ORIGINAL ARTICLE

Synthesis and cytotoxicity of the boron carrier pentagamaboronon-0-ol for boron neutron capture therapy against breast cancer

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ABSTRACT

Boronic acid-containing curcumin analog, pentagamaboronon-0 (PGB-0), acts as a potential boron-carrier agent but has limited water solubility. Thus, a new compound (PGB-0-ol) with better chemical and pharmacological properties than PGB-0 has been synthesized. Molecular docking was performed using a molecular operating environment. Prediction of PGB-0-ol absorption, distribution, metabolism, and excretion (ADME) was performed using pkCSM software. PGB-0-ol was synthesized by adding $NaBH_4$ to PGB-0 and stirring for 1 h. The crude PGB-0-ol was purified using preparative layer chromatography. Cell viability was evaluated using the trypan blue exclusion assay. In comparison to PGB-0 based on molecular docking study, PGB-0-ol could interact in with several cancer biomarkers, such as human epidermal growth factor2 epidermal growth factor receptor, IκB kinase, folate receptor-α, and integrin $\alpha_v\beta_3$. PGB-0-ol also showed an improved ADME profile because of its higher water solubility than PGB-0. PGB-0-ol was synthesized by selective ketone reduction of PGB-0 into primary alcohol by sodium borohydrate producing 30% yield. The cytotoxicity of PGB-0-ol against several breast cancer cells was lower than that of PGB-0. The novel compound PGB-0-ol was synthesized using simple steps. PGB-0-ol has low cytotoxicity against breast cancer cells and could be applied in boron neutron capture therapy as a boron carrier.

Key words: Boron carrier, cytotoxicity, molecular docking, pentagamaboronon-0-ol, synthesis

INTRODUCTION

Since conventional therapy shows nonselective side effect, boron neutron capture therapy (BNCT) provides a new choice by selectively killing cancer cell using α particle as a result from neutron capture reaction of boron-10 atom in its carrier with neutron irradiation.[1] To this extent, the use of an ideal boron carrier (a compound containing boron-10) is critical for the success of BNCT.[2] To date, only two boron carriers, sodium borocaptate (BSH) and

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boronophenylalanine (BPA) have shown promise for biodistribution and clinical therapy studies in humans;[3] however, both exhibit significant variability in uptake by cancer cells.[4]

A boron-containing curcumin analog, pentagamaboronon-0 (PGB-0) [Figure 1] has been synthesized as a boron carrier candidate for BNCT.[5] PGB-0 is reportedly selective for breast cancer cells, having an affinity toward estrogen receptor (ER)-positive, human epidermal growth factor 2 (HER2)-positive, and triple-negative breast cancer (TNBC) cells[5-7] and is thus, a good candidate as a boron carrier for breast cancer therapy. However, this compound is water insoluble[7] that leads to low cell uptake and limited applicability as a boron carrier pharmaceutical (BCP). Hence, we developed an improved PGB-0 through the synthesis of PGB-0-F (fructose) and PGB-0-Sor (sorbitol) complexes.[7] In the present study, we synthesized PGB-0-ol by a simple process using $NaBH_4$ as a reducing agent,[8] and purified the new compound using preparative layer chromatography (PLC). The final product was clarified by IR, NMR, and mass spectrometry. *In silico* studies and *in vitro* cytotoxic evaluation of PGB-0-ol against breast cancer cell lines were performed to evaluate the possibility of using PGB-0-ol as a boron carrier for BNCT to treat breast cancer; the development and potency of this promising novel BCP are discussed in this article.

MATERIALS AND METHODS

Molecular docking

Molecular docking analysis that included simulation of molecular binding, calculation of root-mean-square deviation (RMSD), and visualization of protein-ligand interactions conducted using the licensed software molecular operating environment 2010.10. Protein target was retrieved by considering the presence of native ligand including HER2 (ID: 3PP0), folate receptors α (FR-α) (ID: 4LRH), IκB kinase (IKK) (ID: 4KIK), epidermal growth factor receptor (EGFR) (ID: 1XKK), and integrin αvβ3 (ID: 6AVU).[5,7,9,10] All the docking was simulated on binding site of native ligand using flexible ligand docking with the rigid protein structure. Default settings were used unless otherwise stated. The triangle matcher and London dG were applied for placement setting and scoring method, respectively.[11] To refine the result, force field method was used by calculating from ten retained settings of molecular docking results.

