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Research Article

Synthesis, Anticancer, and Antimicrobial Evaluation of Integerrimide-A

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Integerrimide-A (IG-A) is a cyclic heptapeptide that was recently synthesized after being recovered from the latex of the *Jatropha integerrima* tree. This was achieved by first coupling a tetrapeptide unit (Boc-Gly-L-Leu-L-Leu-L-Leu-OMe) with a tripeptide unit (L-Thr-L-Pro-L-Trp-OMe). The characterization was done by using spectral techniques like FT-IR, ^{1}H-NMR, mass spectrometry, and elemental analysis of the newly synthesized cyclic molecule. Antimicrobial and anticancer properties of IG-A were tested using a biological screening. Gram +ve bacteria (*B. subtilis* and *S. aureus*) and Gram -ve bacteria (*P. aeruginosa* and *E. coli*) were used in the antibacterial testing. Fungal strains such as *C. albicans*, *A. niger*, *T. mentagrophytes*, and *M. audouinii* were used to test the antifungal activities. Antimicrobial activity analysis revealed that cyclic peptide—IG-A (8)—has modest antibacterial activity and antifungal activities, when compared with the standard drugs ciprofloxacin and griseofulvin, respectively. Comparable MTT assays were performed on HCT116 (human colon carcinoma) and B16F10 (melanoma cells) cell lines with doxorubicin as the standard drug to determine the cytotoxic activity of the synthesized cyclic peptide. Inhibition of growth of HCT116 and B16F10 cell lines was used to calculate the cytotoxic effect. At a dosage of 120 μg/mL, the cyclopeptide IG-A (8) inhibited cell proliferation by 87.5 and 72.5 percent, respectively. Cyclopeptide IG-A had CTC_{50} values of 77.65 μM and 68.63 μM against HCT116 and B16F10, respectively. The % growth inhibitions at lesser levels are 72.5 and 50 at 60 μg/mL, respectively. The standard drug inhibited growth by 100 percent with CTC_{50} values of 48.63 μM and 43.25 μM against HCT116 and B16F10, respectively. From the results, it is concluded that IG-A has considerable antimicrobial and cytotoxic effects. Internucleosomal DNA fragmentation may be the underlying mechanism in HCT116 cells, whereas the suppression of eumelanin synthesis in B16F10 cells is another possibility.

1. Introduction

Natural resources are mined for new therapeutic chemicals. Drug development relies on microorganisms to slow microbial proliferation by creating numerous potential drugs [1]. Fungi, bacteria, and plants all produce natural compounds, and they all have unique biological effects. Peptides have recently gained prominence in the field of molecular biology for a variety of reasons, including their ability to stimulate the production of antibodies in animals. Mass spectrometry is a useful tool for detecting these peptides because of their relatively high molecular weights. Peptides have recently been used in the study of protein structure and function. Clinical trials involving inhibitory peptides, which show

promise as potential drugs for treating cancer and other disorders, are now underway [2].

Isolated from their native environments, cyclic peptides serve multiple biological purposes. Cyclic peptides (CP) with novel structures and a wide range of therapeutic actions have been identified from invertebrate animals, marine microorganisms, and higher plants. For a number of reasons, including increased resistance to enzymatic breakdown (*in vivo*) and increased bioavailability, cyclic peptides are emerging as improved therapeutic agents. There are cyclic peptides that are primarily composed of leucine, primarily composed of proline, and primarily composed of a variety of other amino acids [3].

Due to their susceptibility to degradation by peptidase enzymes and the metabolic instability this causes, oral administration of linear peptides is problematic. Another important issue with peptides is their inability to pass the blood-brain barrier. One valuable change of linear peptides is "cyclization," which decreases flexibility, stabilizes secondary structure, and boosts activity [4]. This is just one of several structural modifications that have been studied as a means of overcoming these challenges. Natural congeners found in higher plants have a diverse range of biological functions [5, 6]. Because of their one-of-a-kind structure and broad range of biological activity, cyclic peptides and related congeners have garnered a lot of interest among these naturally occurring congeners [7, 8]. Existing drug resistance can be circumvented by using these cyclic peptides. Integerrimide-A, a new cyclic peptide previously discovered from the latex of *Jatropha integerrima*, is employed as an HSV-1, antifungal, and antimalarial drug (Figure 1).

In this study, CP, IG-A, was synthesized and tested for antibacterial and anticancer activities (MTT assay). The potential mechanism is also discussed.

2. Methodology

This synthesis technique is depicted in Scheme 1. All of the reagents in this investigation were purchased from the Kurukshetra University, Haryana, Institute of Pharmaceutical Sciences' supply room, and were of analytical grade. The uncorrected melting points of compounds were measured by using an open capillary tube method after synthesis. The ^{1}H-NMR spectra were obtained by recording the spectrum in $CDCl_3$ at 400 MHz on an NMR spectrophotometer (BRUKER), and the chemical shifts were reported in ppm relative to tetramethylsilane. Using a potassium bromide pellet method, IR spectra were obtained on a Shimadzu FTIR-8400S. For the chemicals in question, a mass spectrum was acquired using a thermos-Finnigan LCQ Advantage MAX ion trap mass spectrometer. The assigned structures matched the spectral data quite well. Precoated silica gel plates were used in a thin-layer chromatographic (TLC) analysis with a mobile phase of $CHCl_3$: CH_3OH (9.5 : 0.5) to track reaction completions. The UV chamber was used for the spot detection.

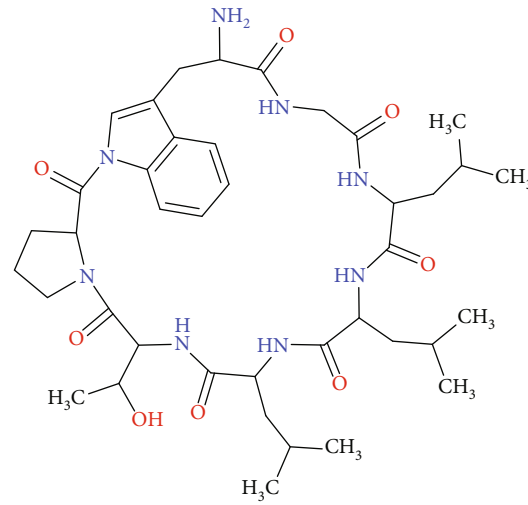


Figure 1: Chemical structure of IG-A.

