Journal of Pharmaceutical Research International

33(46A): 431-446, 2021; Article no.JPRI.75639
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Anti-Cancer Activity of Gabapentin and Chiral Amino Acids-Based Hybrid-Peptides against MCF-7 Breast Cancer Cell-Line

Vraj B. Pansuriya[1], Khushal M. Kapadiya[2], Suryajitsinji L. Rathod[1], Usha B. Prajapati[1], Bhavin M. Vavaiya[3], Jasmin B. Padariya[4], Foram U. Vaidya[5], Chandramani Pathak[5] and Hitesh M. Parekh[1*]

[1]Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad 380009, Gujarat, India.
[2]Department of Chemistry, School of Science, RK University, Rajkot 360020, Gujarat, India.
[3]Department of Chemistry, M. G. Science College, Gujarat University, Ahmedabad 380009, Gujarat, India.
[4]Department of Chemistry, KSKV Kutch University, Bhuj 370001, Gujarat, India.
[5]Department of Cell Biology, School of Biological Sciences and Biotechnology, Indian Institute of Advanced Research, Gandhinagar 382426, Gujarat, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i46A32886
Editor(s):
(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
(2) Dr. Asmaa Fathi Moustafa Hamouda, Jazan University, Saudi Arabia.
Reviewers:
(1) M. Rajeswari Prabha, India.
(2) Mahesh K. Gaidhane, Mandhal RTM Nagpur University, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75639

Original Research Article

Received 06 August 2021
Accepted 12 October 2021
Published 15 October 2021

ABSTRACT

Aims: Herein, we report the cytotoxicity of gabapentin-based peptides (11a-11j) using L-alanine and L-phenyl alanine chiral amino acids for peptide bond formation in ten efficient and straightforward steps. The in vitro MTT assays of derived molecules on the MCF-7 cell line (a human breast adenocarcinoma cell line) exhibited enhanced antitumor activity compared to the control (100% cell proliferation).
Methods: The ten steps synthetic methods were adapted for the synthesis of the Gabapentin-based peptide derivatives through BOC- deBOC methods and using EDC-HCl, DMAP and commercially available solvents. All the synthesized peptides were unambiguously characterized

**Corresponding author: E-mail: hiteshparekh@gujaratuniversity.ac.in;*

with the help of spectroscopic (IR, ^{1}H NMR, ^{13}C-NMR, mass spectra, and elemental) data analysis.
Results: The Compounds 11a, 11b, 11h,11i, and 11j showed a remarkable antiproliferative (cell death) activity, with % cell proliferation values ranging from 25-38 %.
Conclusion: The study showed that the compounds with some specific functionalities like, benzylic and trifluoromethyl functionality enhanced the potency with comparable %cell proliferation and cell death. Based on the findings in this work and their easily accessible molecular structures, compounds 11a and 11j are worthy of further biological investigations.

Keywords: Peptide synthesis; gabapentin; l-alanine; l-phenyl alanine; antiproliferative activity; MCF-7 cell line.

1. INTRODUCTION

Cancer is now reached at the top, threatening human health due to the increasing incidence and mortality. Among various types of cancers, breast cancer is one of the most common types among other malignancies in women and a significant cause of mortality worldwide [1,2]. New cases of breast cancer diagnosed in 2015 accounted for approximately 12% of all new malignancy cases, and mortality accounted for 25% of all cancer cases in women. The worldwide new cases of female breast cancer are estimated to reach nearly 3.2 million per year by 2050 [3-5]. Despite the intensive investigation of breast cancer cell lines, the cellular and molecular mechanisms between the MCF-7 cell line and the drug *polyoxometalate* (POM) are still limited [6]. Anticancer peptides (ACPs) have been proved to be effective small molecules (nearly 50 amino acids) that can act specifically against cancerous cells by either a membranolytic mechanism or disruption of mitochondria [7].

Peptides and proteins are involved in various biochemical processes and physiological functions [8-11]. Approximately 34% of peptide drugs were approved by the US Food and Drug Administrations and were expected to reach USD 23 billion in 2021 [12]. It is currently act as an active hormone [13, 14], neurotransmitters [15,16], growth factors [17], signaling molecules and antibiotics [18-20]. Peptide drugs are applicable as analgesic [21], antimicrobial [22], cardiovascular [23], CNS drugs [24,25], gastrointestinal disorder [26], hematological disorder [27], respiratory disorder [28]and anticancer agents [29-31]. Gabapentin (Gbp) was originally produced in a search for analogues of the neurotransmitter γ-aminobutyric acid (GABA) [32]and later on approved for various therapy by the drug agencies of some countries as therapy for partial seizures, for treatment of post-herpetic neuralgia, for adjunctive therapy for cancer pain despite the known fact of high doses of Gabapentin were associated with pancreatic acinar cell tumour in rats [33,34]. The conformational properties of Gbp in the peptide sequences are the current attention of the researcher [35-38]. In peptide design, Gabapentin may be used as a stereochemically constrained analogue of the parent chiral amino acid to enhance its potency in various cancer treatments and recently introduced as an adjunct in the multimodal management of postoperative pain after breast cancer surgery [39]. The development of gabapentin peptides could transform in vivo to release the active drug. Thus, it could be a feasible strategy to improve the physicochemical, biopharmaceutical or pharmacokinetics, and pharmacological properties of Gbp, thereby increasing its usefulness [40,41]. In November 2019, more than 20 ACPs were approved by the FDA, like Kyprolis, Lutathera, and Gallium Dotatoc Ga68 (Fig. 1) [42,43].

Collectively, ACPs represent a promising alternative to conventional chemotherapy due to their high specificity, reduced side effects, and desired tumour penetration. However, many ACPs also have some disadvantages, including substantial toxicity and poor targeting, which seriously impair their potency. Therefore, mechanisms of effective reconstruction or modification of ACPs to improve their therapeutic properties and reduce their toxicity have become a significant research focus [44-47].

To extend our efforts towards promising anticancer agents using various natural/non-natural precursors [48-50], herein, we have reported a new class of Gabapentin base hybrid-peptide derivatives (11a-11j) and were evaluated for anticancer screening against MCF-7 breast cancer cells line. The structure of newly synthesized peptides was assigned based on IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry analysis.

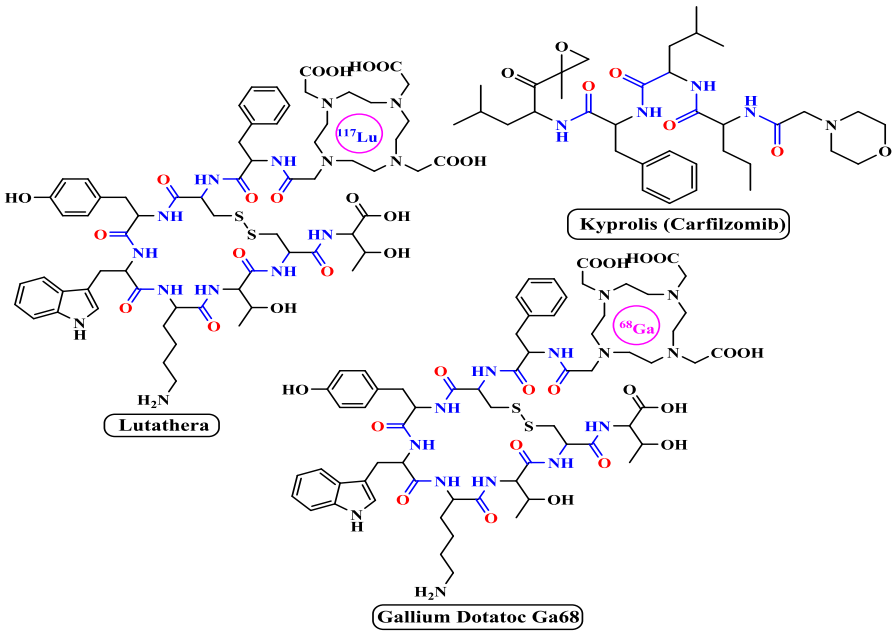


Fig. 1. FDA Approved anticancer peptides

2. MATERIALS AND METHODS

2.1 Materials

The required chemicals and solvents for the synthesis were purchased from JSK Fine, Avra Synthesis, Finar, and Spectrochem. All the chemicals were used without further purification. Precoated plates of silica gel G60 F254 (0.2 mm, Mfg. by Merck) were used for thin-layer chromatography. Visualization was made under UV light (254 and 365nm) or with iodine vapour. Melting points were measured with the help of Optimelt MPA100, an automated apparatus. Spectral analysis of the synthesized compound was done with the help of FTIR-8400 (Shimadzu) using the ATR technique. The ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on the "Bruker AVANCE II Spectrometer" using DMSO-d_6 as solvent and TMS as the internal reference. Mass spectra were recorded on a Jeol-JMSD 300 mass spectrometer at 70ev. Elemental analysis was carried out by a Perkin-Elmer 2400 CHN analyzer.

