Rational Design of a Boron-Modified Triphenylethylene (GLL398) as an Oral Selective Estrogen Receptor Downregulator

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S Supporting Information

ABSTRACT: Development of orally bioavailable nonsteroidal selective estrogen receptor downregulators (SERDs) provides clinical opportunities for the long-term treatment and adjuvant therapy of breast cancer at all stages. We describe the design, synthesis, and identification of a boron-modified GW7604 derivative (GLL398, **9**), a SERD candidate, in which a boronic acid functional group replaces the phenolic hydroxyl group of GW7604. Compound **9** strongly binds to ER α in a fluorescence resonance energy transfer binding assay (IC_{50} = 1.14 nM) and potently degrades ER α in MCF-7 breast cancer cells (IC_{50} = 0.21 μ M). Most importantly, the introduction of the boronic acid group confers superior oral bioavailability of **9** (AUC = 36.9 μ g·h/mL) in rats as compared to GW7604 (AUC = 3.35 μ g·h/mL). The strikingly favorable pharmacokinetic property of **9** makes it a promising oral SERD suitable for clinical evaluation.

KEYWORDS: Hormonal therapy, selective estrogen receptor downregulator (SERD), pseudo-SERD, boronic acid, oral bioavailability

As the sole FDA-approved selective estrogen receptor downregulator (SERD), fulvestrant (**3**) is indicated for hormonally driven metastatic breast cancer in postmenopausal women whose disease has progressed after receiving prior hormonal therapies such as tamoxifen (**1**) or aromatase inhibitors (AIs). However, fulvestrant is limited by its poor oral bioavailability, resulting in a specific administration protocol of intramuscular (i.m.) injection at a monthly dose of 500 mg.^{1,2} Moreover, clinical efficacy of the current injection regimen of fulvestrant is also believed to be negatively impacted by the inferior pharmacokinetic profile of the drug. Thus, the development of highly effective, orally bioavailable SERDs has become a meaningful approach to derive greater clinical benefits of SERD treatment.

In recent years many efforts aiming at developing clinically useful oral SERDs have led to the identification of several nonsteroidal SERD candidates, such as GW5638 (**5**), GDC-810 (**7**), and AZD9496 (**8**).^{3–6} Instead of the long fatty chain of fulvestrant, these recent candidates share a common acrylic acid moiety (Figure 1). Interestingly, GW-5638 embodies a triphenylethylene backbone like tamoxifen, yet it is effective in ER α -positive tamoxifen-resistant breast cancer xenografts.⁷ Although GW5638 was originally investigated as an estrogen receptor (ER) agonist/antagonist, it was subsequently found that the binding of GW5638 with ER α induced a significant destabilization of ER α in MCF-7 cells, the main reason it was later recognized as a SERD.^{4,8} Similar to tamoxifen, GW5638 is considered as a prodrug of its active metabolite GW7604 (**6**).^{5,9} Follow-up studies show that GW7604 is also a distinct SERD

and is significantly more potent than its precursor.⁹ Furthermore, X-ray crystallography of ER α -GW7604 complex reveals the importance of acrylic acid in a conformational change of receptor surface, which results in the degradation of ER.⁸ However, unlike a typical SERD, as it destroys ER in cancer cells, GW5638 maintains estrogen-like effects on bones with minimal stimulation on uterus.^{10,11} Thus, GW5638 is also classified as a pseudo-SERD, which could form the basis of a new paradigm of breast cancer hormonal therapy in its own right. Such pseudo-SERDs are characterized by not only the antiestrogenic and ER-degrading efficacy but also the clinical benefits of preventing osteoporosis, reducing coronary heart disease, and improving gynecological health.¹¹ Unfortunately, the development of GW-5836 was discontinued after a phase I clinical trial. It is believed that the metabolism issue is one of the reasons for terminating the development of GW5638 and GW7604.⁵ Evidence shows that the conversion of GW5638 into GW7604 in human liver microsomes is low (around 16%) and that GW7604 tends to be metabolized rapidly by phase II metabolizing enzymes through glucuronidation and sulfation.⁵ While disappointing, the distinct activities and the clear mechanism of action paved the way to the optimizations of GW5638 and GW7604 to be an orally bioavailable SERD.

