Design, Synthesis and Destructive Dynamic Effects of BODIPY-containing and Curcuminoid Boron Tracedrugs for Neutron Dynamic Therapy

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Abstract. *Background: We previously designed the boron tracedrugs UTX-42, UTX-43, and UTX-44, which possess antioxidant potency. In order to explore their destructive dynamic effects when bombarded by weak thermal neutrons, we performed thermal neutron irradiation of bovine serum albumin (BSA) treated with the boron tracedrugs. Materials and Methods: Boron tracedrugs, including the boron dipyrromethene (BODIPY)-containing compounds UTX-42, UTX-44, and UTX-47 and the curcuminoid compounds UTX-50 and UTX-51, were designed for neutron dynamic therapy based on their molecular orbital calculation. Newly designed UTX-47, UTX-50, and UTX-51 were synthesized. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to detect decomposition by thermal neutron irradiation of BSA treated with these boron tracedrugs. Results: The combination of 1.0 μM BSA with 100 μM of each of the boron tracedrugs showed a decrease in band intensity after irradiation. Conclusion: All boron tracedrugs tested caused destructive dynamic damage of BSA during thermal neutron irradiation, suggesting that boron tracedrugs could be used as dynamic drugs for neutron dynamic therapy.*

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We are currently studying the medicinal chemistry of boron tracedrugs we developed, which are examples of next-generation 'on-demand traceable' drugs (1, 2). In the early stages of drug discovery and development, medicinal chemistry researchers make a great effort to evaluate the pharmacokinetics (PK) of drug candidates. Recently, increased demand for PK studies has encouraged researchers to develop drugs with superior traceability. Traditionally, radiolabeled compounds have been studied for these purposes. These, however, have two inherent problems: their half-life and specific regulations for use by experimental facilities. These problems have increased the need for medicinal chemists to develop traceable drugs that do not require radioisotope labeling themselves. We had the idea of developing tracedrugs with boron atoms embedded in their scaffold. This idea was based on our previous drug design studies for the development of hypoxia-targeting boron-10 (^{10}B) carrier compounds for boron neutron capture therapy (BNCT) (1, 3-6). ^{10}B, a naturally occurring and stable isotope (19.9%), possesses neutron capture activity that produces a prompt γ-ray when irradiated by a thermal neutron. The ^{10}B concentration can be measured by neutron-induced prompt γ-ray spectroscopy (NIPS) to detect the actual localization of boron tracedrugs.

Here, we discuss our boron tracedrug approach to the development of dynamic drugs for neutron dynamic therapy (NDT); we termed these drugs 'neutron dynamic therapeutics'. We also provide an overview of our concept of a boron tracedrug for NDT (Figure 1) (1). Neutrons attack ^{10}B in boron tracedrugs bound to macromolecules such as proteins, DNA/RNAs, sugars, and lipids, and generate a vast amount of energy that subsequently decomposes the boron tracedrugs and their adjacent molecules.

We previously designed the boron tracedrugs UTX-42, UTX-43, and UTX-44, which possess antioxidant potency. In order to explore their additional potential and the

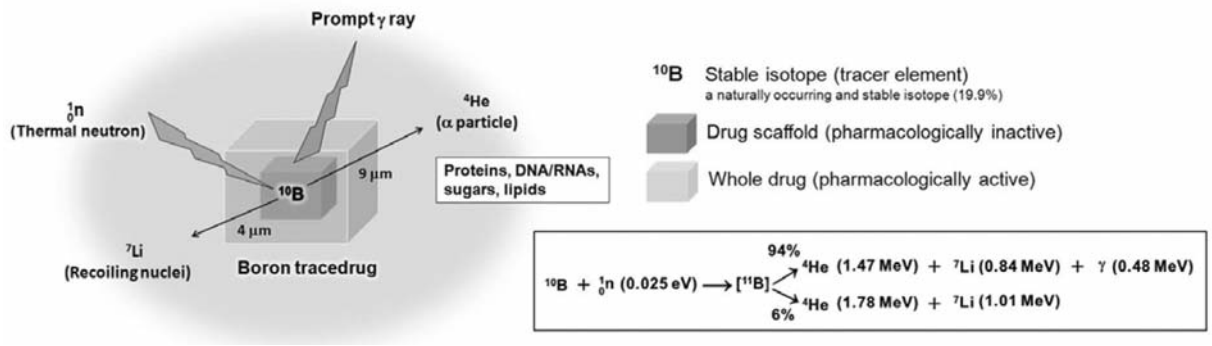

Figure 1. *Overview of the concept of boron tracedrugs as dynamic drugs for neutron dynamic therapy.*

