Research in Pharmaceutical Sciences, August 2019; 14(4): 286-292
Received: November 2018
Accepted: May 2019

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Preparation of pentagamaboronon-0 and its fructose and sorbitol complexes as boron carrier for boron neutron capture therapy (BNCT) application

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Abstract

Development of specific and selective boron carriers is indispensable for boron neutron capture therapy (BNCT) application. Pentagamaboronon-0 (PGB-0) is a promising candidate as boron carrier compound due to the low but selective cytotoxicity in breast cancer cells. Formerly we reported synthesis of PGB-0 which was ineffective due to its low aqueous solubility. In the present study, we, therefore, introduced the new PGB-0 preparation complexed with sugars to increase its solubility in water. By synthesizing at room temperature and using flash chromatography for the purification, we produced PGB-0 with a yield of 40%. PGB-0 fructose complex (PGB-0-F) and PGB-0 sorbitol complex (PGB-0-Sor) were obtained with smaller particle size compared to PGB-0 suspension in water. Based on the MTT assay, the cytotoxicity of PGB-0-F and PGB-0-Sor were higher than PGB-0 even though still categorized as low cytotoxic agents. In conclusion, we provided PGB-0 with a new method and improved its solubility in water. Further investigations are still needed to develop more efficient PGB-0 as boron carrier for BNCT in various cancers.

Keywords: BNCT; Boron carrier; Fructose and sorbitol complexes; PGB-0.

INTRODUCTION

For the successful boron neutron captured therapy (BNCT), an ideal boron carrier is needed. Although numerous boron carriers has been synthesized, there are only sodium borocaptate (BSH) and boronophenylalanine (BPA) that have entered clinical trial as boron carrier for BNCT and promising enough for ensuring clinical biodistribution and therapy (1). In addition, due to the non-selective effect of BPA which highly accumulated in the kidney (2-4), the boron carrier development is still a big challenge to find the more selective and specific target. Pentagamaboronon-0 (PGB-0) (Fig. 1), a code name for curcumin analog bearing boronic acid group on the benzene structure, is proposed as the new candidate of boron carrier.

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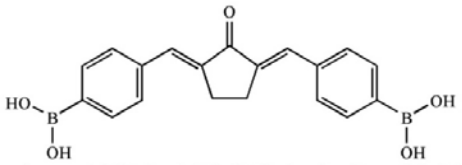

Fig. 1. Pentagamaboronon-0 or 2,5-bis (4-dihydroxyboryl benzylidine) cyclopentanone structure.

The low cytotoxic effect (IC_{50} > 200 μM) on HER2-expressed breast cancer cells, no toxic effect against normal fibroblast cells, and comparable with lapatinib and curcumin on the interaction with HER2 are the current modality of PGB-0 as novel boron carrier (5).

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Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362.263552

On the contrary, the very low water solubility remains an issue on the application of most curcumin analogues including PGB-0 (6,7). Hence, the modification of synthesis method and developing of more soluble derivatives of PGB-0 needs to be introduced.

The previous synthesis method of PGB-0 only gives 30% yields at low temperature condition. Therefore, performing reaction at higher temperature is considered more appropriate method to improve percent yield of PGB-0. Several ways of improving the solubility of curcumin analogue such as liposomal system, and sodium or potassium salt modification have been applied (8-11). However, the more soluble curcumin analogues such as K-PGV-0 correlated with the increasing of cytotoxicity which is prohibited for the development of boron carrier (12). Another possibility on improving PGB-0 solubility is using of fructose and sorbitol which is successfully applied for BPA (4,13). In this study, we synthesized PGB-0 at higher temperature and purified the PGB-0 by flash chromatography. We are also the first researchers who applied fructose and sorbitol to increase the solubility of curcumin analogues. Cytotoxic evaluation of the fructose and sorbitol complex of PGB-0 was also conducted to decide the possibility of using PGB-0 and the fructose or sorbitol complex as boron carrier for BNCT.

