



Research Article

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Selective Cytotoxic Activity of Synthetic Natural Cyclopeptides on HCT11 & B16F10 Cells

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ABSTRACT

Peptides are natural messenger molecules of human body and hence ideal lead compounds for the initiation of drug discovery research. They are the important organic compounds with potent biological activities. Peptides functions as hormones, enzymes enzyme inhibitors, or substrates or growth inhibitors or promoters, neurotransmitters and immunomodulators. Investigation of new and more potent analogs of molecules with already established activities from a key part of research in pharmaceutical field. It's brings many modifications by manipulates the parent molecules structures serves to increase the activity of the compound, also eliminate adverse effect or toxicity associated with the parent drug. Cancer is the leading cause of deaths in world, We evaluated four natural cyclopeptides Diandrine A, Diandrine C, Fanlizhicyclopeptide A, Fanlizhicyclopeptide B, for cytotoxicity against HCT116 (Human Colorectal Carcinoma) & B16F10 (musculus skin melanoma) cells.

Keywords: Cyclopeptides, synthesis, cell line, HCT116, B16F10.

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INTRODUCTION

Plants are a major part of healthcare system in general and always rich in components with greater biological properties. [1-2] Over 70% population of the world follow traditional medicine, mostly based on plant remedies. The natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for development of

new drug leads molecules because of the unmatched availability of chemical diversity. [3-6] Plant-derived cyclopeptides play a vital role in drug discovery and drug design and have provided significant results for future study. [7-10] They have complex structures with modified amino acid moieties and are associated with a number of pharmacological activities including antimicrobial activity [11], tyrosinase inhibitory activity

[12], anti-inflammatory activity [13], antimalarial activity [14], protease inhibitory activity [15], antioxidant and anticancer activity. [16] Only minute quantities of cyclopeptides obtained from natural resources restricted researchers to investigate their biological profiles in detail. Keeping in view broad spectrum of bioactivities exhibited by these natural congeners and in order to obtain a potent bioactive compound in good yield [17-18], present investigation was directed toward *in-vitro* cell line study on synthesized some natural cyclopeptides.

MATERIALS AND METHODS

Melting point was determined by open capillary method and was uncorrected. L-Amino acids and other chemicals used were obtained from Spectrochem Limited (Mumbai, India). IR spectra were recorded on Shimadzu 8700 FTIR spectrophotometer (Shimadzu, Japan) and ¹H and ¹³C NMR spectra were recorded on Bruker AC NMR spectrometer (Bruker, USA) at 300 MHz. FAB-MS was recorded on JMS-DX 303 Mass spectrometer (Jeol, Tokyo, Japan) operating at 70 eV from School of Pharmaceutical Education and Research, Jamia Hamdard University. Purity of all compounds was checked by TLC on precoated silica gel G plates using mixture of chloroform and methanol in different ratios (9:1 intermediate linear peptides and 7:3 for cyclopeptide). Standard MTT assay was used to evaluate cell line viability in the presence of extracts with HCT116 and B16F10 cells.

Synthesis of Natural Cyclopeptides

All linear peptides (0.005 mol) were deprotected at carboxyl end using LiOH (0.18 g, 0.0075 mol) in THF:H₂O (1:1) to get Boc-amino acids-OH. The deprotected peptide unit (0.005 mol) was now dissolved in CHCl₃ (50 mL) at 0°C. To the above solution, pentafluorophenol (1.23 g, 0.0067 mol) and DIPC (0.63 g, 0.005 mol) was added and stirred at RT for 12 h. The reaction mixture was filtered and the filtrate was washed with 10% NaHCO₃ solution (2 × 25 mL) and 5% HCl (3 × 15 mL) to get the corresponding fluorophenyl ester Boc-Amino acids-O-*pfp*. To this compound (0.004 mol) dissolved in chloroform (25 mL), trifluoroacetic acid (0.91 g, 0.008 mol) was added, stirred at RT for 1 h and washed with 10% NaHCO₃ solution (3 × 20 mL). The organic layer was dried over anhydrous Na₂SO₄ to get Amino acids-O-*pfp* which was dissolved in CHCl₃ (25 mL) and TEA/NMM/pyridine (2.8 mL/2.21 mL/1.61 mL, 0.02 mol) was added. Then, whole content was kept for 1 week time at 0°C. The reaction mixture was washed with 10% NaHCO₃ and 5% HCl solutions (3 × 25 mL). The organic layer was dried over anhydrous Na₂SO₄. Finally, chloroform was distilled off and crude cyclized product was crystallized from CHCl₃/*n*-hexane to get pure cyclopeptides.

Diandrine A

Yellowish needles, m.p. 135-137°C, Yield 79.8% (NMM), 70.3% (TEA), 66.8% (C₅H₅N), [α]_D: -67.8° (-

