



Bioactivity assessment of the prepared Chitosan-based Schiff base composite on lung cancer and esophageal cancer

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ABSTRACT

The assessment of bioactivity for 2-hydroxy-5-(naphthalene-1-ylidiazonyl) benzaldehyde (HNDB) on lung cancer and esophageal cancer were investigated. The study aimed to evaluate the biological efficiency of HNDB through various applications and measurements. Previous research on HNDB's efficacy against breast lesions from early lactation human milk and esophageal cancer using the HBL-100 and SKGT-4 cell lines, respectively, was reviewed. HNDB demonstrated significant inhibitory effects on breast and esophageal cancer cells, with optimal concentrations of 500 $\mu\text{g.mL}^{-1}$ and 250 $\mu\text{g.mL}^{-1}$, respectively. Moreover, its effectiveness against lung carcinoma using the A549 cell line was explored, indicating an optimal concentration of 250 $\mu\text{g.mL}^{-1}$. The study employed MTT assays to determine cytotoxicity and inhibition rates, revealing promising anti-proliferative activity of HNDB across various cancer cell lines. Furthermore, response curves illustrated concentration-dependent inhibition, with IC₅₀ values ranging from 148.2 to 261.6 $\mu\text{g.mL}^{-1}$. Box-plot correlations highlighted concentration-dependent inhibition patterns, emphasizing the significance of optimal concentrations in achieving maximal inhibitory effects. Overall, the findings underscore the potential of HNDB as a novel anti-cancer agent against lung and esophageal malignancies, warranting further exploration for therapeutic applications.

1. Introduction

Many cell lines can be used as an in vitro study approach to evaluate, research, discover, and compare their antitumor activities in different cell lines representing different types of human cancers [1]. Some recently synthesized chemical compounds had strong anticancer activity in the lab, but they didn't do well in clinical trials.

Two of the most aggressive and common malignancies in the world are lung and esophageal cancers, with lung cancer accounting for a significant portion of cancer-related deaths [2]. In order to combat these diseases, specialists are looking into the development of novel chemicals with possible anti-cancer characteristics. These substances demonstrate dedication to concentrating on clear pathways involved

in the progression of cancer, providing more effective and targeted treatments with fewer side effects when compared to standard therapy. The assessment of these compounds' bioactivity, which includes their cytotoxicity, ability to induce apoptosis, and impact on cell proliferation, plays a crucial role in determining their actual potential as anti-cancer agents [3]. This work focuses on assessing the bioactivity of a newly prepared substance that was added through synthetic reactions and cleaning techniques in order to elucidate its effects on cell lines related to lung and esophageal cancer. The review aims to contribute to the growing body of knowledge in cancer treatments and identify areas of promising further progress as the enemy of cancer doctors, potentially focusing on comprehension of outcomes and individual fulfilment [4].

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1.1. Lung Cancer And Esophageal Cancer: Two Formidable Challenges in Oncology

1.1.1. Lung Cancer

Cancer is a complicated disease that disrupts normal bodily activities and causes tumors to form due to uncontrolled cell division [5]. It is essential to detect and treat lung cancer, which is quite frequent, as soon as possible. Common symptoms may manifest as a chronic cough, chest discomfort, difficulty breathing, blood in the mucus, and unexplained loss of weight. Reduce the effect of lung cancer by learning about its origins, risk factors, and symptoms and making quitting smoking a priority. An important weapon in the fight against lung cancer is education and knowledge, which can lead to a healthy future.

1.1.2. Esophageal cancer

Over 570,000 new cases of esophageal cancer (EC) are reported each year, making it the sixth most frequent malignancy worldwide. Esophageal squamous cell carcinoma accounts for 90% of cases in China. High mutational loading and an absence of suitable models restrict the genetic causes for ESCC [6]. There is little benefit to survival from the current treatment options, which include targeted therapy, radiation, chemotherapy, and surgery. Cancer immunotherapy, on the other hand, provides remarkable therapeutic advantages for EC patients, including those with ESCC. Recent advances in our knowledge of EC risk factors and therapeutic justifications are covered in this review.

1.2. The Role of the Prepared Compound

The manufactured chemical is highly significant in the battle against oesophagus and lung cancers. The development of this originates from the critical need for therapies that may effectively combat these aggressive cancers [7]. Its potential for specific and targeted action against cancer cells is just one more way this innovative chemical goes above and beyond the scope of conventional treatments. We hope to learn more about its methods of action, such as its capacity to slow tumor development, trigger cell death, and maybe reduce metastasis, by evaluating its bioactivity [8]. With the ability to enhance results and quality of life for individuals fighting esophageal and lung cancer, this chemical stands as a ray of hope in the cancer therapy landscape. This study explores a recently synthesized chemical that shows promise as a therapy for cancers of the oesophagus and lungs.