MATERIALS AND METHODS

Materials

All of the materials and solvents used in this experiment were classified as analytical reagent grade unless otherwise specified. Thin-layer chromatography (TLC) was conducted on glass plate silica gel 60 F254 (Merck KGaA, Darmstadt, Germany) then visualized by UV 254 and 366 nm. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 (^{13}C)-NMR spectra were recorded on a JMTC-400/54/SS (400 MHz, JEOL Ltd., Tokyo, Japan) spectrometer. Infrared (IR) spectra were determined as KBr pellets of the solids on an FT-IR spectrophotometer (Perkin Elmer). Waltham, Massachusetts, USA).ESI–MS measurements were conducted by using a XevoQTof (Waters Corporation, Massachusetts, US). Flash chromatography was performed using a Biotage system (Biotage, Sweden) and melting points were determined using a melting point apparatus (BUCHI Labortechnik AG, Flawil, Switzerland).

General procedure for the synthesis and purification of pentagamaboronon-0

Synthesis of PGB-0 was conducted according to the method proposed by Uttomo *et al.* (5) by modifying the temperature condition from 0 °C to room temperature. Cyclopentanone (270 μL, 3 mmol) was dissolved in ethanol. Then, 2 mL of NaOH 10% was added to the solution. One g (6 mmol) of 4-formylphenyl boronic acid was added to the solution, and then stirred for 1 h. HCl 10% was added dropwise until the pH of the solution was reached to 7. The product was filtered by Whatmann paper then purified by flash chromatography. One gram of impure PGB-0 was dissolved in methanol then mixed with 5 g ISOLUTE® HM-N. The solvent was then dried up by using rotary evaporator. Sample was then applied to the top of silica gel (mesh 0.063-0.200 mm) column. Separation was conducted by using chloroform:acetonitrile as separating solvent with gradient system and using 200-400 nm as the wavelength for detection. The purity of the product was monitored by TLC with silica gel 60 F254 (Merck, Darmstadt, Germany) as stationary phase and chloroform:methanol (9:1) as the mobile phase.

Preparation of pentagamaborono-0 in complex with fructose and sorbitol

Boron compounds in complex with fructose or sorbitol was prepared according to the method of Shull *et al.* (13) with ratio modification. An amount of 33 mg of fructose (0.184 mmol) or 34 mg of sorbitol (0.184 mmol) was diluted in 23 mL of water. Eight mg of PGB-0 (0.023 mmol) was added. After stirring for 1 h, NaOH 10% was added dropwise until pH of 9 was achieved andsubsequently stirred again at room temperature for 1 h. Solution pH was reduced to between 7.5 and 8.0 by addition of Dowex® 50WX4 resin then lyophilized by freeze-drying.

Cell culture

MCF-7/HER2 cell line was kindly gifted from Prof. Yoshio Inouye (Department of Surgery, Toho University School of Medicine, Japan) through Prof. Masashi Kawaichi (NAIST, Japan). The 4T1, T47D, VERO, and NIH3T3 cells were kindly given by Prof. Masashi Kawaichi (NAIST, Japan). All of cells were cultured in Dulbecco's modified eagles medium (DMEM) high glucose (Gibco, New York, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco, New York, USA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium bicarbonate, 150 IU/mL penicillin, 150 µg/mL streptomycin (Gibco, New York, USA) and 1.25 µg/mL amphotericin B (Gibco, New York, USA). Cells were grown at 37 °C with 5% CO_2 in a humidified atmosphere.

MTT assay

The cytotoxicity test was performed by using 3-(4,5-dimetil thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (14) with slight modification. MCF-7/HER2 and 4T1 cells (2×10^3) were grown in 96-well microplates and cultured for 24 h. Cells were treated with PGB-0, PGB-0 fructose (PGB-0-F), and PGB-0 sorbitol (PGB-0-Sor) at several concentrations. After treatment, cells were stained by 0.5 mg/mL of MTT (Biovision, California, USA) and incubated further for 2 h. Cells were lysed using sodium dodecyl sulfate (SDS) stopper containing 0.01 N HCl and incubated in the dark condition overnight. The absorbance was measured by ELISA reader plate at 595 nm (Corona SH-1000, Corona Electric Co. Ltd., Ibaraki-ken, Japan). The absorbance was converted to percent of cell viability by comparing the treated group with the control. Linear regression between concentration and percent of cell viability giving the equation $y = Bx + A$ were used to calculate IC_{50} value.

