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Galaxy Publication

Evaluation of Cytotoxic Activity of Galantamine Peptide Esters: GAL-LEU and GAL-VAL Against PC3 Cell Line

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ABSTRACT

The focus of the present study was to evaluate the cytotoxic effects of novel peptide esters derived from galantamine—GAL-LEU and GAL-VAL—on the PC3 prostate cancer cell line. To assess the cytotoxicity of these compounds, the MTT assay, a method that quantifies cellular metabolic activity through the reduction of MTT to formazan, was used. PC3 cells were exposed to different concentrations (ranging from 1.875 µM to 30 µM) of the peptide esters in triplicate. The absorbance of the formazan product was measured using a spectrophotometer at a wavelength of 570 nm. The results showed that GAL-LEU at a concentration of 30 µM inhibited 55.36% of PC3 cell growth, resulting in a cell viability rate of 44.64%. On the other hand, GAL-VAL showed a relatively weaker effect, with a concentration of 30 µM leading to a 43.96% reduction in cell growth. These findings indicate that both peptide esters exhibited cytotoxic activity on the PC3 cells, with GAL-LEU demonstrating a stronger antiproliferative effect than GAL-VAL. The IC50 value for GAL-LEU was 30.8 µM, indicating a more significant inhibitory effect compared to GAL-VAL (IC50 > 30 µM).

Keywords: PC3 cell line, Peptide esters, MTT, Galantamine

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Introduction

Cancer is characterized by uncontrolled cell proliferation, with age being a significant risk factor for its development [1]. Other contributing factors include smoking [2], exposure to environmental elements such as chemicals, alcohol, drugs, sunlight, ionizing radiation, and electromagnetic fields [3], as well as infections caused by various agents [4]. Chemotherapy, which targets cancer cells, is commonly used in the treatment of lung, pancreatic, and colorectal cancers [5].

Among the most frequently prescribed anticancer drugs are antimetabolites [6], anthracyclines—known for their cytotoxic effects [7]—and topoisomerase inhibitors [8]. For prostate cancer, medications such as Flutamide, Bicalutamide, and Nilutamide are widely utilized [9]. Additionally, research has shown that plant extracts, including those from various Astragalus species, exhibit anticancer properties [10].

Galantamine is a well-known treatment for Alzheimer's disease [11], enhancing cognitive function through several mechanisms: inhibition of acetylcholinesterase [12, 13], stimulation of α7-nicotinic acetylcholine receptor binding [14], and antioxidant effects [15, 16].

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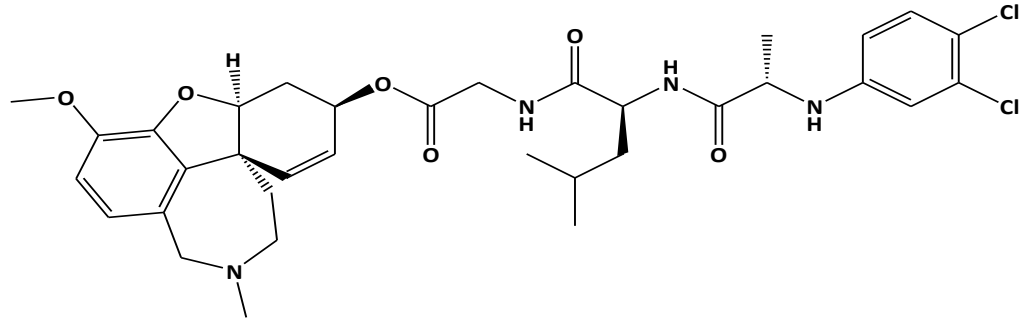
In this context, L-Leucyl-L-Leucine methyl ester has been shown to induce apoptosis in various cell lines [17]. Given that discovering new cytotoxic compounds could open doors to developing effective in-vivo pharmaceutical agents for cancer therapy, this study aims to investigate the cytotoxic effects of two newly synthesized peptide esters: 6-O-N-[N-(3,4-dichlorophenyl)-D, L-Alanylmethyl]-L-Leucyl-Glycil-Galantamine (GAL-LEU) and 6-O-N-[N-(3,4-dichlorophenyl)-D, L-Alanylmethyl]-L-Valil-Glycil-Galantamine (GAL-VAL) [18-20]. These compounds have been found to demonstrate inhibitory activities against both acetylcholinesterase and γ-secretase [21], while also exhibiting antioxidant properties as measured by the FRAP assay [22, 23].
The focus of the present study was to evaluate the cytotoxic effects of novel peptide esters derived from galantamine—GAL-LEU and GAL-VAL—on the PC3 prostate cancer cell line.