2. Literature Review

Ageing and obesity are major contributors to the rising cancer rates that affect people around the world. Risk and tumor behavior are strongly influenced by environmental factors, one of which is dietary intakes [9]. Nutrition as a tool for cancer prevention through the use of bioactive food components is the central theme of this book. A total of thirty-three chapters authored by industry leaders evaluate potential for cancer prevention through the use of bioactive food components and survey the pertinent literature. In its many parts, the book covers topics such as cancer response, omics methods, cellular cancer processes, molecular targets, and those who may gain advantage from certain genetic variations.

The native Indonesian plant *Phaleria macrocarpa* contains gallic acid (GA), an antioxidant that occurs naturally in its fruits. It causes esophageal cancer cells to undergo apoptosis and stops cancer cell lines from proliferating [10]. In cancer cells, GA triggers caspase-cascade activity by up-regulating the pro-apoptosis protein Bax and down-regulating the anti-apoptotic proteins Bcl-2 and Xiap. Additionally, it suppresses the survival Akt/mTOR receptor. It takes longer for non-cancerous cells to express proteins that promote cell death. These data provide support for GA's putative anticancer activities; nevertheless, more *in vivo* research is necessary.

The prognosis for esophageal squamous cell carcinoma (ESCC), a prevalent form of cancer, is dismal [11]. From a chemical library comprising 429 natural compounds, this study seeks to find prospective anticancer medicines. Discovered to cause apoptosis and limit proliferation and colony-formation abilities in ESCC, echinatin is a chemical isolated from the Chinese herb *Glycyrrhiza uralensis* Fisch. Autophagy in cells was similarly increased, however bafilomycinA1 reduced its effects. Constitutive activation nullified the effects of echinatin, which worked by deactivating the AKT/mTOR signalling pathway. The results suggest that echinatin may represent a new approach to treating ESCC.

As an alternative to traditional cancer medications, natural compounds with anti-cancer properties hold great promise in the fight against lung cancer, a huge public health problem around the world [12]. One potential source of these bioactive chemicals is algae, which are known to contain high-value compounds. These peptide, carbohydrate, and lipid secondary metabolites show reduced tumor angiogenesis. The type and chemical structure of algal bioactive are strongly related to how they work. The complementary nature of microalgae

bioactive compounds and traditional pharmaceuticals is the subject of this literature review.

Black South Africans have an especially high risk of developing esophageal cancer. The multi-use tree *Moringa oleifera* has a long history of medical and nutritional uses, notably in the treatment of cancer. Researchers discovered that malignant oesophagus cell lines (SNO) saw a dramatic uptick in lipid peroxidation, DNA breakage, and apoptosis induction when exposed to *Moringa oleifera* crude aqueous leaf extract (MOE) [13]. The 24-kDa fragment was upregulated because MOE boosted PARP-1 cleavage and Smack/DIABLO protein production.

Cancer prevention may be possible by the use of bioactive substances such as glucosinolates, carotenoids, and polyphenols. For more stable and precisely delivered biomolecules, researchers have turned to nano-delivery technologies, such as solid lipid nanoparticles, polymeric nanoparticles, and nanostructured lipid carriers. Breast, prostate, colorectal, and lung cancers are only some of the many cancers that have benefited from these systems enhanced therapeutic efficacy [14]. The potential for certain bioactive chemicals to enhance tumor efficiency while decreasing negative effects makes them attractive candidates for combination therapy with traditional chemotherapeutic drugs.

The wide variety of ingredients, intricate processes, and inherent uncertainties in TCMs make quality control a formidable obstacle. In order to tackle this, a comprehensive QC method was created by utilizing an anti-lung-cancer-guided spectrum-effect connection strategy [15]. The 42nd batch of WEZ's volatile oil was chemically profiled using GC-MS, flow cytometry, the CCK-8 assay, SIMCA-P software, and the PLSR model. Leading bioactive compound quantitation and bioactive compound-based fingerprinting technologies were created. Previous study used chemical compounds for adsorption of drug in drug delivery system [16].