2.2 Synthesis

2.2.1 Synthesis of 2-(1-(((tert-butoxycarbonyl)amino)methyl)cyclohexyl)acetic acid (KSM-1)

In 250 ml RBF, Gabapentin (10 gm, 0.58 mol) and sodium hydroxide (2.6 gm, 0.65 mol) solution dissolved in water (50 ml) was added along with slowly and dropwise addition of Boc anhydride (15.2 gm, 0.69 mol) at 0-5^{o}C temperature. It was stirred at RT overnight (12 hours). The progress of the reaction was monitored by TLC using Dichloromethane: methanol (9:1) as a mobile phase (Rf: 0.2). After completing the reaction, the 3-4 pH was maintained using diluted hydrochloric acid [6:4, HCl: Water] at below 5°C temperature. The reaction mixture was filtered under vacuum and dried in a vacuum dryer. It was found as a white crystalline powder with a 92% of yield.

2.2.2 Synthesis of 2-((tert-butoxycarbonyl)amino)propanoic acid (KSM-2)

In 250 ml RBF, L-alanine (10 gm, 1.12 mol) and sodium hydroxide pellets (5.4 gm, 1.35 mol) dissolve in water (50 ml) was added along with slowly and dropwise addition of Boc anhydride (29.3 gm, 1.35 mol) at 0-5^{o}C temperature. It was stirred at RT overnight. The progress of the reaction was monitored by TLC using Dichloromethane: methanol (9:1) as a mobile phase (Rf: 0.3). After completing the reaction, the pH (3-4) was maintained by dropwise addition of diluted hydrochloric acid [6:4, HCl: Water] at below 5°C temperature. The separated solid was filtered off under vacuum and was dried in a vacuum dryer at 55^{o}C temperature for further reaction. It was found as a white crystalline powder with a 90% of yield.

2.2.3 Synthesis of methyl-2-amino-3-phenylpropanoate hydrochloride (4)

To a solution of L-phenylalanine 3 (10 gm, 0.60 mol) in methanol (50 ml), $SOCl_2$ (7.2 gm, 0.90 mol) was added in a dropwise manner by maintaining the temperature below 0°C. After addition, it was stirred at RT for 12 hours and was monitored by TLC with mobile phase Dichloromethane: methanol (8:2 ml). It was distilled off under reduced pressure and washed hexane with vigorous stirring to afford the product (4). It was used instantly in the next step without further purification.

2.2.4 Synthesis of methyl-2-(2-(1-(((tert-butoxycarbonyl)amino)methyl)cyclohexyl) acetamido)-3-phenylpropanoate (5)

The KSM-1 (3.0 gm, 0.11 mol) was dissolved in DMF (30 ml) and carried out at a temperature below 5°C. To this mixture, EDC·HCl (2.53 gm, 0.13 mol) and 4-Dimethylaminopyridine (4.5 gm, 0.36 mol) were added by maintaining the temperature below 5°C and stirred well for 10 minutes. It was fed by previously prepared intermediate 4 (2.39 gm, 0.11 mol) and was stirred overnight at RT. After completion of the reaction, monitored on TLC using Dichloromethane: methanol (7:3) as mobile phase, the product was poured into cold water and stirred vigorously for 1 h. Extract the product with MDC (50 ml × 3). The organic layer was washed with brine followed by dried using sodium sulfate and distilled off to get the oily mass of product 5 with 88% yield.

2.2.5 Synthesis of methyl-2-(2-(1-(aminomethyl) cyclohexyl)acetamido)-3-phenylpropanoate hydrochloride salt (6)

In a 250 ml RBF, a solution of compound 5 (3.8 gm, 0.87 mol) was dissolved in dioxane (30 ml) and cooled to 0°C temperature. After sometimes the reaction mass appeared cleared and was purged by HCl gas (1.0 hour). The reaction mixture was stirred for 3-4 hours at RT. The reaction was monitored by TLC using mobile phase Dichloromethane and methanol (9:1). It was distilled off under reduced pressure and washed twice with hexane with vigorous stirring to afford the product (6) and was found white hygroscopic solid material.

2.2.6 Synthesis of methyl-2-(2-(1-(2-((tert-butoxycarbonyl)amino)propanamido)methyl)cyclohexyl) acetamido)-3-phenylpropanoate (7)

The KSM-1 (1.48 gm, 0.78 mol) was dissolved in DMF (15 ml) and carried out at a temperature below 5°C. To this reaction mixture, EDC·HCl (1.80 gm, 0.94 mol) and 4-Dimethylaminopyridine (2.87 gm, 2.35 mol) were added by maintaining the temperature below 5°C and stirred well for 10 minutes. It was fed by previously prepared intermediate compound 6 (2. 9 gm, 0.78 mol) and was stirred overnight at RT. After completion of reaction monitored on TLC using Dichloromethane: methanol (7:3) as mobile phase, the product was poured into cold water and stirred vigorously for 1 hour. Extract the product with MDC (35 ml × 3). The organic layer was washed with brine followed by dried using sodium sulfate and distilled off to get the yellowish oily mass of product 7 with 86% yield.

2.2.7 Synthesis of methyl-2-(2-(1-((2-aminopropanamido) methyl) cyclohexyl) acetamido) -3-phenyl propanoate hydrochloride salt (8)

To a solution of compound 7 (3.4 gm, 0.67 mol) was dissolved in dioxane (30 ml) and cooled to 0°C temperature. After sometimes the reaction mass appeared cleared and was purged by HCl gas (1.0 hour). The reaction mixture was stirred for 3-4 hours at RT. The reaction was monitored by TLC using mobile phase Dichloromethane and methanol (9:1). It was distilled off under reduced pressure and washed twice with hexane with vigorous stirring to afford the product (8) and was found while solid material.

2.2.8 General procedure for the synthesis methyl-(2-(1-(2-(substituted benzamido) propanamido) methyl) cyclohexyl) acetyl)-L-phenylalaninate (9a-9c)

In round bottom flask (moisture-free), aromatic acid (0.23 mol) was dissolved in DMF (10 ml) and was charged with EDC·HCl (0.35 mmol) and 4-Dimethylaminopyridine (0.73 mol) at below 5°C temperature. It was stirred for 10 minutes at RT and added compound 8 (0.23 mol) portion-wise. The reaction mass was stirred at RT overnight and was monitored by TLC using Dichloromethane and methanol (8:2) mobile phase system. and after completing the reaction. The reaction mass was poured into crushed ice and stirred vigorously for 1 hour. Extract the

product with MDC (50 ml × 3). The organic layer was washed with brine followed by dried using sodium sulfate and concentrated under reduced pressure to get the oily mass of product 9a-9c with 72% yield.

2.2.9 General procedure for the synthesis of (2-(1-((2-(substitutedbenzamido) propanamido) methyl) cyclohexyl) acetyl)-L-phenylalanine (10a-10c)

For the hydrolysis of ester, 9a-9c (0.21 mol) was dissolved in the binary solvent system [water: THF (1:2)] (12 ml). To this, lithium hydroxide (0.32 mol) was added and the mass was stirred at RT overnight. The progress of the reaction was carried out by TLC. After completing the reaction, it was extracted with ethyl acetate (50 ml × 3) to remove unreacted material and impurity. An aqueous layer was treated with 2N HCl (20 ml) to adjust pH 2. It was further extracted with MDC (50 ml × 3). The MDC layer was washed with brine followed by dried using sodium sulfate and concentrated under reduced pressure to get the product 10a-10c with 79% yield.

2.2.10 General procedure for the synthesis of N-(1-oxo-1-(((1-(2-oxo-2-((-1-oxo-3-substituted phenyl-1-(substitutedphenylamino) propan-2-yl)amino)ethyl) cyclohexyl)methyl)amino)propan-2-yl) benzamide (11a-11j)

For the final product formation, the previously isolated 10a-10c (0.1 mol) was dissolved in DMF (5 ml) and added *N*,*N*-Diisopropylethylamine (3 mmol) in a slow manner. It was charged with EDC·HCl (0.15 mmol) and 4-Dimethylaminopyridine (0.48 mol) at below 5°C temperature. It was stirred for 10 minutes at RT and added various aromatic amine (0.11 mol) portion-wise. The reaction mass was stirred at RT overnight and was monitored by TLC using Dichloromethane and methanol (8:2 ml) mobile phase system. The reaction mass was poured into crushed ice and stirred vigorously for 1 h. Extract the product with MDC (50 ml × 3). The organic layer was washed with brine followed by dried using sodium sulfate and concentrated under reduced pressure to final product 11a-11j. The crude material was purified by column purification using MDC and methanol mobile phase using silica 60-120 as stationary phase.