destructive physical power exerted by weak thermal neutrons, thermal neutron irradiation of bovine serum albumin (BSA) treated with UTX-42 (2) and UTX-44 (2) and the newly designed boron tracedrugs UTX-47, UTX-50, and UTX-51 was performed (Figure 4).

Materials and Methods

Materials. All chemicals were purchased from Tokyo Chemical Industry Co. Ltd, Wako Pure Chemical Industries Ltd., and Sigma-Aldrich.

Molecular orbital (MO) calculation. The *ab initio* MO calculation was performed with the B3LYP hybrid density function in conjugation with the 6-31G(d) basis set using the Gaussian 03 suite of programs (7). The molecular geometries calculated by Gaussian 03 were visualized using MOLKEL 4.3 (8).

*Synthesis of UTX-47 (**4**), UTX-50 (**7**), and UTX-51 (**9**)*. Newly designed boron tracedrugs **4**, **7**, and **9** were synthesized according to the method reported previously with slight modifications: **4** with reference to (9) and **7** and **9** with reference to (10) (Figures 2 and 3, respectively).

*Synthesis of 2-methylpyrrole (**2**)*. Pyrrole-2-carboxyaldehyde (**1**) (2.4 g, 25 mmol) and KOH (4.3 g) were dissolved in ethylene glycol (30 ml). Hydrazine monohydrate (4.5 ml) was then added, at which point the reaction mixture became a yellow slurry. The reaction mixture was refluxed for 2 h, during which time the yellow solid was dissolved. Distillation under reduced pressure produced water and 2-methyl pyrrole. The organic layer was extracted with ether and dried with $MgSO_4$. The solvent was evaporated to give 2-methylpyrrole (1.1 g, 48.8%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (s, 1H), 5.97 (s, 1H), 5.80 (s, 1H), 2.27 (s, 3H).

Synthesis of UTX-47. 2-Methylpyrrole (220 μl, 1.3 mmol) and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (**3**; 300 mg, 2.6 mmol) were dissolved in dry CH_2Cl_2 (64 ml) under an N_2 atmosphere. One drop of trifluoroacetic acid (TFA) was added, and the solution was stirred overnight at room temperature (r.t.). After complete consumption of the aldehyde, a solution of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ; 291 mg, 1.3 mmol) in CH_2Cl_2 was added with stirring for 1 h. *N,N*-Diisopropylethylamine (DIEA; 2.0 ml, 13 mmol) was added to the resulting solution with stirring for 20 min followed by boron trifluoride etherate (BF_3·OEt_2) (1.6 ml, 13 mmol). The mixture was stirred for 2 h, and then washed with aqueous saturated $NaHCO_3$ and brine. The combined organic fractions were dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The residue was purified by basic alumina column chromatography (eluant of ether:hexane 8:1) to give UTX-47 (46 mg, 8.5%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 6.78 (d, 2H, *J*=3.6 Hz), 6.28 (d, 2H, *J*=3.6 Hz), 5.53 (s, 6H), 1.47 (s, 18H); HRMS (FAB$^+$) calculated for $C_{25}H_{31}BF_2N_2O_4$ (M^+) *m/z* 424.2497. found 424.2527; Anal. calculated for $C_{25}H_{31}BF_2N_2O$: C: 70.76, H: 6.60, N: 7.36; found: C: 70.55, H: 7.37, N: 6.50.