3. Methodology

The aim of this study is to assess its biological effectiveness synthesized a Schiff base chemical called 2-hydroxy-5-(naphthalene-1-ylidiazonyl) benzaldehyde (HNDB) [17] against lung and esophageal malignancies using diverse applications and measurements. When evaluating the effectiveness of the synthetic compound 2-hydroxy-5-(naphthalene-1-ylidiazonyl) benzaldehyde (HNDB) as an anti-proliferating agent against certain types of cancers, specifically breast lesions from early lactation human milk, using the HBL-100 cell line, the findings indicated that the most effective concentration for inhibiting human breast carcinoma was 500 $\mu\text{g}\cdot\text{mL}^{-1}$

[18]. This concentration demonstrated the highest average inhibition rate of 76.3% at an IC₅₀ value of 148.2 $\mu\text{g}\cdot\text{mL}^{-1}$. In this investigation, it was observed that the gold nanoparticles generated from grapes (GAuNPs, GSH-GAuNPs, LA-GAuNPs) had a significant affinity towards human breast cancer cells (HBL-100). The primary assays employed were centered around quantifying cellular growth, necrosis, apoptosis, and identifying DNA damage. We conducted an investigation to examine the detrimental impacts of GAuNPs, GSH-GAuNPs, and LA-GAuNPs on HBL-100 cells in a laboratory setting. We analyzed the decrease in MTT and the leakage of LDH as indicators of the cells' toxicity [19]. The cytotoxicity of cells exposed to varying volumes of GAuNPs, GSH-GAuNPs, and LA-GAuNPs for a duration of 24 hours was evaluated by quantifying the number of viable cells using the colorimetric MTT assay. Additionally, a study conducted by Wu et al. demonstrated that the highest enhancement in cell proliferation occurred at a concentration of 1.0×10^{-6} mol. L⁻¹ of BPA. Bisphenol A (BPA) at concentrations of 1.0×10^{-10} , 1.0×10^{-8} , and 1.0×10^{-6} mol. L⁻¹ caused an increase in the number of cells transitioning into the G₂/M phase in the HBL-100 cell line. Additionally, Abzianidze et al. discovered that the IC₅₀ value of the compounds for both the sensitive HBL-100 cells and the resistant variant HBL-100/Dox was 161.5 $\mu\text{g}\cdot\text{mL}^{-1}$.

During the investigation of a synthetic compound's effectiveness as an anti-proliferating agent against certain types of cancers, specifically breast lesions from early lactation samples of human milk, the SKGT-4 cell line was used. The results indicated that the most effective concentration for combating esophageal cancer was 250 $\mu\text{g}\cdot\text{mL}^{-1}$. This concentration demonstrated the highest average inhibition rate of 71.5% at an IC₅₀ value of 261.6 $\mu\text{g}\cdot\text{mL}^{-1}$. These findings are consistent with a study conducted at doses of around 240 $\mu\text{g}\cdot\text{mL}^{-1}$, it induced a reduction in the number of cells and distinct alterations in cell shape that are typically linked to apoptosis. Cell death beginning coincided with a reduction in the electrical potential across the mitochondrial membrane. The chemical compound accumulated during the G₂/M phase of the cell cycle and enhanced radiosensitivity in SKGT-4 cells [20]. This finding is consistent with the study conducted by Jassem et al., who assessed the refined compounds for their anti-esophageal cancer properties on various human esophageal cancer cell lines, including SKGT-4. The cytotoxic and apoptotic effects of these drugs were evaluated using MTT and AO/EB assays in vitro. The compounds 9e and 10b, which are derivatives of thiazolidin-4-one, had the

greatest efficacy against SKGT-4 human esophageal cells compared to the other compounds that were evaluated. The IC₅₀ values of derivatives 9e and 10b were 17.6 ± 0.06 and $18.5 \pm 0.01 \mu\text{g.mL}^{-1}$, respectively, when compared to the reference standard cisplatin, which had an IC₅₀ of $5.21 \pm 0.41 \mu\text{g.mL}^{-1}$. The data derived from the AO/EB experiment demonstrated that these chemicals elicit apoptosis in SKGT-4 cells by a distinct mechanism, which differs from their impact on Vero cells.

