Design, Synthesis and Evaluation of Boron - Containing Drug Having Pharmacological Activity and Physical Destructive Activity

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Contents

Chapter 1: Evaluation of destructive effects of boron tracedrug UTX-51 for glycated BSA as a AGE

1. Introduction

Proteins are constantly 'dancing', are stabilized or modified by other proteins in their vicinity. Under conditions of stress, including oxidation, pH fluctuations, or glycation, the equilibrium is irreversibly lost and proteins become denatured and lose their functionality (1). Denatured proteins form aggregates those organize into complex assemblies, such as those observed in amyloid-β and tau proteins in Alzheimer's disease (2), α-synuclein in Parkinson's disease (3), and p53 in cancer (4). Similar to prion proteins (5), these aggregated proteins are proposed to be self-propagating (6), causing symptoms to progress until these proteins becoame detectable. Clearance therapy, which is focused on clearing, reducing, and removing aggregation-prone proteins, is an important therapeutic strategy.

Advanced glycated end-products (AGEs) are produced by non-enzymatic glycation reaction caused by protein and reducing sugar such as glucose and fructose, and it is known that producing AGEs is significantly increased in aging-related diseases such as diabetes and arteriosclerosis. However, recent studies suggest that AGEs are associated with numerous diseases such as Alzheimer's disease, cardiac dysfunction and non-alcoholic steatohepatitis as well as diabetes. Formation of AGEs in a living body is divided into early stage and advanced stage reactions (Figure 1). In early stage, the carbonyl group of sugar aldehydes reacts with the N-terminus or free amino group of proteins via nucleophilic addition to form Schiff base. These unstable adducts then undergo rearrangements to the more stable Amadori products by acid-base catalyst. In an advanced stage, Amadori compounds further undergo irreversible chemical reactions including oxidation, condensation and cross-linking, eventually generating AGEs. Denatured proteins by glycation, are browned and exhibit fluorescence (excitation: 370 nm, emission: 440 nm). Moreover, these proteins lose their specific function and form insoluble and protease resistant aggregates[8].

Several drugs, such as aminoguanidine, pyridoxamine, and azilsartan, have been developed for the treatment for AGEs. These drugs inhibit AGE formation through their carbonyl trap activity, and blood pressure-lowering activity (Figure 2) (9, 10). These drugs have side-effects and their AGE- inhibiting effects have been proved to be limited in experimental models of diabetes (10). ALT-711, the only AGE crosslink-breaking drug tested in advanced clinical trials, reportedly has limited effects (11) and is an ineffective treatment (12). Most AGE inhibitors are thought to be ineffective because of their limited effect on existing denatured proteins.

To promote the elimination of aggregated and denatured proteins, we have developed a neutron dynamic therapy (NDT) (13, 14). NDT exploits the neutron-capture ability of a stable isotope of boron (B-10), which has been used in boron neutron capture therapy (15, 16). By introducing B-10 into a drug scaffold, we have successfully developed boron tracedrugs possessing molecular disrupting and tracing abilities (Figure 3).

Herein, we evaluated the ability of our NDT to target glycated bovine serum albumin (Gly-BSA) to determine whether the boron tracedrug UTX-51 is an effective treatment for AGEs.

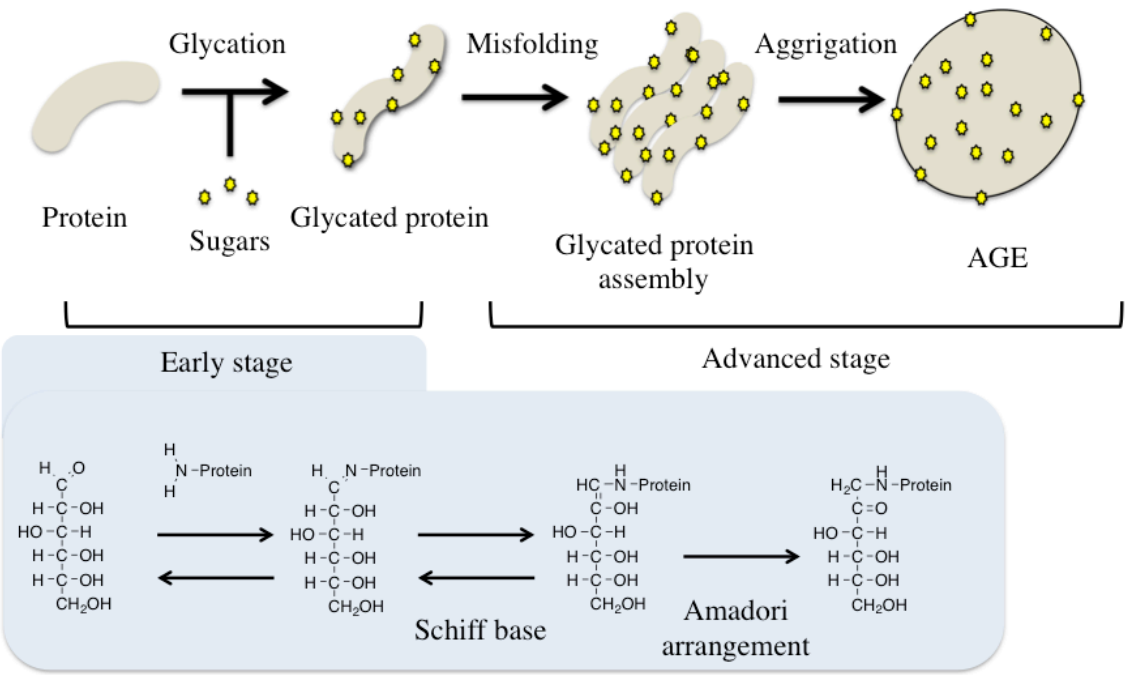


Figure 1. Formation of glycated proteins and advanced glycated end-products (AGEs). In living body, the glycation of a protein with sugars induces denaturation and the formation of aggregates (AGEs).

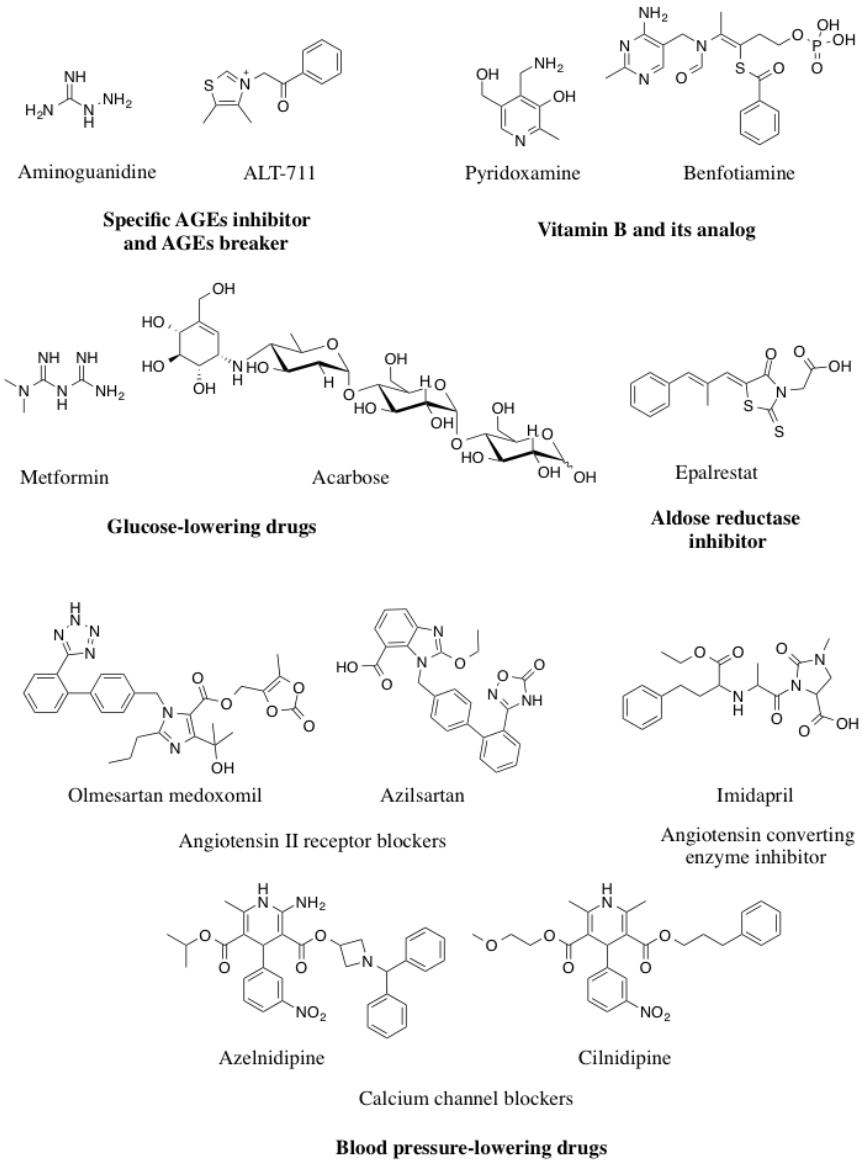


Figure 2. The chemical structure of conventional advanced glycation end-products drugs.