2.2.11 N-(1-oxo-1-(((1-(2-oxo-2-((1-oxo-3-phenyl-1-((pyridin-2-ylmethyl)amino)propan-2-yl)amino)-ethyl) cyclohexyl) methyl)amino) propan-2-yl)-4-(trifluoromethyl)benzamide (11a)

White solid; Yield 39%; mp 256°C; IR (ATR): V_{max}/cm^{-1} = 3635 (NH), 2980, 2852 (2CH), 1633 (C=O), 1531 (C=C), 1350 (CN), 1016 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 1.014-1.133 (m, 3H), 1.231-1.306 (m, 4H), 1.347-1.378 (m, 6H), 1.986-2.084 (m, 2H), 2.751-2.906 (m, 1H), 2.987-3.078 (m, 3H), 4.358-4.372 (d, *J* = 5.6 *Hz*, 2H), 4.469-4.515 (m, 1H), 4.602-4.660 (m, 1H), 7.124-7.159 (m, 1H), 7.167-7.193 (m, 1H), 7.200-7.279 (m, 5H), 7.669-7.708 (td, *J* = 8 *Hz,* 1H), 7.819-7.881 (m, 3H), 8.059-8.090 (d, *J* = 8.4 *Hz,* 2H), 8.316-8.372 (dd, *J* = 14.4 *Hz,* 1H), 8.476-8.492 (dt, 1H, *J* = 4.8 *Hz,* 1H), 8.592-8.630 (q, 1H, *J* = 9.2 *Hz,* 1H), 8.798-8.835 (t, *J* = 14.8 *Hz,* 1H); ^{13}C-NMR (DMSO-d_6): δ_{ppm} = 21.12, 29.52, 29.52, 30.42, 34.65, 34.65, 35.85, 37.86, 41.25, 444.95, 47.01, 52.63, 66.02, 118.35, 121.62, 125.98, 127.12, 127.12, 128.52, 130.15, 130.15, 132.85, 132.85, 134.58, 134.58, 134.94, 136.12, 138.36, 142.86, 150.96, 161.05, 168.32, 170.36, 174.18, 176.35; MS *m/z* (%): 652 (M^+); Anal. Calcd. For $C_{35}H_{40}F_3N_5O_4$: C, 64.50; H, 06.19; N, 10.75; O, 09.82 %; Found: C, 64.54; H, 06.22; N, 10.71; O, 09.78 %.

2.2.12 N-(1-oxo-1-(((1-(2-oxo-2-((1-oxo-3-phenyl-1-(pyridin-2-ylamino) propan-2 yl)amino) ethyl) cyclohexyl) methyl)amino)propan-2-yl)-4-(trifluoromethyl)benzamide (11b)

White solid; Yield 21 %; mp 288°C; IR (ATR): V_{max}/cm^{-1} = 3682 (NH), 2980, 2929 (2CH), 1639 (C=O), 1529 (C=C), 1325 (CN), 1066 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 0.980-1.074 (m, 1H), 1.114-1.365 (m, 6H), 1.305-1.399 (m, 6H), 1.931-2.088 (m, 2H), 2.760-3.139 (m, 4H), 4.425-4.523 (m, 1H), 4.786-4.841 (m, 1H), 7.106-7.264 (m, 4H), 7.332-7.337 (m, 2H), 7.775-7.898 (m, 4H), 8.052-8.107 (m, 3H), 8.328-8.344 (m, 2H), 8.803-8.821 (d, *J* = 7.2 *Hz,* 1H), 10.683-10.696 (d, *J* = 5.2 *Hz,* 1H);^{13}C-NMR (DMSO-d_6): δ_{ppm} = 21.15, 28.53, 28.53, 29.86, 34.46, 34.46, 35.21, 37.36, 42.96, 46.95, 55.23, 59.56, 112.03, 120.45, 126.78, 127.47, 127.47, 128.96, 129.57, 129.57, 130.69, 130.69, 132.58, 132.58, 135.36, 138.47, 141.36, 143.58, 149.95, 153.08, 168.12, 165.62, 173.96, 175.25; MS *m/z* (%): 638 (M^+); Anal. Calcd. For $C_{34}H_{38}F_3N_5O_4$: C, 64.04; H, 06.01; N, 10.98; O, 10.04 %; Found: C, 64.11; H, 06.05; N, 10.92; O, 10.07 %.

2.2.13 N-(1-(((1-(2-((1-((4-fluorobenzyl) amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) cyclohexyl) ethyl)amino)-1-oxopropan-2-yl)-4-(trifluoromethyl)benzamide (11c)

White solid; Yield 53 %; mp 292^{o}C; IR (ATR): V_{max}/cm^{-1} = 3662 (NH), 2980, 2927 (2CH), 1633 (C=O), 1537 (C=C), 1325 (CN), 1128 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 1.007-1.128 (m, 4H), 1.176-1.270 (m, 3H), 1.307-1.399 (m, 6H), 1.983-2.079 (m, 2H), 2.737-2.796 (m, 1H), 2.851-2.899 (m, 1H), 2.980-3.022 (m, 1H), 3.073-3.124 (m, 1H), 4.222-4.261 (d, *J* = 15.6 *Hz,* 2H), 4.472-4.565 (m, 1H), 4.572-4.614 (m, 1H), 7.065-7.156 (m, 2H), 7.163-7.299 (m, 7H), 7.819-7.876 (m, 3H), 8.071-8.091 (d, *J* = 8 *Hz,* 2H), 8.283-8.332 (m, 1H), 8.492-8.535 (q, *J* = 11.2 *Hz,* 1H), 8.795-8.835 (t, *J* = 16 *Hz,* 1H);^{13}C-NMR (DMSO-d_6): δ_{ppm} = 21.58, 25.26, 22.26, 26.35, 29.15, 34.96, 35.02, 39.31, 42.85, 44.30, 45.63, 52.95, 60.85, 117.69, 117.69, 125.45, 126.32, 126.32, 128.08, 130.65, 130.65, 131.89, 131.89, 134.68, 134.68, 135.47, 135.47, 136.65, 137.01, 139.85, 144.30, 165.09, 168.45, 172.35, 174.45, 176.04; MS *m/z* (%): 669 (M^{+}); Anal. Calcd. For $C_{36}H_{40}F_4N_4O_4$: C, 64.66; H, 06.03; N, 08.38; O, 09.57 %; Found: C, 64.60; H, 06.01; N, 08.43; O, 09.61 %.

2.2.14 N-(4-fluorobenzyl)-2-(2-(1-((2-(2-(4-methoxyphenyl) acetamido) propanamido) methyl) cyclohexyl) acetamido)-3-phenylpropanamide (11d)

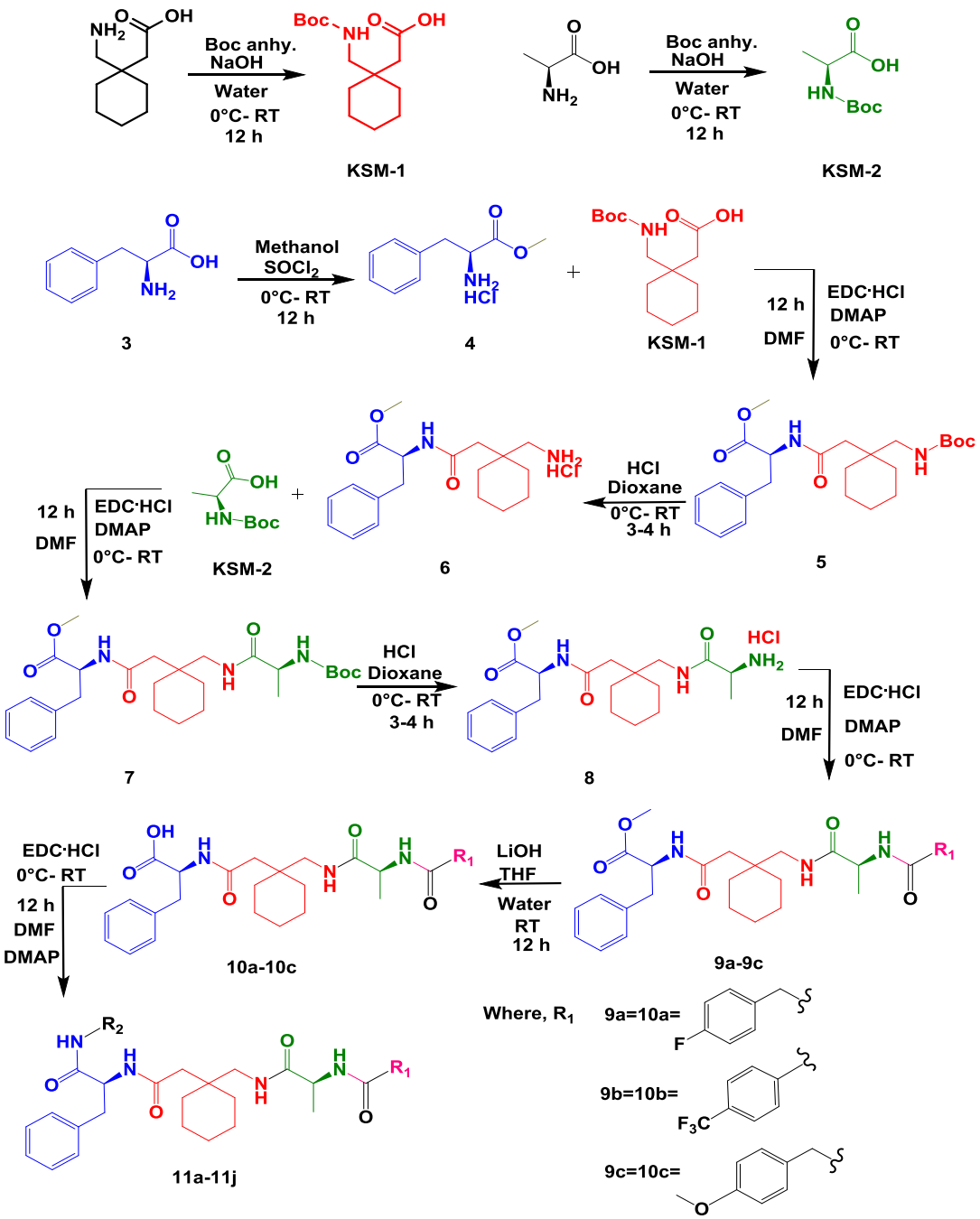
White solid; Yield 63 %; mp 252^{o}C; IR (ATR): V_{max}/cm^{-1} = 3635 (NH), 2980, 2927 (2CH), 1633 (C=O), 1510 (C=C), 1346 CN), 1157 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 0.989-1.163 (m, 4H), 1.195-1.212 (d, *J* = 6.8 *Hz,* 3H), 1.227-1.321 (m, 6H), 1.924-2.023 (q, *J* = 13.6 *Hz,* 2H), 2.769-2.828 (m, 2H), 3.003-3.083 (m, 2H), 3.390 (s, 2H), 3.710 (s, 3H), 4.231-4.308 (m, 3H), 4.580-4.639 (m, 1H), 6.819-6.841 (d, *J* = 8.8 *Hz,* 2H), 7.075-7.153 (m, 2H), 7.162-7.280 (m, 9H), 7.678-7.709 (t, *J* = 6 *Hz,* 1H), 8.196-8.234 (m, 1H), 8.272-8.292 (d, *J* = 8 *Hz,* 1H), 8.524-8.569 (t, *J* = 6 *Hz,* 1H); ^{13}C-NMR (DMSO-d_6): δ_{ppm} = 21.32, 22.20, 22.20, 25.65, 31.45, 31.45, 33.96, 40.11, 41.26, 42.65, 43.89, 45.12, 52.89, 54.36, 57.36, 112.58, 112.58, 114.15, 114.15, 127.68, 128.23, 131.89, 131.89, 133.78, 133.88, 135.23, 135.23, 136.11, 136.11, 136.65, 138.35, 159.37, 162.86, 169.48, 171.85, 173.84, 176.34; MS *m/z* (%): 646 (M^{+}); Anal. Calcd. For $C_{37}H_{45}FN_4O_5$: C, 68.92; H, 07.03; N, 08.69; O, 12.41 %; Found: C, 68.87; H, 07.07; N, 08.72; O, 12.36 %.