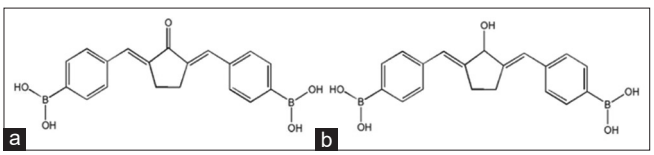

Figure 1: Chemical structure of (a) 2,5-bis (4-boronic acid) benzylidene cyclopentanone (pentagamaboronon-0) and (b) 2,5-bis (4-boronic acid) benzylidene cyclopentanol (pentagamaboronon-0-ol)

Prediction of ADME

The prediction of ADME profile was conducted using pkCSM which is available online from http://biosig.unimelb.edu.au/pkcsm/prediction by inputting the smiles code of PGB-0 and PGB-0-ol.[12] The representative ADME profile such as $CaCO_2$ permeability water solubility, VD_{SS}, clearance, and intestinal absorption was collected for each compound.

Chemistry

All materials and solvents were in analytical grade unless notified. The NMR spectra were recorded on a JEOL JNM-ECZ500R (500 MHz for ^1H and 125 MHz for ^{13}C). The IR spectra were measured using solid KBr pellets on an FT-IR spectrophotometer (Perkin Elmer, USA). Low resolution mass spectroscopy was measured by Thermo scientific Q-Exactive mass spectrometer.

General procedure for the synthesis of pentagamaboronon-0-ol

PGB-0-ol was synthesized by dissolving PGB-0 (100 mg; 0.1 mmol) (collection of CCRC Faculty of Pharmacy, UGM) in ethanol.[7] The sodium borohydride (11 mg; 0.3 mmol) was added followed by stirring for 1 h. The final product was monitored by thin layer chromatography (TLC) and purified by PLC (Silica gel 60 F_{254}, chloroform: methanol 95:5).

Cytotoxicity study

michigan cancer foundation/empty vector (MCF-7/EV), MCF-7/HER2, 4T1, T47D, and HCC1954 cells were cultured as previously described.[12] Cells were seeded in 24-well microplates and incubated overnight. PGB-0 and PGB-0-ol were added to cells at concentrations up to 200 μM. Viable cells were stained with trypan blue and counted after treatment.[13] The IC_{50} value or 50% cell-growth inhibition was calculated using the linear regression of concentration versus cell viability.

Statistical analysis

The molecular docking method was validated by selecting the best conformation of the native ligand after docking simulation and used to calculate the RMSD value. The validity of molecular docking procedure was clarified by the RMSD value of <2 (Supplementary Table 1).

RESULTS

Docking simulation and protein-ligand binding interactions of pentagamaboronon-0-ol and pentagamaboronon-0

PGB-0-ol had lower molecular docking scores than PGB-0 for all proteins, except HER2 [Table 1], but both exhibited subordinate docking scores compared with their native

ligands. The binding affinities against cancer biomarker in this study, such as FR-α, HER2, EGFR, integrin αvβ3, and IKK revealed that PGB-0-ol and PGB-0 exhibited a favorable affinity for all target proteins [Figure 2]. The hydroxyl group on the cyclopentanol structure possibly contributed to the higher affinity of PGB-0-ol for the proteins than PGB-0 as we found in the formation of hydrogen bonding with Thr862 on HER2. We also used lapatinib as a commercial chemotherapeutic drug that targets the HER2 and EGFR proteins for comparison with our compound. In particular, PGB-0-ol interacted more with various amino acids than PGB-0 [Figure 2].

