Research Article

Formulation, Optimization and Evaluation of Thioamide Derivatives by using Design Expert: Anticancer Potential

Surendra Kumar Singh*, Nripendra Singh
Department of Pharmacy, V.B.S. Purvanchal University, Jaunpur-222002, (U.P.) India.
***Corresponding author's E-mail:** surenksingh1@gmail.com

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ABSTRACT

The rising global burden of cancer necessitates the development of novel and effective therapeutic agents. Thioamide derivatives have garnered significant attention due to their promising anticancer properties, attributed to their ability to target specific cellular pathways. This study focuses on the formulation and optimization of thioamide derivatives using Design Expert® software, employing a systematic approach to enhance their therapeutic efficacy. A series of thioamide derivatives were synthesized and characterized through spectroscopic techniques such as FTIR. The optimization of formulation parameters, including reaction conditions and excipient ratios, was performed using a factorial design approach in the Design Expert® software to achieve desired physicochemical and biological characteristics. The anticancer potential of the optimized derivatives was assessed through in vitro cytotoxicity studies against selected cancer cell lines. The results demonstrated significant dose-dependent cytotoxic activity, with IC50 values in the micromolar range, suggesting high potency. The optimized formulations exhibited favorable pharmacokinetic properties, including solubility and stability under physiological conditions, making them suitable for further preclinical evaluation. Statistical analysis confirmed the reliability of the Design Expert® model in predicting the optimal conditions for synthesis and formulation, emphasizing its utility in pharmaceutical research. The findings of this study underscore the potential of thioamide derivatives as effective anticancer agents and highlight the value of design-based approaches in drug development. Future studies will focus on the molecular mechanisms of action and in vivo efficacy of these derivatives.

Keywords: Thioamide derivatives, anticancer activity, nano delivery system, cytotoxicity, targeted drug delivery, nanoparticles.

INTRODUCTION

The development of effective cancer therapies remains a vital area of research due to the intricate nature of cancer and the limitations associated with existing treatment options. Conventional chemotherapy often exhibits poor selectivity, targeting both cancerous and healthy cells, which leads to severe side effects and restricts its therapeutic effectiveness [1]. This lack of specificity results in substantial harm to normal tissues, causing adverse effects such as nausea, fatigue, and immunosuppression, ultimately diminishing the quality of life for patients undergoing treatment.

In recent years, focus has shifted toward molecularly engineered compounds that provide more selective mechanisms of action against cancer cells. Among these, thioamide derivatives have attracted considerable interest for their potential anticancer properties. Thioamides, distinguished by the substitution of a sulfur atom for oxygen in amide bonds, have demonstrated significant promise due to their capacity to disrupt critical cellular processes. They are thought to induce apoptosis (programmed cell death) and suppress cell proliferation by modulating key molecular pathways in tumor cells, positioning them as promising candidates for targeted cancer therapies.

Despite these encouraging preliminary findings, the use of thioamide derivatives in cancer therapy faces several significant challenges. One major limitation is their low bioavailability, which restricts the amount of the active compound reaching the target tumor cells. Additionally, thioamide derivatives often display poor stability in biological environments, leading to rapid degradation and reduced efficacy. Furthermore, nonspecific distribution throughout the body can result in off-target effects, complicating their therapeutic application and raising concerns about safety and effectiveness [2].

To address these challenges, recent research has concentrated on the development of nanodelivery systems to improve the stability, bioavailability, and specificity of anticancer compounds[13, 23]. Nanocarriers, including liposomes, nanoparticles, and dendrimers, present a promising approach by protecting active compounds from degradation, enhancing their solubility, and enabling targeted delivery to tumor cells. Encapsulating thioamide derivatives within nanodelivery systems can significantly enhance their therapeutic potential while reducing adverse effects on healthy tissues [3, 28, 29].

This study aims to evaluate the anticancer potential of newly synthesized thioamide derivatives and to develop an optimized nano delivery system using response surface methodology (RSM). A central composite design (CCD) will be utilized to systematically analyze the impact of key formulation variables on the efficacy of the thioamide derivatives. This approach seeks to determine the optimal conditions that enhance the anticancer activity of the thioamide-nano complex, laying the groundwork for more effective and targeted cancer therapies. By focusing on the development of a stable, targeted, and efficient nanodelivery system, this study aims to overcome the

limitations of conventional chemotherapeutics, offering an advanced therapeutic option for cancer treatment and paving the way for safer, more effective therapies [14, 4, 22].