2.2.15 N-(4-methoxybenzyl)-2-(2-(1-((2-(2-(4-methoxyphenyl) acetamido)propanamido) methyl) cyclohexyl) acetamido)-3-phenylpropanamide (11e)

White solid; Yield 45 %; mp 290^{o}C; IR (ATR): V_{max}/cm^{-1} = 3624 (NH), 2980, 2929 (2CH), 1708 (C=O), 1562 (C=C), 1390 (CN); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 0.976-1.089 (m, 4H), 1.187-1.345 (m, 9H), 1.849-2.012 (m, 2H), 2.748-2.807 (m, 2H), 2.990-3.062 (m, 2H), 3.383 (s, 2H), 3.711-3.731 (s, 6H), 4.204-4.280 (m, 3H), 4.571-4.617 (m, 1H), 6.811-6.862 (m, 4H), 7.105-7.126 (d, *J* = 8.4 *Hz,* 2H), 7.148-7.186 (m, 3H), 7.231-7.273 (m, 4H), 7.669-7.700 (t, 1H), 8.185-8.255 (m, 2H), 8.441-8.471 (t, *J* = 6 *Hz,* 1H);^{13}C-NMR (DMSO-d_6): δ_{ppm} = 21.32, 22.15, 22.45, 24.35, 31.65, 33.65, 33.98, 36.56, 41.85, 42.02, 44.22, 43.98, 50.09, 54.67, 57.65, 57.65, 112.51, 112.51, 115.12, 115.12, 124.37, 126.95, 130.32, 130.32, 132.68, 130.68, 134.41, 134.41, 136.65, 136.65, 137.95, 138.25, 159.45, 161.58, 173.84, 174.98, 175.32, 176.23; MS *m/z* (%): 657 (M^{+}); Anal. Calcd. For $C_{38}H_{48}N_4O_6$:C, 69.49; H, 07.37; N, 08.53; O, 14.62 %; Found: C, 68.87; H, 07.07; N, 08.72; O, 12.36%.

2.2.16 2-(2-(1-((2-(2-(4-fluorophenyl) acetamido) propanamido)methyl) cyclohexyl)acetamido)-3-phenyl-N-(3-(trifluoromethyl) phenyl) propanamide (11f)

White solid; Yield 45 %; mp 274^{o}C; IR (ATR): V_{max}/cm^{-1} = 3639 (NH), 2980, 2929 (2CH), 1656 (C=O), 1537 (C=C), 1382 (CN), 1097 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 1.009-1.208 (m, 4H), 1.217-1.305 (m, 7H), 1.315-1.344 (m, 5H), 2.852-2.906 (m, 2H), 3.020-3.127 (m, 2H), 3.449 (s, 2H), 4.221-4.291 (m, 1H), 4.676-4.732 (m, 1H), 7.165-7.197 (t, 2H), 7.197-7.204 (m, 1H), 7.2287-.282 (q, 4H), 7.320-7.338 (d, *J* = 7.2 *Hz,* 2H), 7.338-7.419 (m, 1H), 7.534-7685 (t, *J* = 8 *Hz,* 1H), 7.685-7.752 (m, 1H), 7.789-7.809 (d, *J* = 4 *Hz,* 1H), 8.059 (s, 1H), 8.291-8.317 (q, 1H), 8.454-8.487 (q, 1H);^{13}C-NMR (DMSO-d_6): δ_{ppm} = 20.25, 21.78, 21.78, 22.36, 31.98, 32.75, 34.65, 35.95, 39.45, 41.85, 43.65, 52.52, 53.95, 111.65, 111.65, 117.35, 121.85, 125.35, 126.14, 126.20, 130.35, 130.35, 132.68, 132.68, 133.58, 134.65, 134.65, 134.89, 135.01, 138.32, 140.96, 160.36, 171.65, 172.33, 174.98, 175.62; MS *m/z* (%): 669 (M^{+}); Anal. Calcd. For $C_{36}H_{40}F_4N_4O_4$: C, 64.66; H, 06.03; N, 08.38; O, 09.57 %; Found: C, 64.69; H, 06.08; N, 08.31; O, 09.53 %.



Scheme 1. Synthesis of Gabapentin-amino acids hybrid peptides (11a-11j)

2.2.17 2-(2-(1-((2-(2-(4-fluorophenyl) acetamido) propanamido) methyl) cyclohexyl) acetamido)-3-phenyl-N-(pyridin-2-ylmethyl) propanamide (11g)

White solid; Yield 35 %; mp 282°C; IR (ATR): V_{max}/cm^{-1} = 3635 (NH), 2980, 2926 (2CH), 1637 (C=O), 1546 (C=C), 1342 (CN), 1047 (CF)]; ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 1.099-1.195 (m, 4H), 1.212-1.475 (m, 9H), 2.019-1.921 (m, 2H), 2.780-2.837 (m, 2H), 3.028-3.096 (m, 2H), 3.45 (s, 2H), 4.248-4.284 (m, 1H), 4.365-4.379 (d, *J* = 5.6 *Hz,* 2H), 4.624-4.683 (m, 1H), 7.005-7.078 (t, *J* = 9.2 *Hz,* 2H), 7.100-7.226 (m, 2H), 7.226-7.296 (m, 7H), 7.668-7.705 (m, 2H), 8.297-8.316 (d, *J* = 7.6 *Hz,* 2H), 8.479-8.490 (d, *J* = 4.4 *Hz,* 1H), 8.639-8.668 (t, *J* = 6 *Hz,* 1H); ^{13}C-NMR (DMSO-d_6): δ_{ppm} = 22.58, 24.01, 24.01, 25.65, 30.95, 30.95, 34.03, 39.58, 41.68, 42.15, 44.35, 45.93, 50.27, 59.34, 110.62, 110.62, 119.58, 125.62, 128.68,

129.52, 129.52, 131.58, 131.58, 133.62, 133.62, 134.65, 139.25, 140.65, 149.36, 162.69, 163.56, 172.65, 174.85, 175.02, 175.92; MS *m/z* (%): 616 (M^+); Anal. Calcd. For $C_{35}H_{42}FN_5O_4$: C, 68.27; H, 06.88; N, 11.37; O, 10.39 %; Found: C, 68.31; H, 06.84; N, 11.33; O, 10.42 %.

