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Boronic Prodrug of Endoxifen as an Effective Hormone Therapy for Breast Cancer

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Abstract

As a prodrug, tamoxifen is activated by the P450 enzyme CYP2D6 that is responsible for converting it to the active metabolites, 4-hydroxytamoxifen and endoxifen. Patients with genetic polymorphisms of CYP2D6 may not receive the full benefit of tamoxifen therapy. There is increasing evidence that poor metabolizer patients have lower plasma concentrations of endoxifen and suffer worse disease outcome, although some clinical studies reported no correlation between CYP2D6 polymorphism and tamoxifen therapy outcome. Endoxifen is currently undergoing clinical trials as a potentially improved and more potent SERM (Selective Estrogen Receptor Modulator) for endocrine therapy that is independent of CYP2D6 status in patients. However, direct administration of endoxifen may present the problem of low bioavailability due to its rapid first-pass metabolism via O-glucuronidation. We have designed and synthesized ZB483, a boronic prodrug of endoxifen suitable for oral administration with greatly enhanced bioavailability by increasing the concentration of endoxifen in mouse blood. Our study demonstrated that ZB483 potently inhibited growth of ER+ breast cancer cells in vitro and was efficiently converted to endoxifen in cell culture media by oxidative deboronation. In vivo this metabolic conversion is equally efficient as indicated in the pharmacokinetic study. Moreover, at the same dose, ZB483 afforded a 30-40 fold higher level endoxifen in mouse blood compared to unconjugated endoxifen administration. The significantly enhanced bioavailability of endoxifen conferred by the boronic prodrug was further validated in an in vivo efficacy study. ZB483 was demonstrated to be more efficacious than endoxifen in inhibiting xenograft tumor growth in mice at equal dosage but more so at lower dosage. Together, these preclinical studies demonstrate that ZB483 is a promising endocrine therapy agent with markedly enhanced bioavailability in systemic circulation and superior efficacy compared to endoxifen.

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Keywords

ZB483; boronic prodrug of endoxifen; CYP2D6 polymorphisms; bioavailability; pharmacokinetics

Introduction

Tamoxifen is extensively metabolized to yield a large number of primary and secondary metabolites with widely varying antiestrogenic activities [1] of which the most potent are two known metabolites, 4-hydroxytamoxifen and endoxifen. As illustrated in Figure 1, the primary metabolic pathway of Tamoxifen, catalyzed by the cytochrome P450 enzyme, CYP3A, is loss of an N-methyl group to form N-desmethyltamoxifen that has a similar antiestrogenic potency as tamoxifen [2-5]. N-Desmethyltamoxifen is then oxidized to yield a secondary metabolite, 4-hydroxy-N-desmethyltamoxifen, also known as endoxifen, which is primarily catalyzed by CYP2D6 [1]. In a separate metabolic pathway, tamoxifen is hydroxylated at the 4- phenyl position to produce the active metabolite, 4-hydroxytamoxifen (4-OHT), which in turn could lose a methyl group to yield endoxifen, a process catalyzed primarily by CYP3A [1, 6-8]. While it has long been established that 4-hydroxytamoxifen is an active metabolite with up to 100 times greater potency than the parent compound [9-11], recent studies have shown that endoxifen is as potent as 4-OHT, as evidenced by its binding affinity to ER α , its ability to inhibit estrogen stimulated breast cancer cell proliferation, and its regulation of estrogen responsive genes [12-14]. Moreover, given that plasma concentrations of endoxifen in patients are 5-7 times higher than those of 4-hydroxytamoxifen, it is now recognized that endoxifen may be a clinically more important active metabolite than 4-hydroxytamoxifen in tamoxifen-based endocrine therapy [15-17]. Indeed, it has been shown that decreased blood level of endoxifen correlates negatively with tamoxifen treatment outcomes [18].