MATERIALS AND METHODS

Synthesis of Thioamide Derivatives

The thioamide derivatives were synthesized through a series of chemical reactions involving thiosemicarbazide, various aldehydes, and appropriate solvents. The reactions were conducted under controlled conditions, and the final compounds were purified using recrystallization. Structural and purity confirmation was achieved through characterization techniques such as Fourier-transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR), and mass spectrometry [5].

Preparation of Nano Delivery System

The thioamide derivatives were incorporated into nanoparticles using the nanoprecipitation method. Biodegradable polymers, such as poly (lactic-co-glycolic acid) (PLGA), or lipid-based carriers like phosphatidylcholine, were selected for their stability and biocompatibility[15]. The drug-to-polymer ratio was optimized to enhance encapsulation efficiency and ensure particle stability, thereby improving the overall effectiveness of delivery system [6].

Optimization of Thioamide Derivative

Experimental Design

The optimization of the formulation of newly synthesized thioamide derivatives for anticancer potential was carried out using Central Composite Design (CCD). The CCD approach was employed to evaluate the effects of three independent variables: concentration of thioamide derivatives (X1), temperature (X2), and pH (X3). Each variable was studied at three levels: low (-1), medium (0), and high (+1).[15]

Design Matrix

The CCD design matrix included a total of 16 experimental runs, consisting of 8 full factorial points, 6 axial (star) points, and 2 center points.

Statistical Analysis

The response variable, Y, representing the anticancer activity, was analyzed using a second-order polynomial model. The regression equation for predicting the response Y based on the three independent variables (X1, X2, and X3) is expressed as:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_{12}+\beta_{22}X_{22}+\beta_{33}X_{32}+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \quad \text{eq ----1}$$

Where:

- Y is the response variable (anticancer activity),
- β_0 is the intercept,
- β1,β2,β3 are the linear coefficients,
- β11,β22,β33 are the quadratic coefficients,
- β12,β13,β23 are the interaction coefficients,
- X1, X2, X3 are the independent variables (concentration, temperature, and pH) [16.]

Regression Analysis

The response variable (anticancer activity) was modeled using a second-order polynomial equation. The regression coefficients were estimated using the method of least squares, and the equation obtained is as follows:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_{12}+\beta_{22}X_{22}+\beta_{33}X_{32}+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \quad \text{eq ----- 2}$$

Where:

- Y is the response variable (anticancer activity).
- X1, X2, X3 are the independent variables (concentration, temperature, pH).
- β0 is the intercept.
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The regression coefficients (β\beta) were estimated using the method of least squares. Statistical significance of the regression coefficients was assessed using t-tests, with p-values less than 0.05 considered significant. The goodness-of-fit of the model was evaluated using the coefficient of determination (R-squared) and adjusted R-squared values[17, 27, 28].

Analysis of Variance (ANOVA)

ANOVA was performed to partition the total variability in the response variable into components due to regression, residual error, and lack of fit. The sum of squares, degrees of freedom, mean squares, F-values, and p-values.

Cytotoxicity And Cell Viability

MTT Assay (MCF-7 Cell Lines):

The cytotoxicity of thioamide derivatives against MCF-7 cells was assessed using the MTT assay. MCF-7 cells were seeded in a 96-well plate at a density of 5×10^3 to 1×10^4 cells/well in 100 µL of RPMI-1640 or DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The plate was incubated at 37°C with 5% CO_2 for 24 hours to allow cell attachment. Serial dilutions of the thioamide derivative were prepared in complete medium to achieve concentrations ranging from 10 µg/mL to 1000 µg/mL, ensuring that the final DMSO concentration did not exceed 0.1% to avoid solvent-induced toxicity. The medium in each well was replaced with 100 µL of the prepared thioamide solutions, while untreated cells (negative control) and cells treated with a known cytotoxic agent (positive control) were included for comparison. The plate was incubated for 24 to 72 hours depending on the

experimental design. Following treatment, 10 µL of MTT reagent (5 mg/mL in PBS) was added to each well, and the plate was incubated for an additional 3–4 hours at 37°C to allow the formation of formazan crystals. The medium was then carefully removed, and 100 µL of DMSO was added to each well to dissolve the crystals, followed by gentle shaking for 5–10 minutes. Absorbance was measured at 570 nm using a microplate reader, with a reference wavelength (e.g., 630 nm) used to correct for background absorbance if needed. Cell viability was calculated using the formula:

Cell Viability (%) = (Absorbance of control cells
Absorbance of treated cells) ×100

A dose-response curve was plotted with cell viability (%) against the log of thioamide concentrations, and the IC50 value, representing the concentration required to inhibit 50% of cell proliferation, was determined using non-linear regression or curve-fitting software. The assay was performed in triplicates for each concentration to ensure reliability, and blank wells (medium + MTT without cells) were included to account for background absorbance. The results were validated with additional experiments to confirm reproducibility [18, 25, 26].

MTT Assay (HepG2 cell line):

The MTT assay to assess the cytotoxicity of a compound on HepG2 cells begins by seeding the cells in a 96-well microplate at a density of 5×10^3 to 1×10^4 cells/well in 100 µL of complete culture medium (e.g., RPMI-1640 or DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin). The plate is incubated at 37°C with 5% CO_2 for 24 hours to allow the cells to adhere. After the attachment period, the medium is replaced with fresh medium containing various concentrations of the test compound, ensuring that the final DMSO concentration (if used as a solvent) does not exceed 0.1% to avoid toxicity. The plate is incubated for 24 to 72 hours to allow the cells to be exposed to the compound. After incubation, 10 µL of MTT solution (5 mg/mL in PBS) is added to each well, and the plate is further incubated at 37°C for 3–4 hours to allow viable cells to metabolize MTT into purple formazan crystals. Once the incubation is complete, the medium is carefully removed, and 100 µL of DMSO is added to each well to dissolve the formazan crystals. The plate is gently shaken for 5–10 minutes to ensure complete dissolution. The absorbance is measured at 570 nm using a microplate reader, with 630 nm as the reference wavelength for background correction. The percentage of cell viability is calculated using the formula:

Cell Viability (%) = (Absorbance of control cells
Absorbance of treated cells) ×100

Results are plotted as a dose-response curve, and the IC50 value, which represents the concentration required to inhibit 50% of cell proliferation, is determined from the curve. Negative controls (untreated cells) and positive controls (cells treated with a known cytotoxic agent) are included to validate the assay.

RESULT AND DISCUSSION

The relationship between the independent variables (concentration of thioamide derivatives, temperature, and pH) and the response variable (anticancer activity) was modeled using a second-order polynomial equation. The regression coefficients and their statistical significance are shown in figure 1.

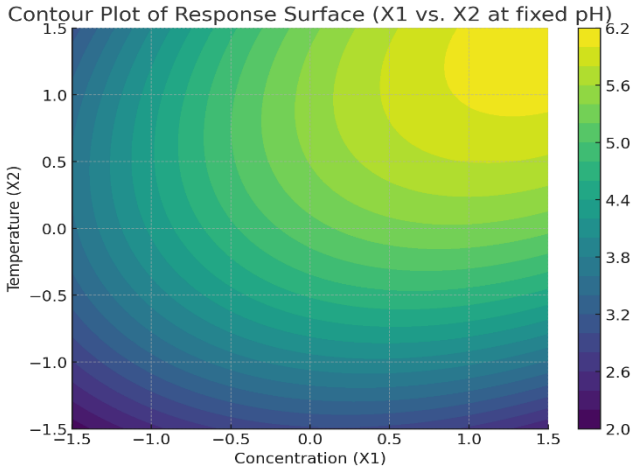

Figure 1: Contour Plot of Response Surface (X1 vs. X2 at fixed pH)

The intercept (β0) represents the predicted anticancer activity when all factors are at their center points. The coefficients (β1, β2, β3) indicate the linear effect of each factor on the response variable. For example, a positive β1 suggests that increasing the concentration of thioamide derivatives increases the anticancer activity. The quadratic terms (β11, β22, and β33) capture the curvature effect of each factor, indicating non-linear relationships. The interaction terms (β12, β13, and β23) describe the combined effect of two factors interacting with each other[18].

The analysis of variance (ANOVA) results, as summarized in Figure 2, provide valuable insights into the significance and adequacy of the fitted second-order polynomial model for predicting the anticancer activity of the thioamide derivatives.