2.2.18 2-(2-(1-((2-(2-(4-fluorophenyl) acetamido) propanamido) methyl) cyclohexyl)acetamido)-3-phenyl-N-(pyridin-2-yl) propanamide (11h)

White solid; Yield 19 %; mp 298°C; IR (ATR): V_{max}/cm^{-1} = 3662 (NH), 2980, 2926 (2CH), 1641 (C=O), 1598 (C=C), 1300 (CN), 1049 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 0.947-1.151 (m, 4H), 1.194-1.240 (m, 8H), 1.298-1.136 (m, 1H), 1.857-2.087 (m, 2H), 2.784-2.885 (m, 2H), 2.877-3.099 (m, 2H), 3.117-3.161 (m, 2H), 4.341-4.403 (m, 1H), 4.827-4.925 (m, 1H), 5.801-5.840 (m, 1H), 7.040-7.135 (m, 3H), 7.156-7.195 (m, 1H), 7.232-7.286 (m, 5H), 7.635-7.728 (m, 1H), 7.757-7.816 (m, 1H), 8.074-8.094 (d, *J* = 8 *Hz,* 1H), 8.293-8.343 (m, 2H), 8.343-8.410 (t, 1H), 10.683-10.730 (m, 1H);^{13}C-NMR (DMSO-d_6): δ_{ppm} = 21.58, 23.68, 23.68, 24.25, 31.70, 31.70, 32.62, 39.82, 42.63, 44.68, 45.32, 50.96, 55.35, 112.95, 114.32, 114.32, 117.36, 124.62, 128.08, 129.62, 130.95, 130.95, 132.65, 132.65, 133.21, 138.32, 140.96, 147.02, 154.63, 162.69, 169.12, 170.65, 172.65, 175.68; MS *m/z* (%): 602 (M^+); Anal. Calcd. For $C_{34}H_{40}FN_5O_4$: C, 67.87; H, 06.70; N, 11.64; O, 10.64 %; Found: C, 67.92; H, 06.66; N, 11.67; O, 10.59 %.

2.2.19 N-(4-fluorobenzyl)-2-(2-(1-((2-(2-(4-fluorophenyl) acetamido) propanamido) methyl) cyclohexyl) acetamido)-3-phenyl propanamide (11i)

White solid; Yield 51 %; mp 270°C; IR (ATR): V_{max}/ cm^{-1} = 3682 (NH), 2980, 2927 (2CH), 1710 (C=O), 1537 (C=C), 1350 (CN), 1122 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 0.995-1.118 (m, 4H), 1.195-1.345 (m, 9H), 1.914-2.011 (m, 2H), 2.759-2.819 (m, 2H), 2.995-3.067 (m, 2H), 3.455 (s, 2H), 4.253-4.284 (m, 3H), 4.573-4.631 (m, 1H), 7.090-7.156 (m, 4H), 7.060-7.296 (m, 9H), 7.677-7.708 (t, *J* = 12.4 *Hz,* 1H), 8.265-8.324 (m, 2H), 8.538-8.567 (t, *J* = 6 *Hz,* 1H); ^{13}C-NMR (DMSO-d_6): δ_{ppm} = 21.65, 23.49, 23.49, 25.02, 31.78, 31.78, 34.32, 37.69, 40.12, 44.29, 45.96, 47.29, 52.62, 56.58, 113.56, 113.56, 117.25, 117.25, 128.37, 132.69, 132.69, 131.23, 131.23, 131.56, 131.56, 134.45, 134.45, 136.52, 138.467, 145.58, 164.31, 165.02, 170.65, 173.45, 174.69, 175.26; MS *m/z* (%): 633 (M^+); Anal. Calcd. For $C_{36}H_{42}F_2N_4O_4$: C, 68.34; H, 06.69; N, 08.85; O, 10.11 %; Found: C, 68.39; H, 06.64; N, 08.88; O, 10.07 %.

2.2.20 2-(2-(1-((2-(2-(4-fluorophenyl) acetamido) propanamido) methyl) cyclohexyl) acetamido)-N-(4-methoxy benzyl)-3-phenylpropanamide (11j)

White solid; Yield 51 %; mp 280°C; IR (ATR): V_{max}/cm^{-1} = 3624 (NH), 2980, 2927 (2CH), 1637 (C=O), 1546 (C=C), 1338 (CN), 1035 (CF); ^{1}H-NMR (DMSO-d_6): δ_{ppm} = 1.009-1.121 (m, 4H), 1.199-1.347 (m, 9H), 1.913-2.011 (m, 2H), 2.752-2.824 (m, 2H), 2.997-3.057 (m, 2H), 3.460 (m, 2H), 3.720-3.730 (d, *J* = 4 *Hz,* 3H), 4.210-4.290 (m, 3H), 4.582-4.639 (m, 1H), 6.826-6.855 (d, *J* = 8.4 *Hz,* 2H), 7.182-7.058 (m, 5H), 7.213-7.300 (m, 6H), 7.0685-7.716 (t, *J* = 6 *Hz,* 1H), 8.285-8.328 (m, 2H), 8.454-8.481 (t, *J* = 5.2 *Hz,* 1H); ^{13}C-NMR (DMSO-d_6): δ_{ppm} = 22.58, 24.65, 25.85, 25.85, 31.30, 32.59, 36.94, 39.36, 40.58, 44.95, 45.62, 46.03, 50.95, 52.45, 54.35, 111.56, 111.56, 114.86, 114.86, 126.82, 129.61, 129.61, 130.58, 130.58, 132.95, 132.95, 133.12, 135.28, 135.28, 137.62, 141.19, 162.68, 164.69, 170.29, 173.65, 174.56, 176.24; MS *m/z* (%): 645 (M^+); Anal. Calcd. For $C_{37}H_{45}FN_4O_5$: C, 68.92; H, 07.03; N, 08.69; O, 12.41 %; Found: C, 68.87; H, 07.08; N, 08.72; O, 12.37 %.

2.3 MCF-7 Brest cancer cell-line study [51, 52]

2.3.1 Materials

All chemicals of molecular biology grade were purchased commercially. Dulbecco's Modified Eagle's Medium (DMEM), Dulbecco's Phosphate Buffer Saline (DPBS), Fetal bovine serum (FBS) and Penicillin- Streptomycin-Neomycin (PSN) antibiotic mixture were purchased from Life Technologies, Invitrogen (USA). Cell culture grade Dimethyl sulfoxide (DMSO) and (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (USA). All other chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.3.2 Cell lines and cell culture

A human breast adenocarcinoma (MCF-7) cell line was obtained from National Center for Cell Science (NCCS), Pune, Maharashtra, India. MCF-7 cells were cultured in DMEM medium

containing L-glutamine (2mmol/l), supplemented with 10% FBS and 1% PSN, an antibiotic mixture (Life technologies, USA). The cells were cultured in a humidified condition of 5% CO2 at 37°C. The exponentially growing cells were used in the entire study.

2.3.3 Treatments of compounds

Compounds were freshly prepared in cell culture grade DMSO at the stock concentration of 100mM. Exponentially growing MCF-7 cells were treated with different Compounds (100µM) for 24 h. Cells treated with DMSO (0.1%) were considered as vehicle control.

2.3.4 Cell proliferation assay

The cell proliferation was examined by MTT assay. Briefly, 2 X 104 MCF-7 cells were treated with series of synthesized compounds for 24 h. Thereafter, the cells were washed with DPBS and incubated with MTT (0.5mg/ml) for 4 h in dark at 37˚C. After the incubation period, the MTT was removed and DMSO was added to each well. The absorbance was recorded at 570 nm with the reference wavelength of 650 nm by using a Multimode microplate reader (SpectraMax M2e, Molecular Devices, USA). The results were represented as a percentage of cell proliferation.

2.3.5 Statistical analysis

The data represented were analyzed by student t-test using Sigma Stat 2.0 statistical analysis software. The normality of data was tested by the Shapiro-Wilk test before the student t-test. p values ***p≤0.001 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic methods adapted for the synthesis of the Gabapentin-based peptide derivatives are represented in Scheme-1. Two parallel reactions were undertaken simultaneously to generate our Key Starting Material, i.e., KSM-1 & 2 (Boc protected KSM) formed by Gabapentin and L-alanine respectively by reaction with NaOH and Boc at 0-5^{o}C followed by 12 hours room temperature stirring. (Herein, we used the term overnight stirring for 12 hours RT reaction conditions). Furthermore, compound 4 was synthesized in methanol using L-phenyl alanine (3) under dropwise addition of $SOCl_2$ in the temperature range between 0^{o}C to RT, and the obtained yield was more than 60%. The C-terminal peptide bond in KSM-1 was furnished by coupling with4in the presence of EDC-HCl and DMAP in the temperature range0^{o}C to RT using DMF as a solvent to form a dipeptide scaffold (5). The deprotected hydrochloride salt of compound 5 was accomplished by dissolved it (5) in DCM followed by acidification with cold HCl and then stirring for 3-4 hours at room temperature. The diverse targeted motif, i.e., Tripeptide (7), was formed by coupling KSM-2 and6 (HCl salt) in the presence of EDC-HCl and DMAP at RT.