ADME profile comparison of pentagamaboronon-0-ol and pentagamaboronon-0

The log *P* of PGB-0-ol (−0.123) was lower than that of PGB-0 (−0.332), implying that this compound would be more dynamic in penetrating the lipid bilayer of most cellular membranes. PGB-0-ol showed more water-soluble properties than PGB-0 indicated by higher water solubility concentrations of −3.339 and −3.415 log mol/L, respectivel [Table 2]. Although the intestinal absorption of PGB-0-ol (56.95% absorbed) was lower than that of PGB-0 (62.23% absorbed), the Caco2 permeability of PGB-0-ol was higher (−0.111 > −0.042 log Papp at 10^{-6} cm/s). In addition, the distribution of PGB-0 (−0.307 log L/kg) was better than that of PGB-0-ol (−0.267 log L/kg) indicated by the higher VDS value, but the clearance of PGB-0 (0.405 log mL/min/kg) was faster than that of PGB-0-ol (0.352 log mL/min/kg).

Table 1: Docking scores of PGB-0 and PGB-0-ol with several protein targetsc

Ligand	ΔG (kcal/mol)				Integrin $\alpha_v\beta_3$
	HER2	EGFR	IKKβ	FRα	
TAK-285	−17.07	-	-	-	-
Lapatinib	−18.19	−15.84	-	-	-
K252a	-	-	−13.40	-	-
Folic acid	-	-	-	−15.86	-
Cyclic RDG	-	-	-	-	−12.26
PGB-0	−11.77	−11.67	−13.57	−13.52	−8.77
PGB-0-ol	−12.13	−11.56	−13.00	−13.47	−8.68

EGFR: Epidermal growth factor receptor, HER2: Human epidermal growth factor2, PGB: Pentagamaboronon-0, IKKβ: Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta , FRα: folate receptor alpha, RDG: arginine–glycine–aspartic , TAK: TAK-285 is a chemical name

Table 2: ADME predictions for PGB-0 and PGB-0-ol

Parameter	PGB-0	PGB-0-ol
logP	−0.123	−0.332
Water solubility (log mol/L)	−3.415	−3.339
Caco2 permeability (log Papp in 10^{-6} cm/s)	−0.042	−0.111
Intestinal absorption (percentage absorbed)	62.23	56.95
Skin permeability (log Kp)	−2.798	−2.792
VDss (log L/kg)	−0.307	−0.267
Total clearance (log ml/min/kg)	0.405	0.352

PGB: Pentagamaboronon-0

Synthesis of pentagamaboronon-0-ol

The reduction of PGB-0 to PGB-0-ol was carried out using $NaBH_4$ in ethanol to produce PGB-0-ol with a yield of 30%. The molecular formula of PGB-0-ol was confirmed by ESI (+) mass spectrum showing [M + 1] ion peak at m/z 350.17 (calculated for $C_{19}H_{20}O_5B_2$: 349.13) [Figure 3]. The loss of the C = O vibrational band at 1619 cm^{-1} in the IR spectrum of PGB-0-ol indicated that the ketone group of PGB-0 was reduced to the secondary alcohol group-CH (OH)-. This phenomenon was strengthened by the appearance of a strong OH stretching band at 3415 cm^{-1}. The hydroxyl group attached to the boron of PGB-0 appeared at 3338 cm^{-1} with moderate intensity. The CH band at 2928 cm^{-1} of PGB-0-ol appeared to be more intense than that of PGB-0. The C = C vibration bands of the alkene and aromatic groups were also observed at 1676 and 1588 cm^{-1}, respectively [Figure 4].