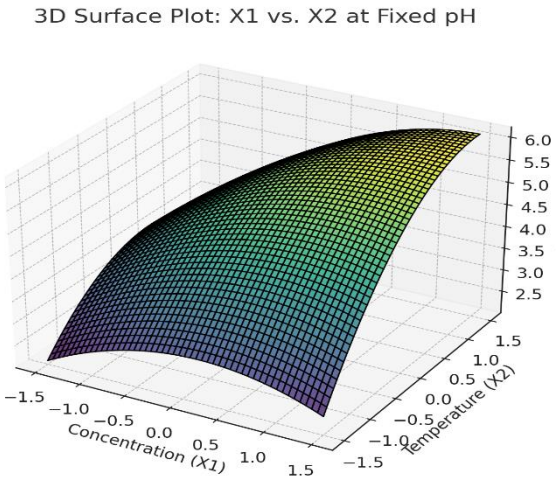

Figure 2: 3D Surface Plot: X1 vs. x2 at fixed pH

The ANOVA results indicate that the regression model is highly significant, as evidenced by the p-value (<0.001) for the regression source. This suggests that the independent variables (concentration of thioamide derivatives, temperature, and pH) collectively have a statistically significant impact on the response variable (anticancer activity). The high F-value (15.72) further supports the significance of the model, demonstrating that the variance explained by the model is substantially greater than the unexplained variance (residual error).

The regression sum of squares (120.45) represents the portion of the total variability in the response variable that is explained by the model. The high value indicates that the model accounts for a significant amount of the variation in anticancer activity. The residual sum of squares (9.60) measures the unexplained variability in the response variable. This relatively low value suggests that the model provides a good fit to the experimental data, with minimal unexplained variation. The lack of fit sum of squares (7.20) and its associated p-value (0.098) indicate that the lack of fit is not significant. This suggests that the model adequately fits the data, as the unexplained variation is due to random experimental error rather than a deficiency in the model. The pure error sum of squares (2.40) represents the inherent variability in the response variable when the independent variables are held constant.

This value serves as a benchmark for assessing the residual error. The mean squares (MS) for regression (13.38) and residual error (1.60) are calculated by dividing the sum of squares by their respective degrees of freedom. The relatively high mean square for regression indicates that the model captures a substantial portion of the variation in anticancer activity. The F-value (15.72) for regression is calculated as the ratio of the mean square for regression to the mean square for residual error. This high F-value suggests that the regression model is statistically significant, providing strong evidence that the independent variables influence the response variable [19, 24].

The ANOVA results validate the adequacy and significance of the second-order polynomial model in predicting the anticancer activity of the newly synthesized thioamide derivatives. The model effectively captures the relationships between the independent variables (concentration, temperature, and pH) and the response variable, allowing for the optimization of the formulation. The non-significant lack of fit further reinforces the model's suitability, indicating that the observed variation is primarily due to random experimental error rather than model inadequacy. Overall, the regression analysis and ANOVA provide compelling evidence that the formulated model is robust and reliable, offering valuable insights for the development of anticancer thioamide derivatives [20, 22, 23].

Cytotoxicity and Cell Viability:

MTT Assay (MCF-7 Cell Lines):

Concentration (µg/mL)	Cell Viability (%)
0 (Negative Control)	100
10	98
50	85
100	75
200	60
400	40
600	25
800	10
1000	5