67.6°), R_f - 0.59; IR (KBr): ν 3472 (m, -NH str, indole ring), 3375 (m/br, -OH str, Tyr), 3129-3125, 3121 (m, -NH str, amide), 3078, 3069-3062 (w, -CH str, aromatic rings), 2998-2992 (m, -CH str, cyclic CH₂ and CH), 2926, 2922, 2917 (m, -CH str, asym, CH₂), 2846, 2837-2833 (m, -CH str, sym, CH₂), 1679-1675, 1640, 1634-1629 (s, -C=O str, 3° and 2° amide), 1560, 1557, 1436-1427 (m, skeletal bands, aromatic rings), 1539, 1535-1532 (m, -NH bend, 2° amide), 718, 698-692 (s, -CH bend, oop, aromatic rings) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 9.89 (1H, br. s, -NH, Tyr), 9.72 (1H, br. s, -NH, Trp), 7.75 (1H, br. s, -NH, Phe), 7.47 (2H, br. s, -NH, indole ring and -OH, Tyr), 7.38-7.36 (1H, d, *J* = 7.75 Hz, α -H, indole ring), 7.25-7.23 (1H, d, *J* = 7.3 Hz, γ -H, indole ring), 7.21-7.15 (4H, m, m-H's, Tyr and Phe), 7.14-7.05 (3H, m, δ - ζ -H's, indole ring), 7.02-6.99 (1H, t, *J* = 6.15 Hz, p-H, Phe), 6.91 (1H, br. s, -NH, Gly), 6.88-6.84 (2H, dd, *J* = 8.55, 5.3 Hz, o-H's, Tyr), 6.83-6.79 (2H, dd, *J* = 8.8, 4.15 Hz, o-H's, Phe), 5.65-5.61 (1H, q, *J* = 5.6 Hz, α -H, Phe), 5.30-5.28 (2H, d, *J* = 4.75 Hz, CH₂, Gly), 4.62-4.58 (1H, q, *J* = 6.2 Hz, α -H, Trp), 4.25-4.21 (1H, q, *J* = 7.85 Hz, α -H, Tyr), 3.92-3.89 (1H, t, *J* = 6.9 Hz, α -H, Pro-2), 3.87-3.84 (1H, t, *J* = 6.75 Hz, α -H, Pro-1), 3.26-3.23 (2H, t, δ -H's, Pro-2), 3.21-3.18 (2H, t, δ -H's, Pro-1), 2.89-2.87 (2H, d, *J* = 5.65 Hz, β -H's, Trp), 2.72-2.63 (4H, m, β -H's, Pro-1 and Pro-2), 2.59-2.57 (2H, d, *J* = 5.45 Hz, β -H's, Tyr), 2.43-2.41 (2H, d, *J* = 5.85 Hz, β -H's, Phe), 1.89-1.82 (4H, m, γ -H's, Pro-1 and Pro-2) ppm; ¹³C NMR (CDCl₃, 300 MHz): δ 173.5, 172.0 (C=O, Pro-1 and Pro-2), 169.9, 169.3 (C=O, Tyr and Trp), 163.1, 162.3 (C=O, Gly and Phe), 154.4 (p-C, Tyr), 137.3 (γ -C, Phe), 135.9 (α' -C, indole ring), 132.2 (γ -C, Tyr), 129.8 (2C, o-C's, Tyr), 128.9 (2C, o-C's, Phe), 128.1 (2C, m-C's, Tyr), 127.4 (2C, m-C's, Phe), 127.0 (β' -C, indole ring), 126.1 (p-C, Phe), 123.3, 121.7 (α -C and ϵ -C, indole ring), 119.8, 118.5 (δ -C and γ -C, indole ring), 111.2, 109.6 (β -C and ζ -C, indole ring), 59.2 (α -C, Pro-2), 57.4 (α -C, Pro-1), 57.8 (α -C, Trp), 54.5 (α -C, Tyr), 53.0 (α -C, Phe), 49.7 (CH₂, Gly), 48.0, 46.9 (δ -C's, Pro-2 and Pro-1), 41.5 (β -C, Phe), 39.6 (β -C, Tyr), 30.3 (β -C, Pro-2), 27.8 (β -C, Pro-1), 27.0 (β -C, Trp), 25.2, 22.7 (2C, γ -C's, Pro-1 and Pro-2) ppm; FAB-MS: *m/z* 748.8 (M + H)⁺, 720.8 (748.8-CO)⁺, 691.8 (Pro-Trp-Pro-Tyr-Phe)⁺, 663.8 (691.8-CO)⁺, 651.7 (Tyr-Phe-Gly-Pro-Trp)⁺, 623.7 (651.7-CO)⁺, 601.6 (Gly-Pro-Trp-Pro-Tyr)⁺, 594.6 (Trp-Pro-Tyr-Phe)⁺, 585.6 (Phe-Gly-Pro-Trp-Pro)⁺, 573.6 (651.7-CO)⁺, 566.6 (594.6-CO)⁺, 557.6 (585.6-CO)⁺, 544.6 (Pro-Trp-Pro-Tyr)⁺, 516.6 (488.5-CO)⁺, 488.5 (Phe-Gly-Pro-Trp)⁺, 465.5 (Tyr-Phe-Gly-Pro)⁺, 460.5 (488.5-CO)⁺, 447.5 (Trp-Pro-Tyr)⁺, 438.5 (Gly-Pro-Trp-Pro)⁺, 437.5 (465.5-CO)⁺, 419.5 (447.5-CO)⁺, 410.5 (438.5-CO)⁺, 381.4 (Pro-Trp-Pro)⁺, 368.4 (Tyr-Phe-Gly)⁺, 341.4 (Gly-Pro-Trp)⁺, 340.4 (368.4-CO)⁺, 313.4 (341.4-CO)⁺, 311.3 (Tyr-Phe)⁺, 302.3 (Phe-Gly-Pro)⁺, 284.3 (Trp-Pro)⁺, 283.3 (311.3-CO)⁺, 256.3 (284.3-CO)⁺, 187.2 (Trp)⁺, 164.2 (Tyr)⁺, 159.2 (C₁₀H₁₁N₂)⁺, 155.2 (Gly-Pro)⁺, 136.2 (C₈H₁₀NO)⁺, 130.1 (C₉H₈N)⁺, 127.2 (155.2-CO)⁺, 120.2 (C₈H₁₀N)⁺, 116.1 (C₈H₆N)⁺, 107.1 (C₇H₇O)⁺, 93.1 (C₆H₅O)⁺, 91.1 (C₇H₇)⁺, 77.1 (C₆H₅)⁺, 70.1 (C₄H₈N)⁺, 58.0

(Gly)⁺, 30.0 (CH₄N)⁺ ppm; Anal. Calcd. for C₄₁H₄₅N₇O₇: C, 65.85; H, 6.06; N, 13.11. Found: C, 65.88; H, 6.05; N, 13.09%.