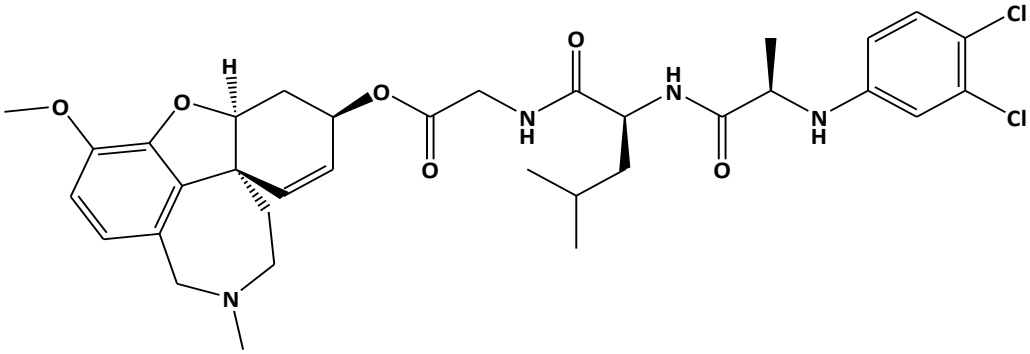
Materials and Methods

Peptide esters analyzed in this study were prepared based on the methodology of Vezenkov *et al*. [20].
Peptide esters GAL-LEU and GAL-VAL were synthesized following the protocol by Vezenkov *et al*. [20] **(Figure 1)**.



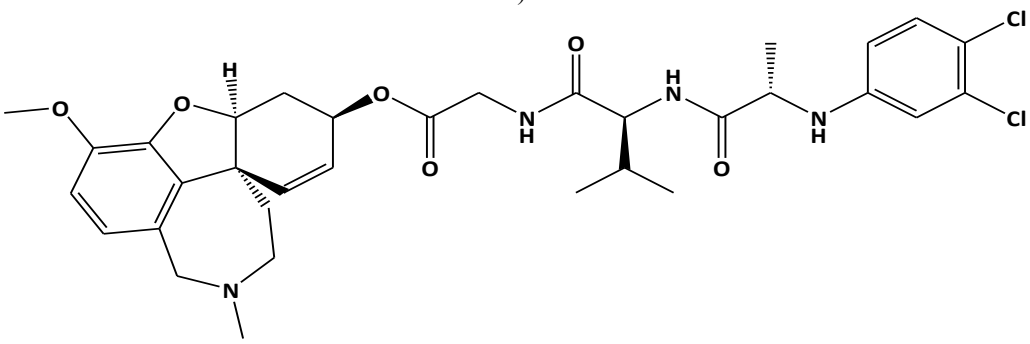
(4a*S*,6*R*,8a*S*)-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-yl (3,4-dichlorophenyl)-*L*-alanyl-*L*-leucylglycinate

a)



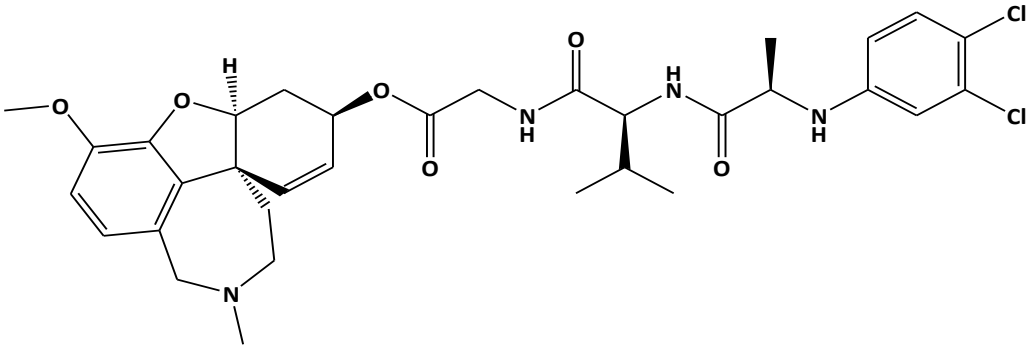
(4a*S*,6*R*,8a*S*)-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-yl (3,4-dichlorophenyl)-*D*-alanyl-*L*-leucylglycinate

b)



(4a*S*,6*R*,8a*S*)-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-yl (3,4-dichlorophenyl)-*L*-alanyl-*L*-valylglycinate

c)



(4a*S*,6*R*,8a*S*)-3-methoxy-11-methyl-4a,5,9,10,11,12-hexahydro-6*H*-benzo[2,3]benzofuro[4,3-*cd*]azepin-6-yl (3,4-dichlorophenyl)-*D*-alanyl-*L*-valylglycinate

d)

Figure 1. Chemical structures of GAL-LEU and GAL-VAL.