The effect of CYP2D6 polymorphism on the plasma level of endoxifen is well understood, but the correlation between poor metabolizer and tamoxifen therapy outcome has been controversial [19-21]. From the standpoint of ensuring therapeutically effective concentration levels in patients, the active metabolites represent desirable alternative regimens to tamoxifen for breast cancer patients lacking the active form of the P450 enzyme CYP2D6 [22, 23]. Moreover, if endoxifen and 4-OHT are nearly 100 times more potent than tamoxifen, it can be expected that lower doses of the active metabolites would suffice to achieve therapeutic efficacy, thus potentially reducing adverse side effects of endocrine therapy in non-target tissues. To this end, two phase I clinical trials are currently being conducted that evaluate the safety and effectiveness of endoxifen in breast cancer patients with hormone receptor positive solid tumors that have not responded to standard treatment [24,25]. As a more polar metabolite, however, endoxifen may not be as readily bioavailable as its parent compound tamoxifen due in part to rapid formation of metabolic conjugates such as O-glucuronidation [26].

To improve the bioavailability of endoxifen as a potential therapeutic agent for the treatment of breast cancer or the prevention of recurrence, we have designed and synthetically

prepared a boronic derivative of endoxifen as a prodrug, ZB483. We assessed the in vitro pharmacology and metabolism using breast cancer cells expressing the estrogen receptor (ER+). Next we used a mouse xenograft tumor model to determine the in vivo efficacy of the prodrug as compared to endoxifen. To investigate the systemic bioavailability and in vivo metabolism we performed pharmacokinetic studies in mice, on both endoxifen and ZB483.

Materials and Methods

Chemicals and reagents

All reagents, solvents, and analytical standards were purchased from Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Fairfield, NJ), AK Scientific (Union City, CA), and CombiPhos Catalysts (Princeton, NJ), and were used as received. (E,Z)-4-(1-(4-(2-((ethoxycarbonyl)(methyl)amino)ethoxy)phenyl)-2-phenylbut-1-en-1-yl)phenyl trifluoromethanesulfonate (**3**) was obtained following the reported synthetic procedure [28]. ¹H and ¹³C NMR spectra were obtained on a Bruker AX 300 spectrometer. Chemical shifts are reported as parts per million (ppm) relative to TMS. Mass spectral data were collected on a Shimadzu LC-MS 2010 instrument and HRMS spectra data were collected on a Thermo LTQ Orbitrap-XL mass spectrometer in positive ion mode. Unless specified otherwise, all tested compounds were confirmed to be >95% pure by HPLC.

Preparation of ZB483

To a 1,4-dioxane solution of (E,Z)-4-(1-(4-(2-((ethoxycarbonyl)(methyl)amino)ethoxy)phenyl)-2-phenylbut-1-en-1-yl)phenyl trifluoromethanesulfonate (**3**, 0.15 g, 0.28 mmol) were added bis(pinacolato)diboron (0.08 g, 0.30 mmol), PdCl₂(dppf) (0.015 g, 5% mol) and KOAc (0.07 g, 0.7 mmol), and the mixture was stirred under N₂ at 80 °C overnight. After the solution was cooled, the dioxane was removed under vacuum, and CH₂Cl₂ and water were added. The resulting mixture was extracted with dichloromethane twice, and the combined organic layer was washed with brine and then dried over MgSO₄. The organic solvent was concentrated in vacuo. The crude was purified by flash chromatography to afford product **4** (0.10 g) in 80 % yield. ¹H-NMR (300MHz, CDCl₃): 7.81 (d, J=8.1 Hz, 1H, Ph-H), 7.46 (d, J=8.4 Hz 1H, Ph-H), 7.25~7.12 (m, 7H, Ph-H), 6.90~6.5 (m, 2H, Ph-H), 6.77 (d, J=8.7 Hz, 1H, Ph-H), 6.53 (d, J=8.7 Hz, 1H, Ph-H), 4.16~3.97 (m, 4H, -O-CH₂), 3.67~3.56 (m, 2H, -N-CH₂), 3.06~2.98 (doubles, 3H, -NCH₃), 2.52~2.43 (m, 2H, CH₃CH₂-), 1.37 (s, 3H, CH₃-), 1.29~1.28 (m, 12H, CH₃-), 0.98~0.90 (m, 3H, CH₃-). ¹³C-NMR (75MHz, CDCl₃): 146.9, 146.4, 142.3, 142.1, 134.6, 133.8, 132.0, 130.7, 130.2, 129.7, 128.9, 127.9, 126.2, 114.0, 113.3, 83.8, 83.7, 83.6, 61.6, 36.0, 29.1, 25.0, 24.9, 24.8, 24.6, 14.7, 13.6. HR-MS (M+H): calculate 556.3234; found 556.3231.

