Research Article

Synthesis and Acute Toxicity Evaluation of Quinoxaline Indandione Hydrazide: A Three Step Synthesis via Formylation and Imine Condensation Derivatives as Potential Cytotoxic Agents for Anti-Lung Cancer Applications

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ABSTRACT

The study concentrated on the design, synthesis, characterization and biological evaluation of new quinoxaline indandione hydrazide compounds as implicit anticancer agents. In this work, a series of compounds was synthesized through a multistep reaction process involving condensation and reflux techniques. The synthesized compounds were purified and characterized using spectroscopic methods such as FT- IR, ^{1}H- NMR,13C NMR and mass spectrometry, which verified the presence of aromatic rings, carbonyl groups, and imine functionalities in the final structures. The results showed a dose-dependent drop in cell viability, with advanced attention flaunting lesser cytotoxic goods. The LC_{50} value was determined to be 224.174 µg/mL, indicating moderate anticancer activity. The study successfully synthesized heterocyclic compounds through multistep reactions, and the final compound was verified by its physical properties. The compound N'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-tetrahydroquinolin-2-yil)benzamidehydrazide showed moderate anticancer exertion against A549 human lung cancer cells, with advanced attention adding cytotoxic goods. ADMET results suggest good drug-like properties, low toxicity, and felicitates for further drug development studies.

Keywords: Cancer, Chemotherapy, MTT Assay, A549 human lung cancer cell line.

INTRODUCTION

Medical science has identified over 100 types of disease and cancer has been second most prevalent cause of mortality in the world. The name "cancer" derives from the Greek word "karkinos" meaning "crab" and was adopted by Hippocrates around 3000 BC. To describe tumors due to their crab like extensions[1]. Chemotherapy was a common treatment for cancer and was generally administered intravenously. Although it was effective, it also caused several side effects. The name "chemotherapy" sometimes referred to as "chemo" was coined in the early 1900s by the German druggist Paul Ehrlich, who termed it "treatment of disease by chemicals"[2].

In cancer treatment, chemotherapy worked by killing or slowing the growth of tumor cells, primarily targeting dividing cells still, due to its nonspecific nature, it also affected normal cells[3,4]. Paul Ehrlich was the first to validate the use of animal models to screen chemicals for remedial exertion, significantly impacting cancer medicine development[5]. By 1908, his work with rabbit models led to arsenical treatments for syphilis. Surgery and radiotherapy dominated cancer remedy until the 1960s, after which chemotherapy gained elevation, frequently administered in cyclic rules with rest ages between treatments[6,7].

The chemotherapy combats cancer growth. Cancer was described as a serious disease characterized by the uncontrolled growth of abnormal cells that invaded nearby tissues and spread throughout the body. Its prevalence had increased over time, with global reports indicating a steady rise in cases and deaths[8].Cancer had been caused by environmental factors such as tobacco, radiation, chemicals, and infections, as well as genetic mutations and immune or hormonal imbalances[9,10]. The disease involved complex mechanisms leading to abnormal cell proliferation. Common symptoms included fatigue, weight loss, lumps, persistent cough, and unusual bleeding[11]. Diagnosis had involved blood tests, imaging techniques, biopsies, and molecular methods[12,13]. Various treatment approaches had been used, including surgery, chemotherapy, radiotherapy, hormone therapy, immunotherapy, targeted therapy, and bone marrow transplantation[14,15]. Chemotherapeutic drugs such as alkylating agents, anti-metabolites, antibiotics, plant-derived compounds, and platinum-based drugs have acted through different mechanisms like DNA damage and inhibition of cell division[16]. Despite their effectiveness, these treatments had caused side effects such as fatigue, hair loss, nausea, infections, and digestive issues[17,18]. Future research had focused on advanced strategies such as artificial intelligence, nanotechnology, gene therapy, and combination therapies to improve outcomes and reduce resistance[19].There are many types of cancer, but this study describes lung cancer. Lung cancer, also known as lung carcinoma, was a malignant tumor that originated in the tissues of the lungs, usually in the lining of the air passages. It had been classified into two main types: small cell lung cancer (SCLC), which accounted for about 15% of cases, and non-small cell lung cancer (NSCLC), which made up approximately 85% of cases[20]. The causes and risk factors of lung cancer have included smoking, which significantly increased the risk, as well as genetic damage to airway cells.