*Synthesis of difluoroboron pentanedione (**6**) (10)*. 2,4-Pentanedione (**5**) (500 μl, 4.9 mmol) and BF_3·OEt_2 (680 μl, 4.9 mmol) were mixed together, and the resulting solution was heated at 60˚C for 3 h. After cooling to r.t., the reaction mixture was subjected to evaporation under vacuum and a pale yellow semisolid was obtained, which solidified after standing at r.t., giving pale yellow needle-like crystals of **6** (676 mg, 94.0%); ^{1}H NMR (400 MHz, CD_2Cl_2): δ 5.96 (s, 1H), 2.30 (s, 6H).

Synthesis of UTX-50. Difluoroboron pentanedione (100 mg, 0.7 mmol) and **3** (318 mg, 1.4 mmol) were dissolved in CH_3CN (3.0 ml). DIEA (520 μl, 2.0 mmol) was then added, and the reaction mixture was stirred at 60˚C for 4 h. The solvent was evaporated, and the residue was purified by silica gel column chromatography (eluant of CH_2Cl_2: hexane 1:1) to give UTX-50 (9.7 mg, 2.5%); ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.94 (d, *J*=15.4 Hz, 2H), 7.39 (s, 4H), 6.49 (d, *J*=15.4 Hz, 2H), 5.99 (s, 1H), 5.65 (s, 2H), 1.40 (s, 36H); HRMS (FAB$^+$) calculated for $C_{35}H_{47}BF_2O_4$ (M^+) *m/z* 580.3535, found 580.3535.

Synthesis of UTX-51. Curcumin (**8**) (50 mg, 0.14 mmol) was suspended in Et_2O (5.0 ml), and BF_3·OEt_2 (50 μl, 0.407 mmol) was added dropwise. The reaction mixture was stirred at r.t. for 20 h. The solvent was evaporated under vacuum. The residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2 eluent) to give UTX-51 (27 mg, 48%); ^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.99 (d, *J*=15.4 Hz, 2H), 7.53-7.61 (m, 1H), 6.96-7.08

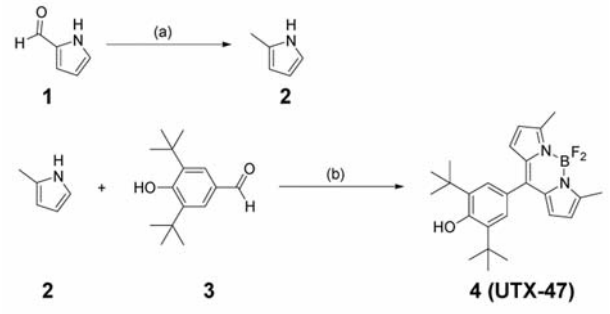

Figure 2. *Synthesis of the newly designed BODIPY-containing boron tracedrug UTX-47* ***(4)****. (a) Hydrazine monohydrate, KOH, ethylene glycol, reflux, 2 h, 48.8%. (b) TFA, DDQ, DIEA, boron trifluoride etherate.*

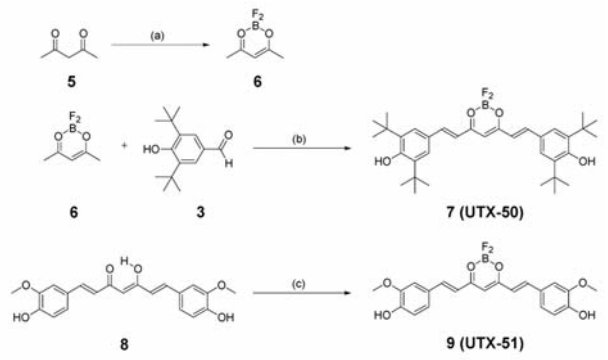

Figure 3. *Synthesis of the newly designed curcuminoid boron tracedrugs UTX-50* ***(7)*** *and UTX-51* ***(9)****. (a) BF_3·OEt_2, 60˚C, 3 h, 94%. (b) DIEA, acetonitrile, 60˚C, 2.5%. (c) BF_3·OEt_2, ether, r.t., 20 h, 48%.*