To a freshly distilled THF solution of (E, Z)-ethyl methyl(2-(4-(2-phenyl-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)but-1-en-1-yl)phenoxy)ethyl)carbamate (**4**, 0.10 g, 0.18 mmol) cooled to -78°C in a dry ice/acetone bath was added 0.125 mL of butyllithium solution (1.6 M in hexanes, 0.2 mmol) dropwise via syringe. After 15 min, the dry ice/acetone bath was removed and the reaction mixture was allowed to warm to room

temperature and stirred overnight. The resulting mixture was quenched with saturated NH_4Cl solution and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over MgSO_4 . The organic solvent was concentrated in vacuo. The crude was purified by flash chromatography to afford **ZB 483** (0.048 g) in 67 % yield. ^1H -NMR(300MHz, CDCl_3): 7.79 (d, J = 7.8Hz, 1H, PhH), 7.43 (d, J = 7.8Hz, 1H, Ph-H), 7.21 (d, J = 7.8Hz, 1H, Ph-H), 7.14~7.09 (m, 6H, Ph-H), 6.93 (d, J = 7.5Hz, 1H, Ph-H), 6.84 (d, J = 8.1Hz, 1H, Ph-H), 6.76 (d, J = 7.5Hz, 1H, Ph-H), 6.59 (d, J = 7.8Hz, 1H, Ph-H), 4.30 (m, 1H, -O- CH_2 -), 4.14 (m, 1H, -O- CH_2 -), 3.70 (s, 3H, -N- CH_3), 3.28 (m, 2H, -N- CH_2 -), 2.44 (m, 2H, CH_3CH_2 -), 1.25 (s, 12H, 4 $\times$ CH_3 -), 0.90 (m, 3H, CH_3 -). ^{13}C -NMR (75MHz, CDCl_3): 156.2, 155.4, 146.2, 142.6, 142.2, 141.9, 138.0, 137.9, 137.0, 134.6, 133.8, 132.0, 130.7, 129.6, 128.8, 127.9, 126.2, 114.4, 113.7, 83.8, 83.6, 67.1, 48.1, 33.3, 29.1, 25.0, 24.9, 13.6, 13.5. HR-MS ($\text{M}+\text{H}$): calculated: 484.3023; found: 484.3060.

In vitro breast cancer cell proliferations assays

The MCF-7 cell line was purchased from ATCC (ATCC #HTB-22, Manassas, VA). The T47D cell line was a gift from Dr. Thomas Wiese of Xavier University of Louisiana. The breast cancer cell lines were routinely cultured in phenol red-free DMEM medium supplemented with 5% FBS, 4mM glutamine, 1mM sodium pyruvate, 100IU/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin and 0.25 $\mu\text{g}/\text{mL}$ amphotericin. Cultures were maintained in 5% carbon dioxide at a temperature of 37°C. For growth assays in the presence of tamoxifen, 4-OHT, endoxifen or ZB483, MCF-7 and T47D cells were plated in six-well plates at a density of 50,000 each well in 5% FBS DMEM medium. The cells were then treated with tamoxifen, 4-OHT, endoxifen, or ZB483 separately at 6 different doses ranging from 10^{-8} M to 5×10^{-6} M for 5 days, while equal volumes of DMSO were used as vehicle controls. Viable cell numbers were counted with a Z Series Coulter Counter instrument (Beckman-Coulter) following manufacturer's instructions. The ratio of drug treated viable cell numbers to vehicle treated viable cell numbers was defined as survival ratio where the control has the survival ratio of 100%. IC_{50} values were obtained from dose-response curves for all treatments. Experiments were conducted in triplicate and data represented as mean $\pm$ SD. For metabolic transformation of ZB483 in cell culture, the drug was incubated with MCF-7 cells in 12- well plates at a concentration of 10^{-6} M. Media samples were collected at 11 time points from 0, 1, 2, 4, 8, 16, 24, 28, 72, 96, 120 hrs. Media samples were immediately mixed with 1 mL of chilled methanol, centrifuged, and brought to a final volume of 3 mL for each sample before HPLC-MS/MS analysis.