The identity of PGB-0-ol was indicated by the absence of the C = O resonance signal at δ 195.4 in the ^{13}C-NMR spectrum [Figure 5] and the presence of a new signal at δ 4.95 (-OCH) and δ 2.75 (br, OH) in the ^{1}H-NMR spectrum of PGB-0-ol [Figure 6]. Methylene protons (H3 and H4) appeared at δ 2.45, whereas alkene

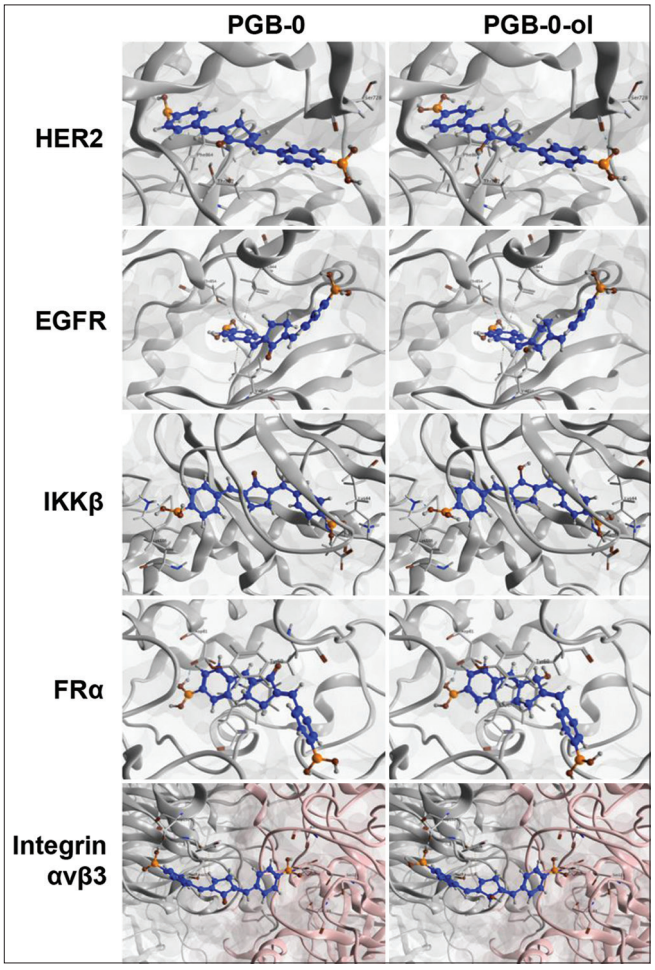

Figure 2: Binding affinities of pentagamaboronon-0 and pentagamaboronon-0-ol to several regulatory proteins