Other contributing factors had been air pollution from vehicle exhaust, exposure to hazardous substances such as asbestos and radon gas, and environmental mutagens[21,22]. The symptoms of lung cancer had included loss of appetite, weight loss, weakness, fever, night sweats, coughing up blood, shortness of breath, chest pain, and persistent cough[23,24]. Various diagnostic methods had been used, including CT scans for staging, biopsies for histological classification, auto fluorescence bronchoscopy for early detection, PET scans for evaluating nodules, pulmonary function tests for assessing lung capacity, and X-rays for detecting lung masses[25,26]. Treatment methods have included surgery, radiation therapy, chemotherapy, and targeted therapies[27,28]. In addition, lifestyle modifications had been recommended, such as quitting smoking, avoiding harmful dietary components, increasing physical activity, and consuming more fruits and vegetables while reducing alcohol, caffeine, and unhealthy fats[29].

MATERIALS AND METHODS

Materials

o-Phenylenediamine, Aniline, Acetone, n-butyl alcohol, Ethanol, Phthalic anhydride, Formic acid, Dimethoxy ethane was procured from standard pharmaceutical suppliers.

Synthesis

Synthesis of compound 1(2{[(quinoxalin-1(4*H*)-yl) amino]methyl}benzoic acid) [Shown in Fig. 1]**:** A mixture of 32.4gm o-phenylenediamine (0.1 mol), 20.7ml Aniline (0.1mol), 30ml n-butyl alcohol in 20.7ml of Formic acid is refluxed for 4 hrs, then cooled and poured over cold water. The crude product was filtered off, washed, recrystallized and dried.The product is light brown solid, water-insoluble in nature, weighs 21gram and has a melting point of 95-98°C.

Synthesis of compound 2 (2-anilino-1*H*-indene-1,3(2*H*)-ione) [Shown in Fig. 2]**:** A mixture of 45.6ml Aniline (0.25 mol) and 74.06gm Phthalic anhydride (0.25 mol) is heated under reflux for 6 hrs in the presence of Acetone, Potassium carbonate. Then the reaction mixture is poured over the cold water. The crude product is filtered off, washed, recrystallized and dried. The product is light gray color, water- soluble in nature, weighs 93.16gm and has a melting point of 105-108°C.

Synthesis of final compound N'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-tetrahyroquinolin-2-yil) benzamide hydrazide [Shown in Fig. 3]**:** Equal amount of compound1 {[(quinoxalin-1(4H)-yl)amino]methyl}benzoic acid and compound2 (2-anilino-1H- indene-1,3(2H)-dione) is triturated and is heated under reflux for 6hrs in the presence of Ethanol and DME. Then the reaction mixture is poured over cold water. The crude product was filtered off, washed, recrystallized and dried. The product is whitish gray colour, soluble in water, DMSO, chloroform, etc...., weighs 1.58gm and has a melting point of 63-65°C.

Yield 76.12%; M.P: 63-65^0 C; IR (KBr, V_{max} Cm^{-1}) 3060 cm^{-1} (Aromatic C-H str), 1777 cm^{-1}, 1733 cm^{-1}, 1702 cm^{-1} (Carbonyl (C=O) Str) ,1590 cm^{-1} and 1493 cm^{-1} (Aromatic C=C Str), 1453 cm^{-1} (C-H Bending Vibration), 1299-1217 cm^{-1} (C–N / C–O Str), 1114-1066 cm^{-1} (C–O or C–N Str), 915 – 845 cm^{-1} (Aromatic C–H Out-of-Plane Bending), 759 – 689 cm^{-1} (Aromatic Ring Substitution).

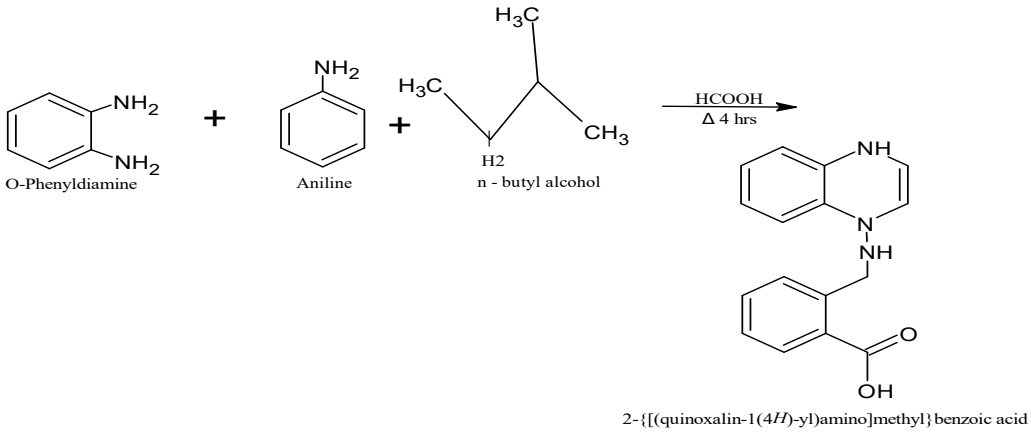


Figure 1: Synthesis of compound 1 (2-{[(quinoxalin-1(4*H*)-yl)amino]methyl}benzoic acid)