RESULTS

Synthesis of pentagamaboronon-0

In this research we modified the reaction condition at room temperature and purified the product by flash chromatography. By using this method, we successfully obtained the PGB-0 with the yield of 40% and the purity was confirmed by TLC with silica gel GF254 as stationary phase and chloroform:methanol (9:1) as mobile phase (Fig. 2). This method produced higher yield than previous method.

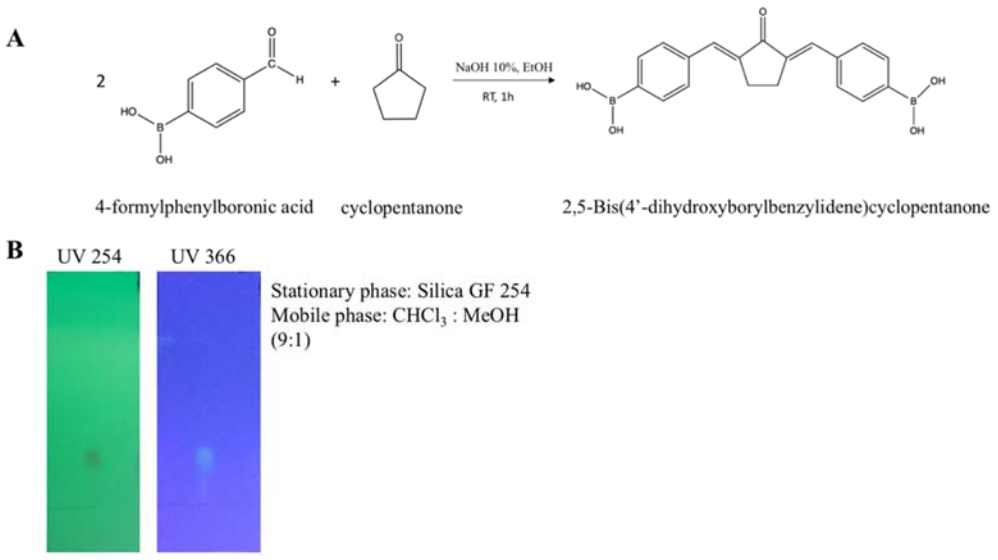

Fig. 2. (A) Synthesis scheme of pentagamaboronon-0 and (B) thin layer chromatography profile of pentagamaboron under UV 254 and 366 nm.

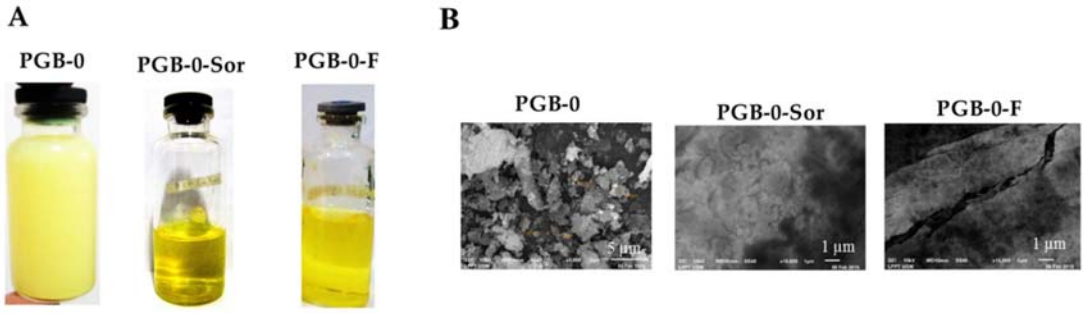

Fig. 3. (A) PGB-0 and its complexes in aqueous solution under visible light and (B) scanning electron microscope analysis of PGB-0 and its complexes. PGB, Pentagamaboronon; F, fructose; and Sor, sorbitol

Table 1. Particle size profile of PGB-0 and its complexes.