Diandrine C

Pale yellow needles, m.p. 114-115°C, yield 83.2% (NMM), 75.7% (TEA), 68.9% (C₅H₅N), [α]_D: +2.1° (+2.2°) (MeOH, c 0.19), R_f - 0.84. IR (KBr): ν 3476 (*m*, -NH str, indole ring), 3372 (*m*, -OH str, Tyr), 3127, 3125-3122 (*m*, -NH str, amide), 3075, 3072 (*w*, -CH str, aromatic rings), 2997, 2994-2989 (*m*, -CH str, cyclic CH₂ and CH), 2928, 2925-2922 (*m*, -CH str, asym, CH₂), 2848-2845, 2842 (*m*, -CH str, sym, CH₂), 1674, 1669, 1635-1632 (*s*, -C=O str, 3° and 2° amide), 1555-1552, 1425-1421 (*m*, skeletal bands, aromatic rings), 1539, 1535 (*m*, -NH bend, 2° amide), 721-717, 695-689 (*s*, -CH bend, oop, aromatic rings) cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ 9.85 (1H, *br. s*, -NH, Tyr), 9.16 (1H, *br. s*, -NH, Gly-2), 7.65 (1H, *br. s*, -NH, Trp), 7.42 (2H, *br. s*, -NH, indole ring and -OH, Tyr), 7.41-7.39 (1H, *d*, *J* = 7.8 Hz, α -H, indole ring), 7.25-7.23 (1H, *d*, *J* = 7.25 Hz, γ -H, indole ring), 7.16-7.07 (3H, *m*, δ - ζ -H's, indole ring), 6.99-6.95 (2H, *dd*, *J* = 8.6, 4.75 Hz, *m*-H's, Tyr), 6.92-6.88 (2H, *dd*, *J* = 8.65, 5.3 Hz, *o*-H's, Tyr), 6.26 (1H, *br. s*, -NH, Gly-1), 5.78-5.74 (1H, *q*, *J* = 6.15 Hz, α -H, Trp), 5.31-5.29 (2H, *d*, *J* = 4.7 Hz, CH₂, Gly-2), 4.23-4.19 (1H, *q*, *J* = 7.75 Hz, α -H, Tyr), 3.96-3.94 (2H, *d*, *J* = 4.75 Hz, CH₂, Gly-1), 3.91-3.86 (2H, *m*, α -H's, Pro-1 and Pro-2), 3.27-3.21 (4H, *m*, δ -H's, Pro-1 and Pro-2), 2.90-2.88 (2H, *d*, *J* = 5.7 Hz, β -H's, Trp), 2.69-2.63 (4H, *m*, β -H's, Pro-1 and Pro-2), 2.61-2.59 (2H, *d*, *J* = 5.65 Hz, β -H's, Tyr), 1.88-1.79 (4H, *m*, γ -H's, Pro-1 and Pro-2) ppm. ¹³C NMR (CDCl₃, 300 MHz): δ 173.2, 172.9 (2C, C=O, Tyr and Pro-2), 171.2, 169.9 (2C, C=O, Pro-1 and Trp), 164.8, 163.2 (2C, C=O, Gly-2 and Gly-1), 154.0 (*p*-C, Tyr), 136.7 (α' -C, indole ring), 133.9 (γ -C, Tyr), 130.2 (2C, *o*-C's, Tyr), 128.7 (2C, *m*-C's, Tyr), 126.7 (β' -C, indole ring), 125.5, 125.9 (2C, α -C and ε -C, indole ring), 120.4, 118.9 (2C, δ -C and γ -C, indole ring), 111.8, 110.3 (2C, β -C and ζ -C, indole ring), 65.4 (α -C, Pro-2), 58.0 (α -C, Pro-1), 57.5 (α -C, Trp), 52.8 (α -C, Tyr), 49.7 (CH₂, Gly-1), 49.1, 47.0 (2C, δ -C's, Pro-2 and Pro-1), 42.4 (CH₂, Gly-2), 37.7 (β -C, Tyr), 33.3 (β -C, Pro-1), 31.5 (β -C, Pro-2), 26.7 (β -C, Trp), 25.0, 23.3 (2C, γ -C's, Pro-2 and Pro-1) ppm. FAB MS: *m/z* 658.7 (M + H)⁺, 630.7 (658.7-CO)⁺, 601.6 (Gly-Pro-Tyr-Trp-Pro)⁺, 573.6 (601.6-CO)⁺, 561.6 (Tyr-Trp-Pro-Gly-Gly)⁺, 533.6 (561.6-CO)⁺, 504.5 (Tyr-Trp-Pro-Gly)⁺, 476.5 (504.5-CO)⁺, 472.5 (Pro-Gly-Gly-Pro-Tyr)⁺, 447.5 (Tyr-Trp-Pro)⁺, 444.5 (472.5-CO)⁺, 419.5 (447.5-CO)⁺, 375.4 (Gly-Gly-Pro-Tyr)⁺, 350.4 (Tyr-Trp)⁺, 347.4 (375.4-CO)⁺, 322.4 (350.4-CO)⁺, 318.3 (Gly-Pro-Tyr)⁺, 309.3 (Pro-Gly-Gly-Pro)⁺, 290.3 (318.3-CO)⁺, 281.3 (309.3-CO)⁺, 212.2 (Pro-Gly-Gly)⁺, 184.2 (212.2-CO)⁺, 164.2 (Tyr)⁺, 159.2 (C₁₀H₁₁N₂)⁺, 155.2 (Pro-Gly)⁺, 136.2 (C₈H₁₀NO)⁺, 130.1 (C₉H₈N)⁺, 127.2 (155.2-CO)⁺, 116.1 (C₈H₆N)⁺, 115.1 (Gly-Gly)⁺, 107.1 (C₇H₇O)⁺, 98.1 (Pro)⁺, 93.1 (C₆H₅O)⁺, 70.1 (C₄H₈N)⁺, 30.0 (CH₄N)⁺ ppm.

Fanlizhicyclopeptide A

Pale yellow solid; m.p. 137-139°C (d); Yield 85 % (C₅H₅N), 78 % (NMM), 68% (TEA); [α]_D = -74.2° (c =