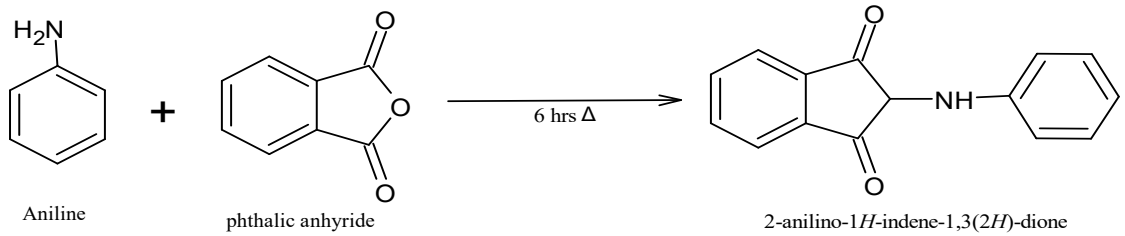
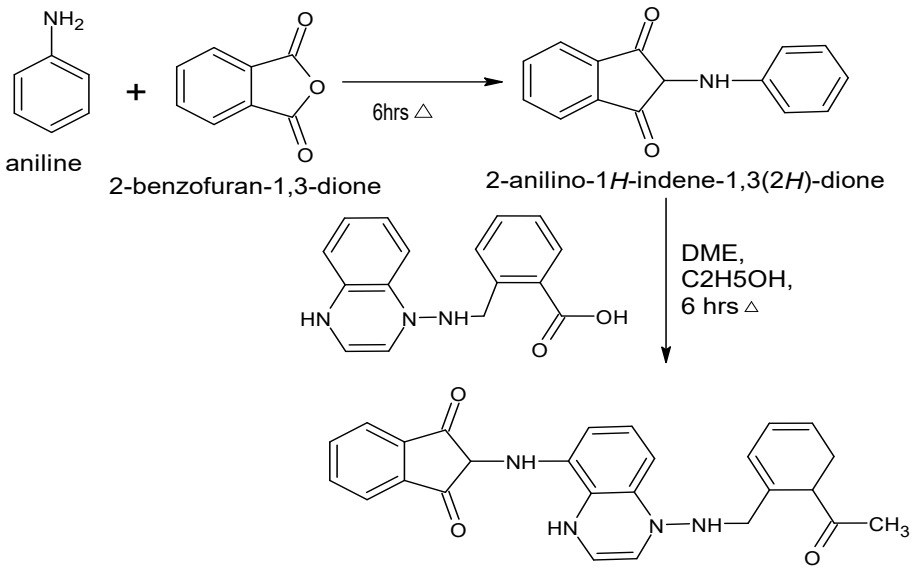


Figure 2: Synthesis of compound 2 (2-anilino-1*H*-indene-1,3(2*H*)-dione)



N'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-*N*-(1,2,3,4-tetrahydroquinolin-2-yil) benzamidehydrazide(carboxilic acid derivatives)

Figure 3: Synthesis of final compound Synthesis of final compound N'-(2-(1,3- dioxoisoindolin -2-yil)phenyl) -N-(1,2,3,4-tetrahydroquinolin-2-yil)benzamidehydrazide)

^{1}H-NMR (DMSO /$CHCL_3$): 7.18-7.20 ppm (Aromatic H, Interpretation:- Benzene ring proton), 7.43 – 7.47 ppm (Aromatic H, Interpretation:- Substituted phenyl ring), 7.52 – 7.60 ppm (Aromatic H, Interpretation:- Coupled aromatic protons), 7.90 – 7.98 ppm (Aromatic H near EWG, Interpretation:- Proton close to carbonyl/imide group), 8.21 ppm (Deshielded aromatic H, Interpretation:- Proton ortho to electron-withdrawing group).