Analytical-grade reagents

The following high-purity reagents were used MTT (3-[4.5-dimethylthiazole-2-yl]-2.5-diphenyl-tetrazolium bromide), dimethylsulfoxide, fetal bovine serum (FBS), 100 IU/ml penicillin, 100 µg/ml streptomycin, and 0.25% trypsin EDTA 1X.

Preparation of peptide ester solutions

Precise amounts of GAL-LEU and GAL-VAL peptide esters were individually dissolved in dimethylsulfoxide to prepare solutions with concentrations of 1.875 µM, 3.75 µM, 7.5 µM, 15 µM, and 30 µM.

MTT solution preparation

MTT was accurately weighed and dissolved in a phosphate buffer to create a 5 mg/ml solution, which remains stable for up to one month when stored at 4 °C.

Cancer cell line cultivation for in vitro testing

For cytotoxic testing, the PC3 prostate cancer cell line was grown in Dulbecco's Modified Eagle Medium (DMEM).

MTT assay

The cytotoxic effect of the galantamine peptide esters on the PC3 cell line was evaluated using the MTT assay by Mosmann [18]. Each well was filled with 200 µl of the respective ester solution at different concentrations (ranging from 1.875 µM to 30 µM) in fresh medium. Three replicates were performed for each concentration. After 48 hours, 200 µl of MTT solution (0.5 mg/ml) was added to each well. The plates were incubated for an additional four hours at 37 °C with 5% CO2. After incubation, the supernatant was removed, and 100 µl of dimethylsulfoxide was added to dissolve the formazan crystals. The absorbance was then measured at 570 nm.

Incubation and culture of PC3 cells

For assessing the cytotoxicity of the compounds, 96-well flat-bottomed microplates were used. The PC3 prostate cancer cells were cultured in 75 cm² flasks in DMEM medium. This medium was supplemented with 5% fetal bovine serum, 100 µg/ml Streptomycin, and 100 IU/ml Penicillin. The cells were incubated in a 37°C humidified incubator with 5% CO2 for 24 hours. After reaching the exponential phase, the cells were trypsinized, and centrifuged, and the cell count was determined using a hemocytometer. The cell concentration was adjusted to 1.10^5 cells/ml, and 100 µl of the cell suspension was added to each well of the 96-well plates. The plates were then incubated for another 24 hours under the same conditions, and the culture medium was removed post-incubation.

Results and Discussion

The cytotoxic effects of GAL-LEU and GAL-VAL peptide esters on the PC3 prostate cancer cell line were evaluated using the standard MTT colorimetric assay. The positive control (A(+)) consisted of PC3 cells treated with MTT alone, while the negative control (A(-)) involved PC3 cells cultured without both MTT and peptide esters. To assess the antiproliferative impact of the peptide esters, the PC3 cells were separately treated with GAL-LEU at concentrations ranging from 1.875 µM to 30 µM, and triplicates were performed with GAL-VAL at 30 µM. Doxorubicin was used as a reference standard. **Table 1** presents the absorbance values for the positive and negative controls, as well as the formazan generated after treatment of the PC3 cells with the tested compounds.

Table 1. Absorbances of controls and formazan produced from the GAL-LEU- and GAL-VAL-treated PC3 cell line.

Cell line			**PC3**		
N	**GAL-LEU**			**GAL-VAL**	
1.	0.947	0.065		1.155	0.064
2.	0.909	0.063		1.007	0.063
3.	0.883	0.061		1.019	0.059
4.	0.930	0.063		1.021	0.065
5.	0.930			1.053	
6.				1.077	
$\bar{X}$	0.920	0.063		1.055	0.063
SD	0.025	0.002		0.055	0.003
$C_{GAL-LEU}$ [µM]	**Absorbances of formazan [AU]**				
	1	2	3	$\bar{X}$	SD
3.75	0.816	0.830		0.823	0.010
7.5	0.864	0.802		0.833	0.044
15	0.751	0.837		0.794	0.061
30	0.465	0.426		0.446	0.028
$C_{GAL-VAL}$ [µM]	**Absorbances of formazan [AU]**				
30	0.666	0.615	0.576	0.619	0.045

Figure 2 demonstrates that as the concentration increases, there is a corresponding reduction in formazan absorbance and a decline in cell viability.