0.54, MeOH) (-74.1° for natural fanlizhicyclopeptide A [11]); R_f = 0.77 (CHCl₃:MeOH - 9:1); IR (KBr): ν = 3372 (O-H_{str}, aromatic ring), 3128-3125, 3123-3119 (N-H_{str}, amide), 3067-3061 (Ar-H_{str}, aromatic ring), 2999, 2996-2991 (C-H_{str}, cyclic CH₂), 2967, 2925-2919 (C-H_{str}, asym, CH₃ and CH₂), 2853, 2949-2843 (C-H_{str}, sym, CH₂), 1668-1664, 1642, 1639 (C=O_{str}, 3° and 2° amide), 1566, 1439 (skeletal bands), 1538, 1532-1529 (N-H_{def}, 2° amide), 1380, 1362 (C-H_{def}, *iso*-propyl), 716, 687 (C-H_{def}, oop, aromatic ring) cm⁻¹; ¹H NMR (CDCl₃): δ = 9.88 (*br. s*, 1 H, NH, Tyr), 9.69 (*br. s*, 1 H, NH, Leu), 9.18 (*br. s*, 1 H, NH, Gly), 7.85 (*br. s*, 1 H, NH, Val), 6.99, 6.95 (*dd*, *J* = 8.6, 5.25 Hz, 2 H, *m*-H's, Tyr), 6.89, 6.86 (*dd*, *J* = 8.55, 4.9 Hz, 2 H, *o*-H's, Tyr), 6.55 (*t*, *J* = 5.9 Hz, 1 H, α -H, Val), 6.33-6.28 (*m*, *J* = 6.7 Hz, 1 H, α -H, Leu), 5.97 (*br. s*, 1 H, OH, Tyr), 4.25 (*t*, 1 H, *J* = 6.85 Hz, α -H, Pro-2), 4.21-4.37 (*q*, *J* = 7.85 Hz, 1 H, α -H, Tyr), 4.02 (*d*, *J* = 5.45 Hz, 2 H, α -H's, Gly), 3.89 (*t*, 1 H, *J* = 6.9 Hz, α -H, Pro-3), 3.75 (*t*, *J* = 6.85 Hz, 1 H, α -H, Pro-1), 3.51 (*t*, 2 H, *J* = 7.2 Hz, δ -H, Pro-2), 3.23 (*t*, *J* = 7.15 Hz, 2 H, δ -H, Pro-3), 2.95 (*t*, *J* = 7.2 Hz, 2 H, δ -H, Pro-1), 2.71-2.65 (*m*, 4 H, β -H's, Pro-1 and Pro-3), 2.64-2.60 (*m*, 2 H, β -H's, Pro-2), 2.57 (*d*, *J* = 5.45 Hz, 2 H, β -H's, Tyr), 1.89 (*t*, 2 H, *J* = 5.85 Hz, β -H's, Leu), 1.87-1.78 (*m*, 6 H, γ -H's, Pro-2, Pro-3 and Pro-1), 1.67-1.62 (*m*, 1 H, β -H, Val), 1.15 (*d*, 6 H, *J* = 4.55 Hz, γ -H's, Val), 0.99 (*d*, 6 H, *J* = 6.25 Hz, δ -H's, Leu), 0.86-0.79 (*m*, 1 H, γ -H, Leu); ¹³C NMR (CDCl₃): δ = 173.9 (C=O, Leu), 172.0, 171.3 (2 C, C=O, Pro-3 and Tyr), 170.8, 170.3, (2 C, C=O, Pro-2 and Val), 169.7, 169.1 (2 C, C=O, Pro-1 and Gly), 153.8 (*p*-C, Tyr), 135.6 (γ -C, Tyr), 131.3 (2 C, *m*-C's, Tyr), 129.1 (2 C, *o*-C's, Tyr), 62.2, 58.4, 56.1 (3 C, *a*-C's, Pro-3, Pro-2 and Pro-1), 55.8 (*a*-C, Val), 54.9, 54.5 (2 C, *a*-C's, Leu and Tyr), 49.3, 46.5, 45.1 (3 C, δ -C's, Pro-3, Pro-2 and Pro-1), 43.6, 42.0 (2 C, β -C's, Leu and Tyr), 40.9 (*a*-C, Gly), 34.6, 33.7, 30.1 (3 C, β -C's, Pro-3, Pro-1 and Pro-2), 29.9 (β -C, Val), 29.0 (γ -C, Leu), 24.1, 23.8 (2 C, γ -C's, Pro-3 and Pro-1), 23.1 (2 C, δ -C's, Leu), 20.7 (γ -C, Pro-2), 18.9 (2 C, γ -C's, Val); MS (FAB, 70 eV): *m/z* (%) = 724 (100) [M + 1]⁺, 696 (11) [724-CO]⁺, 667 (39) [Val-Pro-Pro-Tyr-Leu-Pro]⁺, 639 (17) [667-CO]⁺, 627 (78) [Pro-Tyr-Leu-Pro-Gly-Val]⁺, 625 (49) [Pro-Pro-Tyr-Leu-Pro-Gly]⁺, 611 (64) [Pro-Gly-Val-Pro-Pro-Tyr]⁺, 599 (19) [627-CO]⁺, 597 (16) [625-CO]⁺, 583 (16) [611-CO]⁺, 570 (48) [Val-Pro-Pro-Tyr-Leu]⁺, 568 (37) [Pro-Pro-Tyr-Leu-Pro]⁺, 542 (11) [570-CO]⁺, 540 (13) [568-CO]⁺, 530 (76) [Tyr-Leu-Pro-Gly-Val]⁺, 528 (41) [Pro-Tyr-Leu-Pro-Gly]⁺, 502 (11) [530-CO]⁺, 500 (15) [528-CO]⁺, 471 (76) [Pro-Pro-Tyr-Leu]⁺, 457 (23) [Val-Pro-Pro-Tyr]⁺, 448 (52) [Pro-Gly-Val-Pro-Pro]⁺, 443 (29) [471-CO]⁺, 431 (23) [Tyr-Leu-Pro-Gly]⁺, 429 (16) [457-CO]⁺, 420 (11) [448-CO]⁺, 403 (14) [431-CO]⁺, 374 (48) [Pro-Tyr-Leu]⁺, 358 (61) [Pro-Pro-Tyr]⁺, 351 (72) [Pro-Gly-Val-Pro]⁺, 346 (17) [374-CO]⁺, 330 (14) [358-CO]⁺, 323 (16) [351-CO]⁺, 294 (38) [Val-Pro-Pro]⁺, 277 (41) [Tyr-Leu]⁺, 261 (33) [Pro-Tyr]⁺, 254 (33) [Pro-Gly-Val]⁺, 233 (10) [261-CO]⁺, 226 (14) [254-CO]⁺, 195 (27) [Pro-Pro]⁺, 167 (11) [195-CO]⁺, 155 (29) [Pro-Gly]⁺, 136 (19) [Tyr immonium ion, C₈H₁₀NO]⁺, 127 (21) [155-CO]⁺, 107

(10) [C₇H₇O]⁺, 98 (22) [Pro]⁺, 93 (13) [C₆H₅O]⁺, 86 (21) [Leu immonium ion, C₅H₁₂N]⁺, 72 (26) [Val immonium ion, C₄H₁₀N]⁺, 70 (34) [Pro immonium ion, C₄H₈N]⁺, 57 (14) [C₄H₉]⁺, 43 (28) [C₃H₇]⁺, 30 (16) [Gly immonium ion, CH₄N]⁺, 17 (10) [OH]⁺, 15 (21) [CH₃]⁺; C₃₇H₅₃N₇O₈ (723): calcd. C 61.39, H 7.38, N 13.54; found C 61.41, H 7.36, N 13.55.