Samples	Particle size (nm)	Isoelectric points	Zeta potential (mV)	Electrophoretic mobility (cm2/Vs)
PGB-0	2883.7	0.662	-15.3	-0.000118
PGB-0-Sor	182.0	0.227	-27.9	-0.000216
PGB-0-F	373.5	0.148	-38.3	-0.000296

PGB, Pentagamaboronon; PGB-0-F, PGB-0 fructose; PGB-0-Sor, PGB-0 sorbitol.

PGB-0 was obtained as a yellow powder and decomposed at 253 °C. The molecular formula was determined as $C_{19}H_{18}B_2O_5$ by ESI^+, which showed $(M+H)^+$ ion peak at 349.1426 (calculated for $C_{19}H_{19}B_2O_5$: 349.1426). IR (KBr cm^{-1}): 1619 cm^{-1} (C=O), 1313 cm^{-1} (B-O), 3367 cm^{-1} (-OH), 1546 cm^{-1} (C=C aromatic), 1681 cm^{-1} (C=C alkene). ^{1}H-NMR (500 MHz, DMSO-d_6), δ (ppm): 3.4 (s, 4H, $2CH_2$ alkane), 7.4 (s, 2H), 7.6 (d, 2H, 2CH aromatic, J = 8 Hz), 7.9 (d, 2H, 2CH=CH aromatic, J = 8 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6), δ (ppm): 26.1 (CH_2 alkane), 48.6 (2CH alkene), 129.7 (C-B), 132.5 (2CH=CH aromatic), 134.5 (2CH=CH aromatic), 136.8 (CH=CH aromatic), 138.4 (2CH alkane), 195.4 (C=O).

Complexation of pentagamaboronon-0 by fructose and sorbitol

Strategy by using fructose and sorbitol was widely used to improve the solubility of BPA in water (4,13). The presence of boronic acid groups on PGB-0 possibly can form a fructose or sorbitol complex. After performing several formulas, the ratio of PGB-0 to fructose or sorbitol as 1:8 was found more appropriate. The solution obtained had a yellow color (Fig. 3A). Both PGB-0-F and PGB-0-Sor possessed smaller particle size than PGB-0 with the size of 373.5 and 182.0, respectively (Table 1). Based on the particle size, PGB-0-F and PGB-0-Sor were categorized as possible nano-specific properties (15). In order to obtain the solid form, PGB-0-F and PGB-0-Sor were lyophilized using a freeze dryer. However, after scanning electron microscope analysis, we found that the PGB-0-F and PGB-0-Sor turned into caramelized form (Fig. 3B). It was due to the presence of fructose and sorbitol in a high amount dominating the form of the caramel after freeze drying (16). Then the cytotoxicity of PGB-0, PGB-0-F, and PGB-0-Sor were evaluated against several types of cancer cells as pointed out earlier.

Cytotoxicity study of pentagamaboronon-0 and its complexes

Cyctotoxicity evaluation was conducted to consider the ability of our compounds as boron carrier. Our results showed that PGB-0 and its complexes showed cytotoxic effects in a dose dependent manner on MCF-7/HER2, 4T1, and T47D cells but exhibited no cytotoxic effects againts VERO and NIH3T3 cells (Fig. 4 and Table 2) PGB-0-F cytotoxicity was more potent than PGB-0 and PGB-0-Sor on MCF-7/HER2 cells with the respective IC_{50} values of 40, 62, and 75 μM. Similar pattern also observed on T47D with PGB-0-F as the most potent compound. On the contrary, the cytotoxicity

of PGB-0-F and PGB-0-Sor were less than PGB-0 on 4T1 cells with IC_{50} values of 84, 96, and 50μM, respectively. Compared to BPA which showed IC_{50} > 4 mM (17), PGB-0 and its complexes tended to possess more potent cytotoxicity. However due to the IC_{50} > 50 μM, PGB-0 and its complexes still considered as low cytotoxic agents (18).