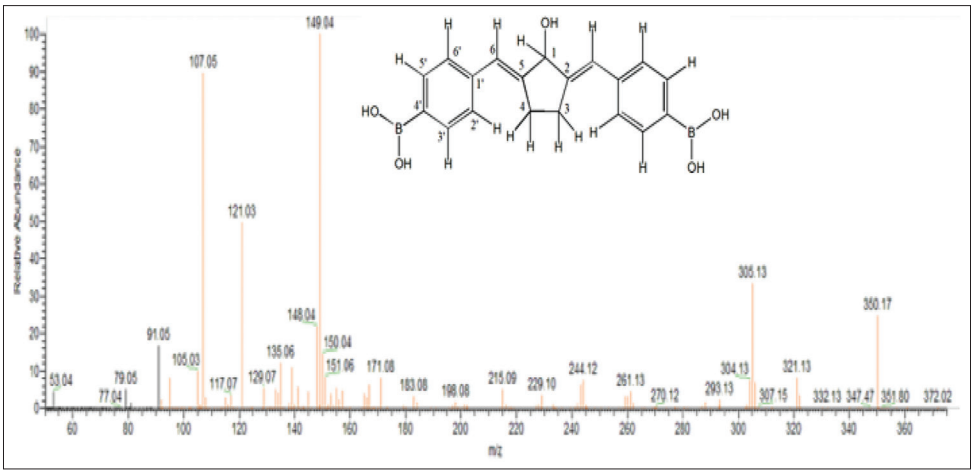

Figure 3: Low resolution mass spectroscopy spectra of pentagamaboronon-0-ol

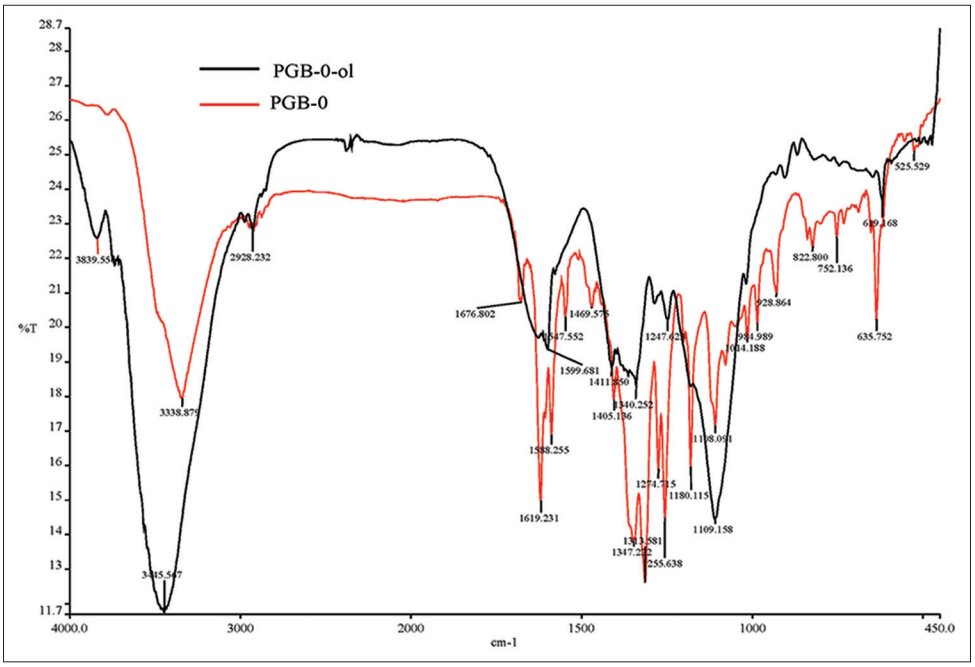

Figure 4: IR spectra of pentagamaboronon-0 and pentagamaboronon-0-ol. Samples were prepared in KBr disks and scanned using an FT-IR spectrophotometer (Perkin Elmer, USA) at 4000 − 1000 cm^{-1}

protons (H7) appeared at δ 6.31. Aromatic proton resonances appeared at δ 7.30 (H3' and H5') and δ 7.75–7.95 (H2' and H6'). In the ^{13}C-NMR spectrum, the new signal at δ 70.0 originated from the resonance of the hydroxy methine carbon caused by the reduction of the carbonyl group. The C2/C5, C3/C4, and C6 resonances of the dimethylene cyclopentanone moiety appeared at δ 134.3, δ 13.3, and δ 125.0, respectively, whereas the signals at δ 134.1, δ 125.6, δ 126.6, and δ 132.0 belonged to C1', C2'/C6', C3'/C5', and C4' of aromatic carbons, respectively. The above spectral analysis proved the structure of PGB-0-ol as the $NaBH_4$ reduction product of PGB-0.

Effect of pentagamaboronon-0-ol and pentagamaboronon-0 on breast cancer cell lines

In vitro cytotoxic evaluation of PGB-0-ol against breast cancer cells was conducted to evaluate its possibility as a boron carrier for BNCT in the treatment of breast cancer. The IC_{50} value of PGB-0-ol was in the range of 60–160 μM 42 [Table 3, Figure 7, and Supplementary Figure 1] against MCF-7/HER2. MCF-7/EV, 4T1, T47D, and HCC1954 cells had respective values of 117, 74, 155, 114, and 61 μM. Overall, PGB-0-ol was more cytotoxic than PGB-0 in MCF-7/HER2, MCF-7/EV, and T47D cells, but not in 4T1 and HCC1954 cells. PGB-0-ol was also more soluble than PGB-0 in the culture medium.