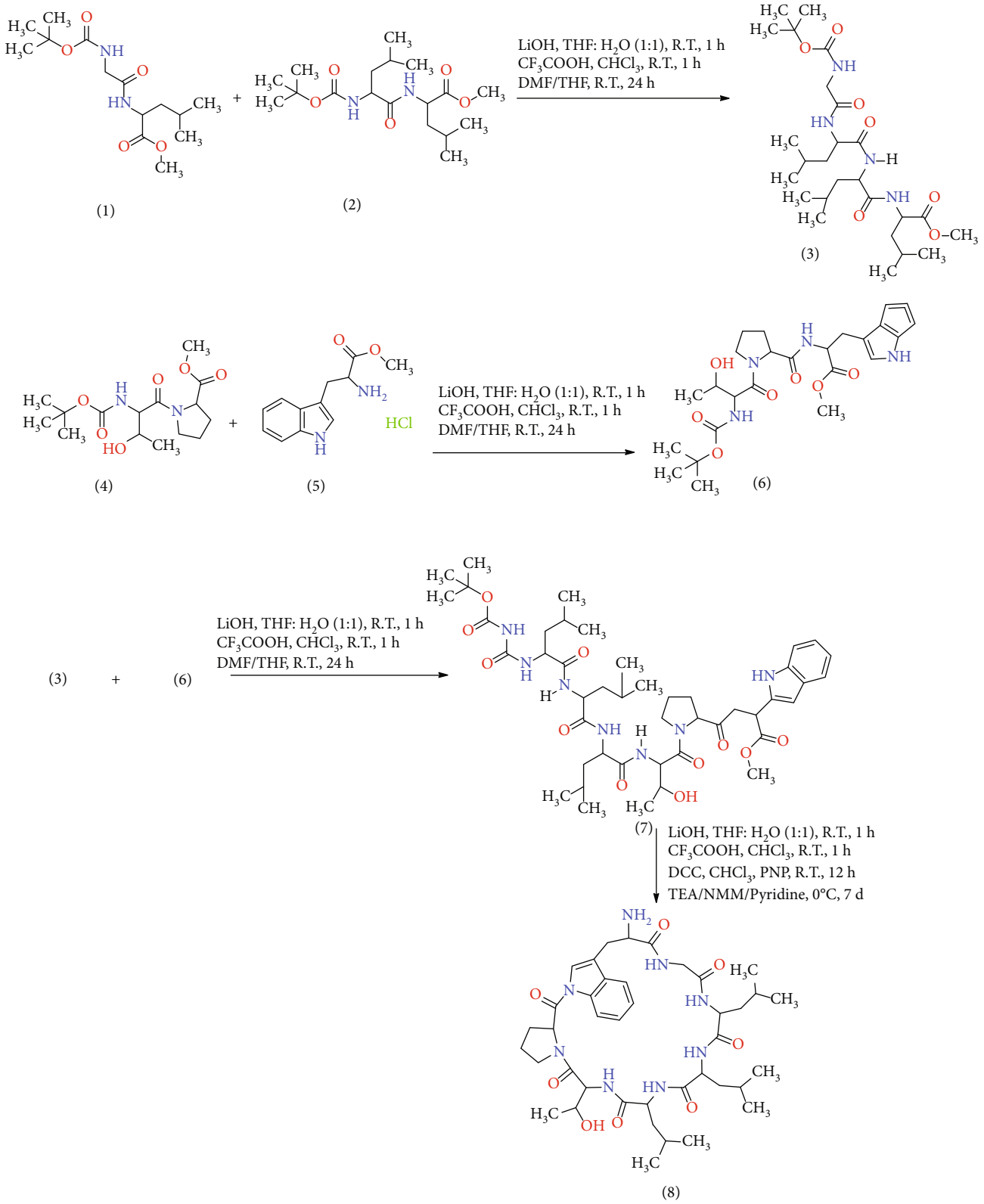
2.1. Experimental

2.1.1. General Approach for the Synthesis of Linear Tri-/Tetrapeptides (1 and 2). Amino acid (10 mmol) and dipeptide methyl ester hydrochloride (10 mmol) were diluted with 20 mL of $CHCl_3$ and poured in a beaker. The mixture was stirred for 15 minutes and kept for 15 minutes at 0°C. Then, 2.3 mL of N-methylmorpholine was added (solution A). Further, 20 mL of $CHCl_3$ and 2.1 g of DCC (10 mmol) were combined with 10 mmol of Boc dipeptide in a separate beaker (solution B). Then, mixture A was agitated for 36 hours; after that, mixture B was added to it. The reaction mixture was filtered, washed with 25 mL of a 5% $NaHCO_3$ solution and 25 mL of 5% NaCl solution, and then cooled to 0°C, where the compound was collected and purified from a chloroform/petroleum ether mixture (b.p. 59-84°C) [9].

2.1.2. Synthesis of Heptapeptide (7). This linear heptapeptide (7) was synthesized by combining the carboxyl-end deprotected tetrapeptide, i.e., Boc-L-Gly-L-Leu-L-Leu-L-Leu-OMe (3), with the amino-end deprotected tripeptide, i.e., Boc-L-Thr-L-Pro-L-Trp-OMe (6). The percentage yield was found to be 64% with melting point 170-172°C. The R_f value was 0.46, when $CHCl_3$: CH_3OH (9.5 : 0.5) was used as TLC system [10, 11].

2.1.3. Synthesis of Cycloheptapeptide (IG-A) (Cyclo(L-Trp-Gly-L-Leu-L-Leu-L-Leu-L-Thr-L-Pro)) (8). The cyclization of linear heptapeptide (7) was taken place by the deprotection of carboxyl end, the cyclic heptapeptide (8) (Scheme 1)[12–13].

The molecular weight was determined to be 780.95, with a percent yield of 60.8% and a melting point of 182-184°C. TLC was used to check the purity of the compound, with $CHCl_3$: CH_3OH (9.5 : 0.5) as the solvent system and iodine vapors as the visualizing agent, and just one spot with R_f value 0.54 was produced [14–16].



Scheme 1: Synthesis of integerrimide-A.

2.2. Biological Activity. CP are now recognized as a major class of chemicals with a diverse biological significance. It is more beneficial to assess its biological profile after its synthesis as well as characterization. Distinctive structures with diverse pharmacological profile characterize CP [17]. CP are the preferred drug in case of drug resistance.