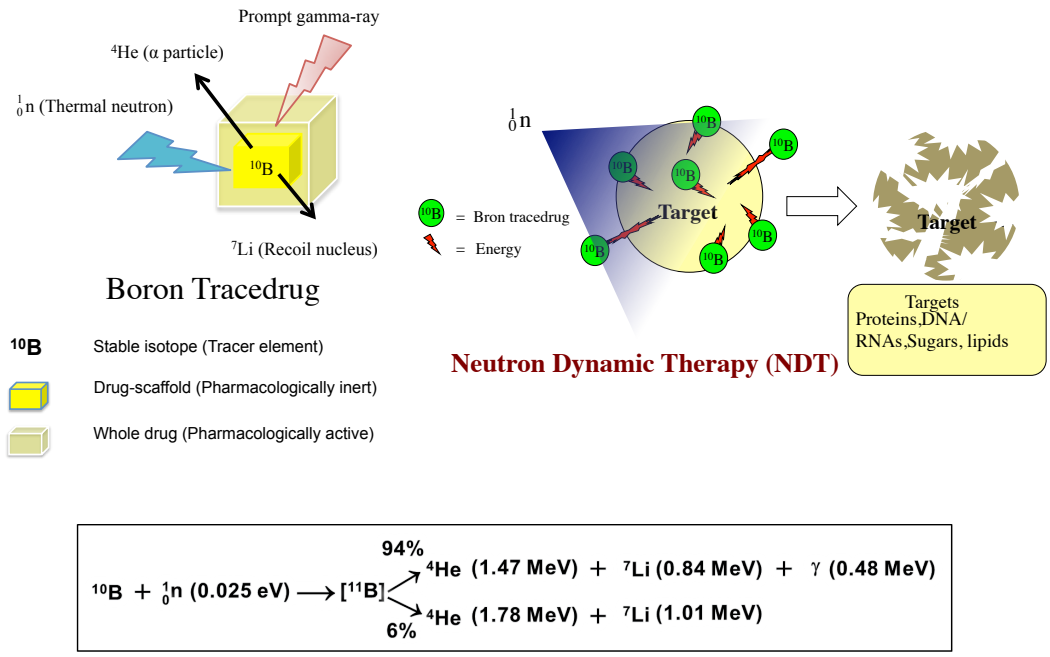
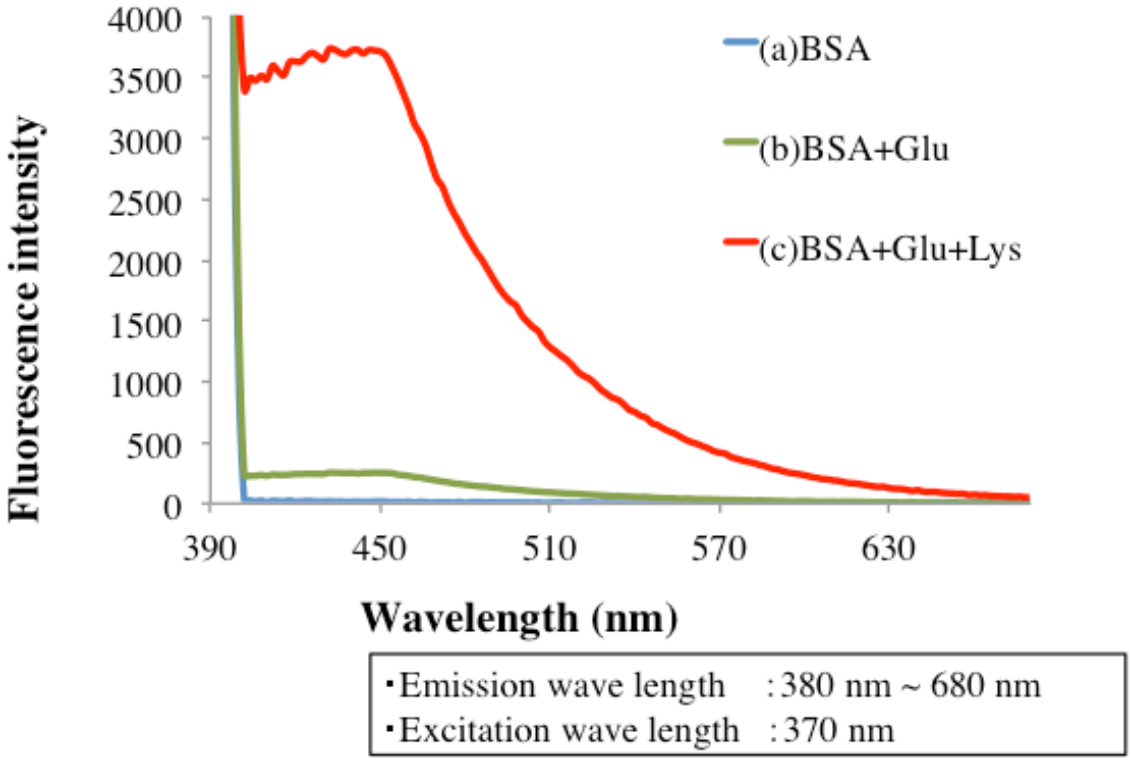


Figure 3. Overview of Neutron dynamic therapy (NDT) and Boron tracedrug.

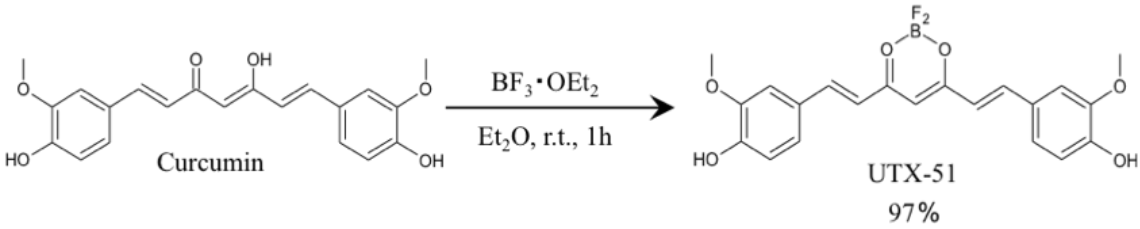
2. Results

Preparation of Gly-BSA. BSA samples incubated in the absence of glucose at 50 ˚C showed no change in their fluorescence intensity at 440 nm, whereas BSA incubated with glucose and lysine showed high fluorescence intensity at 440 nm. As shown in Figure 3, the fluorescence spectrum showed BSA incubated with glucose and lysine had a higher intensity than that of BSA in the presence of glucose, indicating protein glycation had occurred, producing Gly-BSA (19).



Synthesis of UTX-51

Scheme 1 illustrated synthesis of boron tracedrug UTX-51. UTX-51 was obtained by the reaction of curcumin and trifluoroborate in dry diethyl ether.



Scheme 1. Synthesis of UTX-51

NDT experiment and analysis.