Mouse xenograft tumor growth assay

4-6 week old female ovariectomized Nu/Nu mice were purchased from Charles River Laboratories (Wilmington, MA). The mice were given a period of adaptation in a sterile and pathogen-free environment with phytoestrogen-free food and water ad libitum. MCF-7 cells were harvested in the exponential growth phase using a PBS/EDTA solution. The animals were injected bilaterally in the mammary fat pad (MFP) with 5×10^6 viable cells suspended in 50 μL sterile PBS mixed with 100 μL Matrigel (reduced factor; BD Biosciences, Bedford, MA). 17β - Estradiol pellets (0.72 mg, 60-day release; Innovative Research of America, Sarasota, FL) were implanted subcutaneously in the lateral area of the neck using a precision trochar (10 gauge) at the time of cell injection. All procedures in animals were carried out

under anesthesia using a mixture of isoflurane and oxygen delivered by mask. Tumors were allowed to form and at day 15 post cell injection mice were randomized into groups of 10 mice each. Mice were treated daily with oral gavage of either vehicle (1:20 DMSO/PBS for i.p. or 1:10 ethanol/PBS for oral gavage) or a drug for 21 days. For the dose-dependent efficacy study five groups (10 mice/group) of tumor-bearing nude mice (one control and one group each per dose per drug). Tumor size ($\frac{4}{3} \times \pi \times L(\text{larger}) \times S^2(\text{shorter})$) was measured 3 times weekly using digital calipers.

Pharmacokinetics in mice

Female C57BL/6 mice were used for the pharmacokinetic study of ZB483. Mice were given oral gavage containing PBS and ethanol-dissolved ZB483 or endoxifen at a single dose of 1 mg/kg. After oral administration, blood samples were collected from the orbital sinus of the mice at various time points with each group of mice subjected to only one sampling. Murine blood was collected with a capillary into 1.5 ml microcentrifuge tubes containing 0.1 ml of 10 % EDTA anticoagulant. Plasma was then separated from cell pellets by centrifugation in a refrigerated centrifuge at 4 °C and transferred to a separate tube. Plasma samples were frozen at -80 °C until analysis.

Ethical considerations and statistical analysis

All procedures involving the animals were conducted in compliance with State and Federal laws, standards of the U.S. Department of Health and Human Services, and guidelines established by the Institutional Animal Care and Use Committee at Xavier University. All animal experiments were approved by Xavier's Institutional Animal Care and Use Committee. The facilities and laboratory animals program of Xavier University are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Statistical analyses were performed using Microsoft excel software. Pharmacokinetic data analyses were performed using the PKsoftware [27].

HPLC-MS/MS analysis of plasma samples

Plasma samples were extracted with chloroform:methanol (2:1) using traditional Folch method for lipid extraction. Methanol (1 mL) and chloroform (2 mL) were added to each plasma sample followed by addition of 5 ng trans-Tamoxifen-¹³C₂, ¹⁵N to each sample as the internal standard. The mixtures were stored at -20°C overnight. Next the samples were sonicated for 5 min and centrifuged with a Thermo Scientific Heraeus Megafuge16 Centrifuge. The top layer was transferred to another test tube. The bottom layer was washed with 1 mL chloroform:methanol (2:1), centrifuged, and the top layer was transferred and combined with the previous top layer. Eight tenth of a milliliter HPLC grade water was added to the extracts. After vortexing, the mixture was centrifuged. The bottom layer was dried out with nitrogen and re-suspended in 100 µL HPLC grade acetonitrile. An aliquot of 10 µL sample was injected onto a Hypersil Gold column (50×2.1 mm; particle size 1.9 µm, Thermo Scientific) on a Dionex Ultimate 3000 UPLC system equipped with a TSQ Vantage triple quadrupole mass spectrometer for analysis. A binary mobile phase (A: water with 0.05% formic acid B: acetonitrile with 0.05% formic acid) was used to achieve the gradient of initial 30% B for 1 min and then to 80% B at 8 min, to 100% B at 9 min, and returned to 30% B for 4 min. The flow rate was controlled at 0.6 mL/min. The settings of HESI source