DISCUSSION

The development of boron carriers requires the appropriate uptake of boron-10 atoms.[4] Molecular docking studies

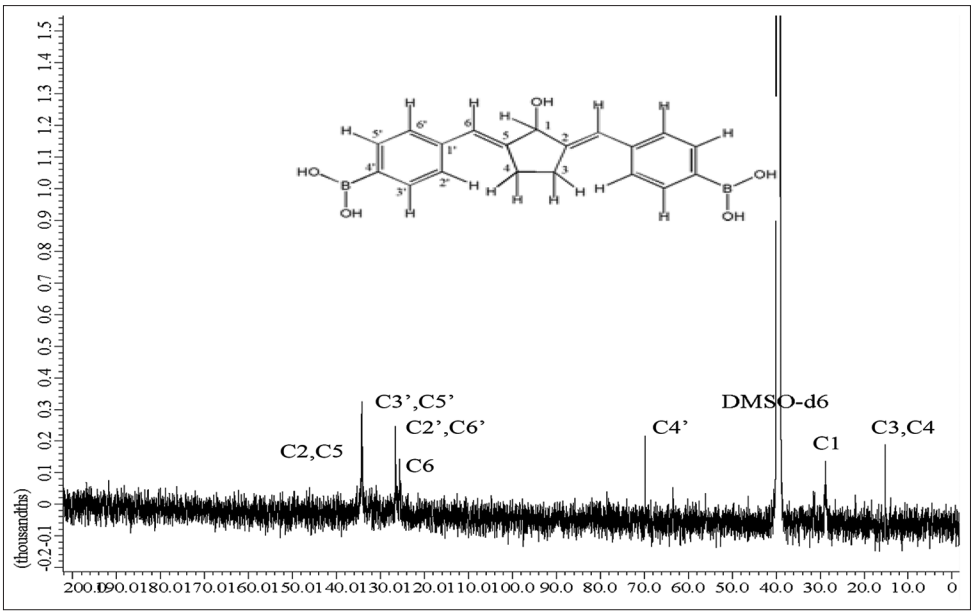

Figure 5: ^{13}C-NMR spectra of pentagamaboronon-0-ol in DMSO-D

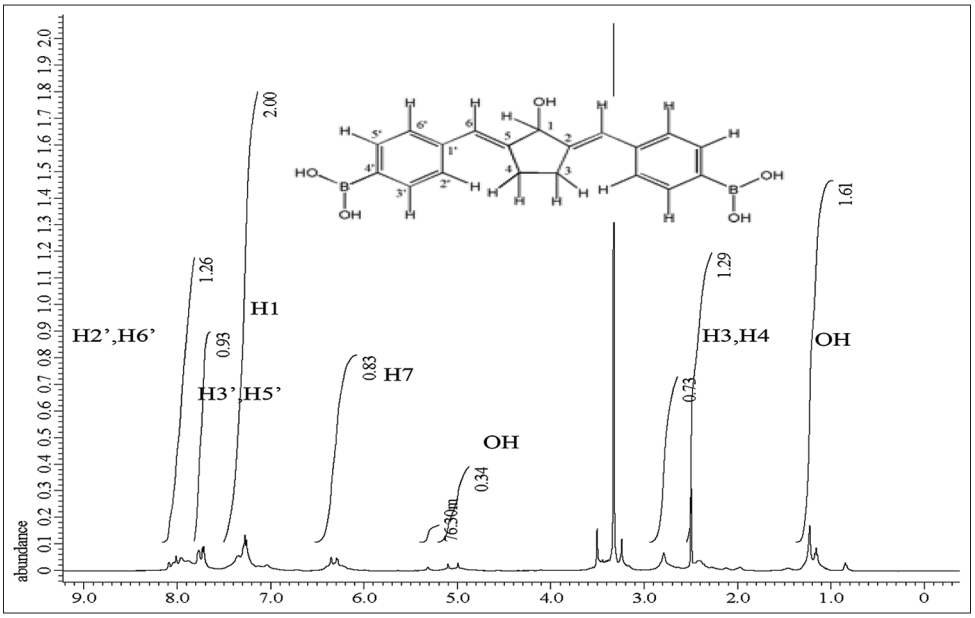

Figure 6: ^{1}H-NMR spectra of pentagamaboronon-0-ol in DMSO-D$_6$

Table 3: The cytotoxicity profile of PGB-0 and PGB-0-ol towards breast cancer and non-cancerous cell lines