Phase II metabolic inactivation and clearance (glucuronidation and sulfation) are common biotransformation pathways of

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Figure 1. Structures of hormonal therapy drugs and candidates for breast cancer.

drugs with a phenol group. This could seriously hamper the bioavailability of GW7604 if it were to be used directly as an oral drug. Therefore, our design is to block the primary metabolizing site by modification of GW7604 with a boronic acid functional group. In our previous studies, we found that boronic derivatives of antiestrogenic compounds significantly reduced first pass metabolism of hydroxylated drug molecules, 4-hydroxytamoxifen and endoxifen, leading to the increased plasma concentrations in the circulation.^{12,13} In summarizing these findings, we believe that the boronic acid or boronate is an oral bioisostere of a phenolic hydroxyl group. Using this knowledge, we have recently identified an orally bioavailable steroidal SERD (fulvestrant-3 boronic acid, ZB716, **4**).¹⁴ Herein, we will report our newest work on a boronic acid-modified nonsteroidal oral SERD (GLL398, **9**), which is derived from GW7604. The boronic acid functional group is expected to block the primary site of phase II metabolism and maintain the pharmacology of the phenolic hydroxyl group in ER recognition and binding (Figure 2).

Figure 2. Design strategy of a boron-modified triphenylethylene, GLL398 (**9**).

The synthetic route of **9** is depicted in Scheme 1. Starting from bis(4-bromophenyl)methanone (**11**), the McMurry reaction with propiophenone afforded the bis(bromobenzene) **12** in 79% yield.^{15,16} The bis(bromobenzene) **12** was then substituted with methyl acrylate in the presence of palladium(II) acetate, triphenylphosphine, and trimethylamine to provide the monoacrylate **13** as the major product (48%) and the diacrylate **14** as the minor (19%). Saponification of monoacrylate **13** with sodium hydroxide in a mixed

Scheme 1. Synthetic Route of GLL398^a

^aReagents and conditions: (a) TiCl₄/Zn, THF; (b) methyl acrylate, Pd(OAc)₂/PPh₃, Et₃N; (c) NaOH in MeOH/THF; (d) bis-(pinacolato)diboron, Pd(dppf)Cl₂/KOAc, 1,4-dioxane; (e) NaIO₄, 1 N HCl, THF/H₂O (4:1).

methanol-tetrahydrofuran (MeOH/THF) solution provided the acrylic acid **15** in a yield of 80%. Borylation of the acrylic acid **15** in the presence of 1,1'-bis(diphenylphosphino)ferrocene)palladium(II) dichloride (Pd(dppf)Cl₂) and potassium acetate gave the pinacol boronic ester **16** in a yield of 81%, which was successfully converted into the free boronic acid **9** (yield, 50%) by using sodium periodate as an oxidant in an acidic condition.^{17,18} The characterization data for desired compound **9** is summarized as follows. Mp 139–142 °C. ESI/MS⁺, 397.0 (M + 1)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.29 (br, 2H), 8.01 (br, 2H),

7.86 (br, 2H), 7.78 (d, $J = 8.0$ Hz, 2H), 7.67 (d, $J = 8.0$ Hz, 2H), 7.58 (d, $J = 16.0$ Hz, 1H), 7.43 (d, $J = 8.0$ Hz, 2H), 7.39 (d, $J = 16.0$ Hz, 1H), 7.32 (d, $J = 8.0$ Hz, 2H), 7.22 (d, $J = 8.0$ Hz, 2H), 7.20–7.09 (m, 12H), 6.84 (d, $J = 8.0$ Hz, 2H), 6.80 (d, $J = 8.0$ Hz, 2H), 6.50 (d, $J = 16.0$ Hz, 1H), 6.34 (d, $J = 16.0$ Hz, 1H), 2.39 (m, 4H), 0.84 (m, 6H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 168.01, 167.96, 145.30, 145.08, 144.64, 144.46, 143.96, 143.89, 142.95, 142.75, 141.79, 141.74, 138.36 (2C), 134.63 (2C), 133.84 (2C), 133.17, 132.14, 131.13 (2C), 130.02 (2C), 129.76 (2C), 129.74 (2C), 129.71 (2C), 128.67 (2C), 128.52 (2C), 128.47 (2C), 128.32 (2C), 127.91 (2C), 127.00, 126.86, 119.48, 119.09, 29.07, 28.97, 13.71, 13.69. HRMS (ESI $^+$): Calcd for $\text{C}_{25}\text{H}_{24}\text{BO}_4$ ($\text{M} + \text{H}$) $^+$, 399.1768; found, 399.1762. Compound **9** is a 1:1 mixture of *E*- and *Z*-isomers whose identity and composition were determined by 1D and 2D NMR spectra in the Supporting Information.