We evaluated the ability of UTX-51 to disrupt Gly-BSA and native BSA by irradiating samples containing UTX-51 and the target proteins with thermal neutrons. We then compared the resultant protein levels by using SDS-PAGE (Figures 4 and 5). As a control, two samples containing Gly-BSA and native BSA were left unirradiated (lane 2). Gly-BSA samples irradiated using thermal neutrons in the presence of 500 nmol UTX-51 (100 nmol B-10) had a band intensity that was 33% lower than that of the control (lane 6, Figure 5), while native BSA had a band intensity that was 54% less than that of the control (lane 6, Figure 6). These data demonstrated the NDT effects of the boron tracedrug molecules embedded in the surface of Gly-BSA.

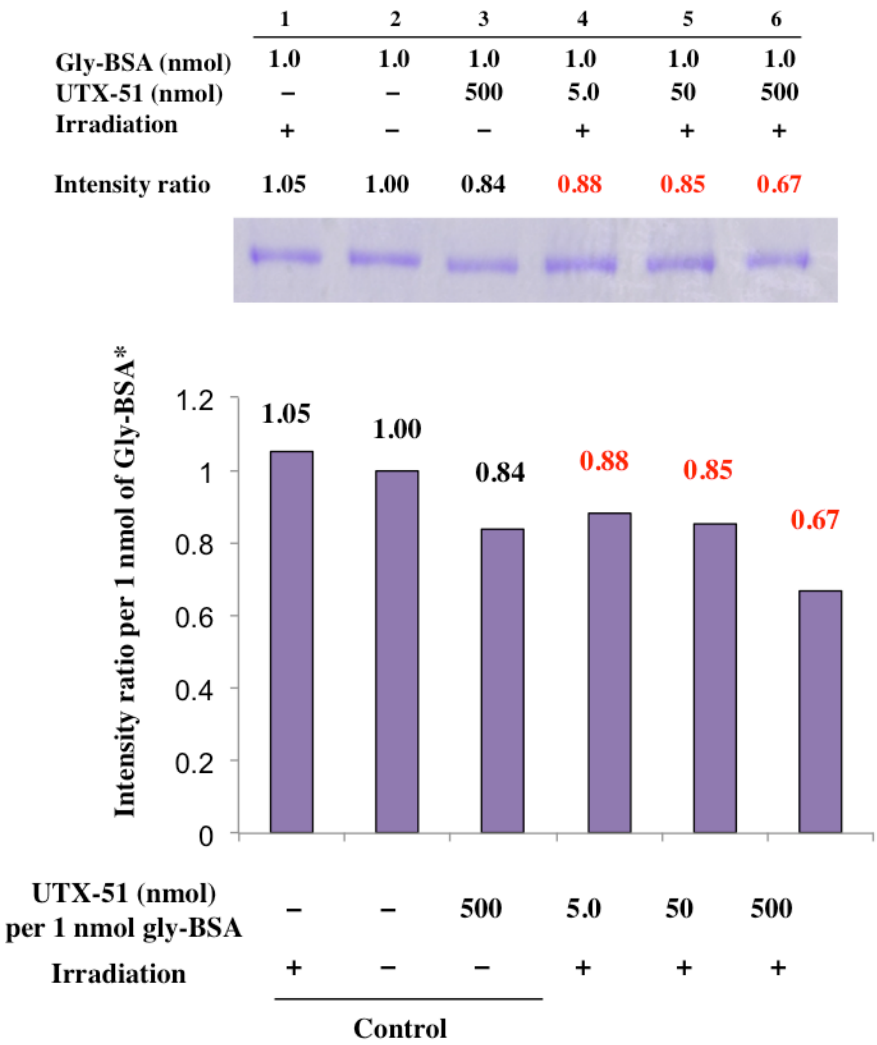


Figure 5. Evaluation of UTX-51 as an neutron dynamic therapy for glycated bovine serum albumin (Gly-BSA) under neutron irradiation. Each lane contained 1 nmol Gly-BSA. Lane 1: Gly-BSA with irradiation; lane 2: Gly-BSA without irradiation; lane 3: UTX-51 (500 nmol) + Gly- BSA without irradiaton; lane 4: UTX-51 (5 nmol) + Gly-BSA with irradiation; lane 5: UTX-51 (50 nmol) + Gly-BSA with irradiation; lane 6: UTX-51 (500 nmol) + Gly-BSA with irradiation. Gly-BSA treated with UTX-51 on SDS-PAGE gel was stained with Coomassie Brilliant Blue. Band intensities were measured and compared using SWEDAY Just TLC software. The band intensity in lane 2 was set at 1.00 as a control. B-10:Gly-BSA=1:1 in lane 4, B-10:Gly-BSA=10:1 in lane 5, B-10:Gly- BSA=100:1 in lane 6.

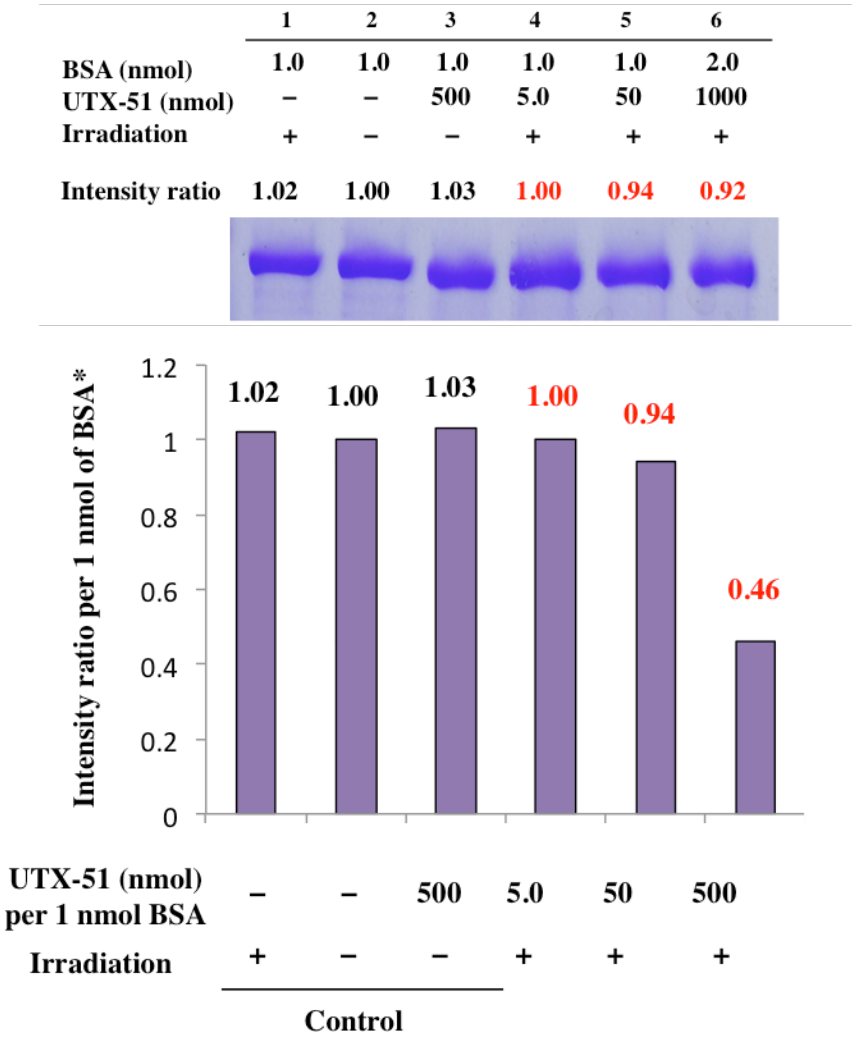


Figure 6. Evaluation of UTX-51 as an neutron dynamic therapy for native bovine serum albumin (BSA) under neutron irradiation. Lane 1: native BSA (0.5 nmol) with irradiation; lane 2: native BSA (0.5 nmol) without irradiation; lane 3: UTX-51 (250 nmol) + native BSA (0.5 nmol) without irradiaton; lane 4: UTX-51 (2.5 nmol) + native BSA (0.5 nmol) with irradiation; lane 5: UTX-51 (25 nmol) native BSA (0.5 nmol) with irradiation; lane 6: UTX-51 (500 nmol) + native BSA (1.0 nmol) with irradiation. Native BSA treated with UTX-51 on SDS-PAGE gel was stained with Coomassie Brilliant Blue. Band intensities were measured and compared using SWEDAY Just TLC software. The band intensity in lane 2 was set at 1.00 as a control. B-10:native BSA=1:1 in lane 4, B-10:native BSA=10:1 in lane 5, B-10:native BSA=100:1 in lane 6.