These unique CP have been synthesized in order to provide the greatest standard of therapeutic potential without

Table 1: Composition of nutrient agar medium.

Sr. no.	Components	Amount
1	Peptone (bacteriological)	5.0 g
2	Yeast extract	1.50 g
3	Sodium chloride	5.0 g
4	Beef extract	1.50 g
5	pH	7.20 ± 0.20
6	Distilled water q.s	1000 mL

Table 2: Composition of Sabouraud's broth.

Sr. no.	Components	Amount
1	Dextrose	40.0 g
2	Peptone (mycological)	10.0 g
3	Agar	15.0 g
4	pH	5.60 ± 0.20
5	Distilled water q.s	1000 mL

side effects [18]. As previously stated, marine peptides naturally impede the proliferation of cancer cells [19]. Therefore, this research has been designed considering the advantages of CP of marine, plants, insects, and pathogenic fungus with modified yield. CP have been assessed against following the activity.

2.2.1. Antimicrobial Activity. Antibacterial activity was evaluated using Gram +ve bacteria, *B. subtilis* and *S. aureus*, and Gram -ve bacteria, *E. coli* and *P. aeruginosa*, and antifungal activity was evaluated using *C. albicans*, *A. niger*, *T. mentagrophytes*, and *M. audouinii* [20–25]. The bacterial and fungal strains were cultivated in the following nutritional media to investigate the antimicrobial effect of newly produced cyclic peptides.

(1) Broth Medium Preparation. The composition of broth in the present work is shown in Tables 1 and 2.

(2) Nutrient Broth Preparation. A total of 13 g of the mixture described above has been mixed with distilled water and makes up to 1000 mL followed by autoclaving at 121°C, 15 psi pressure for 15 minutes.

(3) Sabouraud's Broth Preparation. A total of 65 g mixture has been boiled in distilled water and makes up the volume up to 1000 mL with distilled water followed by autoclaving at 121°C at 15 psi for 15 min.

(4) Microbe Culturing. Microbes were cultured and subcultured under a laminar chamber under UV light and HEPA filters in broth media sterilized above followed by incubation at 37°C and 25°C for 24 h and 48 h for fungus as well as bacterial growth under aerobic conditions.

(5) Antimicrobial Activity: Modified Kirby-Bauer Method. All the treatments at 10 mg/mL concentration have been diluted and inoculated under a laminar chamber under UV light in previously grown microbes into four wells, control, standard, and two for tests. Ciprofloxacin and griseofulvin were used for analysis taking DMF as control, followed by sterilization for 24 h at temperature 37°C and for 48 h at 25°C for fungi [22, 23]. ZOI (mm) has been determined thrice, and average has been noted down (Figure 2). The ZOI has been compared with that of different groups for the assessment of antibacterial and antifungal activities. ZOI is shown in Table 3.

2.2.2. Anticancer Activity. Deshpande Labs Pvt. Ltd., Bhopal, has performed MTT cytotoxicity testing on HCTCL and B16CL using doxorubicin as the reference drug. Human CRC derived HCTCL, while mouse epidermis derived B16CL. The percentages of cell line growth inhibition were determined. The median CTC_{50} values have been calculated. Concentrations of 120-7.5 μg/mL of IG-A, the control, and doxorubicin were utilized for the study [26–36].

(1) Principle. Mitochondrial activity is measured by the colour change that occurs during the conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) into formazan crystals in living cells. Once inside the cells, MTT is changed into an insoluble, dark purple formazan complex. After that, isopropanol is added to the mixture of cells, and the spectrophotometer is used to determine how much of the formazan reagent was produced.

(2) Requirements. Instruments and apparatus required are as follows: plate with 96 wells having a flat bottom and incubator with CO_2 (5%); reagents required are as follows: MTT, phosphate-buffered saline (PBS), and isopropanol with acidic pH.

(3) Procedure. Five thousand to ten thousand cells from the HCT116 (human colon carcinoma) and B16F10 (melanoma cells) cell lines were put into a 96-well flat-bottomed tissue culture plate with 100 L of RPMI-1640 culture medium. Before adding test cyclic peptides to the wells, the above mixture was left alone for one night to let the cells stick together. Then, 50 μL of MTT reagent (5 mg/mL) was added to each well, and the plates were kept at 37°C for 4 hours to let the MTT break down and turn the cells to a different colour. Using a pipette with multiple channels, add 0.1 mL of acidic isopropanol to each well of the above mixture and mix well. When HCl was mixed with phenol red culture media, a yellow colour was made. This made it easy to measure MTT formazan. When formazan and isopropanol were mixed together, they made a blue solution that was uniform throughout. Then, an optical density plate reader was used to measure the test drug at 570 nm and the standard drug at 630 nm. The following formula was then used to figure out what percentage of growth had been inhibited:

$$\%\text{growth inhibition} = \frac{\text{Cell}_{\text{total}} - \text{Cell}_{\text{dead}}}{\text{Cell}_{\text{total}}} \times 100. \quad (1)$$

2.3. Statistical Data Analysis. GraphPad Prism 5 was used to conduct a Student *t*-test or two-way analysis of variance

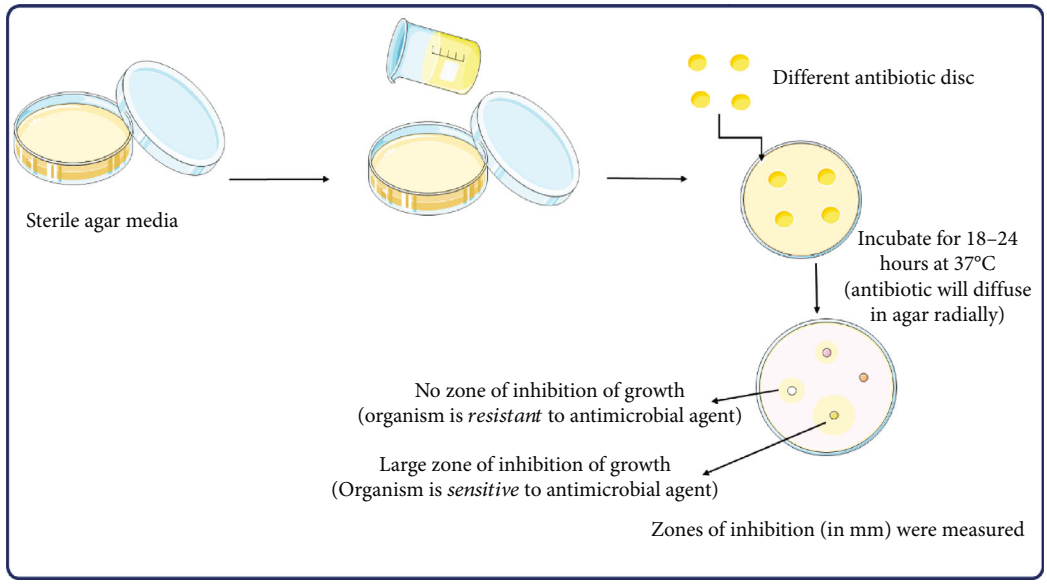


Figure 2: Schematic diagram showing the performance of antibiotic sensitivity testing by disc diffusion method.