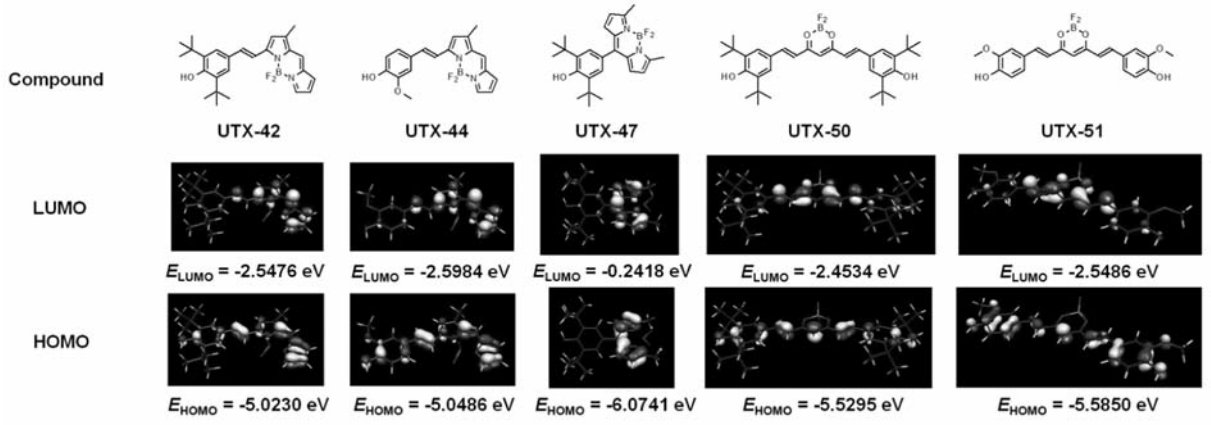

Figure 4. *Molecular orbitals and their energy levels of the BODIPY-containing boron tracedrugs UTX-42, UTX-44, and UTX-47 and the curcuminoid boron tracedrugs UTX-50 and UTX-51.*

(m, 4H), 6.56 (d, *J*=15.4 Hz, 2H), 6.02-6.03 (m, 3H), 3.97 (s, 6H); HRMS (FAB+) calculated for $C_{21}H_{19}BF_2O_6$ (M+) *m/z* 416.1243, found 416.1252.

Neutron irradiation. UTX-42, UTX-44, UTX-47, UTX-50, and UTX-51 were used for the BSA irradiation study. The final concentration of the boron tracedrugs was 100 μM (diluted with pH 7.0 wide-range buffer from 2.0 mM boron tracedrug stock solution in DMSO) and 10 mg/ml BSA stock solution was made up in phosphate-buffered saline (PBS). Thermal neutron irradiation was performed using a reactor neutron beam with a cadmium (Cd) ratio of 9.4. The neutron fluence was measured from the radioactivation of gold foils at the front of the sample tubes, and the average neutron fluence determined from the values measured was used. Contaminating γ-ray doses, including secondary γ-rays, were measured with thermoluminescence dosimeter powder at the front of the sample tubes. The absorbed dose was calculated using the flux-to-dose conversion factor (11).

Results

We performed MO calculations to aid in the design of the boron tracedrugs for NDT. In particular, the MO calculations enabled us to examine the extent to which the boron dipyrromethene (BODIPY) unit in UTX-42, UTX-44, and UTX-47 and the difluoroboronate moiety in UTX-50 and UTX-51 influenced the overall electronic states of the molecules. The MO calculations were performed using Gaussian 03 with B3LYP/6-31G(d). As shown in Figure 4, the lowest unoccupied MO (LUMO) and the highest occupied MO (HOMO) of all the boron tracedrugs tested were not localized at the boron connection site like the general molecules without boron-containing moieties such as BODIPY and difluoroboronate molecules. The energy levels of the LUMOs (E_{LUMO}) and HOMO (E_{HOMO}) of these boron tracedrugs for NDT had general values.