3. Discussion

Herein, we confirmed the effectiveness of the boron tracedrug UTX-51 as an NDT to decompose Gly-BSA and native BSA under thermal irradiation. These results suggest that other denatured proteins implicated in refractory diseases such as amyloid-β and tau proteins in Alzheimer's disease (2), α-synuclein in Parkinson's disease (3), and p53 in cancer (4) can be destroyed in this manner. UTX-51 (500 nmol) was less destructive towards Gly-BSA than native BSA, supporting the results of other studies that showed that aggregated proteins tend to be resistant to proteinase K (20). Thus, AGEs such as Gly-BSA assemblies might be resistant to clearance by immune cells and progressively accumulate in the body.

Our novel NDT involved the use of innovative boron tracedrugs that are markedly different from conventional therapies such as chemotherapy and immunotherapy. AGE drugs that have been tested in clinical trials can be classified into seven types: (i) specific AGE inhibitors and breakers such as aminoguanidine and ALT-711; (ii) B vitamins and analogs; (iii) the aldose reductase inhibitor epalrestat; (iv) glucose- lowering drugs such as metformin; (v) blood pressure- lowering drugs such as angiotensin II receptor blockers, angiotensin-converting enzyme inhibitor, and calcium channel blockers; (vi) lipid-lowering drugs such as atorvastatin; and (vii) anti-rheumatic agents such as methotrexate (10). In clinical trials, almost all these drugs were shown to have limited effects or to be ineffective treatments for AGEs. In particular, aminoguanidine showed an unfavorable-perceived in the risk-to-benefit ratio (21). Therefore, these drugs must be used long-term to attain results (10). With the exception of AGE breakers, these drugs are expected to have preventive effects such as anti-glycation, anti-oxidation, metal chelation, renal protection, and polyol metabolic inhibition, but they have no effect on existing denatured proteins. In preventative therapies, a regular administration of the drugs is necessary, imposing a strain on patients. NDT, on the other hand, has the ability to completely destroy denatured proteins in tissues by using high-energy α-particles produced from B-10-captured thermal neutrons. Thermal neutron irradiation in the presence of NDT drugs requires significantly less time than the aforementioned conventional drug-based AGE therapies. Our NDT strategy has other advantages. i) NDT of low- cost, requiring only B-10; and unlike conventional drugs, expensive production processes can be avoided. ii) No

personalization is required; it is an all-purpose and patient- friendly treatment for a variety of diseases. iii) Unnecessary damage to surrounding normal tissues is minimized because NDT uses thermal neutrons with low energy to irradiate B- 10 atoms, forming a functional drug. iv) NDT requires few to no pharmacokinetic and pharmacodynamic studies, which are typically necessary to develop new drugs.

To the best of our knowledge, our present study is the first to report the destruction of a denatured protein, Gly-BSA, by using energy generated from B-10 captured neutrons. We believe that our physically-powered NDT using boron tracedrugs is a next-generation standard therapy that can replace conventional chemistry-based therapies. However, for NDT to become an established therapy, a compact neutron generator for NDT must be developed.

4. Conclusion

We present our NDT strategy, which has been used for the targeted clearance of Gly-BSA in a model of AGE. Our results suggest that NDT with boron tracedrugs can be used for the treatment of diabetes, Alzheimer's disease, and heart disease.

5. Materials and Methods

Materials.

All chemicals were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan), Wako Pure Chemical Industries Ltd. (Osaka, Japan), and Nacalai Tesque INC. (Kyoto, Japan). Nuclear magnetic resonance (NMR) ^{1}H spectra were obtained using a JNM- EX400 spectrometer (Jeol Ltd., Tokyo, Japan) at 400 MHz. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin- layer chromatography was performed on glass-backed silica gels (Merck 60 F_{254}) and components were visualized using ultraviolet (UV) light. Column chromatography was performed using KANTO Chemical silica gel 60 N (spherical neutral) 40-50 μm.

Preparation of Gly-BSA.

We prepared Gly-BSA, which served as our AGE model, by using the thermal glycation method (17). BSA (2 mg; fatty acid-free; Wako Pure Chemical Industries Ltd.), 0.5 M glucose, and 0.1 M lysine, each dissolved in 0.2 M sodium phosphate buffer (SPB buffer 1.5 ml) (pH 7.5), were incubated at 50 ˚C for 4 days to produce Gly-BSA. The control samples of BSA and BSA with 0.1 M glucose were also incubated under similar conditions.

Analysis.

We confirmed the glycation of BSA by measuring emissions at 440 nm upon excitation at 370 nm by using the Tecan Infinite M200 Microplate reader (Tecan group Ltd., Männedorf, Switzerland).

Synthesis of UTX-51.

Curcumin (1,000 mg, 2.7 mmol; Tokyo Chemical Industry Co. Ltd.) was suspended in diethyl ether (6 ml) under an atmosphere of nitrogen. Boron trifluoride etherate (1 ml, 8.1 mmol) was added drop-wise with stirring at room temperature for 1 h. The solvent was evaporated. The residue was purified using silica gel column chromatography (Eluents: hexane:EtOAc=10:1) to generate UTX-51 as a red solid (1094 mg, 97% yield) (18).

NDT experiment and evaluation.

Samples for NDT analysis were prepared using Gly-BSA and native BSA. The Gly-BSA solution was diluted in 0.2 M SPB (660 ng/μl) and was added 100 μl to Teflon tubes. UTX-51 was stoichiometrically diluted in microtubes to 2 μg/50 μl, 20 μg/50 μl, and 200 μg/50 μl by using dimethyl sulfoxide (DMSO). To each Teflon tube containing Gly-BSA-SPB solution, 50 μl of UTX-51-DMSO solution was added. Control samples of Gly-BSA alone were prepared. BSA was dissolved in SPB buffer (330 μg/ml) and 100 μl of this solution was transferred to each Teflon tube. To each Teflon tube containing BSA-SPB

solution, 25 μl of UTX-51-DMSO solution was added. A sample containing 200 μg of UTX-51-DMSO solution was added to BSA (66 μg/100 μl SPB buffer). Control samples of BSA alone were prepared.

All samples were adjusted using SPB buffer to prepare a solution of 200 μl. A subset of the samples were irradiated. Samples were irradiated using thermal neutrons at the Kyoto University Research Reactor Institute. Irradiation conditions were as follows: time, 45 min; and absorbance dose, 0.31 Gy in the presence of B-10 (100 nmol). Absorbance dose was measured by taping the gold at the front and back sides of the Teflon tubes.

After irradiation, we evaluated the integrity of target molecules by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie Brilliant Blue (CBB). Band intensities were measured and compared using Just TLC software (Sweday., Lund, Sweden).

6. Reference

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Chapter 2: Design, Synthesis, and Biological Evaluation of PARP-Targeted BoronTracedrugs

1. Introduction

In Chapter 1, I reported about destructive activity of boron tracedrug UTX-51 with thermal neutron against glycated BSA. This result suggests that boron tracedrugs are able to destroy a target that is interacted with the drugs. In this chapter 2, I describe that design and synthesis of newly boron tracedrug that having pharmacological activity as well as the physical destructive activity.

Poly (ADP-ribose) polymerase[1] is a intranuclear enzyme and catalyzes the addition of poly(ADP-ribose) molecules to target proteins[2] (Figure 7), and the PARP family is comprised of 18 members. Among them, PARP1 is one of the most abundant proteins in the nuclear and it is clear that the PARP1 is associated with DNA repair of both single-strand breaks and double-strand breaks[3] (Figure 8). Single-strand breaks are repaired by base-excision repair (BER), and double-strand breaks are repaired by homologous recombination (HR)[4]. Inhibiting PARP1 activity causes the unrepaired single-strand breaks to develop into double-strand breaks, and eventually the DNA damages are repaired via HR pathway.