Table 3: Results of antimicrobial activity of cyclopeptide IG-A.

Compounds	Zone of inhibition (mm)							
	Bacterial strains					Fungal strains		
	P. aeruginosa (MUMC-266)	*E. coli* (MUMC 106)	*B. subtilis* (MUMC-408)	*S. aureus* (MUMC 377)	*A. niger* (MUMC-96)	*T. mentagrophytes* (MUMC-665)	*C. albicans* (MUMC-29)	*M. audouinii* (MUMC-545)
IG-A (8)	20.65	15.09	17.80	21.58	19.34	18.56	20.96	18.59
Control (DMF)	—	—	—	—	—	—	—	—
Ciprofloxacin (standard)	25.41	20.68	20.03	19.89	—	—	—	—
Griseofulvin (standard)	—	—	—	—	18.63	21.31	20.58	19.56

(ANOVA) followed by the Tukey test for multiple comparisons of MTT assay data expressed as the mean SEM from three separate trials. The cutoff for significance was $p < 0.05$.

3. Results

3.1. Characterization of Heptapeptide (7)

3.1.1. IR V_{max} (cm^{-1}) (KBr). N-H stretching for amide (3129), Ar-CH stretching (3098), C-H stretching for -CH_2, proline (2975-2980), C-H *asymmetrical* stretching, -CH_3 and -CH_2 (2956, 2918), C-H in -CH_3 stretching (2952), aliphatic C-H stretching of -C-H (2880), C=O stretching, ester (1740), C=O stretching, *tertiary* and *secondary* amide (1682, 1653, 1635), Ar-C=C (1550), N-H deformation, *secondary* amide (1536, 1553), N-H bending (1523), *asymmetrical* -CH_3 bending (1464), C-H deformation, *tertiary* butyl (1389, 1368), C-O stretching ester (1262), C-O stretching (1135) 898, 685 (-C-H bending, Ar rings).

3.1.2. ^{1}H-NMR ($CDCl_3$) (ppm). 8.78 s (6H, amide), 8.67-8.67 d (1H, $J = 7.0$ Hz, δ-H indole ring), 7.77-7.81 d (1H, $J = 7.15$ Hz, β-H, indole ring), 7.46-7.52 m (2H, ε and η-H's, indole ring), 7.15-7.17 t (1H, ζ-H, indole ring), 6.12 br. (3H, S, -NH), 4.54-4.56 t (1H, α-H, proline), 4.32 m (1H, CH, threonine), 4.18-4.20 m (1H, α-H, tryptophan), 4.09 s (3H, methoxy), 3.75-3.77 d (2H, $J = 4.8$ Hz, -CH_2, glycine), 3.77 t (2H, δ H's, proline), 3.52-3.57 t (3H, α-H, leucine), 2.64-2.68 m (4H β- and γ-H's, proline), 2.60-2.63 d (2H, $J = 6.75$ Hz, β-H's, tryptophan), 1.98-2.02 t (6H, β-H's, leucine), 1.62 (9H, s, *tert*-butyl group), 1.44-1.47 d (3H, threonine), 1.02-0.99 d (18H, $J = 6.0$ Hz, H's, leucine).

3.2. Characterization of IG-A (8)

3.2.1. Elemental Analysis. The calculated elemental analysis for integerrimide-A (molecular formula—$C_{40}H_{60}N_8O_8$) was found to be C = 61.52%, H = 7.74%, O = 16.39%, and N = 14.35%, and actual found was C = 61.58%, H = 7.66%, O = 16.44%, and N = 14.33%.

Table 4: Results of cytotoxic effects of cyclopeptide IG-A.

Compound	Conc. (μg/mL)	HCT116 (human colon carcinoma) Live cells counted	No. of dead cells	% growth inhibition	CTC_{50} (μM)	B16F10 (melanoma cells) Live cells counted	No. of dead cells	% growth inhibition	CTC_{50} (μM)
Control (saline)	120	40.0	0	0	—	40.0	0	0	—
	60	40.0	0	0		40.0	0	0	
	30	40.0	0	0		40.0	0	0	
	15	40.0	0	0		40.0	0	0	
	7.5	40.0	0	0		40.0	0	0	
IG-A (8)	120	5.0 ± 1.21	35.0 ± 1.29	87.5 ± 1.35	77.64	7.0 ± 1.69	33.0 ± 2.15	72.50 ± 2.08	68.62
	60	18.0 ± 1.30	29.0 ± 2.01	72.5 ± 2.03		13.0 ± 2.14	27.0 ± 2.15	50.0 ± 1.97	
	30	24.0 ± 1.14	18.0 ± 2.38	45.0 ± 2.14		23.0 ± 2.31	17.0 ± 1.02	35.0 ± 1.69	
	15	31.0 ± 1.59	8.0 ± 1.98	20.0 ± 1.88		31.0 ± 1.05	9.0 ± 02.11	20.0 ± 1.33	
	7.5	35.0 ± 1.87	6.0 ± 2.34	15.0 ± 2.31		33.0 ± 1.08	7.0 ± 2.06	10.0 ± 2.04	
Doxorubicin (standard)	120	0	40.0 ± 1.01	100.0 ± 1.01	48.64	0	40.0 ± 1.01	100.0 ± 1.04	43.24
	60	0	40.0 ± 1.01	100.0 ± 1.02		0	40.0 ± 1.01	100.0 ± 1.03	
	30	12.0 ± 1.17	28.0 ± 1.21	70.0 ± 1.19		12.0 ± 1.11	28.0 ± 1.17	70.1 ± 1.21	
	15	22.0 ± 1.13	18.0 ± 1.14	45.0 ± 1.17		22.0 ± 1.16	18.0 ± 1.19	45.0 ± 1.24	
	7.5	28.0 ± 1.15	12.0 ± 1.27	30.0 ± 1.14		28.0 ± 1.12	12.0 ± 1.18	30.0 ± 1.15	