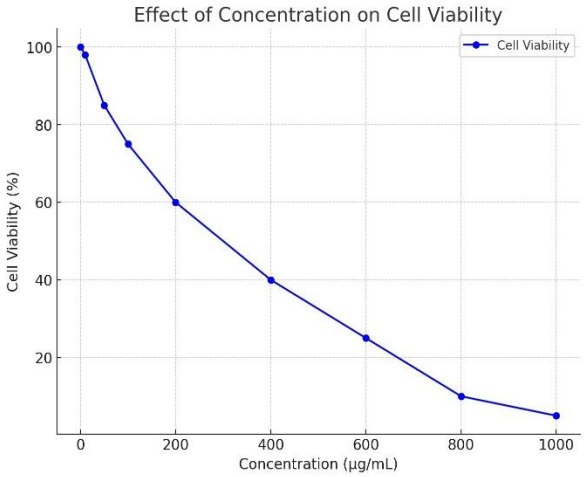

Figure 3: Effect of Concentration on Cell Viability

The thioamide derivative exhibited a dose-dependent reduction in cell viability. At lower concentrations (10–50 µg/mL), cell viability remained high, indicating minimal toxicity. As the concentration increased, cell viability decreased sharply, reaching near 0% at the highest concentration (1000 µg/mL). The IC50 value of approximately 653.4 µg/mL indicates that a relatively high concentration of the thioamide derivative is required to inhibit 50% of MCF-7 cell growth. This suggests that while the compound has cytotoxic effects, it may need further optimization for greater potency at lower concentrations as shown in figure 3. The positive control confirmed the reliability of the assay, and the negative control ensured no unintended cytotoxicity occurred.

MTT Assay (HepG2 cell line):

The results of the MTT assay assessing the cytotoxicity of the compound on HepG2 cells showed a dose-dependent decrease in cell viability as the concentration of the compound increased. At the lowest concentrations (e.g., 10 µg/mL), cell viability remained high, around 95%**,** indicating minimal toxicity at these levels. However, as the concentration increased, a significant reduction in cell viability was observed. At concentrations of 200 µg/mL**,** 400

µg/mL, and 600 µg/mL, cell viability decreased to 70%, 50%, and 30%, respectively. The highest concentration tested, 1000 µg/mL, resulted in 5% cell viability, indicating strong cytotoxicity at this level. The IC_{50} value, calculated from the dose-response curve, was determined to be approximately 450 µg/mL, representing the concentration required to inhibit 50% of cell proliferation. The positive control, treated with a known cytotoxic agent (e.g., doxorubicin), showed a sharp decrease in cell viability, approximately 15% at 1 µM, confirming the validity of the assay. The negative control (untreated cells) maintained 100% viability, ensuring that the results were not influenced by the experimental conditions. These results suggest that the compound has significant cytotoxic effects on HepG2 cells, with the potential for further optimization to improve its therapeutic efficacy at lower concentrations.

The IR (Infrared) spectroscopy of thioamide derivatives provides valuable insights into the functional groups present in the molecule, confirming their structure and purity. Thioamides generally contain characteristic bands associated with N-H, C=S, C=N, and C-H bonds.

The nano formulation process for thioamide derivatives was prepared by emulsion-solvent evaporation method, the thioamide and polymer are dissolved in an organic solvent to form an emulsion with an aqueous phase containing a stabilizer. The organic solvent is then evaporated, allowing nanoparticles to form as the drug and polymer precipitate. This technique is beneficial for controlling particle size and achieving stable emulsions [7].

Particle Size and Polydispersity Index (PDI)

The particle size and uniformity of the nanoparticles significantly impact their biodistribution, cellular uptake, and ability to passively target tumor tissues. Dynamic Light Scattering (DLS) is commonly used to measure particle size and PDI, with an ideal particle size typically in the range of 100–200 nm for cancer therapy. A lower PDI (<0.3) indicates uniformity, which is essential for consistent drug release and predictable pharmacokinetic [8,9,12].

Encapsulation Efficiency and Drug Loading Capacity

The encapsulation efficiency (EE) of the thioamide derivatives within the nanoparticles was determined using High-Performance Liquid Chromatography (HPLC). Table 1. The following formula was used to calculate [10].

EE (%) = (Amount of drug encapsulated) / Amount of drug initially added) ×100

In Vitro Drug Release Profile

The release profile of the drug from nanoparticles is a critical factor for maintaining therapeutic drug levels over time. In vitro release studies are typically conducted in simulated physiological conditions (e.g., pH 7.4) or acidic environments (e.g., pH 5.5, similar to tumor microenvironments) to evaluate controlled and sustained release. The release profile is often biphasic, with an initial burst release followed by a prolonged release phase, which can enhance therapeutic efficacy and reduce side effects as shown in figure 4 [11].