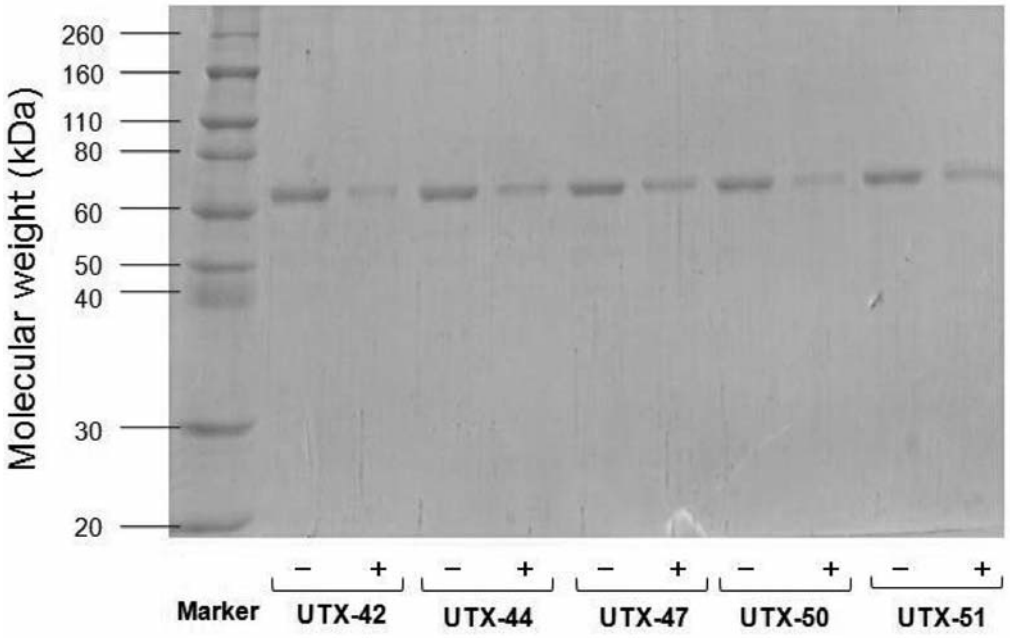

Figure 5. *BSA treated with boron tracedrug on SDS-PAGE gel stained with Coomassie Brilliant Blue (CBB) (boron tracedrug:BSA=100:1). The molecular weight of BSA was 66.0 kDa. +, 100 μM of the boron tracedrug (0.2 ppm ^{10}B) +1.0 μM BSA with thermal neutron irradiation. –, 100 μM of the boron tracedrug (0.2 ppm ^{10}B) +1.0 μM BSA without thermal neutron irradiation. The absorbed dose was 2.1 Gy, with an irradiation time of 45 min.*

Synthesis of new boron tracedrugs for NDT, such as BODIPY-containing boron tracedrugs like UTX-47 and curcuminoid boron tracedrugs like UTX-50 and UTX-51, was accomplished with reasonable and moderate yield, as shown in Figure 2 and Figure 3, respectively.

Thermal neutron irradiation was conducted at the Kyoto University Research Reactor Institute (KURRI). SDS-PAGE was performed to detect the decomposition of BSA treated with the five boron tracedrugs (UTX-42, UTX-44, UTX-47, UTX-50, and UTX-51) and thermal neutron irradiation. Electrophoresis-based analysis indicated a decrease in the intensity of the bands for BSA treated with the boron tracedrugs and thermal neutron irradiation.

Discussion

We present here the destructive dynamic effects of boron tracedrugs such as BODIPY-containing and curcuminoid compounds for NDT. We recognize that NDT using boron tracedrugs conceptually resembles BNCT using a boron carrier for cancer treatment. NDT, however, can be applied for all macromolecule-related diseases, whereas BNCT can only be used for cancer treatment targeting DNA in cancer cells. A similar concept to our NDT is a bone-specific directed radiopharmaceutical for palliation of painful bone metastases (12). Our boron tracedrugs contain stable isotope boron only and no radioisotope; this is beneficial for the development of clinical drugs. Our idea of using these boron tracedrugs for NDT was stimulated by the progress of BODIPY-containing bioimaging probes, especially a report on difluoroboron-derivatized curcumins as near-infrared probes for *in vivo* detection of amyloid deposits (10). We believe that these boron tracedrugs for NDT are prototypes of dynamic drugs that will be available in the future.

Conclusion

To the best of our knowledge, herein, we present data showing for the first time that boron tracedrugs such as UTX-42, UTX-44, UTX-47, UTX-50, and UTX-51 cause destructive dynamic damage of BSA during thermal neutron irradiation. We suggest that boron tracedrugs can be used as dynamic drugs for NDT.

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