During the investigation of a synthetic compound's effectiveness as an anti-proliferating agent against certain types of cancers, specifically breast lesions, using the A549 cell line and a sample of human milk from early lactation, the results showed that the most effective concentration for inhibiting human lung carcinoma was $250 \mu\text{g.mL}^{-1}$. This concentration had the highest average inhibition concentration of 43.67% at an IC₅₀ value of $217.9 \mu\text{g.mL}^{-1}$. These findings align with a study by Sak et al., Compound 5, which has a PI of 4.95 and contains 2 fluorine atoms, exhibited the highest level of potency in inhibiting the growth of A549 cells. Apoptosis induction effectively resulted in cellular demise. In addition to the presence of cleaved caspase-3, the cells exhibited chromatin condensation, marginalization, and apoptotic bodies. Besides, Zuliani et al. concentrate on introduced an immediate and proficient strategy for blending a gathering of 5-benzylidene hydantoin in arrangement, which were then assessed for their ability to restrain cell development in the A549 cell line of human lung adenocarcinoma. An examination was led on the substituents situated at positions 1, 3, and 5 on the hydantoin core. Compounds containing a 5-benzylidene bunch and a lipophilic substituent at position 1 successfully repressed cell multiplication at a grouping of $20 \mu\text{M}$ [21]. The compound 7 (UPR1024), which has 1-phenethyl and (E)- 5-p-Goodness benzylidene, not set in stone to be the most impressive subsidiary in the series. It inhibited the autophosphorylation of EGFR and induced DNA damage in A549 cells. Compound 7 and other derivatives of 5-benzylidene hydantoin increased p53 levels, suggesting that compound 7 and other compounds in the series have a similar dual mode of action. Butcher et al. discovered that the IC₅₀ value of S-Me-DDC combined with Cu ($158,191 \text{ nM}$) is 1250 times higher than that of DDC combined with Cu (125 nM), and significantly higher than the IC₅₀ values of other substances (ranging from 3 to 3285 nM). The TMDS, in which the ethyl groups in DS are replaced with methyl groups, remains highly toxic to A549 cells when paired with copper. The IC₅₀ value of TMDS/Cu (112 nM) is significantly higher than that of DS/Cu (3 nM)

with statistical significance ($p < 0.01$). Cu-DDC and DDC/Cu exhibit lower cytotoxicity in A549 cells as compared to TMDS/Cu and DS/Cu [22]. Azo compound, Schiff base and other polymers have been utilized in several previous study as efficient compounds for several applications[23-32].

4. Anticancer Assay

Involving MTT examine to decide the manufactured compound movement as hostile to multiplying specialist against specific sorts of cancers, for example, Human Throat Carcinoma utilizing SKGT-4 cell line, the outcomes found from rehashing the examination multiple times to get the mean absorbance in the wake of finishing the measure with standard deviation, however the relapse degree and the R square worth showed that there is a low level of linearity and first request relationship between the underlying focuses (0.573), and the half-maximal inhibitory fixation (IC₅₀) which report the cytotoxicity degree for the compound at various fixations was $216.6 \mu\text{g.mL}^{-1}$ with a surmised fixation as apoptotic inhibitory focus, with various focus slopes, the outcomes uncovered that the most ideal and appropriate fixation utilized as hostile to Human Throat Carcinoma was $250 \mu\text{g.mL}^{-1}$, it has the mean hindrance fixation with less S.D when contrasted with its underlying focus, as in raising the fixation the extent of restraint expanded a smidgen with expanding S.D also aside from $750 \mu\text{g.mL}^{-1}$ which showed the least S.D yet with low cytotoxic action when contrasted with $500 \mu\text{g.mL}^{-1}$.

The preceding figure provides a summary of the five primary concentrations of the compound that was synthesized, as well as the relationship between its various concentrations and the percentage of inhibition to SK-GT-4 cancer cells. The findings indicated that there was no significant difference between the main four high concentrations, which were 250, 500, 750, and $1000 \mu\text{g.mL}^{-1}$. This indicates that $250 \mu\text{g.mL}^{-1}$ is the optimal concentration that should be assigned as the optimal concentration for higher inhibition activity to cancer cells.

According to the findings of this study, the response curve exhibited a delayed response to inhibition when the initial concentration reached Log 0.5. Subsequently, as the concentration increased, the response of inhibition for tumor cells increased rapidly until it reached approximately Log 2.2. This occurred until the steady state was reached with an IC₅₀ of $216.6 \mu\text{g.mL}^{-1}$ and a poor linearity degree ($R^2 = 0.5834$). Any increase in the compound concentration will not result in any change in the inhibition degree.