3.2.2. IR V_{max} (cm^{-1}) (KBr). N-H stretching, indole ring of tryptophan (3477), O-H stretching, hydroxyl group of threonine (3368), N-H stretching, amide (3176-3156), C-H stretching, Ar ring (3079), asymmetrical C-H stretching, CH_3 and CH_2 (2895), C-H stretching, C-H (2876-2857), C=O stretching, amide (1677, 1643-1631), N–H deformation, amide (1549, 1539-1533), C-H bending, CH_2 (1468), asymmetrical bending, CH_3 (1454), symmetrical C-H bending, *iso*-propyl in leucine (1383 and 1374), C-O stretching, alkyl ether (1099), C-H def. oop, Ar ring (927, 683).

3.2.3. ^{1}H-NMR ($CDCl_3$) (ppm). 10.11 s (1H, NH for indole), 7.63 d (1H, J = 8.6 Hz, CH-Ar for indole), 7.33 d (1H, J = 7.6 Hz, CH-Ar for indole), 7.19 s (1H, CH-NH for indole), 7.13 d (2H, J = 6.4 Hz, CH-Ar for indole), 4.30-4.24 m (1H, CH for threonine), 3.87 t (1H, J = 8.5 and 8.9 Hz, CH for tryptophan), 3.65 t (1H, J = 4.7 and 4.5 Hz, CH for proline), 3.60 s (2H, CH_2 glycine), 3.59 s (1H, OH for threonine), 3.54 d (1H, J = 8.7 Hz, CH-N for threonine), 3.47 t (3H, J = 4.5 and 0.5 Hz, CH for leucine), 3.34 d (2H, J = 8.6 Hz, CH_2 for tryptophan), 2.78-2.71 m (2H, CH_2 for proline), 2.02 s (6H, for NH), 1.98-1.94 m (2H, CH_2 for proline), 1.78 t (6H, J = 7 and 5.4 Hz, CH_2 for leucine), 1.67-1.61 m (2H, CH_2 for proline), 1.50-1.43 m (3H, CH for Leu), 1.19 d (3H, J = 7.7 Hz, CH_3 for threonine), 0.93 d (18H, J = 8.6 Hz, CH_3 for leucine)

3.2.4. FABMS m/z. $(M + H)^+ = 781.8$, $(781.9 - CO)^+ = 753.8$; $(H - Trp - Gly - Leu - Leu - Leu - Thr)^+ = 684.9$; $(H - Pro - Trp - Gly - Leu - Leu - Leu)^+ = 680.9$; $(H - Thr - Pro - Trp - Gly - Leu - Leu)^+ = 668.9$; $(684.8 - CO)^+ = 656.9$; $(680.8 - CO)^+ = 652.9$; $(668.8 - CO)^+ = 640.9$; $(H - Leu - Leu - Thr - Pro - Typ)^+ = 611.8$; $(H - Pro - Thr - Leu - Leu - Leu - Gly)^+ = 595.8$; $[(611.7 - CO)^+ = 583.8$; $(595.7 - CO)^+ = 567.8$; $(H - Leu - Leu - Leu - Gly - Trp)^+ = 563.8$; $(H - Leu - Leu - Gly - Trp - Pro)^+ = 567.8$; $(583.7 - CO)^+ = 555.8$; $[(567.7 - CO)^+ = 563.8$; $(H - Leu - Leu - Leu - Thr - Pro)^+ = 538.8$; $(H - Thr - Leu - Leu - Leu - Gly)^+ = 498.7$; $(538.7 - CO)^+ = 510.8$; $(498.6 - CO)^+ = 470.7$; $(H - Thr - Pro - Typ - Gly)^+ = 442.6$; $(H - Leu - Leu - Leu - Thr)^+ = 441.6$; $(442.5 - CO)^+ = 414.6$; $(441.5 - CO)^+ = 413.5$; $(H - Leu - Leu - Leu - Gly)^+ = 397.5$; $(H - Thr - Pro - Typ)^+ = 385.9$; $(397.5 - CO)^+ = 369.5$; $(385.9 - CO)^+ = 357.9$; $(H - Leu - Gly - Typ)^+ = 357.0$; $(H - Leu - Leu - Leu)^+ = 340.4$; $(357.0 - CO)^+ = 329.1$; $(340.4 - CO)^+ = 312.5$; $(H - Leu - Leu)^+ = 227.4$; $(227.3 - CO)^+ = 199.4$; $(H - Leu - Gly)^+ = 171.9$; $(171.8 - CO)^+ = 143.90$; (Typ immonium ion $C_{10}H_{11}N_2)^+ = 159.3$; $(H - Leu)^+ = 114.3$; Leu immonium ion $(C_5H_{12}N)^+ = 86.2$; (Thr immonium ion $C_3H_8NO)^+ = 74.2$; $(C_6H_5)^+ = 71.2$; (Pro immonium ion $C_4H_8N)^+ = 70.2$; $(H - Gly)^+ = 58.2$; $(C_4H_9)^+ = 57.2$; $(C_2H_5O)^+ = 45.2$; $(C_3H_7)^+ = 43.2$; (Gly immonium ion $CH_4N)^+ = 30.2$; $(C_2H_5)^+ = 29.3$; $(CH_3)^+ = 15.2$.