BRCA1 are closely associated with DNA repair via HR pathway, and mutation of *BRCA1* gene lead to an increased risk for familial breast cancer and ovarian cancer[5,6]. Besides, high proportion of *BRCA1* mutation is found triple-negative breast cancer that is negative for expression of estrogen receptor (ER), expression of progesterone receptor (PR) and expression HER2. Whereas *BRCA1* mutation becomes the cause of some cancers, BRCA1 dysfunction is served as an important therapeutic target for *BRCA1*-associated cancer[7,8]. In *BRCA1*-deficient cell, inactivation of PARP1 causes the multiple double-strand breaks to form, and results in leading to cell death that called synthetic lethality. Thus, the PARP inhibitor has great potential for the *BRCA*-mutated cancer[9,10].

Previously developed PARP inhibitors designed based on the NAD+ that is substrate for PARP. Among them, a scaffold of 1*H*-benzimidazole-4-carboxamide is one of the highest inhibitory activities against PARP (Ki=95 nM), and some PARP inhibitors were developed based on the scaffold (Figure. 9).

Especially, based on 1*H*-benzimidazole-4-carboxamide PARP inhibitor Veliparib (ABT-888) is in advanced clinical trials[11]. In this study, I choose Veliparib as a lead compound of the novel boron tracedrug and tried to design and synthesize boron modified PARP inhibitors. Herein, I reported novel boron tracedrugs having PARP-1 inhibition activity.

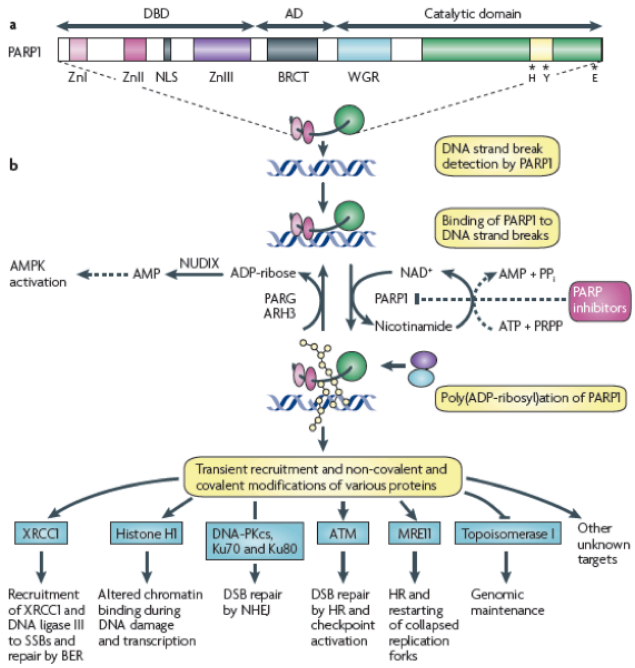


Figure 7. Structural and functional characteristics of PARP1.

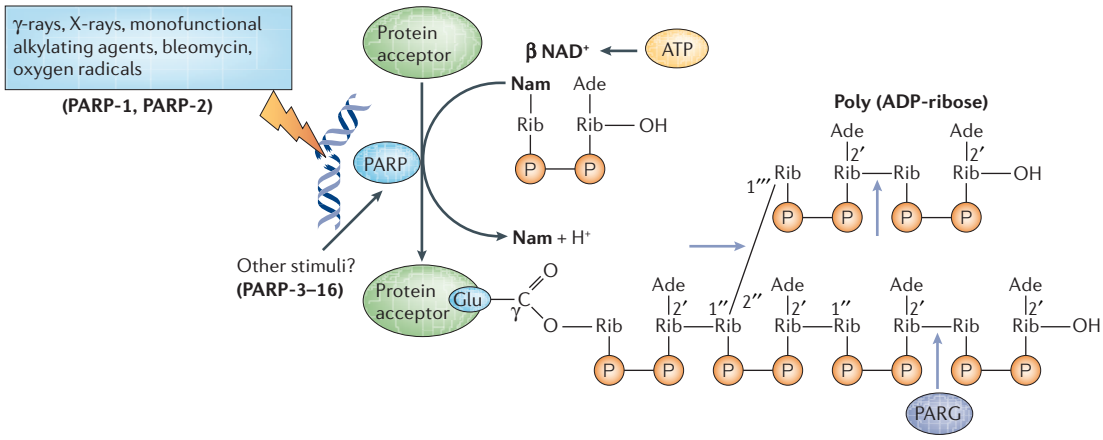


Figure 8. Metabolizm poly(ADP-ribose) during DNA damage and repair.

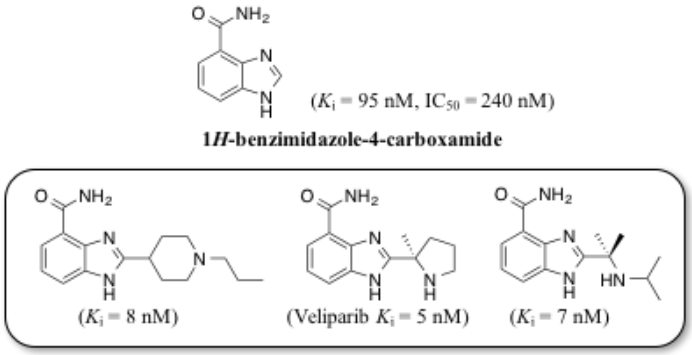
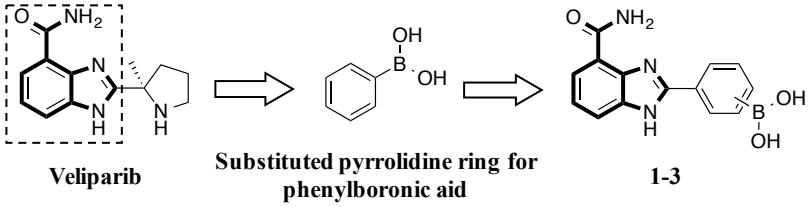


Figure 9. Structure of 1*H*-benzimidazole-4-carboxamide and its analogue.

2. Drug Design

To design of boron modified PARP inhibitor, as shown in the scheme 2. I selected phenylboronic acid as the boron-introducing unit and designed boron modified PARP inhibitors *o*-, *m*- and *p*-ABIP.

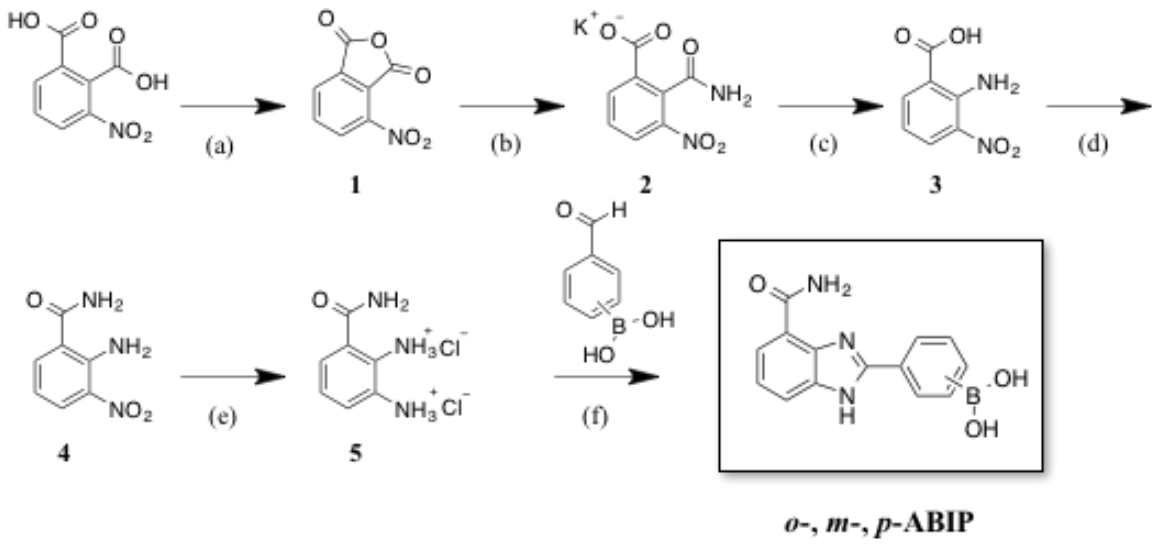


Scheme 2. Design of Boron modified PARP inhibitors based on Veliparib.