Cell type	IC_{50} (μM)	
	PGB-0	PGB-0-ol
MCF7/HER2	166	117
MCF7/EV	87	74
4T1	118	155
T47D	172	114
HCC1954	36	61

PGB: Pentagamaboronon-0

revealed that PGB-0-ol improved protein interaction with several marker proteins of PGB-0 including HER2, EGFR, IKK, FR-alpha, and integrin αvβ3. PGB-0-ol showed greater molecular docking scores than PGB-0 for HER2 and similar scores for EGFR. This finding is in agreement with a previous finding that PGB-0 exhibits cytotoxic activity in HER2-positive breast cancer cells.[5,7,14] Our compound also interacted with an essential nuclear factor kappa B related protein, IKK,[15] with an analogous docking score to its native ligand. Recent studies have explored various FR[16] and integrin αvβ3-targeted cancer therapies[17] including BNCT. The results of molecular docking analysis highlighted the potency of PGB-0-ol as a boron carrier because of the interaction between PGB-0-ol and these proteins that might enhance its cellular uptake through receptor-mediated endocytosis.

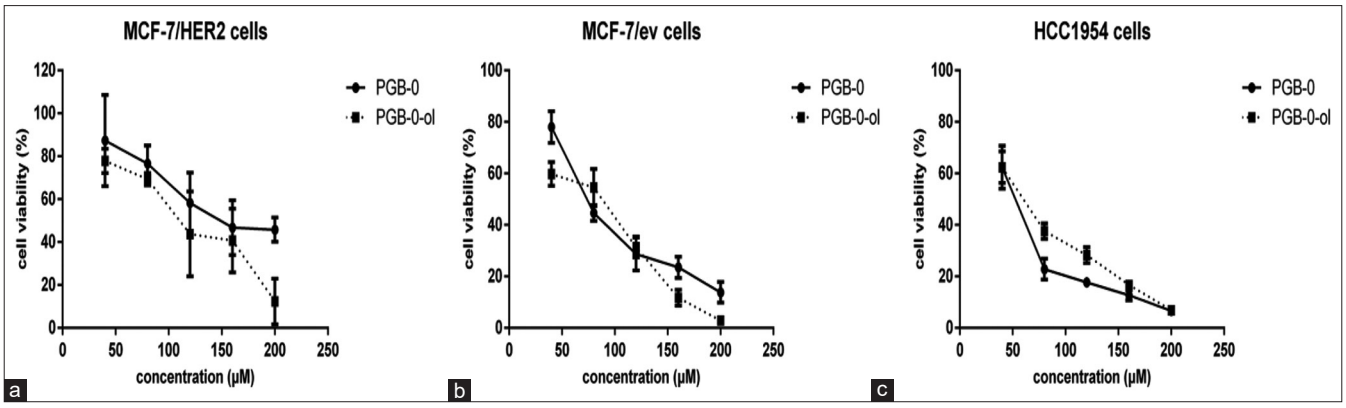

Figure 7: Cytotoxicity profiles of pentagamaboronon-0-ol and pentagamaboronon-0 against (a) MCF-7/human epidermal growth factor2, (b) MCF-7/ev, and (c) HCC1954 cells. pentagamaboronon-0 and pentagamaboronon-0-ol were added to cells prior to the incubation for 24 h as described in the methods; cell viability was measured using the trypan blue assay. Data are shown as the mean ± standard error (n = 3)

The synthesis of a new compound derived from PGB-0 with better chemical and physical properties is an accession to the advancement of BCPs. Compared with the synthesis of BPA, BSH,[2,18,19] and even the sugar complexation of boron carriers,[7] this method is rather simple, quick, and requires less effort. Only one-step reduction using $NaBH_4$ is relatively available and inexpensive, providing a low-cost and simple synthesis.[20]