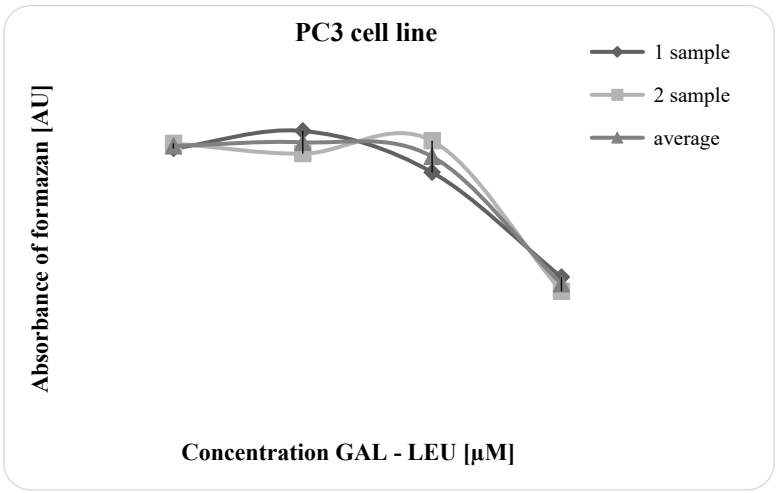


Figure 2. Absorbance–concentration relation for GAL-LEU.
Experiments were applied in duplicate.
Sample 1 = 1st experiment
Sample 2 = 2nd experiment

Table 2 presents a summary of the ester activity in terms of the percentage inhibition of cell growth.

Table 2. Effect of GAL-LEU and GAL-VAL on inhibition of PC3 cell growth.

$C_{GAL\text{-}LEU}$ [µM]	Inhibition PC3 cell growth [%]				
	1	2	3	$\bar{X}$	SD
3,75	12.11	10.48		11.30	1.15
7,5	6.51	13.75		10.13	5.12
15	19.70	9.66		14.68	7.10
30	53.08	57.63		55.36	3.22
$C_{GAL\text{-}VAL}$ [µM]	Inhibition PC3 cell growth [%]				
	1	2	3	$\bar{X}$	SD
30	39.22	44.36	48.29	43.96	4.55

Figure 3 shows the cytotoxic effect of GAL-LEU against the PC3 cell line.

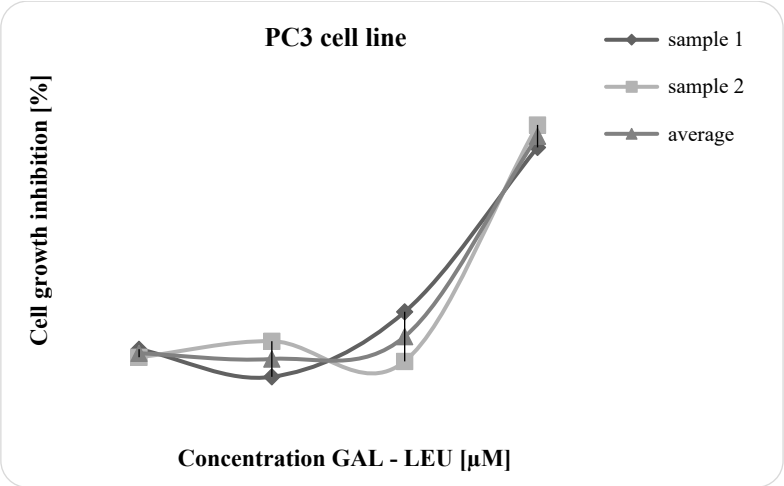


Figure 3. Cytotoxic effect of GAL-LEU against PC3 cell line.
Experiments were applied in duplicate.
Sample 1 = 1st experiment
Sample 2 = 2nd experiment

The results for the inhibition concentration (IC50) are as follows: GAL-LEU at 30.8 µM and GAL-VAL at >30 µM. **Table 3** displays the data regarding the impact of the esters on the cell viability index (V %).

Table 3. Effect of GAL-LEU and GAL-VAL on proliferation of PC3 cell line.