Fanlizhicyclopeptide B

Pale yellow solid; m.p. 121-123°C (d); Yield 87% (C₅H₅N), 79% (NMM), 73% (TEA); [α]_D = -113.5° (c = 0.41, MeOH) (-113.6° for natural fanlizhicyclopeptide B [14]); R_f = 0.68 (CHCl₃:MeOH - 9:1); IR (KBr): ν = 3375 (O-H_{str}, aromatic ring), 3128-3124, 3121 (N-H_{str}, amide), 3068-3063 (Ar-H_{str}, aromatic ring), 2998-2992 (C-H_{str}, cyclic CH₂), 2969, 2925, 2918 (C-H_{str}, asym, CH₃ and CH₂), 2842, 2837 (C-H_{str}, sym, CH₂), 1668, 1645-1639 (C=O_{str}, 3° and 2° amide), 1567, 1435 (skeletal bands), 1535, 1531-1527 (N-H_{def}, amide), 714, 685 (C-H_{def}, oop, aromatic ring) cm⁻¹; ¹H NMR (CDCl₃): δ = 8.68 (br. s, 1 H, NH, Ile), 8.35 (br. s, 1 H, NH, Tyr), 7.72 (br. s, 1 H, NH, Ala), 7.25 (br. s, 1 H, NH, Gly), 6.99, 6.96 (dd, J = 8.6, 4.9 Hz, 2 H, o-H's, Tyr), 6.88, 6.85 (dd, J = 8.6, 5.3 Hz, 2 H, m-H's, Tyr), 5.97 (br. s, 1 H, OH, Tyr), 5.94-5.89 (m, 1 H, α-H, Ala), 5.68-5.64 (q, J = 7.85 Hz, 1 H, α-H, Tyr), 5.29 (d, J = 5.5 Hz, 2 H, α-H's, Gly), 3.89 (t, 1 H, J = 6.9 Hz, α-H, Pro), 3.81 (t, J = 8.6 Hz, 1 H, α-H, Ile), 3.25 (t, 2 H, J = 7.15 Hz, δ-H, Pro), 2.68-2.64 (m, 2 H, β-H's, Pro), 2.37 (d, J = 5.5 Hz, 2 H, β-H's, Tyr), 1.85-1.79 (m, 2 H, γ-H's, Pro), 1.63-1.58 (m, 2 H, γ-H's, Ile), 1.53-1.48 (m, 1 H, β-H's, Ile), 1.44 (d, 3 H, J = 5.85 Hz, β-H's, Ala), 1.01 (d, J = 5.9 Hz, 3 H, γ'-H's, Ile), 0.96 (t, 3 H, J = 7.8 Hz, δ-H's, Ile); ¹³C NMR (CDCl₃): δ = 173.3 (C=O, Ala), 172.1 (C=O, Tyr), 170.7 (C=O, Ile), 170.2 (C=O, Pro), 163.5 (C=O, Gly), 152.6 (p-C, Tyr), 133.7 (γ-C, Tyr), 129.2 (2 C, o-C's, Tyr), 127.9 (2 C, m-C's, Tyr), 59.0, 56.2, 53.7 (3 C, a-C's, Ile, Pro and Tyr), 49.2, 48.7 (2 C, a-C's, Gly and Ala), 48.0 (δ-C, Pro), 39.9, 36.4, 32.7 (3 C, β-C's, Tyr, Ile and Pro), 24.4, 22.8 (2 C, γ-C's, Ile and Pro), 17.8 (β-C, Ala), 16.9 (γ'-C, Ile), 10.6 (δ-C, Ile); MS (FAB, 70 eV): m/z (%) = 502 (100) [M + 1]⁺, 474 (14) [502-CO]⁺, 431 (64) [Gly-Pro-Ile-Tyr]⁺, 403 (15) [431-CO]⁺, 389 (38)

[Tyr-Ala-Gly-Pro]⁺, 377 (11) [405-CO]⁺, 374 (76) [Pro-Ile-Tyr]⁺, 361 (18) [389-CO]⁺, 348 (59) [Ile-Tyr-Ala]⁺, 346 (15) [374-CO]⁺, 339 (49) [Ala-Gly-Pro-Ile]⁺, 320 (16) [348-CO]⁺, 311 (14) [339-CO]⁺, 292 (61) [Tyr-Ala-Gly]⁺, 277 (28) [Ile-Tyr]⁺, 268 (46) [Gly-Pro-Ile]⁺, 240 (13) [268-CO]⁺, 235 (45) [Tyr-Ala]⁺, 211 (39) [Pro-Ile]⁺, 207 (11) [235-CO]⁺, 198 (10) [226-CO]⁺, 183 (10) [211-CO]⁺, 155 (29) [Gly-Pro]⁺, 136 (28) [Tyr immonium ion, C₈H₁₀NO]⁺, 129 (19) [Ala-Gly]⁺, 127 (10) [155-CO]⁺, 107 (10) [C₇H₇O]⁺, 93 (13) [C₆H₅O]⁺, 86 (21) [Ile immonium ion, C₅H₁₂N]⁺, 70 (38) [Pro immonium ion, C₄H₈N]⁺, 57 (18) [C₄H₉]⁺, 44 (18) [Ala immonium ion, C₂H₆N]⁺, 30 (16) [Gly immonium ion, CH₄N]⁺, 29 (12) [C₂H₅]⁺, 17 (11) [OH]⁺, 15 (24) [CH₃]⁺; C₂₅H₃₅N₅O₆ (501): calcd. C 59.87, H 7.03, N 13.96; found C 59.88, H 7.05, N 13.95.

Evaluation of Cytotoxic Activity

Synthesized cyclopeptides were subjected to short term *in vitro* cytotoxic study (from Deshpande Laboratories Pvt. Ltd., Bhopal) at 120-7.5µg/ml using Doxorubicin as reference compound. Activity was assessed by determining the percentage inhibition of HCT116 and B16F10 Cellline. Standard MTT assay was used to evaluate cell line viability in the presence of extracts. In 96 well plate, 100µl medium (RPMI 1640) was poured in each well and selected with 5000-10,000 HCT116 and B16F10 cells. Cells were allowed to attach overnight and then various concentration of the crude extract were added to respective wells. After 24 h incubation at 37°C, 5% CO₂ and relative humidity 20µl of MTT (5 mg/ml) was added to each cell. After further 4 h incubation at 37°C, 100µl of DMSO solutions was added to each well to solublize MTT crystals. The plates were again incubated overnight at conditions mentioned above. The plates were read for optical density at 570 nm as test wave length and 630 nm as the reference using using a plate reader. Percentage inhibition was calculated by following formula.

$$\text{Percentage of cytotoxicity} = \frac{\text{Control} - \text{Test Sample} \times 100}{\text{Control}}$$

Table 1: Cytotoxic activity data of Diandrine A

Compd.	Conc. (µg/ml)	HCT116			^b CTC ₅₀ (µM)	B16F10			^b CTC ₅₀ (µM)
		Live cells counted	No. of dead cells	% growth inhibition ^a		Live cells counted	No. of dead cells	% growth inhibition ^a	
Diandrine A	120	04 ± 1.21	36 ± 1.29	90 ± 1.35	20.16	05 ± 1.69	35 ± 1.15	87.5 ± 2.08	20.91
	60	08 ± 1.30	32 ± 2.01	80 ± 2.03		10 ± 2.14	30 ± 1.35	75 ± 1.97	
	30	14 ± 1.14	26 ± 2.38	65 ± 2.14		15 ± 2.31	25 ± 1.02	62.5 ± 1.69	
	15	22 ± 1.59	18 ± 1.98	45 ± 1.88		23 ± 1.05	17 ± 02.11	42.5 ± 1.33	
	7.5	29 ± 1.87	11 ± 2.34	27.5 ± 2.31		30 ± 1.08	10 ± 2.06	25 ± 2.04	
Control	120	40	0	0	0	40	0	0	0
	60	40	0	0		40	0	0	
	30	40	0	0		40	0	0	
	15	40	0	0		40	0	0	
	7.5	40	0	0		40	0	0	
Doxorubicin	120	0	40 ± 1.01	100 ± 1.01	7.05	0	40 ± 1.01	100 ± 1.04	6.51
	60	0	40 ± 1.01	100 ± 1.02		0	40 ± 1.01	100 ± 1.03	
	30	08 ± 1.17	32 ± 1.21	80 ± 1.19		10 ± 1.11	30 ± 1.17	75 ± 1.21	
	15	16 ± 1.13	24 ± 1.14	60 ± 1.17		17 ± 1.16	23 ± 1.19	57.5 ± 1.24	
	7.5	22 ± 1.15	18 ± 1.27	45 ± 1.14		24 ± 1.12	16 ± 1.18	40 ± 1.15	