Table 1. The compound's reaction with the SKGT-4 cell line

TITLE	COMPOUND				
Concentration $\mu\text{g. mL}^{-1}$	125	250	500	750	1000
Technical replicates	4	4	4	4	4
Mean absorbance	0.093	0.028	0.026	0.026	0.026
Mean inhibition%	71.5	91.5	92.2	92	92.1
SD \pm	17.57	1.5	2.3	0.26	1.2
IC50	~ 216.6	$\mu\text{g. mL}^{-1}$			

Were the concentration of control $0.32785 \mu\text{g. mL}^{-1}$, $R^2 = 0.5834$, type of test is MTT assay, absorbance at 620nm, cell line SKGT-4 human esophagus carcinoma

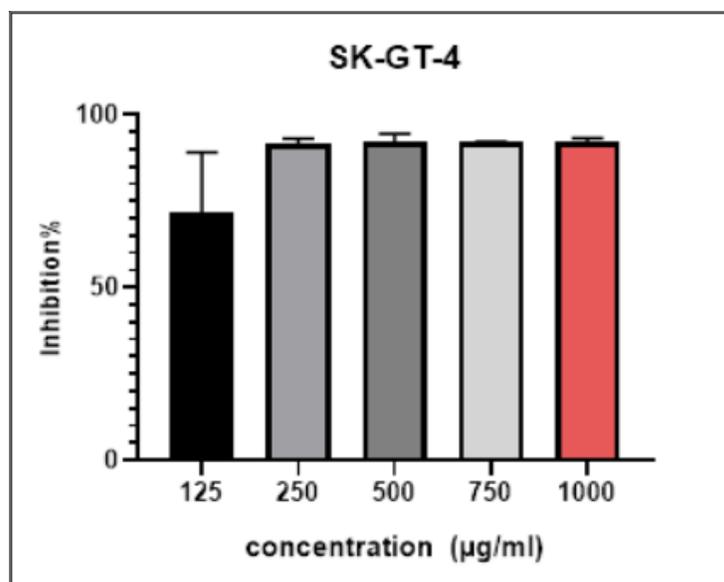


Fig. 1. The percentage of inhibition and concentration of the synthesized chemical against SKGT-4 cancer cells are correlated in a box plot.

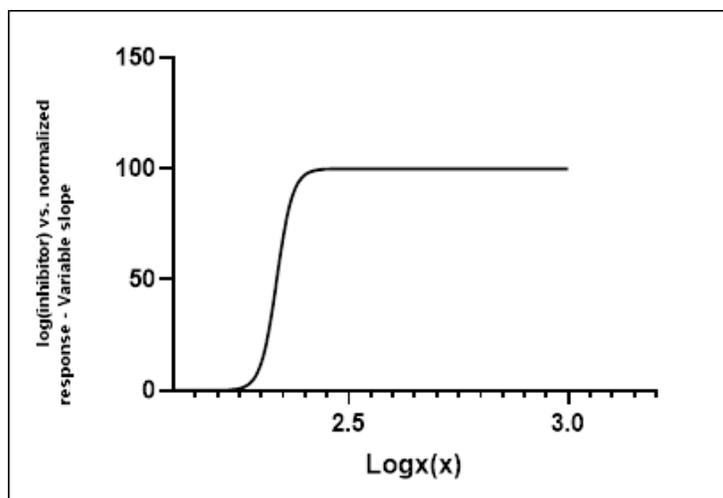


Fig. 2. The compound's response curve from log concentration to inhibitory activity

Involving MTT assay to determine the synthetic compound action as anti-multiplying specialist against certain sorts of tumors, for example, bosom injury from an early lactation test of human milk utilizing HBL-100 cell line, utilizing more fixations proportions beginning from 15.6 to 500 $\mu\text{g.mL}^{-1}$ for giving a more extensive scope of focuses with low harmfulness and greater selectivity, the outcomes found from rehashing the trial multiple times to get the mean absorbance in the wake of finishing the assay with standard deviation, the regression degree and the R square worth showed that there is a serious level of linearity and first request association between the underlying focuses (0.952), the half-maximal inhibitory focus (IC50) which report the cytotoxicity degree for the compound at various fixations was 148.2 $\mu\text{g.mL}^{-1}$ with a surmised fixation as apoptotic inhibitory fixation, with various fixation slopes, the outcomes uncovered that the most ideal and appropriate fixation utilized as anti-Human bosom Carcinoma was 500 $\mu\text{g.mL}^{-1}$, it has the most elevated mean hindrance fixation (76.31 $\mu\text{g.mL}^{-1}$) which get multiple times more than the portion of its focus (250 $\mu\text{g.mL}^{-1}$) with less S.D when contrasted with its underlying fixations, the little S.D assured that the dependability and accuracy of the test duplicates too at higher focuses, the least cytotoxic action was displayed in 31.25 $\mu\text{g.mL}^{-1}$ focus which

showed a relative high hindrance rate when contrasted with ensuing focus.