The deprotection with HCl salt formation of compound 7 followed by N-terminal amide bond formation of compound 8 in the presence of EDC-HCl and DMAP at below 5^{o}C temperature to afford intermediate compound 9a-9c. Basic hydrolysis of compounds 9a-9c was carried out by dissolved in THF, the addition of LiOH, and stirred at RT for overnight to form compounds 10a-10c. For the synthesis of final adducts (11a-11j), the amidic bond was generated by DMAP/EDC.HCl at RT stirring for 12 hours.

The structure of synthesized compounds 11a-11j was confirmed through various spectral data. The IR spectrum of compound 11a-11jshoweda strong absorption band at ~3635 cm^{-1} due to N-H stretching, secondary amine. The absorption band appeared at ~2980 cm^{-1} due to stretching vibrations of aromatic hydrogen. Sharp absorption peak observed at ~2927 cm^{-1} for –C-H stretching of a methylene group. The intense absorption peak at ~1656 cm^{-1}was obtained due to >C=O stretching of amide carbonyl. Moreover, absorption bands at ~1537, ~1325 cm^{-1} corresponding to C=C, C-N stretching, respectively. Compound 11a-11j showed a characteristic peak at ~1047 cm^{-1} assignable to the C-F bond. In ^{1}H NMR spectra, the appearance of multiplet at δ = ~1.95 ppm value in compounds 11a-11j is confirmed to the presence of methylene proton in the cyclohexane ring. The presence of -CO-C$\underline{H_2}$-NH- linkage was showed a singlet peak at ~2.78 ppm. Aromatic protons appeared as multiplet in the region δ = ~7.14 to ~8.45 ppm. The absorption peak at δ = ~8.66 ppm executed a proton of secondary amine in the structure. The remaining substituent's protons were in good agreement with theoretical values. ^{13}C NMR spectra helped us to identify the formation of the final adducts. The characteristic value δ= ~22 to ~34 ppm showed the presence of methyl group in cyclohexane ring. The signal obtained at δ= ~44 ppm can be assigned to the presence of

methylene carbon between cyclohexane and amide linkage. The aromatic ring carbons were in decent covenants with the theoretical values (δ= ~124 ppm). The characteristic value at δ= ~175 ppm showed carbonyl carbon (>C=O) in compounds 11a-11j. The mass spectrum revealed a molecular ion peak in compound 11a-11j at m/z = ~601 to ~668 in mass spectra; molecular ion peak was in agreement with the proposed molecular weight and elemental analysis.

In the entire chemistry, the simple and traditional methods were used to derived novel scaffolds and were further characterized by various spectroscopic techniques. The initial results provoked us to explore the synthetic applicability of 11a-11j to attain new derivatives of gabapentin-amino acid-based hybrid peptide molecules of expected anticancer activity. Thus, introducing a gabapentin core into the peptide framework may provide products with potential anticancer activity from a structure-activity perspective.

3.2 Anticancer Evaluation

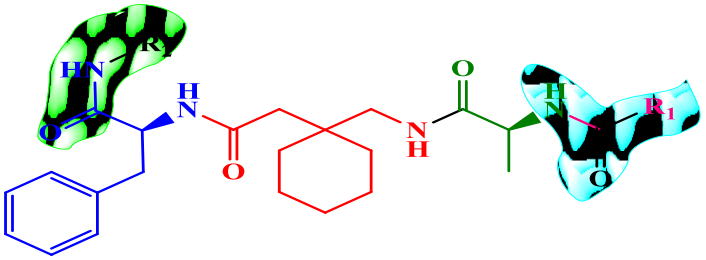
MCF-7 cancer cell line is valuable for *in vitro* breast cancer studies because of several ideal characteristics, particularly the mammary epithelium, including estrogen process and sensitivity to cytokeratin. Herein, we observed that five molecules (11a, 11b, 11h, 11i, and 11j) are more active than their corresponding peptides (11c, 11d, 11e, 11f, 11g). The biological effect is dependent on the substituent present on R_1 and R_2 (C & N terminal sides). However, a clear structure-activity relationship between the size/substitutions of the moiety and the antiproliferative effect of the MCF-7 human breast cancer cell line is not observed. The most active compound (11j), which presents a methoxybenzene and fluorobenzyl group as a substituent at two terminals of peptide core, shows significant results with % cell proliferation, 25.62% and antiproliferation/cell death, 74.38%. The following two more active compounds, 11a, and 11h, present substituents as the 2-methyl pyridine (R_2 in both) and 4-CF_3 benzene and 4-F benzyl (as R_1, respectively) showed antiproliferative activity nearly 71%. Compounds 11b, which feature a 2-pyridine (R_2) heterocycle and 4-CF_3 benzene, exhibited similar activity with the 11i, featured with 4-F benzyl (as R_1& R_2) with cell proliferation nearly 38% and 34% respectively (Table 1 and Fig 2).

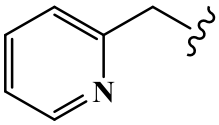
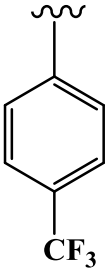
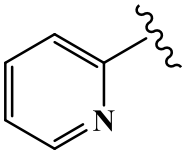
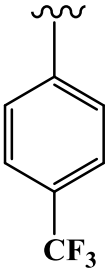
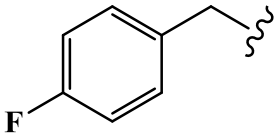
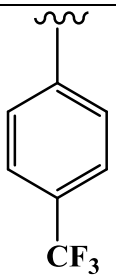
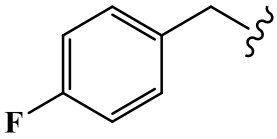
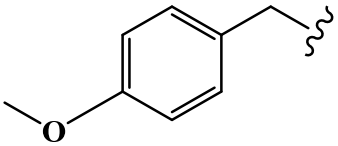
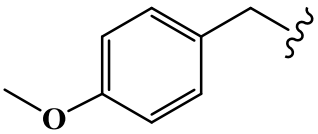
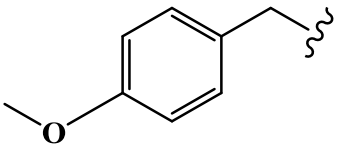
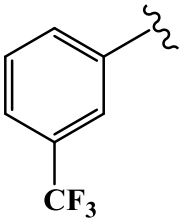
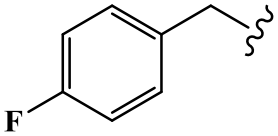
Generally, in all derivatives, the three powerful combinations: a) pyridine and 4-CF_3 benzene, b) 4-F benzyl and 4-F benzyl, and c) 4-methoxybenzene and 4-F benzyl are essential for the antitumor activity of similar kinds of molecules. Furthermore, if we compare derivatives 11a and 11b with 11f, the substitution of the benzene ring by its isosteric "3-CF_3" and "4-CF_3", the latter one has led to an improvement in the activity.

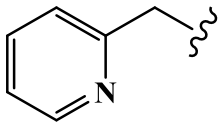
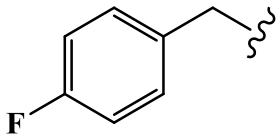
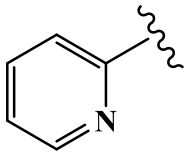
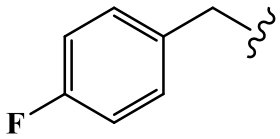
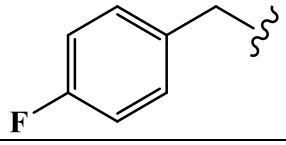
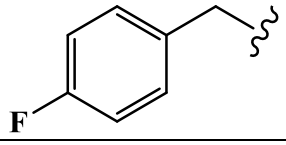
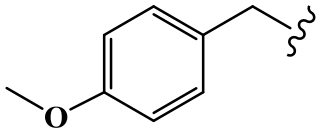
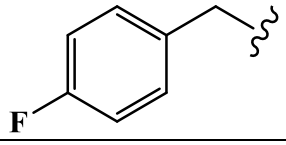
3.3 SAR (Structure-Activity Relationship) for used Substituents

Literature-based findings directed us to choose specific -R_1 and -R_2 groups to enhance the potency of derived peptides. The epidermal growth factor receptor (EGFR) is one of the transmembrane receptor tyrosine kinase ErbB family. The EGFR also stimulates vascular endothelial growth factor (VEGF), which helps to induce tumour angiogenesis and these both are important targets in cancer therapy [53]. It plays a crucial role in regulating cell proliferation, apoptosis, and migration [54]. The unique properties of fluoro organic molecules may arise due to some factors to affect the pharmacological properties of the fluorinated molecules such as electronegativity, strength in the carbon-fluorine bond, low polarizability, the smallest atomic radius of the fluorine atom, in well studied anti-cancer agents (for example, 5-fluorouracil (5-FU) and 5-fluoro-2-deoxyuridine) [55-58]. The bioavailability of the pyridine ring has been evaluated and was found to be most active against two human BC cell lines and was explained the cytotoxic potency to induce the apoptosis of MCF-7 cells (for example, Trovirdin hydrochloride as anticancer agent contains two pyridine rings) [59,60]. Previous research has suggested contrasting effects of methoxy substitutions in chemical entities: it has been reported that they may have unfavorable steric effects and the enhanced potency of some of the lead scaffold is most likely the result of methoxylation leading to improved pharmacokinetic properties and increased stability [61]. Moreover, it has been reported that upon delivery, methoxylated compounds are targeted by tumour-specific *O*-demethylases that provide free hydroxyl groups and hence an increase in redox properties [62]. Therefore, it seems reasonable that methoxylated novel compounds could make promising candidates as potential chemotherapeutic agents, providing improved pharmacological attributes, including cancer-specific activation [63].