^{13}C NMR (125Mhz, DMSO-D6) δC: Carbonyl Carbons (Highly Deshielded: Imide C=O (phthalimide): δ 165–170 ppm (2signals), Amide C=O: δ 167–172 ppm, Ketone (acetyl group): δ 195–200 ppm.

Aromatic Carbons (Phenyl + Quinoline Rings): Quaternary aromatic C (C–N, C–C=O): δ 140–155 ppm, Protonated aromatic carbons: δ 120–135 ppm.

Heterocyclic / Quinoline Ring Carbons:C attached to nitrogen (C–N): δ 145–155 ppm, Saturated ring carbons (tetrahydroquinoline CH_2/CH): δ 25–55 ppm,

Aliphatic Carbons: CH_2 near –NH–N– linkage: δ 40–55 ppm, Other aliphatic CH_2: δ 25–40 ppm.(ESI-MS); m/z =278.0782 (base peak) corresponds to the $[M+H]^+$ molecular ion.

Anti- lung cancer activity

Determination by MTT assay

A549 (Human Lung Cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, (DMEM).The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator. The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate[30,31]:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10^3 cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of compound stock:

1mg of sample was weighed and dissolved in 1ml 0.1% DMSO using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Anticancer Evaluation:

After 24 hours the growth medium was removed, freshly prepared each compound were added at concentrations of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml of DMEM. Each concentration was added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator. Untreated control cells were also maintained.

Anticancer Assay by Direct Microscopic observation:

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Anticancer Assay by MTT Method[32]:

15mg of MTT was reconstituted in 3ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37◦C in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT solubilization solution Dimethyl sulfoxide (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm.

Calculation

The percentage of growth inhibition was calculated using the formula,

$$\% \text{ of viability} = \frac{Mean\ OD\ samples \times 100}{Mean\ OD\ of\ control\ group}$$

Toxicological evaluation:

The in-silico toxicity evaluation of the synthesized compound, namely *N*'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-tetrahydroquinolin-2-yil)benzamidehydrazide, was carried out using computational toxicity prediction approaches analogous to ProTox-II/ProTox 3.0 platforms. The predicted toxicity profile suggested that the compound possesses moderate acute oral toxicity with an estimated LD_{50} value of approximately 700 mg/kg in rats, thereby classifying the molecule under toxicity class IV according to the Globally Harmonized System (GHS).

This classification indicates that the compound may be harmful if swallowed but does not exhibit extremely severe acute toxicity. The presence of aromatic hydrazide and heterocyclic nitrogen-containing pharmacophores appears to contribute significantly to the observed toxicological behavior. Computational analysis further predicted acceptable pharmacological safety for preliminary drug development while emphasizing the necessity for experimental validation.

The organ toxicity prediction revealed a moderate probability of hepatotoxicity, which may arise from metabolic activation of the hydrazide linkage and aromatic imide system. Such functional groups are known to generate reactive intermediates during hepatic biotransformation, potentially leading to oxidative stress and hepatocellular injury. In addition, the compound demonstrated a possible tendency toward immunotoxicity and weak mutagenic potential.

The mutagenicity prediction may be attributed to the presence of conjugated aromatic systems and hydrazide-associated reactive centers capable of interacting with nucleophilic biomolecules. However, the carcinogenicity prediction indicated a comparatively low probability of tumorigenic effects, suggesting that the compound may not possess strong carcinogenic liabilities under normal exposure conditions. The predicted absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics suggest favorable oral absorption and moderate tissue permeability.

The tetrahydroquinoline scaffold may facilitate partial blood–brain barrier penetration owing to its lipophilic nature, whereas the aromatic nitrogen atoms may contribute to cytochrome P450 enzyme inhibition, particularly CYP3A4 and CYP2D6 isoforms. Furthermore, a low-to-moderate risk of hERG channel inhibition was predicted, indicating a comparatively lower possibility of severe cardiotoxicity. Renal toxicity was estimated to be minimal, whereas mild skin and eye irritation potential was observed due to the carbonyl-rich imide functionalities present in the molecular framework.

Structural alert analysis identified several toxicophoric fragments within the molecule, including the hydrazide linker, quinoline-like nitrogen system, aromatic ketone, and phthalimide nucleus. These moieties are frequently associated with reactive metabolite generation, oxidative stress induction, and enzyme interaction pathways. Despite these observations, the overall toxicity assessment indicated that the synthesized derivative remains a promising lead molecule for medicinal chemistry optimization.