This synthetic compound movement as an anti-multiplying specialist against certain sorts of tumors utilizing a HBL-100 cell line, utilizing more fixation proportions beginning from 7.8 to 500 $\mu\text{g.mL}^{-1}$ for giving a more extensive scope of focuses with low harmfulness and greater selectivity, the outcomes found from rehashing the trial multiple times to get the mean absorbance subsequent to finishing the assay with standard deviation, the outcomes uncovered that the most ideal and reasonable fixation utilized as anti-Human Throat Carcinoma was 250 $\mu\text{g.mL}^{-1}$, it has the most elevated mean restraint fixation (76.31 $\mu\text{g.mL}^{-1}$) when contrasted with 500 $\mu\text{g.mL}^{-1}$ which showed a little expansion in its hindrance rate with multiplying the fixation.

It has been seen that the reaction bend showed a postponed reaction to hindrance with the underlying fixation compasses to Log 1.9, from that point onward, with expanding the focus, the reaction of restraint for cancer cells gets raised quickly till approx. Log 2.3, to arrive at the consistent state with IC50 148.2 $\mu\text{g.mL}^{-1}$ and moderate linearity degree (R²= 0.952) any expansion in the compound focus won't roll out any improvement in the restraint degree.

Table 2. The effects of the chemical exposure to the HBL-100 cell line on the breast lesion

Title	Compound					
Concentration $\mu\text{g. mL}^{-1}$	15.6	31.25	62.5	125	250	500
technical replicates	4	4	4	4	4	4
mean absorbance	0.312	0.307	0.314	0.293	0.258	0.086
mean inhibition %	13.96	15.13	13.18	19.04	28.88	76.31
SD \pm	7.1	7.6	4.8	7.4	9.6	4.0
IC50	148.2	$\mu\text{g. mL}^{-1}$				

Were the concentration of Control 0.36, R² = 0.952, type of test is MTT assay, absorbance at 620nm, cell line HBL100

Table 3. The outcomes of the compound's exposure to the HBL-100 cell line with

Concentration	7.8	15.6	31.25	62.5	125	250	500
Inhibition%	6.92	10.23	18.52	29.84	41.44	79.01	76.80
	15.48	22.94	6.92	13.82	21.00	77.07	77.63
	23.21	7.19	13.18	14.65	31.22	78.73	78.18
	10.23	20.17	14.10	17.83	21.83	70.45	78.18
Mean Inhibition%	13.96	15.13	13.18	19.04	28.88	76.31	77.70

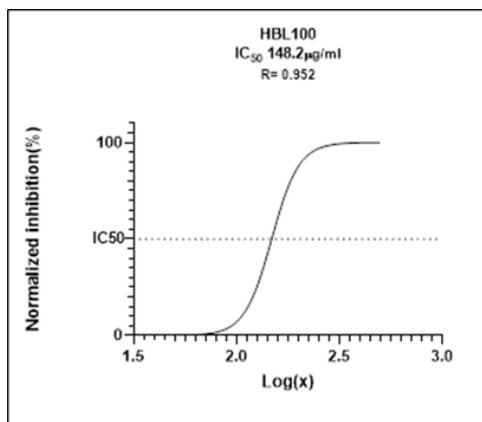


Fig. 3. The compound's response curve from log concentration to inhibitory activity

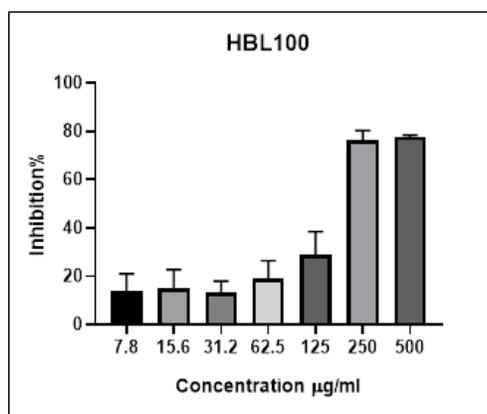


Fig. 4. The percentage of inhibition and concentration of the synthetic substance against HBL-100 cancer cells are correlated in a box-plot fashion.

This figure sums up the main seven focuses beginning from $7.8 \mu\text{g.mL}^{-1}$ to $500 \mu\text{g.mL}^{-1}$ concerning the IC_{50} of the combined compound and the connection between its various fixations and the level of restraint to HBL-100 malignant growth cells, the outcomes showed a slight contrast somewhere in the range of 250 and $500 \mu\text{g.mL}^{-1}$ in their hindrance rate, to uncover that $250 \mu\text{g.mL}^{-1}$ fixation is more ideal with reasonable cytotoxicity and viability. Too, the $15.6 \mu\text{g.mL}^{-1}$ focus shows a promising action when contrasted with $7.8 \mu\text{g.mL}^{-1}$, $31.2 \mu\text{g.mL}^{-1}$, and $62.5 \mu\text{g.mL}^{-1}$.