DISCUSSION

This new approach of PGB-0 synthesis led to the more effective and efficient PGB-0 production. The reaction steps are also relatively short and simple compared to the synthesis of BPA which required five steps reaction from 4-formylphenyl boronic acid (19,20). For further development of boron carrier, it needs to consider the use of ^{10}B which is relatively cheap and also available for the successful BNCT application (21). Using of fructose and sorbitol also successfully increased the solubility of PGB-0 by reducing the particle size. Compared to the liposomal and salt modification, the fructose and sorbitol complexes provide more eligible and efficient BNCT application due to the using of simple materials for the production. The mechanism of complex formation was possibly similar to other metals which act as chelating agent (22,23). Another mechanism was the formation of hydrogen binding between carbons from benzene and boron (13,24).

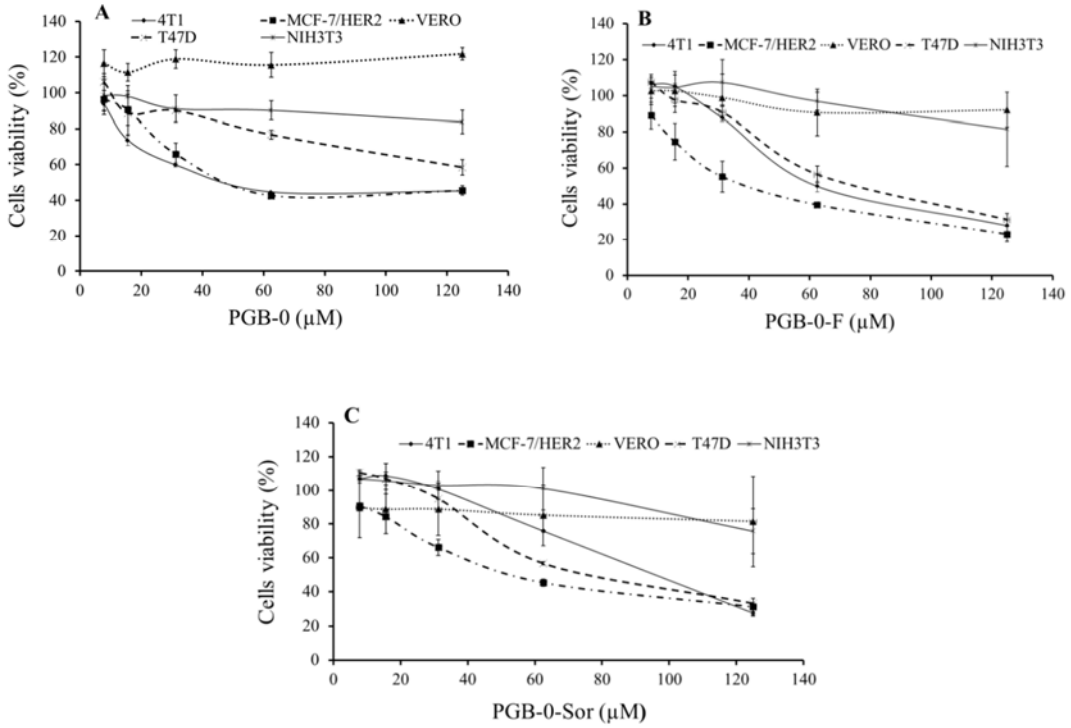

Fig. 4. Cytotoxicity of (A) PGB-0, (B) PGB-0-F, and (C) PGB-0-Sor on several types of cell lines. Cells were treated by PGB-0 and its complexes for 24 h as described in the method section. The graph represents mean ± SD from three independent experiments. PGB, Pentagamaboronon; PGB-0-F, PGB-0 fructose; PGB-0-Sor, PGB-0 sorbitol.

Table 2. Cytotoxicity of PGB-0 and its complexes on several types of cell lines.

Compounds	IC_{50} (μM)				
	MCF-7/HER2	4T1	T47D	VERO	NIH3T3
PGB-0	62 ± 4.9	50 ± 2.1	170 ± 28.3	> 250	> 250
PGB-0-F	40 ± 4.6	84 ± 1.4	80 ± 1.4	> 250	> 250
PGB-0-Sor	75 ± 3.5	96 ± 1.4	95 ± 3.5	> 250	200 ± 23.8

PGB, Pentagamaboronon; PGB-0-F, PGB-0 fructose; PGB-0-Sor, PGB-0 sorbitol.