Table 1. Result of anticancer activity of synthesized compounds (11a-11j) by MTT assay method



Entry	-R_2	-R_1	%Cell Proliferation	%Cell Death
11a			28.29	71.71
11b			38.48	61.52
11c			57.95	42.05
11d			44.17	55.83
11e			40.80	59.2
11f			64.18	35.82

Entry	-R_2	-R_1	%Cell Proliferation	%Cell Death
11g			40.85	59.15
11h			28.32	71.68
11i			34.47	65.53
11j			25.62	74.38

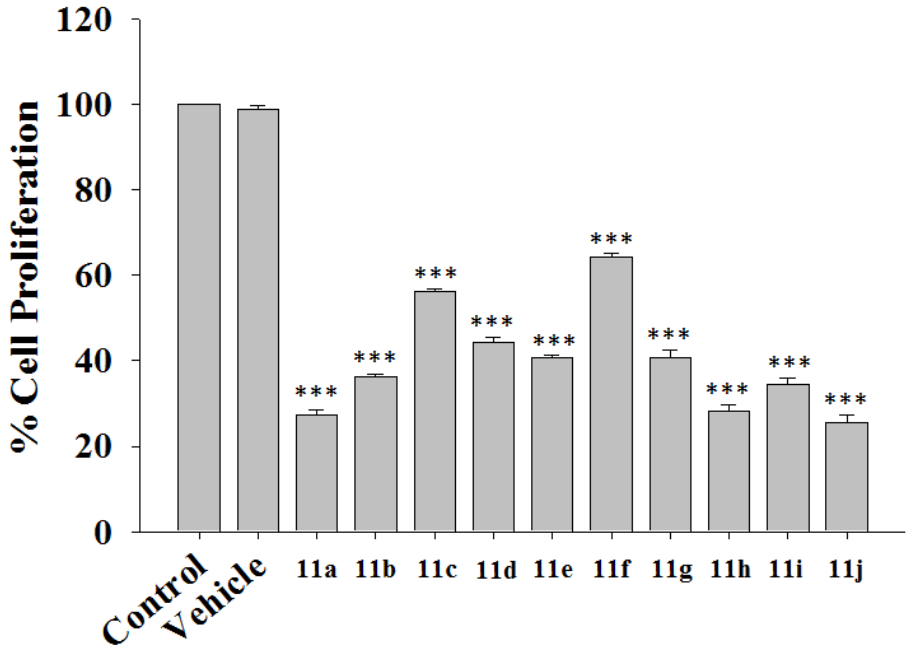


Fig. 2. Evaluation of cell proliferation by MTT assay for synthesized compounds (11a-11j) at 100 micromolar concentration; The bar graphs represent the percentage of cell proliferation. Vehicle control contained 0.1% DMSO, and control represents untreated cells. Error bars represent ± SEM of three independent experiments. Significance indicated as ***p ≤ 0.001 between untreated cells and treated cells

4. CONCLUSIONS

In this study, ten peptide derivatives having Gabapentin and chiral amino acid as core were synthesized, purified, and characterized by various analysis methods. *In vitro* cytotoxic activities were screened against breast (MCF-7) cancer cell lines by MTT assay. Five of the ten compounds showed high cytotoxic activity against tested cell lines. It was summarized that the compounds with benzylic and trifluoromethyl functionality enhanced the potency with

comparable %cell proliferation and cell death. Based on the findings in this work and their easily accessible molecular structures, compounds 11a and 11j are worthy of further biological investigations.

SUPPLEMENTARY INFORMATION

Supplementary files contain ^{1}H NMR, ^{13}C NMR, IR, and mass of 11a-11j and required NMR of intermediate compounds.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The author would like to express their utmost gratitude and appreciation to the four various organizations contributing to carrying out this research work in terms of financial, laboratory work, biological study, writing part, and end support. (Dedicated to Department of Chemistry, Gujarat University, Ahmedabad; School of Science, RK University, Rajkot; M. G. Science College, Gujarat University, Ahmedabad; KSKV Kutch University, Bhuj and Cell Biology Department, Indian Institute of Advanced Research, Koba, Gandhinagar).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Xie M, Liu D, Yang Y, Anti-cancer peptides: classification, mechanism of action, reconstruction and modification. Open Biol. 2020; 10: 200004-200013.
2. Worm DJ, Els-Heindl S, Beck-Sickinger AG, Targeting of peptide-binding receptors on cancer cells with peptide-drug conjugates. J. Pept. Sci. 2020; 112: 24171-24192.
3. Cao H, Li C, Qi W, Meng X, Tian R, Qi Y, et al. Synthesis, cytotoxicity and antitumour mechanism investigations of polyoxometalate doped silica nanospheres on breast cancer MCF-7 cells. PLoS ONE. 2017; 12: 0181018.
4. Tao Z, Shi A, Lu C, et al. Breast Cancer: Epidemiology and Etiology. Cell Biochem Biophys. 2015; 72: 333-338.
5. Cui YX, Bradbury R, Flamini V, et al. MicroRNA-7 suppresses the homing and migration potential of human endothelial cells to highly metastatic human breast cancer cells. Br J Cancer. 2017; 117: 89-101.
6. Gaidhane M, Ghatole A, Lanjewar K, Hatzade K. Innovational combination of hetero-bifunctional N-PEG quinoline scaffolds derivatives with improved anticancer activity against breast and colon cancer cell lines and P-glycoprotein, cytochrome p450 enzyme activity prediction. Turk J Chem. 2020; 44(6): 1495-1514.
7. Lunagariya J, Xiaojian Liao X, Weili Long W, et al. Cytotoxicity Study of Cyclopentapeptide Analogues of Marine Natural Product Galaxamide towards Human Breast Cancer Cells. Oxid. Med. Cell. Longev. 2017; 2017: 8392035.
8. Penke B, Bogar F, Paragi G, Gera J, Fulop L, Key Peptides and Proteins in Alzheimer's Disease. Curr. Protein Pept. Sci. 2019; 20: 577-599.
9. Bhandari D, Rafiq S, Gat Y, Gat P, Waghmare R, Kumar V, A Review on Bioactive Peptides: Physiological Functions, Bioavailability and Safety. Int. J. Pept. Res. Ther. 2020; 26: 139-150.
10. Pihlanto A, Korhonen H. Bioactive peptides and proteins. Adv Food Nutr Res. 2003; 47: 175-276.
11. Jo C, Khan FF, Khan MI, Iqbal J, Marine bioactive peptides: Types, structures, and physiological functions. Food Rev. Int. 2017; 33:44-61.
12. Hauser AS, Attwood MM, Rask-Andersen M, Schioth HB, Gloriam DE, Trends in GPCR drug discovery: new agents, targets and indications. Nat. Rev. Drug Discov. 2017; 16: 829-842.