^a % growth inhibition = 100 - [(Celltotal - Celldead) × 100] / Celltotal; ^bCTC₅₀ = conc. inhibiting 50% of percentage growth (n = 3)

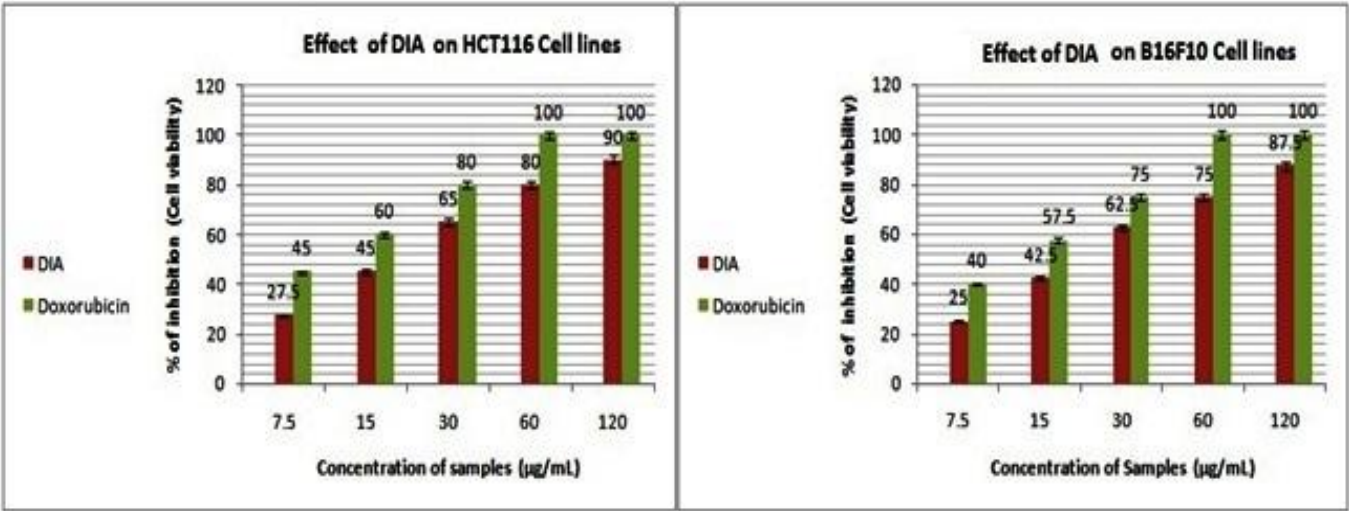


Fig. 1: Cytotoxic activity of Diandrine A

Table 2: Cytotoxic activity data of Diandrine C

Compd.	Conc. (µg/ml)	HCT116			^b CTC ₅₀ (µM)	B16F10			^b CTC ₅₀ (µM)
		Live cells counted	No. of dead cells	% growth inhibition ^a		Live cells counted	No. of dead cells	% growth inhibition ^a	
Diandrine C	120	06 ± 2.13	34 ± 2.19	85 ± 2.16	16.42	08 ± 2.15	32 ± 2.23	80 ± 2.87	17.73
	60	11 ± 2.25	29 ± 2.21	72.5 ± 2.18		12 ± 02.24	28 ± 2.19	70 ± 2.21	
	30	16 ± 2.27	24 ± 2.24	60 ± 3.21		17 ± 3.22	23 ± 2.26	57.5 ± 2.23	
	15	23 ± 1.98	17 ± 2.22	42.5 ± 2.24		25 ± 3.23	15 ± 2.24	37.5 ± 2.17	
	7.5	31 ± 1.94	9 ± 2.25	22.5 ± 3.28		2 ± 2.21	08 ± 2.27	20 ± 2.19	
Control	120	40	0	0	0	40	0	0	0
	60	40	0	0		40	0	0	
	30	40	0	0		40	0	0	
	15	40	0	0		40	0	0	
	7.5	40	0	0		40	0	0	
Doxorubicin	120	0	40 ± 1.12	100 ± 1.14	5.973	0	40 ± 1.19	100 ± 1.14	6.51
	60	0	40 ± 1.11	100 ± 1.20		0	40 ± 1.13	100 ± 1.14	
	30	17 ± 1.14	33 ± 1.22	82.5 ± 1.19		18 ± 1.11	32 ± 1.14	80 ± 1.12	
	15	12 ± 1.15	28 ± 1.19	70 ± 1.24		14 ± 1.16	26 ± 1.21	65 ± 1.21	
	7.5	22 ± 1.11	18 ± 1.26	45 ± 1.21		23 ± 1.12	17 ± 1.18	42.5 ± 1.23	

^a % growth inhibition = 100 - [(Celltotal - Celldead) × 100]/Celltotal; ^bCTC₅₀ = conc. inhibiting 50% of percentage growth (n = 3)