$C_{GAL\text{-}LEU}$ [µM]	Index of cell viability of PC3 cell line [%]				
	1	2	3	$\bar{X}$	SD
3.75	87.89	89.52		88.70	1.15
7.5	93.49	86.25		89.87	5.12
15	80.30	90.34		85.32	7.10
30	46.92	42.37		44.64	3.22
IC_{50}	29.84	31.76		30.80	1.38
$C_{GAL\text{-}VAL}$ [µM]	Index of cell viability of PC3 cell line [%]				
	1	2	3	$\bar{X}$	SD
30	60.78	55.64	51.71	56.04	4.55

Figure 4 depicts how the cell viability index (V %) correlates with varying concentrations of GAL-LEU.

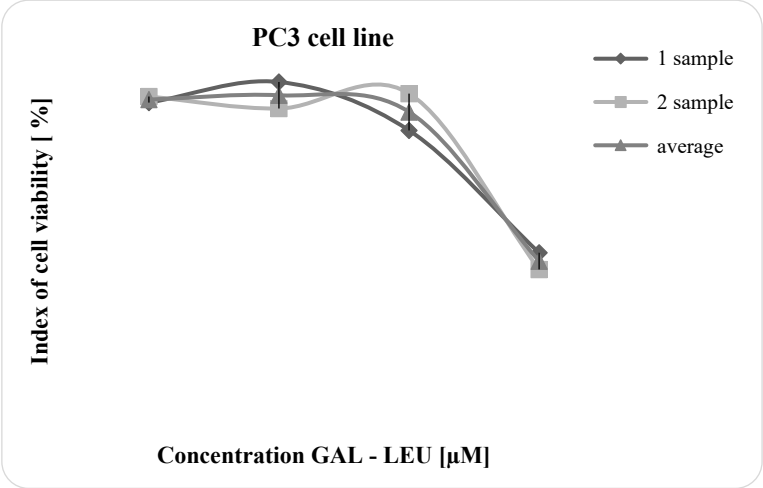


Figure 4. Effect of GAL-LEU on PC3 cell viability.
Experiments were applied in duplicate.
Sample 1 = 1st experiment
Sample 2 = 2nd experiment

Prostate adenocarcinoma ranks as the second most prevalent cancer diagnosis and is the second leading cause of cancer deaths among men [24, 25]. The androgen receptor regulates prostate cancer cell proliferation by androgens [26], and aggressive forms of the disease are often linked to the up-regulation of caveolin-1 [27]. The standard treatment for metastatic prostate cancer generally involves a combination of docetaxel and prednisone [28], while other approaches, such as surgery, radiation, and hormone therapy, may also be used. Natural compounds like lycopene, curcumin [25], genistein [29], and gallic acid [30] have also been explored for their potential benefits in prostate cancer treatment. Liposomal hydroxy aluminum phthalocyanine gel has shown promise in photodynamic therapy for prostate carcinoma [31]. Additionally, vitamin D analogs are being considered as potential treatments for prostate cancer due to the association between vitamin D deficiency and an increased risk of prostate cancer development [32, 33].

Cell lines like PC3, LNCaP, and DU145 [34] are widely used to study prostate cancer progression both in vitro and in vivo [35]. The PC3 cell line, which was established in 1979 [36], is particularly useful for examining the biochemical changes in advanced prostate cancer and assessing responses to chemotherapy.

To evaluate cell viability and proliferation, the MTT assay by Mosmann [18] is commonly employed. This method relies on the reduction of yellow MTT to purple formazan by metabolically active cells, with the absorbance at 570 nm serving as a measure of cell viability [18]. From these readings, the percentage of cell viability (V %) and inhibition of cell growth (I %) are calculated. Lower absorbance values correlate with decreased cell growth, while higher absorbance indicates enhanced cell proliferation.

Various substances have been shown to inhibit prostate cancer cell proliferation. For instance, indole-3-carbinol has been found to inhibit PC3 and DU145 cell growth and induce apoptosis [37, 38]. Other compounds, such as Pinocembrin (5, 7-dihydroxy flavanone), exhibit strong antiproliferative effects on PC3, DU145, and LNCaP cells [39], while propolis extracts also suppress the viability of prostate cancer cells [40].

Arachidonic acid and its metabolites, produced by 5-lipoxygenase, contribute to prostate cancer cell proliferation. Natural products like caffeic acid [41], curcumin [42], quercetin [43], luteolin [44], resveratrol [45], rosmarinic acid [46], and nordihydroguaiaretic acid [47] serve as inhibitors of this enzyme and may prevent prostate cancer cell growth.

