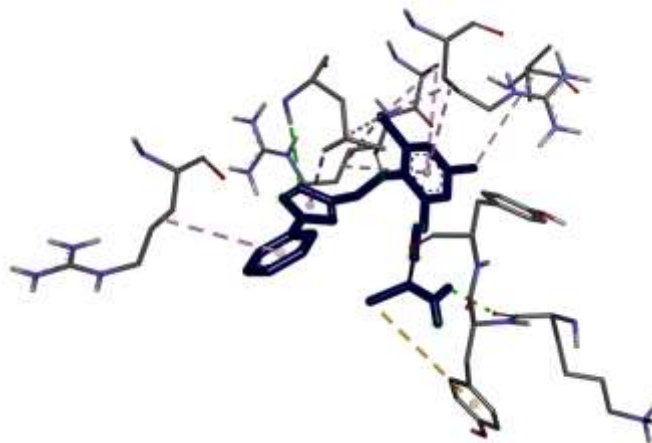
2D interaction image of M5 to CKD2



3D interaction image of M5 to CKD2



3D interaction image showing hydrogen bond of M5 to CKD2



3D interaction image showing amino acids of M5 to CKD2

The molecular docking analysis revealed that all five molecules (M1-M5) exhibited significant binding affinities with the CDK2 protein [19]. Molecule 5 (M5) demonstrated the highest binding energy of -6.56 kcal/mol, followed closely by Molecule 4 (M4) with a binding energy of -6.46 kcal/mol [20]. These findings suggest that Molecules 4 and 5 may be particularly effective inhibitors of CDK2, making them strong candidates for further development as therapeutic agents for colon cancer [21].

Hydrogen bonding plays a crucial role in the stability and specificity of the binding interactions [22]. Molecules 1, 2, and 3 formed conventional hydrogen bonds with key residues in the CDK2 active site, enhancing their binding affinity [23]. Hydrophobic interactions also significantly contribute to the binding energy and stability of the molecule-CDK2 complex [24]. All molecules exhibited multiple hydrophobic interactions, with Molecule 2 having the most (16 in total), which compensated for its relatively lower binding energy compared to the others [25].

Electrostatic interactions and other types of non-covalent interactions, such as Pi-Pi T-shaped and Pi-Sulfur bonds, also contributed to the binding affinity [26]. These interactions were observed in Molecules 2 and 3, providing additional stabilization to the complex [27].

Visualizations of the 2D and 3D interactions of each molecule with CDK2 are essential for a comprehensive understanding of the binding interactions [28]. These images highlight the specific amino acids involved in hydrogen bonds, hydrophobic interactions, and other binding interactions [29].

In conclusion, the molecular docking analysis suggests that all five molecules (M1-M5) show promising binding affinities with the CDK2 protein, with Molecules 4 and 5 exhibiting the highest binding energies [30]. The interaction profiles indicate that these molecules may be effective inhibitors of CDK2,

potentially making them valuable candidates for further development as therapeutic agents for colon cancer [31].

CONCLUSION

The synthesis and evaluation of novel thiosemicarbazone-1, 2, 3-triazole hybrids as potential anticancer agents present a promising approach in the fight against cancer. These hybrid compounds combine the unique chemical properties of thiosemicarbazones and 1, 2, 3-triazoles, both of which are known for their biological activities. Thiosemicarbazones exhibit metal chelation properties and have demonstrated cytotoxic effects against various cancer cell lines, while 1, 2, 3-triazoles serve as stable linkers and enhance the bioavailability and pharmacokinetics of drug molecules. Through targeted synthesis, the hybrid molecules have shown increased anticancer activity, often improving selectivity toward cancer cells while minimizing toxicity to normal cells. These compounds act by inhibiting key biological processes in cancer cells, such as cell proliferation, DNA synthesis, and metal ion metabolism, leading to apoptosis or cell cycle arrest. Structural modifications of these hybrids have been explored to enhance their anticancer efficacy and solubility, with promising results in preclinical studies.

In conclusion, thiosemicarbazone-1, 2, 3-triazole hybrids hold significant potential as novel anticancer agents. However, further in-depth studies, including in vivo testing and clinical trials, are required to fully understand their mechanisms of action, optimize their pharmacological profiles, and assess their therapeutic potential in cancer treatment.

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NIL

CONFLICT OF INTREST

NIL

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article and its supplementary information files.

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