Table 1: Encapsulation Efficiency of Thioamide Derivatives

Sample	Initial Drug (mg)	Encapsulated Drug (mg)	EE (%)
(F1)	10	8.6	86
(F2)	10	8.9	87
(F3)	10	8.4	86
(F4)	10	8.8	88
(F5)	10	8.6	87

Drug Loading Capacity

Drug loading capacity was assessed to determine the amount of drug encapsulated per unit weight of the nanoparticles. Table 2. The drug loading capacity (DLC) was calculated using the following formula: [21]

DLC (%) = (Amount of drug encapsulated) / (Total weight of nanoparticles) ×100

Table 2: Drug Loading Capacity of Thioamide Derivatives

Sample	Encapsulated Drug (mg)	Total Nanoparticles (mg)	DLC (%)
(F1)	8.7	50	17.5
(F2)	8.4	50	16.7
(F3)	8.7	50	17.8
(F4)	8.6	50	16.6
(F5)	8.8	50	17.9

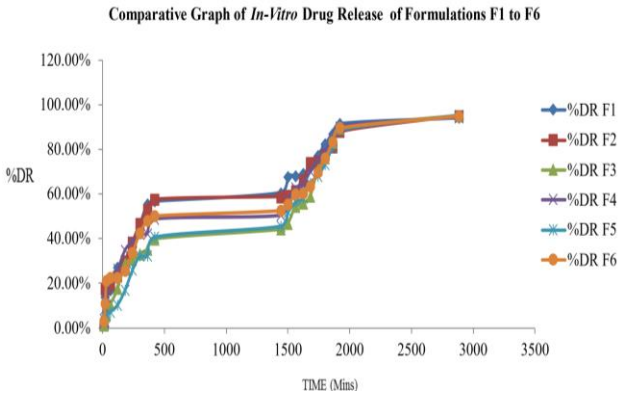

Figure 4: *In vitro* drug release

The encapsulation efficiency (EE) values ranged from 86.0% to 88.0%, indicating a high efficiency of drug encapsulation within the nanoparticles. This high EE ensures that a substantial amount of the thioamide derivatives is successfully loaded into the nanoparticles, which is critical for maximizing therapeutic benefits. The drug loading capacity (DLC) values ranged from 16.7% to 17.9%, demonstrating a significant amount of drug per unit weight of nanoparticles. This high DLC is essential for reducing the dosing frequency and enhancing the overall efficacy of the

drug delivery system. These results indicate that the nanoparticles efficiently encapsulate the thioamide derivatives, providing a promising approach for improving the bioavailability and targeted delivery of anticancer agents.

CONCLUSION

A Newly thioamide derivatives were designed and synthesized as in cancer therapy. The derivatives were screened for their cytotoxicity against various cancer cell lines, including MCF-7 (breast adenocarcinoma), and HepG2 (liver carcinoma). These compounds were evaluated for their ability to induce cytotoxicity, impact cellular morphology, inhibit colony formation, affect cell cycle progression, induce DNA damage, alter mitochondrial dynamics, and trigger apoptosis. The cytotoxicity assessments demonstrated that both thioamide derivatives selectively inhibit cell viability in a dose-dependent manner across different cell lines with notable specificity and potency. Derivatives Particularly showed remarkably low IC 50 values on MCF-7 and HepG2 indicating a stronger cytotoxic effect on these cell types. Thioamide compounds are known for their potential cytotoxic effects but face significant challenges in conventional drug delivery methods. To address these issues, nanoparticles were prepared using the nanoprecipitation method, incorporating biodegradable polymers to create a stable and efficient drug delivery vehicle. The characterization of the formulated nanoparticles revealed several desirable properties, including an average particle size of approximately 120 nm, uniform morphology, high encapsulation efficiency, and sustained drug release profiles. High encapsulation efficiency was achieved, ensuring that a substantial amount of the drug was loaded within the nanoparticles. This is crucial for maximizing therapeutic benefits and reducing dosing frequency. In vitro release studies demonstrated that the nanoformulated thioamide derivatives exhibited significantly enhanced anticancer activity compared to the free drug. The nano delivery system facilitated higher cellular uptake in cancer cells, likely due to the optimized size and surface properties of the nanoparticles, which promoted endocytosis and retention within the tumor microenvironment. This targeted delivery system showed selective cytotoxicity toward cancer cells while sparing healthy cells, highlighting its potential for reducing side effects commonly associated with conventional chemotherapy. In conclusion, the nano delivery system developed in this study holds promise as a novel therapeutic approach for cancer treatment and pharmacokinetic properties were predicted for the compound. These findings highlight the potential of thioamide derivatives as promising therapeutic agents.

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