3.3. Results of Antimicrobial Activity. The results of antimicrobial activity are shown in Table 3.

3.4. Results of Anticancer Activity. The results of anticancer activity are shown in Table 4 and Figures 3–5.

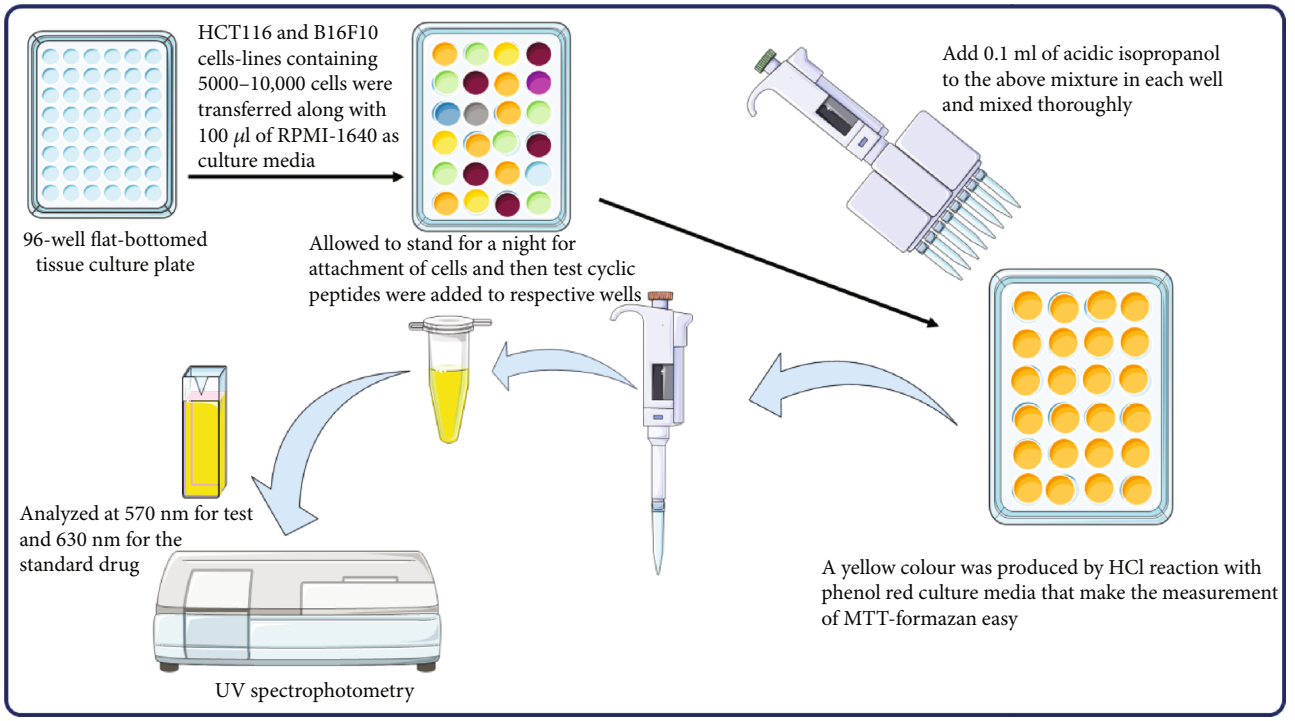


Figure 3: In vitro anticancer activity.

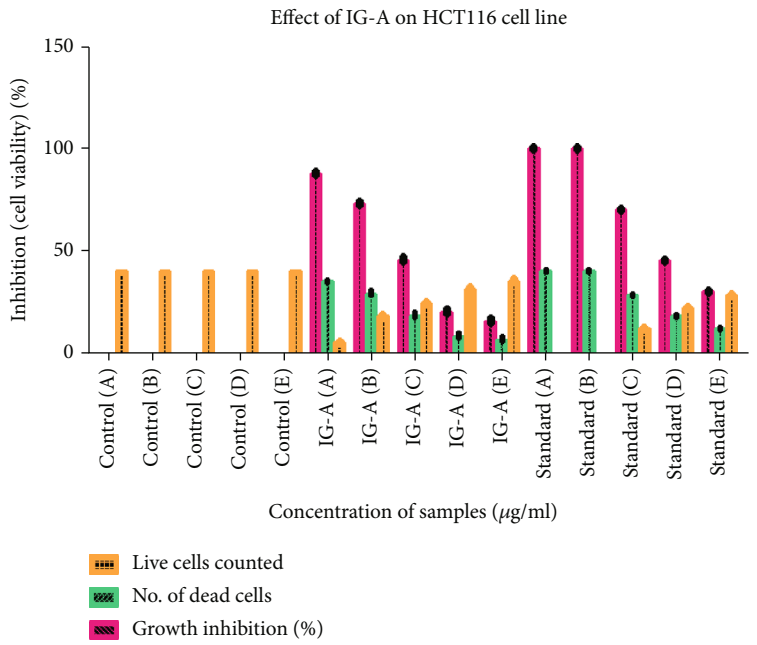


Figure 4: Anticancer activity of IG-A on HCT116 (human colon carcinoma) cell line, where A (120 μg/mL), B (60 μg/mL), C (30 μg/mL), D (15 μg/mL), and E (7.5 μg/mL) CTC_{50} values are expressed as mean ± standard error of the mean (SEM) of quintuplicate determinations. Different letters represent statistically significant differences determined by one-way ANOVA ($\rho < 0.0001$).

4. Discussion

The solution phase approach was used to create the cyclic peptide since it is simpler and less expensive than solid-phase synthesis. Cyclic peptide was synthesized with a high yield utilizing NMM as the cyclization base and DCC as the coupling agent.

4.1. Chemical Examination. Spectral data was used to characterize the synthesized cyclopeptide IG-A. The synthesis

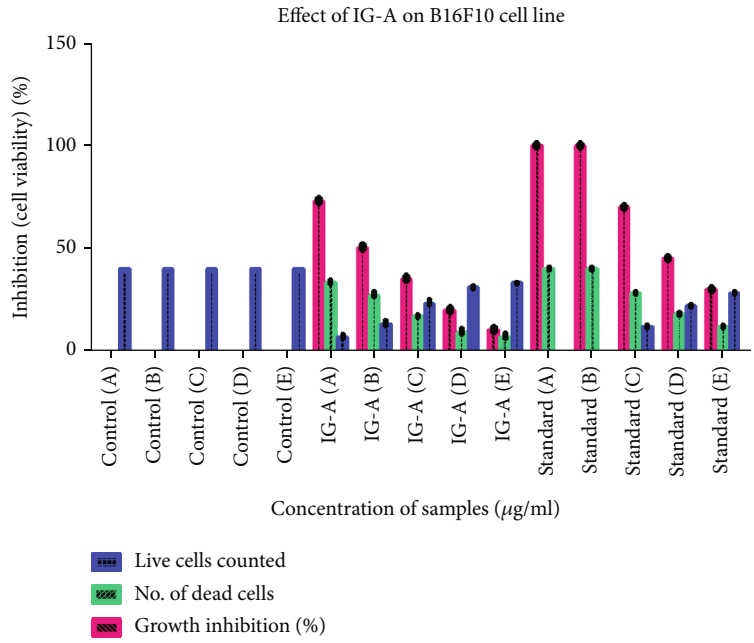


FIGURE 5: Anticancer activity of IG-A on B16F10 (melanoma cells) cell line, where A (120 μg/mL), B (60 μg/mL), C (30 μg/mL), D (15 μg/mL), and E (7.5 μg/mL) CTC_{50} values are expressed as mean ± standard error of the mean (SEM) of quintuplicate determinations. Different letters represent statistically significant differences determined by one-way ANOVA ($\rho < 0.0001$).