Nevertheless, additional experimental investigations such as Ames mutagenicity assay, HepG2 cytotoxicity studies, OECD acute oral toxicity testing, cytochrome P450 inhibition assays, and hERG screening are strongly recommended to validate the computational toxicity predictions and establish the safety profile of the compound for further pharmacological development.

Acute Toxicity Classification

According to Protox / GHS scale:

Table 1: Toxicity classification according to Globally Harmonized System (GHS)

Toxicity Class	LD50 Range
Class 1	≤5 mg/kg
Class 2	5–50 mg/kg
Class 3	50–300 mg/kg
Class 4	**300–2000 mg/kg**
Class 5	2000–5000 mg/kg
Class 6	>5000 mg/kg

So this compound fits:

$LD_{50} \approx 700$ mg/kg

Therefore:

- Harmful if swallowed
- Moderate acute toxicity

Table 2: Toxicity profile Predicted by ProTox-II/ProTox3.0 platform

Toxicity Parameter	Predicted Result	Interpretation
Acute Oral Toxicity (LD_{50}, rat)	~450–900 mg/kg	Moderately toxic
GHS Toxicity Class	Class 4	Harmful if swallowed
Hepatotoxicity	Moderate risk	Aromatic hydrazide may form reactive metabolites
Cardiotoxicity (hERG inhibition)	Low–moderate	Aromatic nitrogen system may weakly interact
Mutagenicity (AMES)	Possible weak positive	Hydrazide fragment sometimes linked to genotoxicity
Carcinogenicity	Low–moderate concern	Long-term aromatic hydrazides require testing (Inactive/low possibility).
Skin Irritation	Mild	Aromatic ketone + imide may irritate.
Eye Irritation	Moderate	Carbonyl-rich scaffold.
Respiratory Toxicity	Low	No highly volatile groups.
CYP450 Inhibition	Possible CYP3A4/CYP2D6 inhibition.	Quinoline-like systems often inhibit CYP enzymes
Bioaccumulation	Moderate	Aromatic lipophilic scaffold
Blood Brain Barrier Penetration	Moderate	Tetrahydroquinoline ring increases lipophilicity

RESULTS

The anticancer activity of N'-(2-(1,3-dioxoisoindolin-2-yil) phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamide hydrazide was evaluated against the A549 human lung cancer cell line using the MTT assay. The cytotoxic effect of the compound was determined by measuring the optical density (OD) at 540 nm, which reflects the number of viable cells after treatment. All experiments were performed in triplicates, and the results were expressed as mean percentage cell viability ± standard error (SE).

Absorbance and Cell Viability

The absorbance values obtained from the microplate reader and the calculated percentage viability of A549 cells after treatment with different concentrations of N'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil) benzamidehydrazide are presented in table 3.

LC_{50} Value

LC_{50} value of N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil) benzamidehydrazide = 224.174 µg/ML.

This indicates that a concentration of approximately 224.174 µg/mL of N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide is required to inhibit 50% of A549 lung cancer cells. The absorbance values obtained from the microplate reader and the calculated percentage viability of A549 cells after treatment with different concentrations of N'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide are. The results clearly demonstrate a dose-dependent decrease in cell viability of A549 lung cancer cells following treatment with N'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide.

The statistical analysis was performed using triplicate values for each concentration. The low standard deviation and standard error values indicate good reproducibility and consistency between the experimental replicates shown in table 3.

Since the highest tested concentration in this experiment was 100 µg/mL, the LC_{50} lies beyond the tested range, suggesting that N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide exhibits moderate cytotoxic activity against A549 human lung cancer cells.

Table 3: Absorbance value, cell viability and Statistical analysis

A549 – human lung cancer cell line						
Sample: N'-(2-(1,3-dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide						
Sample concentration (µg/mL)	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
OD1	0.7956	0.7859	0.7541	0.7152	0.6528	0.6252
OD2	0.8013	0.7912	0.7498	0.7068	0.6625	0.6189
OD3	0.8064	0.7925	0.7426	0.7148	0.6571	0.6241
Average Absorbance @ 540nm	0.8011	0.7899	0.7488	0.7123	0.6575	0.6227
Percentage viability 1	100	98.7808	94.7838	89.8944	82.0513	78.5822
Percentage viability 2	100	98.7395	93.5729	88.2067	82.6781	77.237
Percentage viability 3	100	98.2763	92.0883	88.6409	81.4856	77.7375
Average	100	98.5989	93.4817	88.914	82.0717	77.7375
Std dev	0	0.28013	1.3507	0.8764	0.59653	0.73569
Std error	0	0.16173	0.77947	0.50599	0.34441	0.42475