3. Results and Discussion

The synthesis of *o*-, *m*-, p-ABIP were shown in scheme 3. Starting from 3-nitrophathalic acid was reacted with acetic anhydride under reflux condition to give 1 in 77.4% yield. 1 was treated with 28% NH_3 aq and acetic anhydride, and then KOH aq, ethanol and diethyl ether were added to give 2 in 83.8% yield. 3 was obtained from 2 via Hofmann rearrangement in 67.8% yield. 3 was reacted with thionyl

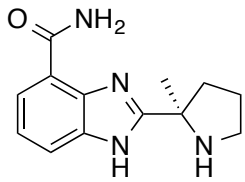
chloride and NH3 aq to give 4 in 57.5% yield. The key intermadiate 5 was obtained from 4 through Pd/C hydrogenation in 68.1% yield. The target compounds *o*-, *m*- and *p*-ABIP were obtained from 5 via one-pot three step reactions including protection of phenylboronic acid by *N*-methyliminodiacetic acid in 15.6-25.6% yield.



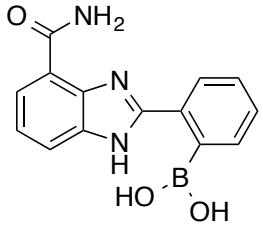
Regent and condition: (a) Ac_2O, reflux, 40 min y.89% (b) (i) NH_3 aq, -5˚C, 1.5 h (ii) KOH, r.t., 5min, y.46% (c) (i) Br_2, KOH, water, r.t. 15 min (ii)55˚C, 30 min (iii) HCl, r.t. y.88%(d)(i) $SOCl_2$ THF, DMF, 55˚C, 1 h. (iii) NH_3 aq 0˚C, y. 57%(e) (i)Pd/C, H_2,6 h.(ii)HCl gas, 5min, r.t. y. 50% (f) (i) MIDA, DMF, 120˚C, 6 h, (ii) 2,3-diaminobenzamide 2HCl, KI, 80˚C, (iii) NaOH, 0˚C, 3 h.

Scheme 3. Synthesis of *o*-, *m*-, *p*-ABIP

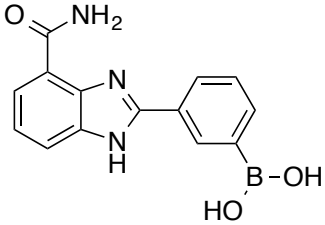
All the target compounds were screened in vitro for their PARP-1 inhibiting activity, and determined IC_{50} values. The results were shown figure 9. Veliparib, as the positive control, was the highest inhibition activity (IC_{50}: 0.034 μM), and *o*-ABIP was the lowest inhibition activity (27 μM). On the other hand, both *m*- and *p*-ABIP were not able to determine their IC_{50} values in my set condition (both IC_{50} values: <1 μM).



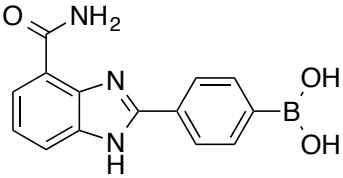
Veliparib
IC_{50}: 0.034 μM



o-ABIP
IC_{50}: 27 μM



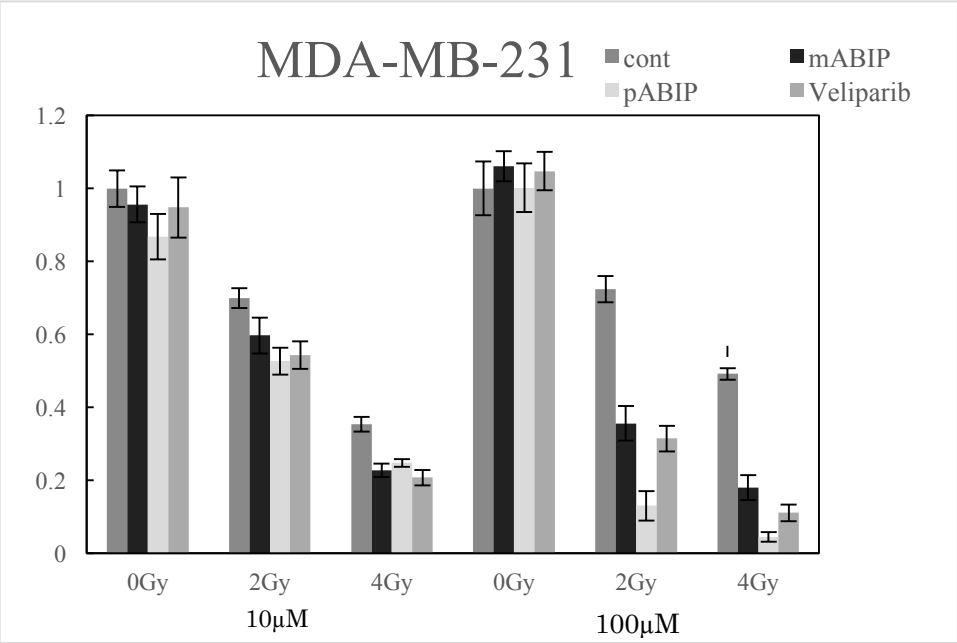
m-ABIP
IC_{50}: 58.4%/1.0 μM



p-ABIP
IC_{50}: 56.0%/1.0 μM

Figure 9. The structure and PARP-1 enzyme inhibition activity of compounds.

Inactivation of PARP reduced the single-strand breaks repair in the result that increased sensitivity to ionizing radiation or alkylating agents[12-14]. To evaluate with a cell level of the PARP inhibition activity of the target compounds *m*- and *p*-ABIP, I evaluated radiosensitivity effect of target compound through colony formation assay with human breast cancer MCF7 and MDA-MB-231 cells. MCF7 cells are negative for expression of ER and PR, while MDA-MB-231 cells were triple negative. Both breast cancer cells express wild-type BRCA1 while MDA-MB-231 cells loss of one *BRCA1* allele and the non-mutated allele is containing. The results were shown in figure 10.