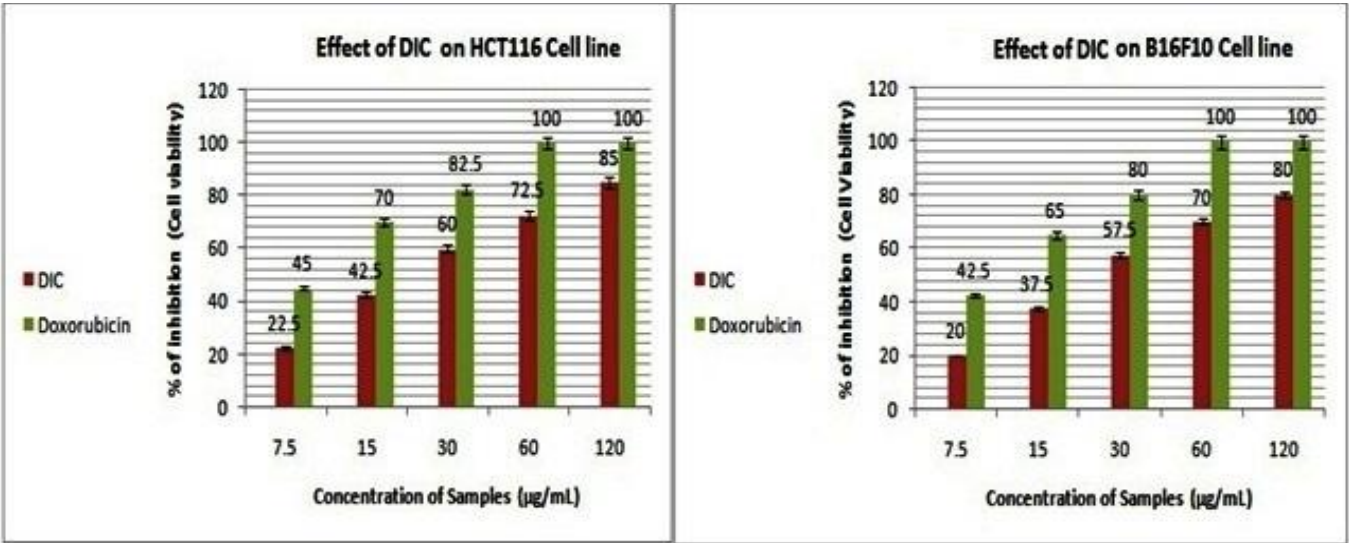


Fig. 2: Cytotoxic activity of Diandrine C

RESULTS AND DISCUSSION

The synthesized all four natural cyclopeptides, Diandrine A, Diandrine C, Fanlizhicyclopeptide A and Fanlizhicyclopeptide B was accomplished with good yields and pyridine was proved to be an effective base for Cyclization of all the four linear peptides units.

Cyclization of the all linear peptide fragment was supported by the disappearance of absorption bands and confirmation by IR, ¹H and ¹³C NMR and FAB Mass spectroscopy.

Table 3: Cytotoxic activity data of Fanlizhicyclopeptide A

Compd.	Conc. ($\mu\text{g/ml}$)	HCT116			^b CTC ₅₀ (μM)	B16F10			^b CTC ₅₀ (μM)
		Live cells counted	No. of dead cells	% growth inhibition ^a		Live cells counted	No. of dead cells	% growth inhibition ^a	
Fanlizhicyclopeptide A	120	07 $\pm$ 2.21	33 $\pm$ 2.31	82.5 $\pm$ 2.31	23.136	09 $\pm$ 1.87	31 $\pm$ 2.23	77.5 $\pm$ 2.42	25.35
	60	14 $\pm$ 2.25	26 $\pm$ 2.14	65 $\pm$ 2.14		16 $\pm$ 2.24	24 $\pm$ 2.19	60 $\pm$ 2.21	
	30	21 $\pm$ 2.30	19 $\pm$ 2.19	47.5 $\pm$ 1.97		22 $\pm$ 2.22	18 $\pm$ 2.26	45 $\pm$ 2.23	
	15	28 $\pm$ 2.26	12 $\pm$ 1.98	30 $\pm$ 2.17		27 $\pm$ 2.23	13 $\pm$ 2.24	32.5 $\pm$ 2.17	
	7.5	33 $\pm$ 2.22	07 $\pm$ 1.99	17.5 $\pm$ 2.34		34 $\pm$ 2.21	6 $\pm$ 2.27	15 $\pm$ 2.19	
Control	120	40	0	0	0	40	0	0	0
	60	40	0	0		40	0	0	
	30	40	0	0		40	0	0	
	15	40	0	0		40	0	0	
	7.5	40	0	0		40	0	0	
Doxorubicin	120	0	40 $\pm$ 1.22	100 $\pm$ 1.15	8.14	0	40 $\pm$ 1.19	100 $\pm$ 1.14	8.68
	60	0	40 $\pm$ 1.31	100 $\pm$ 1.21		0	40 $\pm$ 1.13	100 $\pm$ 1.14	
	30	14 $\pm$ 1.13	26 $\pm$ 1.22	65 $\pm$ 1.13		16 $\pm$ 1.11	24 $\pm$ 1.14	60 $\pm$ 1.12	
	15	21 $\pm$ 1.21	19 $\pm$ 1.19	47.5 $\pm$ 1.22		22 $\pm$ 1.16	18 $\pm$ 1.21	45 $\pm$ 1.21	
	7.5	27 $\pm$ 1.14	13 $\pm$ 1.24	32.5 $\pm$ 1.24		28 $\pm$ 1.12	12 $\pm$ 1.18	30 $\pm$ 1.23	

^a % growth inhibition = $100 - \{[(\text{Celltotal} - \text{Celldead}) \times 100] / \text{Celltotal}\}$; ^bCTC₅₀ = conc. inhibiting 50% of percentage growth (n = 3)

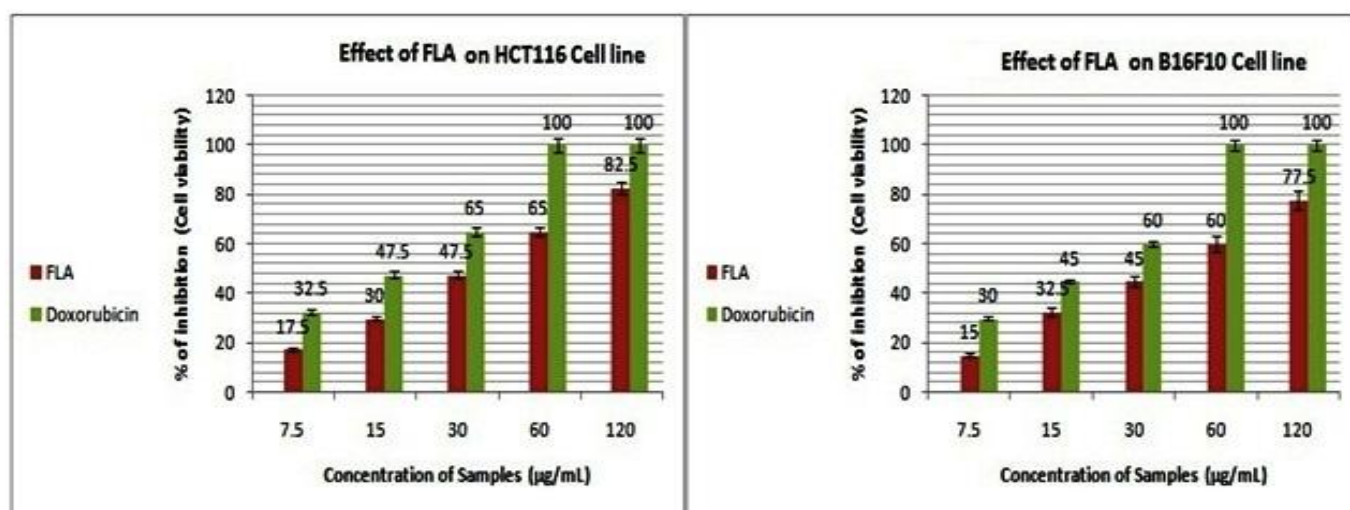


Fig. 3: Cytotoxic activity data of FLA (20)