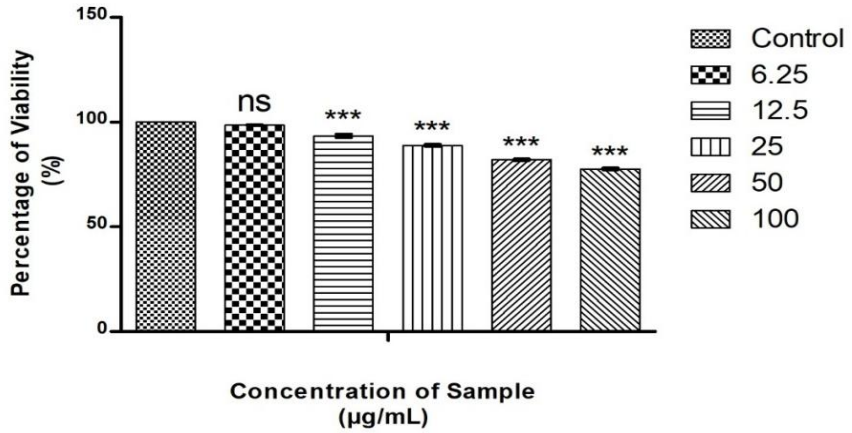


Figure 4: Graphical representation depicting the anticancer effect of N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide by MTT assay- Along Y axis Percentage viability, Along X axis varied concentration of N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. ***p< 0.0001 compared to control group, ns-non significant compared to control group. (Calculated using ED50 PLUS V1.0 Software).

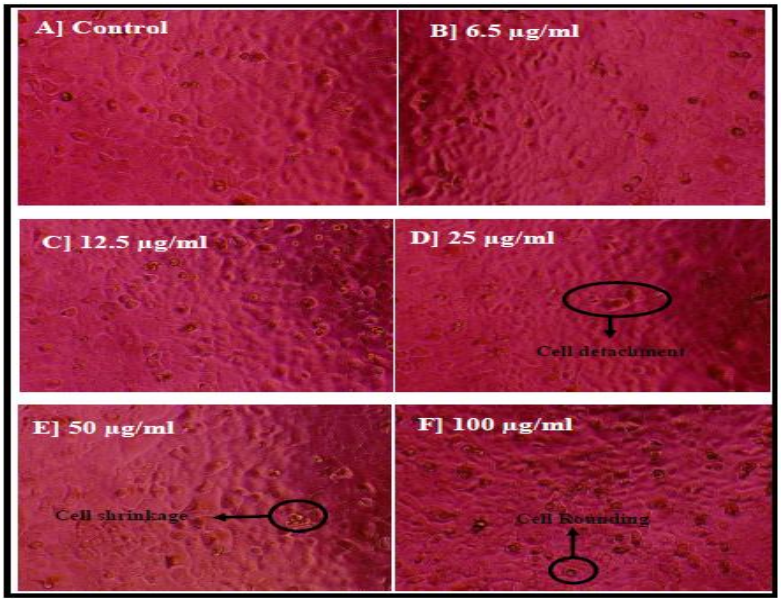


Figure 5: Human lung cancer A549 cells treated with different concentrations of N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil) benzamide hydrazide (6.25–100 µg/mL) for 24 h. Control cells show normal morphology, whereas treated cells with compound N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-tera hyroquinolin-2-yil) benzamidehydrazide exhibit concentration-dependent morphological changes such as cell rounding, shrinkage, detachment, and reduced cell density, indicating cytotoxic activity, although the effect is moderate as indicated by the relatively high LC_{50} value (224.174 µg/mL). Which demonstrated a gradual decrease in cell viability from 98.60% at 6.25 µg/mL to 77.73%at100µg/mL. (Microscopic examination using an inverted phase-contrast microscope (10×magnification).

Microscopic Observation Results

Microscopic examination using an inverted phase-contrast microscope (10× magnification) revealed morphological changes in treated cells compared to the control group.

Control cells

- Normal epithelial morphology
- Intact cell membrane
- Uniform monolayer growth

The test compound N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamide hydrazide exhibited dose-dependent cytotoxic activity against the A549 human lung cancer cell line as determined by the MTT assay. Treatment with increasing concentrations (6.25–100 µg/mL) resulted in a gradual decrease in cell viability from 98.60% to 77.73%. The calculated LC_{50} value was 224.174 µg/mL, indicating moderate anticancer.