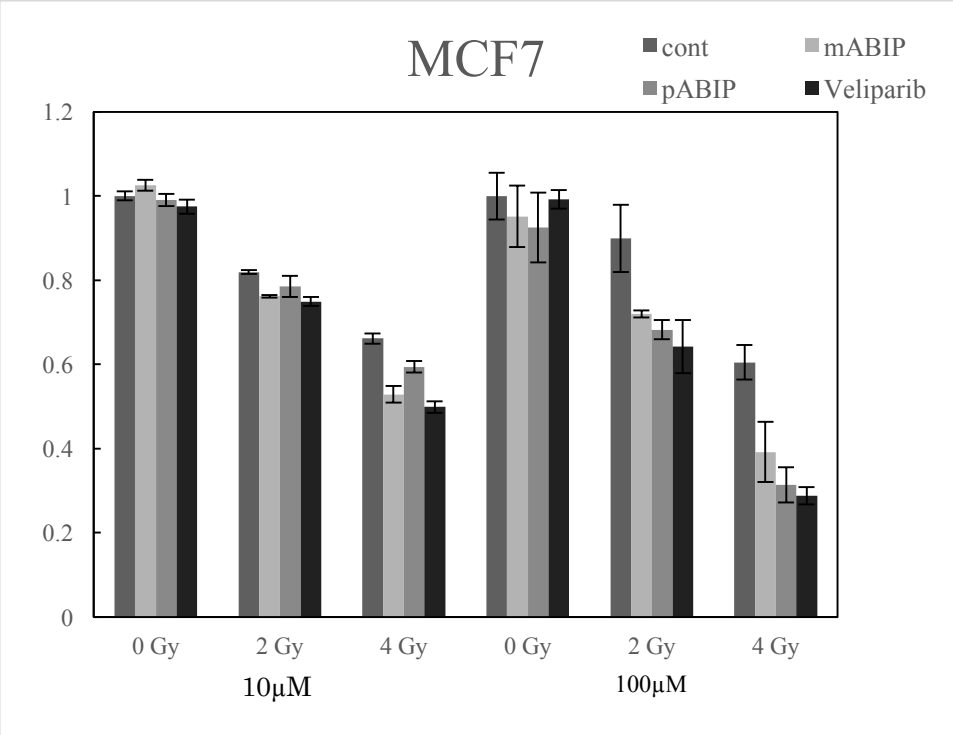
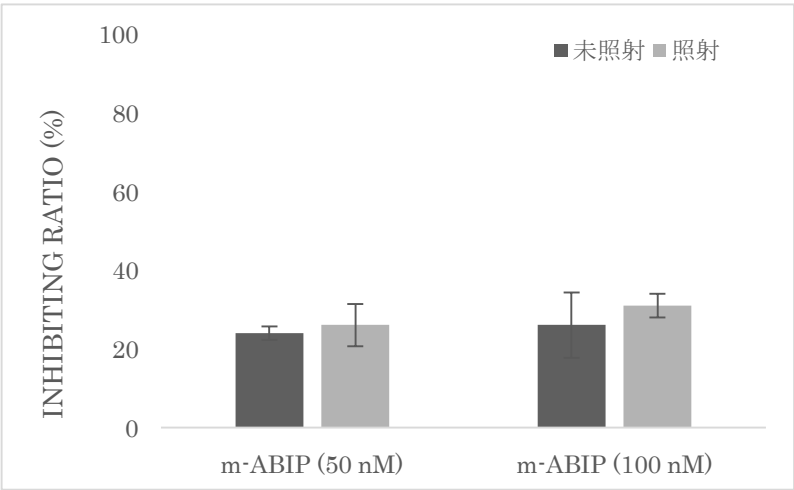


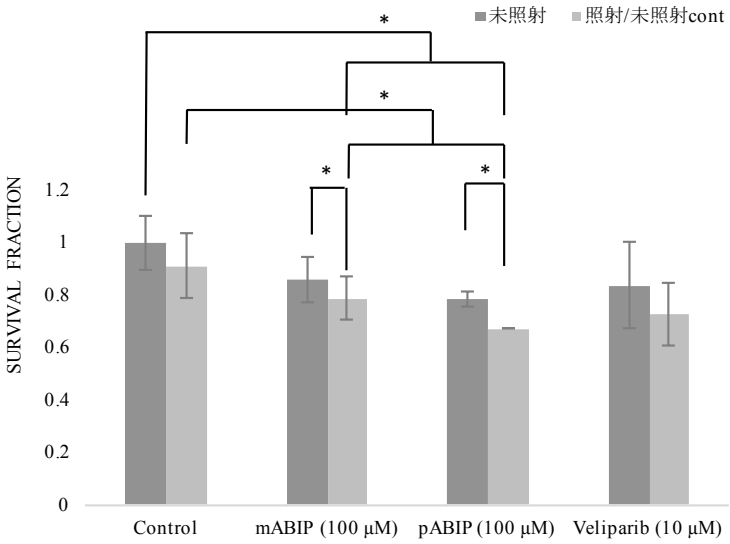
Figure 10. Radio sensitizing activity against MDA-MB-231 and MCF7.

As well as screening results, Veliparib is the highest radiosensitizing effect. Both *m- and p*-ABIP was exhibited radio sensitizeing activity against MCF7 and MDA-MB-231. *p*-ABIP was higher radio

sensitizing effect than m-ABIP in 100 μM. MCF7 lacked caspase-3, exhibit resistance to cell death by ionizing radiation[15].

Finally, I evaluated neutron sensitizing activity of m-ABIP against PARP enzyme and MDA-MB-231 cells and as neutron source, IEC fusion devises was used in this experimental. Neutron sensitizing activity against enzyme was for 10 min irradiation, and against cell was for 2 h irradiated (Figure 11).





*: P<0.05

Figure 11. Neutron sensitizing activity of PARP enzyme and MDA-MB-231

PARP inhibitory activity of m-ABIP against PARP enzyme was enhanced by neutron irradiation for 10 min, And Antitumor activity was also enhanced neutron irradiation for 60 min.

4. Conclusion

In contrast, I described here in the design and synthesis of novel boron tracedrugs having PARP inhibiting activity (*m*-ABIP: inhibitory ratio 58.4%/1.0 μM, *p*-ABIP: inhibitory ratio 56.0%/1.0 μM). And the compounds showed significant radiationsensitizing activity against MCF7 and MDA-MB-231 cells. Moreover, it is observed that PARP inhibitory activity of the *m*-ABIP and antitumor activity of *m*-, *p*-ABIP against MDA-MB-231 cells were enhanced by neutron irradiation. These results suggest that PARP targeted-boron tracedrugs with neutron irradiation have a great potential for BRCA-mutated cancer.

5. Material and methods

Material and method

General.

All chemicals were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan), Wako Pure Chemical Industries Ltd. (Osaka, Japan), and Sigma Aldrich Japan (Tokyo, Japan). All reaction carried out under nitrogen and atmosphere. Nuclear magnetic resonance (NMR) ^{1}H spectra were obtained using a JNM- EX400 spectrometer (Jeol Ltd., Tokyo, Japan) at 400 MHz. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin- layer chromatography was performed on glass-backed silica gels (Merck 60 F_{254}) and components were visualized using ultraviolet (UV) light. Column chromatography was performed using KANTO Chemical silica gel 60 N (spherical neutral) 40-50 μm. X-ray irradiator was MX-160 Labo (MediXtec Japan Co., Chiba, Japan).

Synthesis of 4-nitro-2-benzofuran-1,3-dione (1)

3-nitrophathalic acid (5.05 g, 23.9 mmol) and acetic anhydride (2.52 μl 23.9 mmol) were refluxed for 30

min. The reaction mixture was cooled to room temperature. The precipitation was filtered and washed with diethyl ether to give the product as white yellow solid (3.91 g, 18.5 mmol).

Yield: 77.4%; ^{1}H NMR (400MHz, CD_3OD) δ8.37-8.39 (m, 2H), 7.79 (t, 1H, J = 8.24Hz).

Synthesis of 2-(aminocarbonyl)-3-nitorobenzoic acid-K (2)

To a round-bottle flask was added 28% NH_3 aq (14.5 ml, 0.133 mmol) at -5℃, and then, 4-nitro-2-benzofuran-1,3-dien (7.73 g, 0.04 mmol) and acetic anhydride (2.52 ml, 23.9 mmol) was added slowly. After added, KOH in 5 ml of water (2.65 g, 0.04 mol) is added and stirred for 5 min at room temperature. And then ethanol and diethyl ether were added each 3 ml, the precipitate was collected and dried to give the product as yellow solid (6.13 g, 0.024).

Yield: 83.8% (400MHz, CD_3OD) δ7.95-8.00 (m, 2H), 7.57 (t, 1H, J = 8.24Hz).

Synthesis of 2-amino-3-nitorobenzoic acid (3)

To a round-bottle flask was added KOH (462 mg, 8.24 mmol) and water (4 ml),

the mixture were stirred at 0℃. And then, Br_2 (248 mg, 79.5 μl) was added slowly. After 5 min, 2-(aminocarbonyl-3-nitorobenzoic acid-K (444 mg, 1.78 mmol) was added and stirred for 15 min at room temperature. And then the mixture was heated to 55℃ for 30 min. After 30 min, the mixture stirred for overnignt at room temperature. The reaction mixture was added 20 ml of water, and acidification with HCl, the precipitate was collect and dry to give the product (149 mg, 0.82 mmol).