MTT assay was also used to determine the synthetic compound action as anti-multiplying specialist against certain sorts of diseases, for example, human lung carcinoma utilizing A549 cell line, utilizing more focuses proportions beginning from 7.8 to $500 \mu\text{g.mL}^{-1}$ for giving a more extensive scope of fixations with low harmfulness and greater selectivity, the outcomes found from rehashing the examination multiple times to get the mean absorbance in the wake of finishing the assay with standard deviation, the regression degree and the R square worth showed that there is a moderate level of linearity and first request association between the

underlying fixations (0.848), the half-maximal inhibitory fixation (IC_{50}) which report the cytotoxicity degree for the compound at various fixations was $217.9 \mu\text{g.mL}^{-1}$ with a surmised focus as apoptotic inhibitory focus, with various focus slopes, the outcomes uncovered that the most ideal and appropriate focus utilized as anti-Human Lung Carcinoma was $250 \mu\text{g.mL}^{-1}$, it has the most elevated mean hindrance focus ($43.67 \mu\text{g.mL}^{-1}$) as respected to the IC_{50} of the compound which is close to $217 \mu\text{g.mL}^{-1}$, also, the underlying convergence of the compound $7.8 \mu\text{g.mL}^{-1}$ is promising as it has a little level of poisonousness with high hindrance rate when contrasted with its three back to back focuses.

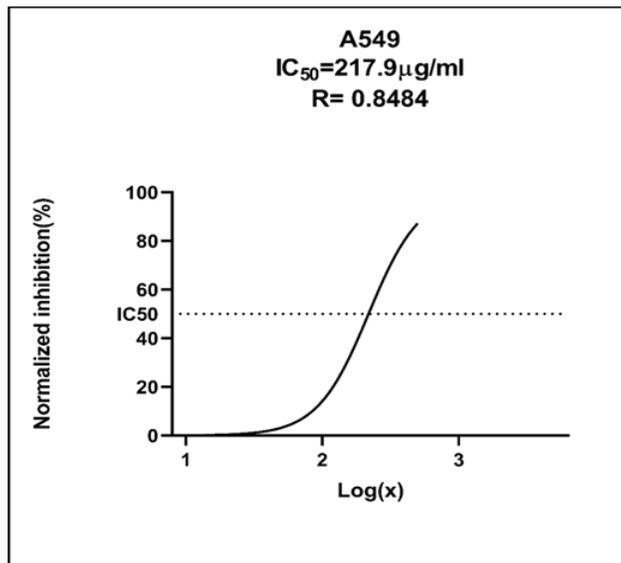
It was noticed that the reaction bend showed a postponed reaction to restraint with the underlying focus scopes to Log 1.8, from that point forward, with expanding the fixation, the reaction of hindrance for cancer cells gets raised quickly till approx. Log 2.4, to arrive at close to the consistent state with IC_{50} $217.9 \mu\text{g.mL}^{-1}$ and moderate linearity degree ($R^2= 0.8484$) and it has been proposed that any expansion in the compound focus won't roll out any improvement in the restraint degree.

Table 4. The outcomes of the compound's exposure to the A549 Cell line

Concentration $\mu\text{g.mL}^{-1}$	7.8		15.6	31.25	62.5	125	250	500
Technical Replicates	4		4	4	4	4	4	4
Mean Absorbance	0.340		0.360	0.361	0.337	0.278	0.224	0.068
Mean Inhibition%	14.51		9.29	9.11	15.20	30.03	43.67	82.91
Sd \pm	4.65		2.68	11.29	18.61	4.78	11.72	5.17
Ic50	217.9		$\mu\text{g.mL}^{-1}$					
Control	0.397							
R Squared	0.848							
Type Of Test		MTT assay						
Absorbances Nm		620						
Cell Line		A549		HUMAN LUNG CARCINOMA				

Table 5. The A549 Cell line exposure compound's results on four comprehensive replicates