were as follows: spray voltage (3200 volt); vaporizer temperature (365 °C); sheath gas pressure (45 psi); auxiliary gas pressure (10 psi); capillary temperature (330 °C). Nitrogen was used as the sheath gas and auxiliary gas. Argon was used as the collision gas.

Results

Structure and synthesis of ZB483, a boronic prodrug of endoxifen

As outlined in Figure 2, the monoalkylation of **1** by ethyl(2-hydroxyethyl)(methyl)carbamate prepared by reacting the alcohol with ethylchloroformate/triethylamine in dichloromethane yielded ethyl(2-(4-(1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)(methyl) (carbamate) (**2**). The compound **2** was then converted into the triflate **3** by reacting with trifluoromethanesulfonic anhydride in dichloromethane. The PdCl₂ (dppf)-catalyzed borylation of the triflate **3** formed the pinacolyl boronate ester **4** using a diboron reagent in the presence of KOAc. The removal of ethoxycarbonyl moiety from **4** with methyllithium at -78 °C afforded the endoxifen derivative **ZB483**.

The compound consists of an equimolar mixture of the E- and Z- isomers which upon oxidative hydrolysis is converted to an equimolar mixture of E- and Z- endoxifen. The use of an isomeric mixture instead of a single isomer for the prodrug composition is based on the fact that E- and Z- endoxifen isomers are roughly equipotent in their antiestrogenic effects on estrogen (E2)-induced gene expression [12,13,26].

ZB483 acts as a potent antiestrogen in vitro, comparable to endoxifen and 4OHT, but more active than tamoxifen

To determine the antiestrogenic activity of the boronic prodrug in breast cancer cells, we exposed two estrogen receptor-expressing breast cancer cell lines, MCF-7 and T47D to varying concentrations of ZB483. These cell lines are roughly representative of Luminal A and Luminal B ER α -positive breast cancers, as MCF-7 is p53-wild type and expresses high levels of ER α , while T47D is p53 mutant and expresses lower levels of ER α . The results, as shown in Figure 3, suggest that ZB483, like 4-hydroxytamoxifen and endoxifen, is a potent SERM. In MCF-7 cells, tamoxifen inhibited cell growth to a range of 64% (5 μ M) to 78% (0.1 μ M) whereas 4-OHT and endoxifen inhibited cell growth to a range of 48% (5 μ M) to 74% (0.1 μ M) and 33% (5 μ M) to 67% (0.1 μ M), respectively. With treatment of ZB483, growth of MCF-7 cells was decreased to 69% at 0.1 μ M, and to 31% at 5 μ M.

We observed a similar trend in T47D cells (Figure 3) with all drugs exhibiting lower potency, consistent with previous reports that T47D cells are less sensitive to antiestrogens than MCF-7 [29]. Again, ZB483 behaved as a SERM, in ways similar to endoxifen and another active metabolite of tamoxifen, 4-OHT.

The dose-response study of the antiestrogenic effects of ZB483 on MCF-7 and T47D cells as compared with tamoxifen, 4-OHT, and endoxifen yielded IC₅₀ values as listed in Table 1. The IC₅₀ concentration of ZB483 in MCF-7 cells was observed at 0.708 μ M, comparable to that of endoxifen (0.675 μ M), but nearly 40 times more potent than that of tamoxifen (25.77 μ M). In T47D cells, the IC₅₀ concentration of ZB483 was measured to be 8.73 μ M, compared to that of endoxifen at 4.29 μ M.