Potential of N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide against A549 lung cancer cells. Morphological changes such as cell shrinkage, rounding, and cytoplasmic vacuolization further confirmed the cytotoxic effect of the compound. These morphological alterations indicate cytotoxic stress and reduced proliferation of A549humanlungcancercelllines.

CONCLUSION

The study successfully synthesized a series of heterocyclic quinoxaline indandione compounds mixes through multistep reactions involving condensation, cyclization, and nucleophilic condensation reactions. Firstly, o-Phenylenediamine replied with aniline in the presence of formic acid and n- butyl alcohol under reflux to yield 2-{((quinoxalin- 1(4H)- yl) amino)methyl} benzoic acid via formylation and intramolecular cyclization, establishing the quinoxaline . Latterly, aniline and Phthalic anhydride passed nucleophilic acyl substitution and cyclization in acetone with potassium carbonate to form 2- anilino- 1H- indene-1,3(2H)-dione. In the final step, these intermediates were condensed in ethanol and the target hybrid compounds through C– N bond conformation. All compounds were attained as crystalline solids and verified by their physical properties. Biological evaluation using the MTT assay on the A549 human lung cancer cell line revealed that the test compound N'-(2-(1,3- dioxoisoindolin-2-yil)phenyl)-N-(1,2,3,4-terahyroquinolin-2-yil)benzamidehydrazide displayed concentration- dependent cytotoxicity, reducing viability to 77.73 at 100 µg/ mL, with observable morphological changes similar as cell loss and vacuolization the LD_{50} value of 224.174 µg/ mL indicates moderate anticancer exertion likewise, in- silico ADMET analysis of N-(2-(1,3-dioxoisoindolin-2-yil) phenyl) -N-1,2,3,4-tetrahyroquinolin-2-yil) benzamidehydrazide demonstrated favorable drug-like properties, including good oral absorption, moderate lipophilicity, respectable metabolic stability, low mutagenic and carcinogenic threat, and a predicted LD_{50} of 700 mg/ kg (OECD Class IV), suggesting slight toxicity with no major safety enterprises. Overall, the synthesized compound shows promising outcomes for further pharmacological development, although molecular and in- vivo studies are necessary to validate its remedial efficacy and safety profile.