Caffeic acid phenethyl ester has been shown to inhibit the growth of LNCaP, DU145, and PC3 cells in a dose-dependent manner [48]. Additionally, curcumin from *Curcuma longa* and isoflavones like genistein, daidzein, and glycitein can reduce LNCaP and DU145 cell growth, with the combination of curcumin and isoflavones exhibiting stronger effects than curcumin alone [49]. Curcumin has been found to reduce Bcl-2 and Bcl-x proteins, which inhibit apoptosis, and to activate pro-apoptotic proteins and caspases to induce cell death [50]. Luteolin is also known to suppress human prostate tumor growth [43], and quercetin promotes prostate cancer cell death by stimulating caspases [44]. Moreover, nordihydroguaiaretic acid increases calcium ion concentrations by releasing calcium from the endoplasmic reticulum, thereby affecting prostate cancer cell function [47].

Tumors may arise from mutations in stem cells [51], and the MTT assay remains a valuable tool for assessing the cytotoxic effects of plant extracts against various cancers. While cancers such as lung, breast, prostate, and cervical are common, others like primary squamous cell carcinoma of the urinary bladder are rare [52]. In Saudi Arabia, there is a significant prevalence of cervical cancer, thalassemia, and sickle-cell anemia [53].

Previous studies have applied the MTT assay to evaluate the cytotoxicity of plant extracts, such as those from Rheum ribes L. against A-549 and KB cell lines [16], Acalypha wilkesiana against HeLa cells [19], and MCF-7 breast cancer cells [23]. Additionally, the effects of ginger on breast and pancreatic cancer cell viability have been studied [54], with specific cell-line-growth media used for these assays [55].

In our earlier studies, GAL-VAL demonstrated cytotoxic effects on 3T3 cells, inhibiting 88.32% of cell growth at 30 µM with an IC50 of 23.17 µM [56]. GAL-LEU at 30 µM suppressed 99.9% of 3T3 cell growth, with an IC50 of 19 µM [57].

In the current study, we evaluated the effects of the Galantamine peptide esters GAL-LEU and GAL-VAL [58] on PC3 cell growth inhibition. The PC3 cells were treated in triplicate with varying concentrations (1.875 µM to 30 µM) of each peptide ester. The MTT assay was used to measure formazan production, correlating it with cell viability. The cytotoxicity was determined by the concentration that inhibited 50% of the PC3 cells (IC50), and cell growth inhibition (I %) and viability index (V %) were calculated using standard formulas.

$$\mathrm{I}\ (\%) = 100 - \frac{\mathrm{A(t)} - \mathrm{A(-)}}{\mathrm{A(+)} - \mathrm{A(-)}} \quad (1)$$

$$\mathrm{V}\ (\%) = \frac{\mathrm{A(t)} - \mathrm{A(-)}}{\mathrm{A(+)} - \mathrm{A(-)}} \quad (2)$$

The following parameters were considered: V (%) representing the cell viability index, I (%) denoting cell growth inhibition, At as the mean absorbance value of formazan after treating cells with test compounds, A (+) as the mean absorbance of formazan in the positive control group (cells treated with MTT solution only), and A (-) as the mean absorbance in the negative control group (cells treated with MTT but without the test compounds).

Experimental findings indicate that GAL-LEU, at a concentration of 30 µM, leads to a 55.36% reduction in PC3 cell growth, resulting in a cell viability index of 44.64%. In comparison, GAL-VAL at the same concentration of 30 µM inhibits 43.96% of cell growth, showing a cell survival index of 56.04%. These outcomes highlight that GAL-VAL has a weaker antiproliferative effect compared to GAL-LEU.

Conclusion

The application of peptide esters on the PC3 cell line led to a decrease in the formazan absorbance, signifying their ability to inhibit cell proliferation. The data from the experiment highlighted that GAL-LEU significantly reduced the growth of PC3 cancer cells. Both GAL-LEU and GAL-VAL exhibited cytotoxic effects on the PC3 cells, but GAL-LEU showed a more pronounced effect, with a lower IC50 value of 30.8 µM, compared to GAL-VAL, which had an IC50 greater than 30 µM.

The antiproliferative activity of these peptide esters was found to be less potent than that of doxorubicin, which displayed an IC50 of 1.698 µM ± 0.285 µM.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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