Concentration $\mu\text{g.mL}^{-1}$	7	15	31	62	125	250	500
Inhibition%	8.59	10.10	20.68	3.56	28.35	55.68	87.64
	13.13	11.11	7.46	-4.00	36.54	41.96	84.36
	18.92	10.61	14.07	36.04	25.21	48.82	84.11
	17.41	5.32	-5.76	25.21	30.03	28.23	75.55
Mean Inhibition%	14.51	9.29	9.11	15.20	30.03	43.67	82.91

**Fig. 5.** The compound's response curve between its log₅₀ concentration and its inhibitory effect on A549 cancer cells

This figure sums up the initial six focuses beginning from $7 \mu\text{g.mL}^{-1}$ to $250 \mu\text{g.mL}^{-1}$ concerning the IC50 of the combined compound and the connection between its various fixations and the level of restraint to A549 disease cells, the outcomes showed a slight distinction between $125 \mu\text{g.mL}^{-1}$ and $250 \mu\text{g.mL}^{-1}$ in their hindrance rate, to uncover that $125 \mu\text{g.mL}^{-1}$ focus is more ideal with reasonable cytotoxicity and viability. Too, the $7 \mu\text{g.mL}^{-1}$ fixation shows promising action when contrasted with other continuous focuses.

While adding the seventh fixation ($500 \mu\text{g.mL}^{-1}$), the restraint rate seemed, by all accounts, to be extremely high and significant when contrasted with the other six focuses concerning the IC50 of this incorporated build and the connection between its various focuses and the level of hindrance to A549 malignant growth cells.

5. Conclusion

The compound HNDB showed significant inhibitory activity against lung and oesophageal cancer cells, suggesting its potential as an anticancer agent. The optimal concentration of HNDB for maximum inhibitory effect against lung cancer cells was found to be $250 \mu\text{g.mL}^{-1}$, while the optimal concentration for esophageal cancer cells was also $250 \mu\text{g.mL}^{-1}$. MTT tests showed the compound ability to induce antiproliferative effects in different cancer cell lines, supporting its potential as a promising anticancer therapy. The results call for further research to explore the potential of HNDB in future therapeutic applications against lung and oesophageal cancers.

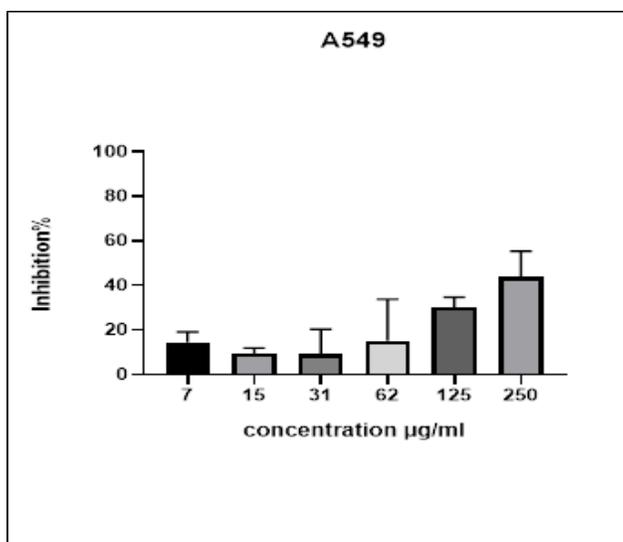


Fig. 6. The percentage of inhibition and the first six concentrations of the synthetic compound's box-plot correlation

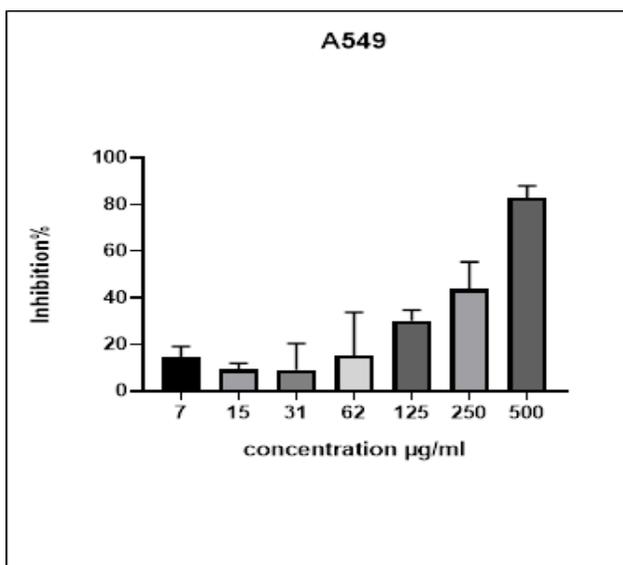


Fig.7: The synthesized compound's box-plot connection with all investigated concentrations and the percentage of inhibition

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