13. Rougeot C, Rosinski-Chupin I, Mathison R, Rougeon F, Rodent submandibular gland peptide hormones and other biologically active peptides. Peptides. 2000; 21: 443-455.
14. Kim KH, Seong BL, Peptide amidation: Production of peptide hormones in vivo and in vitro. Biotechnol. Bioprocess Eng. 2001; 6: 244-251.
15. Snyder SH, Innis RB, Peptide Neurotransmitters. Annu. Rev. Biochem. 1979; 48: 755-782.
16. Leng G, Ludwig M, Neurotransmitters and peptides: whispered secrets and public announcements. J. physiol. 2008; 586: 5625-5632.
17. Kane MT, Morgan PM, Coonan C, Peptide growth factors and preimplantation development. Hum. Reprod. Update. 1997; 3: 137-157.
18. Kaniusaite M, Tailhades J, Kittilä T, et al. Understanding the early stages of peptide formation during the biosynthesis of teicoplanin and related glycopeptide antibiotics. FEBS J. 2021; 288: 507-529.
19. Narayana JL, Mishra B, Lushnikova T, et al. Two distinct amphipathic peptide antibiotics with systemic efficacy. Proc. Natl. Acad. Sci. 2020; 117: 19446-19454.
20. Hyun S, Choi Y, Jo D, et al. Proline Hinged Amphipathic α-Helical Peptide Sensitizes Gram-Negative Bacteria to Various Gram-Positive Antibiotics. J. Med. Chem. 2020; 63: 14937-14950.
21. Scuteri D, Berliocchi L, Rombola L, et al. Effects of Aging on Formalin-Induced Pain Behavior and Analgesic Activity of Gabapentin in C57BL/6 Mice. Front. Pharmacol. 2020; 11: 663-669.
22. Chen WF, Huang SY, Liao CY, Sung CS, Chen JY, Wen ZH, The use of the antimicrobial peptide piscidin (PCD)-1 as a novel anti-nociceptive agent. Biomaterials. 2015; 53: 1-11.
23. Henning RJ, Sawmiller DR, Vasoactive intestinal peptide: cardiovascular effects. Cardiovasc. Res. 2001; 49: 27-37.
24. Banks WA, The CNS as a target for peptides and peptide-based drugs. Expert Opin. Drug Deliv. 2006; 3: 707-712.
25. McGonigle P, Peptide therapeutics for CNS indications. Biochem. Pharmacology. 2012; 83: 559-566.
26. Playford RJ, Macdonald CE, Johnson WS, Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. Am. J. Clin. Nutr. 2000; 72: 5-14.
27. Klausen U, Holmberg S, Holmström, et al. Novel Strategies for Peptide-Based Vaccines in Hematological Malignancies. Front. Immunol. 2018; 9: 2264-2271.
28. Braide-Moncoeur O, Tran NT, Long JR, Peptide-based synthetic pulmonary surfactant for the treatment of respiratory distress disorders. Curr. Opin. Chem. Biol. 2016; 32: 22-28.
29. Yaghoubi A, Khazaei M, Avan A, Hasanian SM, Cho WC, Soleimanpour S, p28 Bacterial Peptide, as an Anticancer Agent. Front. Oncol. 2020; 10: 1303-1312.
30. Horibe T, Kohno M, Haramoto M, Ohara K, Kawakami K, Designed hybrid TPR peptide targeting Hsp90 as a novel anticancer agent. J. Transl. Med. 2011; 9: 1-12.
31. Lu S, Wang H, Sheng Y, Liu M, Chen P, Molecular binding of self-assembling peptide EAK16-II with anticancer agent EPT and its implication in cancer cell inhibition. J. Control. Release. 2012; 160: 33-40.
32. Reddy KM, Mallikarjunasarma D, Bulliraju K, Sreelatha V, Kumari YB, Dandala R, Ananda K, Solid Phase and Solution Phase Synthesis of Gamma Amino Acid Homo-Oligomers and Mixed Oligomers. Int J Pept Res Ther. 2011; 17: 113-121.
33. Shah SB, Hariharan U, Bhargava AK, Recent trends in anaesthesia and analgesia for breast cancer surgery. Trends Anaesth. Crit. Care. 2018; 20: 11-20.
34. Irizarry MC, Udaltsova N, Webb DJ, Boudiaf N, Logie J, Habel LA, Friedman GD. Risk of cancer in patients exposed to gabapentin in two electronic medical record systems. Pharmacoepidemiol Drug Saf. 2012; 21: 214–225.
35. Guo L, Zhang W, Reidenbach AG, et al. Characteristic structural parameters for the γ-peptide 14-helix: importance of subunit preorganization. Angew. Chem. 2011; 50: 5843-5846.
36. Chatterjee S, Vasudev PG, Raghothama S, Ramakrishnan C, Shamala N, Balaram P, Expanding the Peptide β-Turn in αγ Hybrid Sequences: 12 Atom Hydrogen Bonded Helical and Hairpin Turns. J Am. Chem. Soc. 2009; 131: 5956-5965.
37. Vasudev PG, Chatterjee S, Ananda K, Shamala N, Balaram P, Hybrid αγ

polypeptides: structural characterization of a C12/C10 helix with alternating hydrogen-bond polarity. Angew. Chem. 2008; 47: 6430-6432.
38. Iryna O. Lebedyeva IO, David A. Ostrov DA, et al. Gabapentin hybrid peptides and bioconjugates. Bioorg. Med. Chem. 2014; 22: 1479-1486.
39. Jiang Y, Li J, Lin H, Huang, et al. The efficacy of gabapentin in reducing pain intensity and morphine consumption after breast cancer surgery. Medicine. 2018; 97: 11581-11591.
40. Rautio J, Kumpulainen H, Heimbach T, et al. Prodrugs: design and clinical applications. Nat Rev Drug Discov. 2008; 7: 255-270.
41. Liu J, Bu W, Shi J, Chemical Design and Synthesis of Functionalized Probes for Imaging and Treating Tumor Hypoxia. Chem. Rev. 2017; 117: 6160-6224.
42. Pan X, Xu J, Jia X, Research progress evaluating the function and mechanism of anti-tumor peptides. Cancer Manag. Res. 2020; 12: 397-409.
43. Xie M, Liu D, Yang Y, Anti-cancer peptides: classification, mechanism of action, reconstruction and modification. Open Biol. 2020; 10: 200004-200013.
44. Qiao X, Wang Y, Yu H, Progress in the mechanisms of anticancer peptides. Chin. J. Biotechnol. 2019; 35: 1391-1400.
45. Hilchie AL, Hoskin DW, Coombs MR, Anticancer activities of natural and synthetic peptides. Antimicrob. Pept. 2019; 1117: 131-147.
46. Pan X, Xu J, Jia X, Research progress evaluating the function and mechanism of antitumor peptides. Cancer Manag. Res. 2020; 12: 397-409.
47. Vasudev PG, Chatterjee S, Shamala N, Balaram P, Gabapentin: A Stereochemically Constrained γ Amino Acid Residue in Hybrid Peptide Design. Acc. Chem. Res. 2009; 42: 1628-1639.
48. Pandya MK, Dholaria PV, Kapadiya KM, Synthesis of Lanso Aminopyrimidines as Dominant Chemotherapeutic Agents for Leukaemia. Russ. J. Org. Chem. 2020; 56: 1995-2004.
49. Pandya MK, Kapadiya KM. A Study on Leukemic and Non-small Cell Lung Cancer Efficacy of Novel Isoxazoles Synthesized by Microwave Irradiation. Lett Drug Des Discov. 2021; 18: 1-9.
50. Kapadiya KM, Khunt RC, Discovery of Hybrid Purine-quinoline Molecules and Their Cytotoxic Evaluation. Lett Drug Des Discov. 2019; 16: 21-28.
51. Bondock S, Alqahtani S, Fouda A, Synthesis and anticancer evaluation of some new pyrazolo[3,4-d][1,2,3]triazin-4-ones, pyrazolo[1,5-a]pyrimidines and imidazo[1,2-b]pyrazoles clubbed with carbazole. J Heterocyclic Chem., 2021; 58: 56-73.
52. Vaidya F, Sharma R, Shaikh S, Ray D, Aswal V, Pathak C, Pluronic micelles encapsulatedcurcumin manifests apoptotic cell death and inhibits pro-inflammatory cytokines in human breast adenocarcinoma cells. Cancer Reports., 2019; 2: 1133-1149.
53. Liew S, Malagobadan S, Arshad N and Nagoor N.A Review of the Structure–Activity Relationship of Natural and Synthetic Antimetastatic Compounds. Biomolecules. 2020;10 (1): 138-166.
54. Wieduwilt M and Moasser M.The epidermal growth factor receptor family: Biology driving targeted therapeutics. Cell. Mol. Life Sci. 2008; 65(10): 1566-1584.
55. Ghorab M, Alsaid M and El-Gaby M.Antimicrobial and anticancer activity of some novel fluorinated thiourea derivatives carrying sulfonamide moieties: synthesis, biological evaluation and molecular docking. Chemistry Central Journal. 2017;11(1):32-46.
56. Hu J and Zeng Y. Recent advances in green fluorine chemistry. Rep Org Chem.2015; 5:19-39.
57. Müller K, Faeh C and Diederich F. Fluorine in pharmaceuticals: looking beyond intuition. Science. 2007;317(5846):1881-1886.
58. Chkanikov ND, Golubev AS and Belyaeva EV. New Approaches to the Synthesis of CF2X-Substituted Heterocyclic Antitumor Cytostatic Agents. INEOS OPEN. 2019; 2(2): 33-40
59. Pang C, Sun C, Wang J, Xiao D, Ding L and Bu H.Novel 2H-pyrazolo[4,3-c]hexahydropyridine derivatives: synthesis, crystal structure, fluorescence properties and cytotoxicity evaluation against human breast cancer cells. Sci. China: Chem. 2013; 56:702-715.
60. Liu J, Ming B, Gong G, Wang D, Bao G and Yu L.Current research on anti-breast cancer synthetic compounds. RSC Adv. 2018; 8: 4386-4416.

61. Martínez-Pérez C, Ward C and Turnbull A.Antitumour activity of the novel flavonoid Oncamex in preclinical breast cancer models. Br J Cancer. 2016;114(8): 905-916.
62. Lavigne J, Goodman J, Fonong T, Odwin S, He P, Roberts D and Yager J. The effects of catechol-O-methyltransferase inhibition on estrogen metabolite and oxidative damage levels in estradiol-treated MCF-7 cells. Cancer Res.2001; 61(20):7488-7494.
63. Arroo R,Androutsopoulos V, Beresford K, Ruparelia K, Surichan S, Wilsher N and Potter G. Phytoestrogens as natural prodrugs in cancer prevention: dietary flavonoids. Phytochem Rev.2009; 8(2):375-386.

© 2021 Pansuriya et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75639