Since it was conformed that, BCP exhibited low toxicity on normal cells in the BNCT application (12), our synthesized compounds were examined *in vitro* for their cytotoxicity against breast cancer cells such as MCF-7/HER2 ($HER2^+$, ER^+, PR^+), 4T1 ($HER2^-$, ER^-, PR^-), and T47D cells (ER^+, PR^+), while for the selectivity toward normal cells, we used VERO and NIH3T3 cells. $HER2^+$ breast cancer was found in 30% cases. HER2 itself is a class of tyrosine kinase receptor responsible for cancer progression, proliferation, and metastasis (25,26). On the other hand, triple negative breast cancer cells also caused the high mortality on cancer patients due to the inability of hormonal and targeted chemotherapy to treat this cancer types (27,28). The results showed that both of our compounds exerted selective cytotoxic effects against several breast cancer cell lines, while they showed no toxic effect against normal cell models. Cellular cytotoxicity is also possibly due to the increase of uptake in the cancer cells (29,30). Therefore, the measurement of boron microdistribution needs to be conducted.

CONCLUSIONS

In conclusion, new preparation method for PGB-0 synthesis was successfully applied. Compared to the previous method (5), the current method provided higher yields and purity. PGB-0 also successfully modified into PGB-0-F and PGB-0-Sor complexes which were categorized as nano-specific properties. Though fructose and sorbitol complexes increased the cytotoxicity of PGB-0 on MCF-7/HER2 and 4T1 but still considered as low cytotoxic agents. Therefore, PGB-0 and its fructose and sorbitol complex are potential to be developed as boron carrier for BNCT application against breast cancer.

ACKNOWLEDGEMENTS

This project was financially supported by the Ministry of Research, Technology and Higher Education, the Republic of Indonesia in collaboration with BNCT Research Centre, Osaka Prefecture University, Osaka, Japan.

REFERENCES

1. Barth RF, Zhang Z, Liu T. A realistic appraisal of boron neutron capture therapy as a cancer treatment modality. Cancer Commun (Lond). 2018;38(1): 36-42.
2. Takahara K, Inamoto T, Minami K, Yoshikawa Y, Takai T, Ibuki N, *et al*. The anti-proliferative effect of boron neutron capture therapy in a prostate cancer xenograft model. PLoS One. 2015;10(9):e0136981.
3. Bendel P, Margalit R, Salomon Y. Optimized 1H MRS and MRSI methods for the in vivo detection of boronophenylalanine. Magn Reson Med. 2005;53(5):1166-1171.
4. Watanabe T, Hattori Y, Ohta Y, Ishimura M, Nakagawa Y, Sanada Y, *et al*. Comparison of the pharmacokinetics between L-BPA and L-FBPA using the same administration dose and protocol: a validation study for the theranostic approach using [18F]-L-FBPA positron emission tomography in boron neutron capture therapy. BMC Cancer. 2016;16(1):859-868.
5. Utomo RY, Putri H, Pudjono P, Susidarti RA, Jenie RI, Meiyanto E. Synthesis and cytotoxic activity of 2,5-bis(4-boronic acid)benzylidine cyclopentanone on HER2 overexpressed-cancer cells. Indones J Pharm. 2017;28(2):74-81.
6. Cunico LP, Acosta MC, Turner C. Experimental measurements and modeling of curcumin solubility in CO2-expanded ethanol. J Supercrit Fluids. 2017;130:381-388.
7. Xu DH, Wang S, Mei XT, Luo XJ, Xu SB. [Studies on solubility enhancement of curcumin by Polyvinylpyrrolidione K30]. Zhong Yao Cai. 2008;31(3):438-442.
8. Istyastono EP, Siwi SU, Utama AA, Supardjan AM. Synthesis new potential anti-inflammatory agent sodium salt of pentagamavunon-0. Indones J Chem. 2004;4(3):180-185.
9. Margono SA, Verawati EY. Synthesis of potassium pentagamavunonat-0 using potassium hydroxyde and solvent tertrahydrofuranethanol. Indonesian J Pharm. 2005;16:239-245.
10. Feng T, Wei Y, Lee RJ, Zhao L. Liposomal curcumin and its application in cancer. Int J Nanomedicine. 2017;12:6027-6044.
11. Emami J, Mohiti H, Hamishehkar H, Varshosaz J. Formulation and optimization of solid lipid nanoparticle formulation for pulmonary delivery of budesonide using Taguchi and Box-Behnken design. Res Pharm Sci. 2014;10(1):17-33.
12. Barth RF, Vicente MGH, Harling OK, Kiger WS, Riley KJ, Binns PJ, *et al*. Current status of boron neutron capture therapy of high grade gliomas and recurrent head and neck cancer. Radiat Oncol. 2012;7(1):146-166.