of cycloheptapeptide, IG-A (8), was completed with a yield of 60.8% and was illustrious by prominent peaks at 3476 cm^{-1} (due to N-H stretching of indole ring present in tryptophan), 3369 cm^{-1} (due to O-H stretching of the hydroxyl group present in threonine), and 3175-3155 cm^{-1} (due to N-H stretching of amide). The ^{1}H-NMR signal shows a singlet of peptide bond at 2.01 (s, 6H, and NH) and by the presence of pseudomolecular ion peak at m/z 781.1.

4.2. Biological Evaluation. The biological evaluation of synthesized cyclic peptide—IG-A (8)—for antibacterial, antifungal, and cytotoxic effects was carried out, and the results are as follows.

Gram +ve strains, *B. subtilis*, and Gram -ve strains, *E. coli*, *P. aeruginosa*, and *S. aureus*, were used to test antibacterial activity. Antifungal strains *C. albicans*, *A. niger*, T. mentagrophytes, and *M. audouinii* were used to test the antifungal activity. The antimicrobial activity was determined using the modified Kirby-Bauer method with ciprofloxacin as the standard drug for antibacterial activity and griseofulvin as the standard drug for antifungal activity at 10 mg/mL conc. Antimicrobial activity analysis revealed that IG-A (8) has modest antibacterial activity against *S. aureus*, with an inhibition value of 21.59 percent in comparison with the standard drug ciprofloxacin 19.89 percent. The antifungal activity revealed an inhibition value of 20.96 percent against C. albicans in comparison to standard drug griseofulvin 20.58 percent.

Deshpande Laboratories Pvt. Ltd., Bhopal, conducted cytotoxic research of manufactured IG-A (8) on HCT116 (human colon carcinoma) and B16F10 (melanoma cells) cell lines utilizing MTT test and doxorubicin as standard drug. The cytotoxic activity was determined by calculating the percentage suppression of growth of the HCT116 and B16F10 cell lines. A concentration of 7.5-120 μg/mL was utilized for the test, control, and standard drug. The possible mechanism of HDT-116 cells is shown in Figure 6. The morphological features of apoptosis, including phosphatidylserine externalization and internucleosomal DNA fragmentation, were observed to be induced in HCT116 cells [29, 30, 31, 32].

HCT116 cell lines are derived from human colorectal carcinoma, whereas B16F10 cell lines are derived from mouse cutaneous melanoma. At a dosage of 120 μg/mL, the cyclopeptide IG-A (8) inhibited cell proliferation by 87.5 and 72.5 percent, respectively. IG-A (8) had CTC_{50} values of 77.65 μM and 68.63 μM against HCT116 and B16F10, respectively. The % growth inhibitions at lesser levels are 72.5 and 50 at 60 μg/mL, respectively. Doxorubicin, Std. drug, inhibited growth by 100 percent with CTC_{50} values of 48.63 μM and 43.25 μM against HCT116 and B16F10, respectively. There is no inhibition in the control sample. Specifically, ultraviolet (UV) light triggers the release of α-melanocyte stimulating hormone (α-MSH), which has a negative impact on epidermal cell activity. Increased amounts of cyclic adenosine monophosphate (cAMP) are caused by α-MSH activation of adenylate cyclase, which in turn activates protein kinase A and cAMP-response element binding protein, which in turn activate the microphthalmia-associated transcription factor gene promoter. De novo transcription of tyrosinase, tyrosinase-related protein 1, and tyrosinase-related protein 2 is triggered by an upregulation of microphthalmia-associated transcription factor. Within melanocytes, eumelanin is generated by an enzymatic cascade regulated by

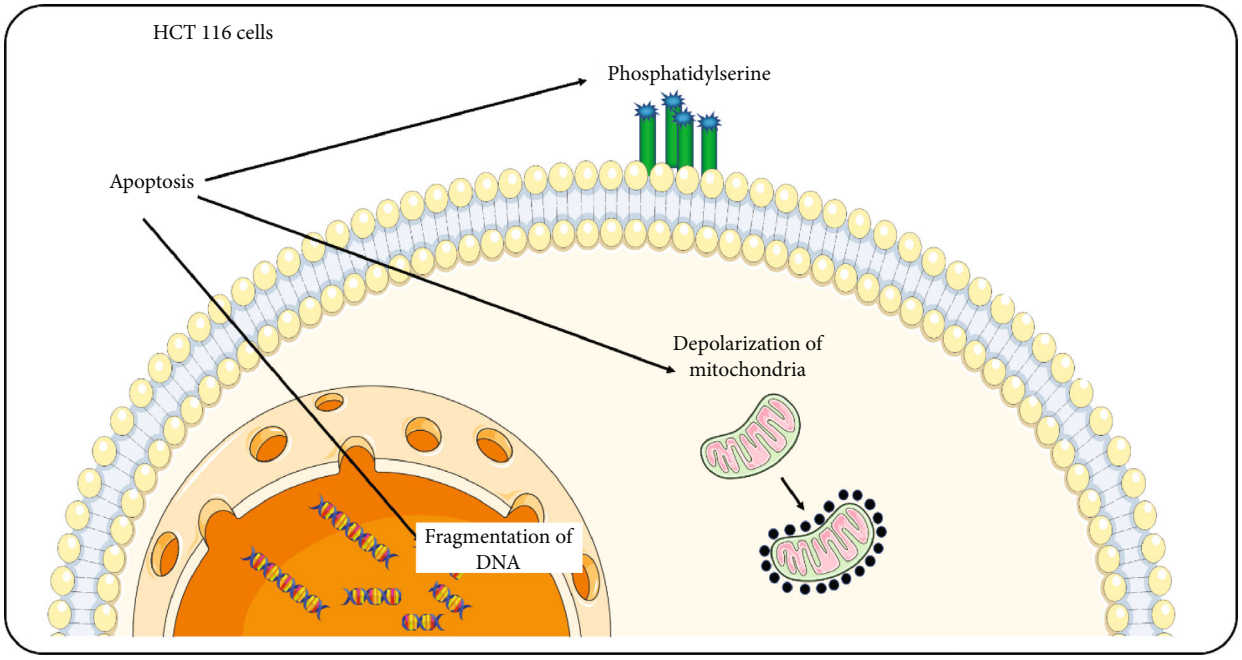


Figure 6: Possible mechanism of HCT 116 cells by internucleosomal DNA fragmentation.