To determine the binding affinity of tested compounds to ER α , a TR-FRET competitive binding assay was performed. Generally, ER α was labeled by a terbium-labeled anti-GST antibody in this assay. When a fluorescent ligand (tracer) was bound to the receptor, fluorescence resonance energy transfer (FRET) from the antibody to the tracer occurred. The test compounds competed with the tracer, and the percent displacement was quantitatively correlated with the loss of FRET signal between the antibody and the tracer. Figure 3

Figure 3. Competitive binding curves of 17 β -estradiol, 4-hydroxytamoxifen, GW7604, and **9**.

shows the competitive binding curves of 17 β -estradiol, 4-hydroxytamoxifen, GW7604, and **9**, with IC $_{50}$ values measured at 0.41, 0.53, 13.8, and 1.14 nM, respectively. 17 β -Estradiol and 4-hydroxytamoxifen served as the positive controls in this assay. As measured by the ability of competitive binding with ER α , **9** exhibits a nearly 10-fold higher binding affinity toward ER α as compared to GW7604, which suggests that the boronic acid group of **9** interacts more tightly with the residues Glu351 and Arg394 of ER α protein by hydrogen bond(s) than the hydroxyl group of GW7604, which observed previously.¹⁹ Thus, this binding assay at the molecular level supports our hypothesis that a boronic acid group could be treated as an isostere of the hydroxyl group at GW7604, although binding affinity does not necessarily reflect the potency and efficacy of a compound behaving as an ER modulator or downregulator.²⁰

The effects of GW7604 and **9** on breast cancer cell growth were evaluated in MCF-7 cell line and its tamoxifen-resistant variant MCF-7/TamR, which was developed in our lab.²¹ Cells were treated with vehicle or five different doses (ranging from 10 $^{-9}$ to 10 $^{-5}$ M) of 4-hydroxytamoxifen, GW-7604, or **9** for 5 days before counting of survived cells. 4-Hydroxytamoxifen was included as the control, which showed a strong inhibitory

activity against MCF-7 (IC $_{50}$, 0.0034 μM) but low efficacy against MCF-7/TamR (IC $_{50}$, 20 μM). The dose–response curves in Figure 4 indicate that **9** exhibits comparable growth

Figure 4. Effects of GW7604 and **9** on breast cancer cell proliferation in MCF-7 (A) and MCF-7/TamR (B). Data are presented as percent of vehicle treated samples. IC $_{50}$ s of three different independent experiments are reported. 4-Hydroxytamoxifen, **2**.

inhibition of both tamoxifen-sensitive and resistant breast cancer cells as GW7604. This result is consistent with the previous observation that GW7604 is effective in tamoxifen-resistant breast cancer cells⁷ and provides key evidence that **9** possesses the same efficacy as GW7604 in inhibiting endocrine resistant breast cancer cell proliferation.

The ER α degradation effect of **9** was determined in order to elucidate its mechanism of action as compared to GW7604. MCF-7 breast cancer cells were treated with GDC-810, GW7604, or **9** at increasing concentrations from 10 $^{-10}$ to 10 $^{-6}$ M. GDC-810 served as a positive control since it is also a triphenylethylene-derived SERD. The Western blots of ER α and percent degradation curves are exhibited in Figure 5. GW7604 and **9** downregulated ER α at approximately the same level in MCF-7 cells, with IC $_{50}$ values of 0.22 and 0.21 μM , respectively. These results indicate that **9** is as potent as GW7604 in its action as a SERD in MCF-7 cells, although **9** has a much higher binding affinity toward ER α than GW7604.