Table 4: Cytotoxic activity data of Fanlizhicyclopeptide B

Compd.	Conc. ($\mu\text{g/ml}$)	HCT116			^b CTC ₅₀ (μM)	B16F10			^b CTC ₅₀ (μM)
		Live cells counted	No. of dead cells	% growth inhibition ^a		Live cells counted	No. of dead cells	% growth inhibition ^a	
Fanlizhicyclopeptide B	120	09 $\pm$ 0.23	31 $\pm$ 0.32	77.5 $\pm$ 0.28	25.5	08 $\pm$ 0.19	32 $\pm$ 0.28	80 $\pm$ 0.35	26.05
	60	15 $\pm$ 0.28	25 $\pm$ 0.30	62.5 $\pm$ 0.24		13 $\pm$ 0.25	27 $\pm$ 0.17	67.5 $\pm$ 0.31	
	30	22 $\pm$ 0.19	18 $\pm$ 0.29	45 $\pm$ 0.26		19 $\pm$ 0.13	19 $\pm$ 0.23	47.5 $\pm$ 0.27	
	15	30 $\pm$ 0.14	10 $\pm$ 0.24	25 $\pm$ 0.28		28 $\pm$ 0.22	12 $\pm$ 0.29	30 $\pm$ 0.34	
	7.5	36 $\pm$ 0.15	04 $\pm$ 0.25	10 $\pm$ 0.27		33 $\pm$ 0.34	7 $\pm$ 0.37	17.5 $\pm$ 0.36	
Control	120	40	0	0	0	40	0	0	0
	60	40	0	0		40	0	0	
	30	40	0	0		40	0	0	
	15	40	0	0		40	0	0	
	7.5	40	0	0		40	0	0	
Doxorubicin	120	0	40 $\pm$ 0.01	100 $\pm$ 0.01	9.23	0	40 $\pm$ 0.01	100 $\pm$ 0.04	9.77
	60	0	40 $\pm$ 0.01	100 $\pm$ 0.02		0	40 $\pm$ 0.01	100 $\pm$ 0.03	
	30	12 $\pm$ 0.17	28 $\pm$ 0.21	70 $\pm$ 0.19		13 $\pm$ 0.11	27 $\pm$ 0.17	67.5 $\pm$ 0.21	
	15	22 $\pm$ 0.13	18 $\pm$ 0.14	45 $\pm$ 0.17		21 $\pm$ 0.16	19 $\pm$ 0.19	47.5 $\pm$ 0.24	
	7.5	28 $\pm$ 0.15	12 $\pm$ 0.27	30 $\pm$ 0.14		27 $\pm$ 0.12	13 $\pm$ 0.18	32.5 $\pm$ 0.15	

^a % growth inhibition = $100 - \{[(\text{Celltotal} - \text{Celldead}) \times 100] / \text{Celltotal}\}$; ^bCTC₅₀ = conc. inhibiting 50% of percentage growth (n = 3)

Diandrine A, Diandrine C, Fanlizhicyclopeptide A and Fanlizhicyclopeptide B these all the synthesized cyclopeptides were evaluated for their in-vitro Cytotoxic activity using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) against HTC116 and B16F10 Cellline. The life span was increased for the compounds, when compared to

Doxorubicin for the selected concentration from 7.5 $\mu\text{g/mL}$ to 120 $\mu\text{g/mL}$. The CTC₅₀ value against HTC116 and B16F10 Cellline was calculated for Diandrine A, 20.16 & 20.21, for Diandrine C, 16.42 & 17.73 for Fanlizhicyclopeptide A, 23.12 & 25.35, and Fanlizhicyclopeptide B, 25.5 & 26.05 respectively.

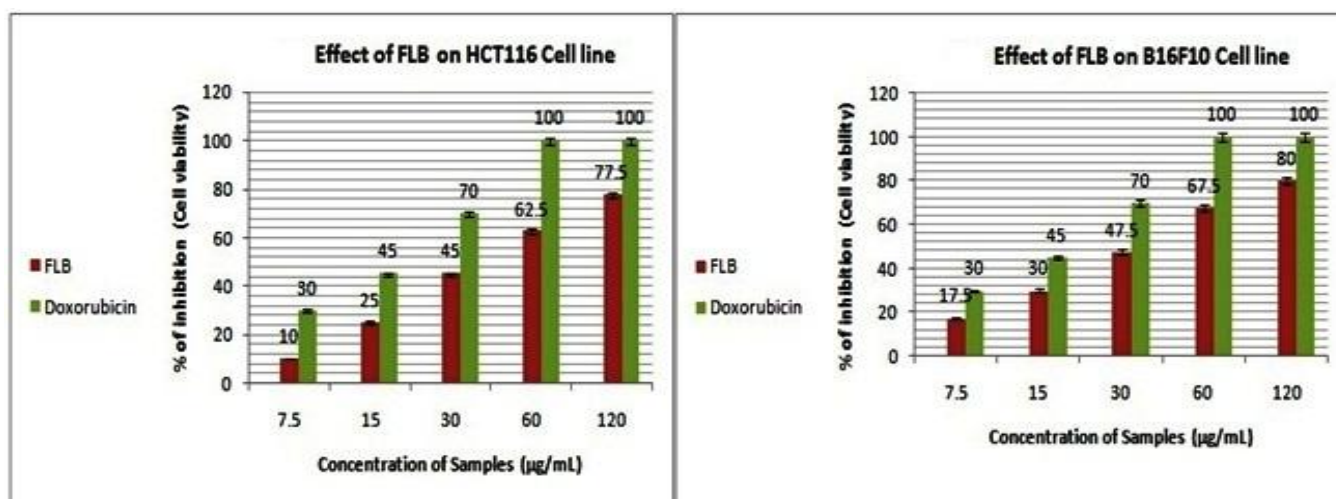


Fig. 4: Cytotoxic activity data of Fanlizhicyclopeptide B

All four synthesized natural cyclopeptides were shows cytotoxic effect against HTC116 and B16F10 Cellline. The cytotoxic activity of these natural cyclopeptides could be due to the presence of bioactive structure.

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