ZB483 is converted to endoxifen in breast cancer media culture

As ZB483 is intended as a prodrug of endoxifen aimed at providing a therapeutically effective concentration of endoxifen, the in vitro metabolism of ZB483 when incubated with breast cancer cells was first examined. As shown in Figure 4, in cell culture, ZB483, the pinacol ester form of the prodrug is rapidly hydrolyzed to boronic acid of endoxifen (B401), which is then efficiently deboronated to form endoxifen (Figure 4A). At 4 hours after incubation, 60% of the prodrug has been converted to endoxifen (Figure 4B). This observation provided evidence that the activity of ZB483 in vitro as an antiestrogen is primarily due to the effective concentration of endoxifen.

ZB483 inhibits tumor growth in mice (MCF-7 xenografts) and is more efficacious than endoxifen in a dose-dependent manner

To determine if the in vitro antiestrogenic activity of ZB483 and its metabolic conversion to endoxifen can be extended to an in vivo animal model, endoxifen or its prodrug ZB483 was orally administered to mice bearing MCF-7 breast tumor at two doses, 1 mg/kg and 0.1 mg/kg. Results shown in Figure 5 demonstrate that the prodrug was more effective in inhibiting the tumor growth at both dose levels than endoxifen. It is noted that at 0.1 mg/kg, oral endoxifen was not statistically superior to control, suggesting that this dose may represent the lower limit of endoxifen efficacy in this model. Importantly, ZB483 also showed dose-dependence at the two dose levels where the 1 mg/kg treatment showed significantly better efficacy than that of 0.1 mg/kg. At both doses neither endoxifen nor ZB483 showed any acute toxicity in mice treated with the drugs over a period of at least 21 days.

Boron-endoxifen-treated mice have significantly higher serum concentrations of endoxifen than mice treated with endoxifen

As shown in Figure 6, oral administration of endoxifen resulted in a peak endoxifen concentration of 1.0 ng/mL in plasma. In contrast, ZB483, the boronic prodrug of endoxifen afforded a peak endoxifen concentration of 43 ng/mL, more than 40 times higher than endoxifen administration. Moreover, the plasma concentration of the boronic prodrug fraction itself was also significantly higher than that of endoxifen in mice treated with endoxifen. This sharp difference in bioavailability between endoxifen administration and ZB483 administration is reflected in the pharmacokinetic parameters presented in Table 2. $C_{\max}$ was reached for endoxifen administration at 2 hours whereas for ZB483 treatment the $C_{\max}$ of endoxifen was reached at 24 hours. Largely due to the 40-fold increase in peak endoxifen concentration achieved by ZB483 administration, the AUC (area under curve) value was 2419 ng/mL*h with ZB483, vs. 31.8 ng/mL*h with endoxifen.

Discussion

Endoxifen, or 4-hydroxy-N-desmethyltamoxifen, was first identified as a tamoxifen metabolite in 1988 [6,7]. The antiestrogenic activity and pharmacological characterization of endoxifen, however, came at a much later time [12,13,30], followed by the recognition of its important role in the therapeutic effect of tamoxifen treatment [23,31,32]. There have been intensified efforts to explore the potential of endoxifen as an alternative endocrine therapy to

tamoxifen [24,25,33]. The advantages of endoxifen include independence of CYP2D6 status, greater potency as a SERM with 100-time stronger binding affinity towards ER than tamoxifen. However, being a hydroxylated metabolite, endoxifen has a higher first-pass metabolism than tamoxifen, in part due to glucuronidation [26,34]. Thus direct use of endoxifen as an oral regimen may be challenged by poor bioavailability and inactivation by glucuronidation. Indeed, at an oral dose of 1 mg/kg, endoxifen administration to mice yielded a modest peak plasma concentration of 1 ng/mL (Figure 6).