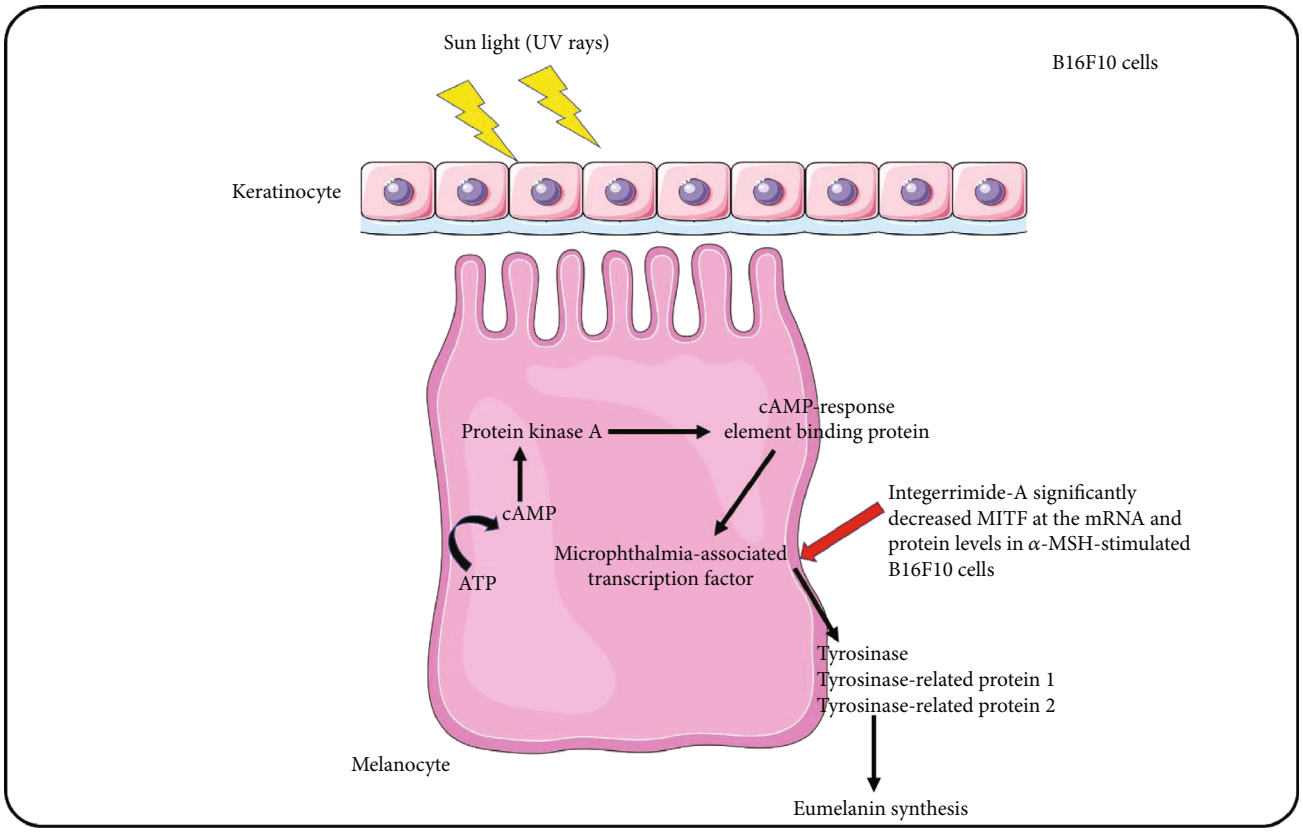


Figure 7: Possible mechanism of B16F10 cells by the inhibition of eumelanin synthesis.

tyrosinase, tyrosinase-related protein 1, and tyrosinase-related protein. We postulated that the microphthalmia-associated transcription factor signalling cascade was implicated in the suppression of melanogenic enzymes by IG-A as shown in Figure 7 [35, 36].

5. Conclusion

IG-A is a cyclic heptapeptide isolated from the latex of *Jatropha integerrima*. It is synthesized by combining a tetrapeptide fragment (Boc-Gly-L-Leu-L-Leu-L-Leu-L-Leu-OMe) with a tripeptide fragment (L-Thr-L-Pro-L-Trp-OMe) and then cyclizing the linear heptapeptide unit under alkaline conditions. FT-IR, ^{1}H-NMR, mass spectroscopy, and elemental analysis were used to analyze the structure of the newly synthesized chemical. The antibacterial and anticancer properties of IG-A were investigated. The antibacterial activity was assessed using Gram +ve and Gram -ve bacteria, whereas the antifungal activity was determined using fungal strains such as *Candida albicans*, *Aspergillus niger*, *T. mentagrophytes*, and *M. audouinii*. Similarly, the cytotoxic activity of doxorubicin was determined using the MTT assay on HCT116 and B16F10 cell lines. This cyclic peptide was found to have considerable antibacterial and cytotoxic effects against cancer cell types. The possible mechanism of HCT116 cells may be internucleosomal DNA fragmentation and B16F10 cells by the inhibition of eumelanin synthesis.

Abbreviations

Boc_2O: Di-*tert*-butyl dicarbonate
$CHCl_3$: Chloroform
CH_3OH: Methanol
$CDCl_3$: Deuterated chloroform
CO_2: Carbon dioxide
DCC: Dicyclohexylcarbodiimide
IG-A: Integerrimide-A
KBr: Potassium bromide
LiOH: Lithium hydroxide
MHz: Megahertz
mL: Milliliter
NMM: N-Methylmorpholine
μg: Microgram
UV: Ultraviolet.

Data Availability

Data will be available on request.

Conflicts of Interest

The authors confirm that this article content has no conflict of interest.

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Supplementary Materials

The supplementary material included the IR, ^{1}H-NMR, and mass spectra of the final product of cyclic peptide IG-A. *(Supplementary Materials)*

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