Subsequently, we conducted pharmacokinetic studies of GW7604 and **9** in rats to determine how the boronic acid moiety enhanced the oral bioavailability of **9**. After a single oral dose of 10 mg/kg, blood samples were collected from rats, and plasma preparations were analyzed for concentrations of GW7604 and **9** at 1, 3, 6, and 24 h post drug administration. Figure 6A,B shows the plasma drug concentrations achieved after oral administration of GW7604 and **9**, respectively. Compound **9** reached 3.51 $\mu\text{g/mL}$ peak concentration at 1 h

Figure 5. Dose-dependent ER- α degradation by GDC-810, **9**, and GW7604 in MCF-7 cells. DMSO treated groups served as negative controls. Cells were treated with increasing concentrations (10^{-10} – 10^{-6} M) of test compounds in DMSO. (A) Western blots showing ER α protein downregulated by GDC-810, **9**, and GW7604 in a dose-dependent manner. (B) Dose–response curves of percent ER α protein degradation.

Figure 6. Pharmacokinetics of GW7604 (A) and **9** (B). Rats ($n = 3$) were given oral gavage containing 5% DMSO, 40% PEG 400, and 55% saline and dissolved GW7604 or **9** at a single dose of 10 mg/kg.

after administration, a level 13-fold higher than that achieved by GW7604 (0.27 $\mu\text{g/mL}$) at 3 h after administration, providing definitive evidence that oral bioavailability of **9** is far superior. We also note that in Figure 6B the peak plasma concentration (0.56 $\mu\text{g/mL}$) of GW7604 in rats treated with **9** is higher than that in GW7604 treated rats. Indeed, our data demonstrate that

9 can be partially converted into GW7604 ($\sim 15\%$) in rat plasma. This result meets our initial expectation and is consistent with our observation in the recent study on fulvestrant-3 boronic acid.¹⁴ Pharmacokinetic parameters of orally administered GW7604 and **9** in rats are provided in Table 1.

Table 1. Oral Pharmacokinetic Parameters of GW7604 and **9** in Rats

oral drug	GW7604	compd 9	
active ingredient	GW7604	GW7604	9
$t_{1/2}$ (h)	7.0	4.5	3.9
C_{max} ($\mu\text{g/mL}$)	0.27	0.56	3.51
AUC ($\mu\text{g}\cdot\text{h/mL}$)	3.35	8.67	36.9

Antiestrogen and aromatase inhibitor therapies are two of the most effective treatments for hormone receptor (HR) positive breast cancers. However, acquired resistance to these endocrine therapy agents often develop, accompanied by various alterations in ER signaling, including ER up-regulation and ligand-independent activation. Thus, SERD has become a standard of care for metastatic breast cancers that have progressed on tamoxifen and/or aromatase inhibitors. However, the intrinsic mechanism of action of SERDs involving degrading the estrogen receptors near completely in all body tissues and organs is also central to serious adverse side effects such as bone and joint pain and urinary tract infections. As a result, fulvestrant can only be used in postmenopausal patients.²² In contrast, GW7604, an exemplary pseudo-SERD, will in principle offer superior side effect profile to fulvestrant since it does not increase the risks of coronary heart disease and osteoporosis while improving gynecological health in both pre- and postmenopausal women. Therefore, in order to develop an oral pseudo-SERD we incorporated a boronic acid functional group into the primary glucuronidation and sulfation site of GW7604 to provide a new SERD candidate, **9**, which retained a GW7604-like action in breast cancer cells and exhibited markedly enhanced bioavailability in rats. These preliminary results suggest that **9** could be a promising orally bioavailable GW7604-like SERD candidate for treating advanced HR-positive breast cancers in pre- and postmenopausal women.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.6b00410.

Complete experimental procedure and characterization data for all compounds and original ^1H , ^{13}C , and 2D NMR spectra of desired compounds (PDF)

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Notes

The authors declare no competing financial interest.

The authors declare that a patent application (US 62/332,541) is filed on compound 9.

ABBREVIATIONS

ER, estrogen receptor; SERD, selective estrogen receptor downregulator; AI, aromatase inhibitor; i.m., intramuscular; FRET, fluorescence resonance energy transfer; HR, hormone receptor

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