To address this issue, we have designed and synthesized a boronic prodrug of endoxifen (Figure 2) which can be efficiently converted to endoxifen after entering systemic circulation. The substitution of the hydroxyl group with boronic acid pinacol ester prevents endoxifen from undergoing O-glucuronidation catalyzed by glucuronosyltransferase. Once reaching systemic circulation, the prodrug is efficiently deboronated to yield endoxifen. Our pharmacokinetic study demonstrated that the conversion from the boronic prodrug to endoxifen is about 80-90% complete within 24 hours (Figure 6), compared to 70% conversion in vitro incubation with MCF-7 cell culture (Figure 4). Moreover, the boronic prodrug afforded a 40-fold increase in C_{max}, and 80-fold increase in the AUC value (Table 2).

The marked improvement of bioavailability in oral administration of ZB483 largely accounted for the increased efficacy of the prodrug at comparable doses (Figure 5). A previous mice efficacy study of oral endoxifen used a dose range of 2 to 8 mg/kg [33]. Given the superior endoxifen bioavailability of ZB483, we sought to determine the therapeutic effect of ZB483 at 1 mg/kg and 0.1 mg/kg doses that are lower than most drug doses used in animal studies. At both 1.0 and 0.1 mg/kg doses employed, ZB483 was more effective than endoxifen in inhibiting xenograft tumor growth in mice. Thus, the boronic prodrug of endoxifen, ZB483 represents a promising therapeutic agent that acts as a potent antiestrogenic agent both in vitro against ER+ breast cancer cells and in vivo against MCF-7 tumors in mice. Moreover, the boronic structure of ZB483 afforded a significantly enhanced bioavailability, evidenced by a 40-fold increase in peak plasma concentration of endoxifen and an 80-fold increase in AUC value.

Conclusion

The preclinical studies demonstrate that ZB483 is an effective endoxifen prodrug capable of delivering a markedly increased plasma level of endoxifen compared to direct administration of endoxifen. Because endoxifen is an active metabolite of tamoxifen, ensuring a sustained plasma level of endoxifen by its orally available prodrug can effectively circumvent the requirement for a functioning CYP2D6 enzyme necessary for metabolizing tamoxifen to endoxifen. Our study shows that a novel boronic prodrug of endoxifen that can be readily prepared from commercially available materials, can deliver a therapeutically effective concentration in systemic circulation at a lower dosage than endoxifen and holds great potential for further clinical development as an improved hormonal regimen for treatment and prevention of breast cancer.

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Figure 1. Major metabolic pathways of tamoxifen

Figure 2. Synthetic route for ZB483, a boronic prodrug of endoxifen

Figure 3. ZB483 inhibits growth of MCF-7 and T47D breast cancer cells

Figure 4.

A. Metabolic conversion of ZB483 to endoxifen, B. Time-course of ZB483 to endoxifen conversion in cell culture.

Figure 5. Endoxifen and its boronic prodrug ZB483 inhibit xenograft tumor growth in nude mice (n=7-9) in a dose dependent manner. On day 37, in the order of decreasing efficacy, ZB483 at 1 mg/kg>endoxifen at 1 mg/kg>ZB483 at 0.1 mg/kg>endoxifen at 0.1 mg/kg, $p<0.01$.

Figure 6. Pharmacokinetic profiles of orally administered endoxifen and the boronic prodrug of endoxifen (ZB483).

Table 1
IC₅₀ values for Tamoxifen, 4-OHT, endoxifen, and ZB483 in MCF-7 and T47D breast cancer cells

	IC ₅₀	
	MCF-7 (μM)	T47D (μM)
Tamoxifen	25.8	11.9
4-OHT	4.22	2.16
Endoxifen	0.675	4.29
ZB483	0.708	8.73

Table 2
Pharmacokinetic parameters of endoxifen and its boronic prodrug, ZB483 following single dose, oral administration

	Endoxifen (1 mg/kg)	ZB483 (1 mg/kg)
$t_{\max}$ (hrs)	2	24 (endoxifen) 2 (B401)
$t_{1/2}$ (hrs)	31	18.8 (endoxifen) 21.2 (B401)
$C_{\max}$ (ng/mL)	1.0	42.3 (endoxifen) 15.7 (B401)
AUC (ng/mL*h)	31.8	2419 (endoxifen) 374 (B401)