13. Shull BK, Spielvogel DE, Head G, Gopalaswamy R, Sankar S, Devito K. Studies on the structure of the complex of the boron neutron capture therapy drug, L-p-boronophenylalanine, with fructose and related carbohydrates: chemical and 13C NMR evidence for the beta-D-fructofuranose 2,3,6-(p-phenylalanylorthoboronate) structure. J Pharm Sci. 2000;89(2):215-222.
14. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1):55-63.
15. Yinghuai Z, Cheng Yan K, Maguire JA, Hosmane NS. Recent developments in boron neutron capture therapy driven by nanotechnology. Curr Chem Biol. 2007;1(2):147-163.
16. Roos Y, Karel M. Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. J Food Sci. 1991;56(1):38-43.
17. Hattori Y, Kurihara K, Kondoh H, Asano T, Kirihata M, Yamaguchi Y, *et al*. Biological evaluation of fluorinated p-boronophenylalanine derivatives as a boron carrier. Protein Pept Lett. 2007;14(3): 269-272.
18. Sebaugh JL. Guidelines for accurate EC50/IC50 estimation. Pharm Stat. 2011;10(2):128-134.
19. Kirihara M, Morimoto T, Ichimoto I. A Simple and improved synthesis of p-boronophenylalanine, a boron carrier for the boron-neutron capture therapy. Biosci Biotechnol Biochem. 1993;57(11):1940-1941.
20. Harada S, Kajihara R, Muramoto R, Jutabha P, Anzai N, Nemoto T. Catalytic asymmetric synthesis of α-methyl-p-boronophenylalanine. Bioorg Med Chem Lett. 2018;28(10):1915-1918.
21. Soloway AH, Tjarks W, Barnum BA, Rong FG, Barth RF, Codogni IM, *et al*. The chemistry of neutron capture therapy. Chem Rev. 1998;98(4):1515-1562.
22. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. 2014;9(6):385-406.
23. Mousavi S, Forootanfar H, Faramarzi M, Ameri A, Shakibaie M. Metal nanoparticle production assisted by α-amylase. Res Pharm Sci. 2012;7(5):S218.
24. Chen BG, Zhang MY, Zhao YY, Zhang J, Sun CC. Structural and electronic properties of BPA-fructose complex used in boron neutron capture therapy. J Mol Struct THEOCHEM. 2006;766(1):35-40.
25. English DP, Roque DM, Santin AD. HER2 expression beyond breast cancer: therapeutic implications for gynecologic malignancies. Mol Diagn Ther. 2013;17(2):85-99.
26. Mitri Z, Constantine T, O'Regan R. The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy. Chemother Res Pract. 2012;2012. Article ID 743193. DOI: 10.1155/2012/743193.
27. Gupta S. Triple negative breast cancer: A continuing challenge. Indian J Med Paediatr Oncol. 2013;34(1):1-2.
28. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13(11):674-690.
29. Hattori Y, Kusaka S, Mukumoto M, Uehara K, Asano T, Suzuki M, *et al*. Biological evaluation of dodecaborate-containing L-amino acids for boron neutron capture therapy. J Med Chem. 2012;55(15):6980-6984.
30. Miyahara T, Ueda K, Akaboshi M, Shimada Y, Imamura M, Utsumi H. Hyperthermic enhancement of cytotoxicity and increased uptake of cis-diamminedichloroplatinum(ii) in cultured human esophageal cancer cells. Jpn J Cancer Res. 1993;84(3):336-340.