Yield: 67.8% (400MHz, CD_3OD) δ8.23-8.28 (m, 2H), 7.57 (t, 1H, J = 8.24Hz)

Synthesis of 2-amon-3-nitrobenzoic acid (4)

To a 100ml round-bottle flask was added 2-amino-3-nitro-benzoic acid (2.2 g, 12.1 mmol), THF (10ml) DMF (10 drop). The mixture was cooled to 0℃ and added $SOCl_2$ followed by stirred for 10 min. And then, the reaction mixture was refluxed for 3.5 h. The mixture was cooled to 0℃, and added NH_3 aq (20 ml) and then stirred for 30 min. The reaction mixture was filtered. The precipitate was washed with water

and CH_2Cl_2. The residue was collected and dried to give the product as yellow solid (1.25 g, 6.9 mmol).

Yield: 57.5% (400MHz, CD_3OD) δ8.26 (dd, 1H, *J*=8.72 Hz, 1.82 Hz), δ7.88 (dd, 1H, *J* = 7.56 Hz, 1.56 Hz), δ6.67 (t, 1H, J=7.80 Hz). EI-MS *m/z* 181 (M^+).

Synthesis of 2,3-diaminobenzamide 2HCl (5)

To a side arm flask was added 2-amon-3-nitrobenzoic acid, MeH, THF and Pd/C (10% activity). The mixture was stirred under the H_2 gas for 6.5 h. The reaction mixture was added EtOAc (20 ml) and filtered by celite. The filtrate was flowed HCl gas for 15 min. The precipitate was collected and dried.

Yield 68.1% (400MHz, CD_3OD) δ7.02(dd, 1H, *J*=8.72 Hz, 1.36 Hz), δ6.81 (dd, 1H, *J* = 7.72 Hz, 6.67 Hz), δ6.67 (t, 1H, J=7.80 Hz). EI-MS *m/z* 56 (M^+).

Synthesis of (2-(4-carbamoyl-1*H*-benzo[*d*]imidazol-2-yl) phenyl) boronic acid

To a solution of 2-Formylphenylboronic acid (75 mg, 0.5 mmol) in DMF (4 ml) was added MIDA and 4 Å molecular sieves (200 mg). The reaction mixture heated to 120℃ for 3h. The reaction mixture was added MIDA (222 mg, 1.5 mmol) and KI (16.6 mg, 0.1 mmol). The reaction mixture was exposed air and allow to stir at 80℃ in an open flask for 4h. The mixture was cooled to room temperature and added NaOH (3 ml, 1 N). The reaction mixture was diluted with 5 ml of water, and wash with CH_2Cl_2. The combined basic aqueous layer was filtered. The filtrate was acidification with HCl (1 N) until the pH 7 and extracted with EtOAc. The organic layer was dried with sodium sulfate and concentrated. The residual solution was added CH_2Cl_2. The precipitate was collected by filtration and purified by ODS column chromatography with MeOH and water to give the product as white solid.

Yield 25.6% (400MHz, CD_3OD) δ8.08-8.06 (d, 1H, *J*=8.00 Hz), δ7.95-7.93 (d, 1H, *J* = 8 Hz), δ7.84 (s, 1H, J=8.00 Hz) δ7.55-7.51 (m, 3H) δ7.45-7.41 (dd, 1H, *J*=8.00 Hz).

Synthesis of (3-(4-carbamoyl-1*H*-benzo[*d*]imidazol-2-yl) phenyl) boronic acid

To a solution of 3-Formylphenylboronic acid (75 mg, 0.5 mmol) in DMF(4 ml) was added MIDA and

4 Å molecular sieves (200 mg). The reaction mixture heated to 120℃ for 3h. The reaction mixture was added MIDA (222 mg, 1.5 mmol) and KI (16.6 mg, 0.1 mmol). The reaction mixture was exposed air and allow to stir at 80℃ in an open flask for 4h. The mixture was cooled to room temperature and added NaOH (3 ml, 1 N). The reaction mixture was diluted with 5 ml of water, and wash with CH_2Cl_2. The combined basic aqueous layer was filtered. The filtrate was acidification with HCl (1 N) until the pH 7 and extracted with EtOAc. The organic layer was dried with sodium sulfate and concentrated. The residual solution was added CH_2Cl_2. The precipitate was collected by filtration and purified by ODS column chromatography with MeOH and water to give the product as white solid.

Yield 23.7% (400MHz, CD_3CN) δ8.17-8.15 (d, 2H, *J*=8.00 Hz), δ7.94-7.92 (d, 3H, *J* = 8.00 Hz), δ7.84 (dd, 1H, *J*=8.00 Hz).

Synthesis of (4-(4-carbamoyl-1H-benzo[d]imidazol-2-yl) phenyl) boronic acid

To a solution of 4-Formylphenylboronic acid (75 mg, 0.5 mmol) in DMF (4 ml) was added MIDA and 4 Å molecular sieves (200 mg). The reaction mixture heated to 120℃ for 3h. The reaction mixture was added MIDA (222 mg, 1.5 mmol) and KI (16.6 mg, 0.1 mmol). The reaction mixture was exposed air and allow to stir at 80℃ in an open flask for 4h. The mixture was cooled to room temperature and added NaOH (3 ml, 1 N). The reaction mixture was diluted with 5 ml of water, and wash with CH_2Cl_2. The combined basic aqueous layer was filtered. The filtrate was acidification with HCl (1 N) until the pH 7 and extracted with EtOAc. The organic layer was dried with sodium sulfate and concentrated. The residual solution was added CH_2Cl_2. The precipitate was collected by filtration and purified by ODS column chromatography with MeOH and water to give the product as white solid.

Yield 15.6% (400MHz, CD_3OD) δ8.18-8.16 (d, 2H, *J*=8.00 Hz), δ7.96-7.95 (d, 1H, *J*=4.00 Hz), δ7.82-7.80 (d, 2H, J=8.00 Hz) δ7.77-7.75 (d, 1H, *J*=8.00 Hz) δ7.40-7.36 (dd, 1H, J=4.00 Hz).

Cell lines.

The MCF7 and MDA-MB-231 human breast cancer cell lines were supplied by Mr. Yoshio Endo,

Kanazawa University, Ishikawa, Japan. The MCF7 cells were cultured in EMEM medium, 10% phosphate buffered saline and 1% penicillin-streptomycin. The MDA-MB-231 cells were cultured in RPMI-1640 medium, 10% phosphate buffered saline and 1% penicillin-streptomycin. Both cell lines were incubated at 37˚C in a humidified atmosphere of 5% CO_2.

Evaluation of PARP inhibiting activity.

PARP inhibiting activity was assayed using the Travigen Universal Colorimetric PARP Assay kit w/Histone Coated Strip Wells (Travigen Inc., Gaithersburg, MD, USA) according to the manufactuer's instruction. All drugs were dissolved in DMSO and the stock solution was diluted to final concentration of 1%DMSO.

Evaluation of radiation sensitizing activity of boron tracedrugs.

Brast cancer cells were plated into a φ6 cell culture dish and incubated 24 h. The final drug concentration was 100 μM, 10 μM, 10 μM or 1 μM, and final DMSO concentration was 1% in all plates (*m*-, *p*-ABIP: 100 μM or 10 μM. Veliparib: 10 μM or 1 μM). The cells were then immediately irradiated with 0 Gy, 2 Gy, 4 Gy by MX-160 Labo irradiator (MediXtec Japan Co., Chiba, Japan). Cells were incubated for 6 h, medium was aspirated, and fresh medium (3 ml) was then added. MDA-MB-231 cells were incubated for 10 days, MCF7 cells were incubated for 12 days. Cells were washed with 1xPBS, and then fixed MeOH for 10 min. After fixed MeOH was aspirated and cells were stained with Giemsa stain solution for 1 h. After stained colonies were counted by the naked eye.

Evaluation of neutron sensitizing activity of boron tracedrugs.

MDA-MB-231 cells were plated 24 multiwell plate and incubated for 12 h. The final drug concentration was 100 μM or 10 μM, and final DMSO concentration was 1% in all plates (*m*-, *p*-ABIP: 100 μM. Veliparib: 10 μM). After drug added for 12 h, neutron irradiated for 2 h ($2x10^7$ n/s,) and room temperature was 30˚C. After 5h, the medium was changed to fresh and incubated for 48h. The cell viability was

measured by WST-1 assay. The absorbance was monitored at 450 nm.

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