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REFERENCES

1. Marshall Jr EK. Historical perspectives in chemotherapy. Advances in chemotherapy. 1964;13:1-8.
2. Torino F, Barnabei A, Paragliola R, Baldelli R, Appetecchia M, Corsello SM. Thyroid dysfunction as an unintended side effect of anticancer drugs. Thyroid. 2013 Nov; 23(11):1345-66.
3. PostelVinay S, Vanhecke E, Olaussen KA, Lord CJ, Ashworth A, Soria JC. The potential of exploiting DNA-repair defects for optimizing lung cancer treatment. Nature reviews Clinical oncology. 2012 Mar; 9(3):144-55.
4. Devita Jr VT, Young RC, Canellos GP. Combination versus single agent chemotherapy: a review of the basis for selection of drug treatment of cancer. Cancer. 1975 Jan;35(1):98-110.Papac RJ. Origins of cancer therapy. The Yale journal of biology and medicine. 2001 Nov; 74(6):391.
5. DeVita Jr VT. The evolution of therapeutic research in cancer. New England Journal of Medicine. 1978 Apr 20; 298(16):907-10.
6. DeVita Jr VT, Chu E. A history of cancer chemotherapy. Cancer research. 2008 Nov 1; 68(21):8643-53.
7. Venugopal S, Kaur B, Verma A, Wadhwa P, Magan M, Hudda S, Kakoty V. Recent advances of benzimidazole as anticancer agents. Chemical biology & drug design. 2023 Aug;102(2):357-76.
8. Wright JC. Cancer chemotherapy: past, present, and future—Part I. Journal of the National medical Association. 1984 Aug; 76(8):773.
9. Gibbs WW. Untangling the roots of cancer. Scientific American. 2003 Jul 1; 289(1):56-65.
10. Chabner BA, Longo DL. Cancer chemotherapy and biotherapy: principles and practice. Lippincott Williams & Wilkins; 2011 Dec 7.
11. Deol GJ, Priyadarsini PI, Nallagattla VG, Amarendra K, Seelam K, Latha BR. A novel SegNet segmentation with MobileNet brain tumor classification using MRI images. SN Computer Science. 2025 May 19;6(5):477.
12. Swain S, Kumar Sahu P, Beg S, Manohar Babu S. Nanoparticles for cancer targeting: current and future directions. Current drug delivery. 2016 Dec 1;13(8):1290-302.
13. Zaimy MA, Saffarzadeh N, Mohammadi A, Pourghadamyari H, Izadi P, Sarli A, Moghaddam LK, Paschepari SR, Azizi H, Torkamandi S, Tavakkoly-Bazzaz J. New methods in the diagnosis of cancer and gene therapy of cancer based on nanoparticles. Cancer Gene Therapy. 2017 Jun;24(6):233-43.
14. Masood I, Kiani MH, Ahmad M, Masood MI, Sadaquat H. Major contributions towards finding a cure for cancer through chemotherapy: a historical review. Tumori Journal. 2016 Jan;102(1):6-17.
15. Dong L, Wang W, Li A, Kansal R, Chen Y, Chen H, Li X. Clinical next generation sequencing for precision medicine in cancer. Current genomics. 2015 Aug 1;16(4):253-63.
16. Alexanian R, Haut A, Khan AU, Lane M, McKelvey EM, Migliore PJ, Stuckey WJ, Wilson HE. Treatment for multiple myeloma: combination chemotherapy with different melphalan dose regimens. Jama. 1969 Jun 2;208(9):1680-5.
17. Katzung BG, Masters SB, Trevor AJ, editors. Basic & clinical pharmacology. 2004.
18. Carey MP, Burish TG. Etiology and treatment of the psychological side effects associated with cancer chemotherapy: a critical review and discussion. Psychological bulletin. 1988 Nov;104(3):307.
19. Frei III E. Clinical cancer research: an embattled species. Cancer. 1982 Nov 15;50(10):1979-92.
20. Weinstein IB. Cancer prevention: recent progress and future opportunities. Cancer research. 1991 Sep 15;51(18_Supplement):5080s-5s.
21. Ali NA, Elsayed GH, Mohamed SH, AbdElkarim AS, Aly MS, Elgamal AM, Elsayed WM, El-Newary SA. Chia seed (Salvia hispanica) attenuates chemically induced lung carcinomas in rats through suppression of proliferation and angiogenesis. Pharmaceuticals. 2024 Aug 27;17(9):1129.

22. Christiani DC, Amos CI. Lung Cancer: Epidemiology. Murray &Nadel's Textbook of Respiratory Medicine (7th ed.). Elsevier. 2022:1018-28.
23. Holm SM, Balmes JR. Systematic review of ozone effects on human lung function, 2013 through 2020. Chest. 2022 Jan 1;161(1):190-201.
24. Pastis NJ, Gonzaloz AV, Silvestri GA. Lung cancer diagnosis and staging,2022; 1039-1051
25. Lam S, Kennedy T, Unger M, Miller YE, Gelmont D, Rusch V, Gipe B, Howard D, LeRiche JC, Coldman A, Gazdar AF. Localization of bronchial intraepithelial neoplastic lesions by fluorescence bronchoscopy. Chest. 1998 Mar 1; 113(3):696-702.
26. Hammerschmidt S, Wirtz H. Lung cancer: current diagnosis and treatment. Deutsches Arzteblatt International. 2009 Dec 4; 106(49):809.
27. Brown JS, Eraut D, Trask C, Davison AG. Age and the treatment of lung cancer. Thorax. 1996 Jun 1; 51(6):564-8.
28. Hoy H, Lynch T, Beck M. Surgical treatment of lung cancer. Critical Care Nursing Clinics. 2019 Sep 1;31(3):303-13.
29. Bade BC, Cruz CS. Lung cancer 2020: epidemiology, etiology, and prevention. Clinics in chest medicine. 2020 Mar 1; 41(1):1-24.
30. Jerard C, Michael BP, Chenicheri S, Vijayakumar N, Ramachandran R. Rosmarinic Acid-Rich Fraction from Menthaarvensis Synchronizes Bcl/Bax Expression and Induces Go/G1 Arrest in Hepatocarcinoma Cells ProcNatlAcadSci, India, Sect B BiolSci, 2020 Sep;90(3):515–22.
31. MosmannT.Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983 Dec; 65(1–2):55–63.
32. Talarico LB, Zibetti RG. Anti-herbes simplex virus activity of sulfate galactans, 2004 Apr 1; 34(1-2):63-71.