The molecular interaction models of PGB-0-ol are fundamental for underscoring its potency as a BCP. Our data support the hypothesis that PGB-0-ol is more soluble in water, making PGB-0-ol more effective than PGB-0 in killing cancer cells. In this regard, we compared PGB-0 and PGB-0-ol in terms of their cytotoxicity toward several breast cancer cells, including HER2-positive (MCF-7/HER2 and HCC1954), ER-positive (T47D and MCF-7/EV), and TNBC (4T1). BCPs should exhibit low toxicity to cells in BNCT.[6] Overall, PGB-0-ol exhibited low cytotoxicity against cancer cells. This result is consistent with the docking results and a previous study on PGB-0. The strongest cytotoxic activity of PGB-0-ol was observed in the HCC1954 cells. Compared with BPA, which has an IC_{50} >4 mM,[21] our compounds possess more potent cytotoxicity. However, given that their IC_{50} was >50 μM,[22] PGB-0 and PGB-0-ol are still considered to be low-cytotoxicity agents. The increased cellular cytotoxicity of PGB-0-ol is possibly due to its increased solubility and uptake in cancer cells.[7,23] Therefore, boron-10 micro-distribution measurements must be conducted in further studies.

CONCLUSION

The novel compound PGB-0-ol was synthesized through simple steps. The molecular docking and ADME prediction of PGB-0-ol indicated that it possesses better properties than its lead compound. Thus, PGB-0-ol may be considered an agent with low cytotoxicity toward breast cancer cells and could be developed as a boron carrier for the application of BNCT. Further investigations of the micro-distribution and cellular uptake of PGB-0-ol are warranted for further development and use as a boron carrier.

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Conflicts of interest
There are no conflicts of interest.

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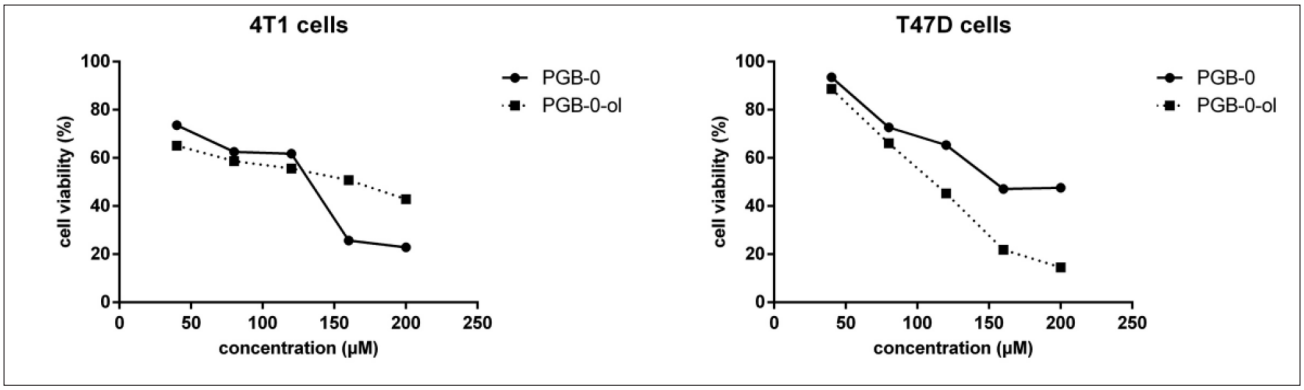

Supplementary Figure 1: Cytotoxicity profiles of pentagamaboronon-0-ol and, pentagamaboronon-0 against (a) 4T1 and (b) T47D cells

Supplementary Table 1: RMSD value of PGB-0 and PGB-0-ol on molecular docking

Ligand	RMSD (Å)				
	HER2	EGFR	IKKβ	FRα	integrin αvβ3
TAK-285	0376	-	-	-	-
Lapatinib	1.899	1.079	-	-	-
K252a	-	-	0.266	-	-
Folic acid	-	-	-	0.525	-
Cyclic RDG	-	-	-	-	0.794
PGB-0	1.290	1.242	0.918	1.662	1.106
PGB-0-ol	1.077	1.032	1.776	0.628	0.814