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Ester-to-Amide Rearrangement of Ethanolamine-Derived Prodrugs of Sobetirome with Increased Blood-Brain Barrier Penetration

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Abstract

Current therapeutic options for treating demyelinating disorders such as multiple sclerosis (MS) do not stimulate myelin repair, thus creating a clinical need for therapeutic agents that address axonal remyelination. Thyroid hormone is known to play an important role in promoting developmental myelination and repair, and CNS permeable thyromimetic agents could offer an increased therapeutic index compared to endogenous thyroid hormone. Sobetirome is a clinical stage thyromimetic that has been shown to have promising activity in preclinical models related to MS and X-linked adrenoleukodystrophy (X-ALD), a genetic disease that involves demyelination. Here we report a new series of sobetirome prodrugs containing ethanolamine-based promoieties that were found to undergo an intramolecular O,N acyl migration to form the pharmacologically relevant amide species. Several of these systemically administered prodrugs deliver more sobetirome to the brain compared to unmodified sobetirome. Pharmacokinetic properties of the parent drug sobetirome and amidoalcohol prodrug **3** are described and prodrug **3** was found to be more potent than sobetirome in target engagement in the brain from systemic dosing.

Graphical abstract

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A. Supporting Information

Additional figures illustrating the details of multiple reaction monitoring in the LC-MS/MS method and NMR spectra and HPLC chromatograms of the lead compound and representative examples are contained within. This material is free of charge via the Internet at <http://>

1. Introduction

Thyroid hormone is an essential regulatory molecule in vertebrate physiology and homeostasis. In the central nervous system (CNS) thyroid hormone plays an integral role in development and maintenance of brain function. Myelination of nerve fibers and neuronal and glial cell differentiation are processes in which thyroid hormone plays a key regulatory role.¹ Thyroid hormone prompts the maturation of oligodendrocytes (OLs) from oligodendrocyte progenitor cells (OPCs),² promotes the expression of oligodendrocyte-specific genes that activate the production of myelin,³ and has been shown to play a role in stimulating myelin repair in response to demyelination.^{4–6} Currently, the only treatment options for multiple sclerosis (MS), the most prevalent demyelinating neurological disorder,⁷ target the autoimmune inflammatory process of the disease that causes demyelination but do not address myelin repair.^{8,9} The endogenous thyroid hormone is not a viable candidate for myelin repair as it lacks a therapeutic index (TI) separating desirable therapeutic effects from deleterious systemic thyrotoxic effects, particularly on heart, bone, and skeletal muscle.¹⁰ The thyromimetic sobetirome (**1**, also known as GC-1) displays selective tissue action with a TI separating beneficial from adverse effects and has progressed to clinical studies in hyperlipidemia.¹¹ In terms of potential for CNS disorders, sobetirome has been studied in pre-clinical models of X-linked adrenoleukodystrophy (X-ALD), a lipid storage disease that produces severe neurological phenotypes involving demyelination.¹² In addition, sobetirome has been shown to promote oligodendrogenesis from human and rodent OPCs *in vitro*, and enhance oligodendrogenesis during development with attending increased production of myelin proteins *in vivo*, supporting the idea that thyromimetic agents that distribute to the central nervous system (CNS) may be useful candidates for treating demyelinating disorders.¹³

Most thyromimetics, including sobetirome, contain inner-ring, negatively charged carboxylate groups at physiological pH. These carboxylate groups are crucial for high affinity binding to the thyroid hormone receptor, but are a known liability for CNS drug distribution due to their inherent lipophobic character and electrostatic repulsion at negatively charged tight junctions of blood-brain barrier (BBB) endothelial cells.^{14,15} While sobetirome does distribute to the CNS,^{16,17} employing a prodrug strategy which masks these carboxylate groups should, in theory, provide greater access to the CNS and could potentially limit peripheral exposure of the parent drug. After crossing the BBB, these prodrugs can be hydrolyzed to the parent drug sobetirome. Recently, an *in vivo* study evaluating the brain exposure of ester-based prodrugs of sobetirome confirmed this strategy

to be effective.¹⁸ In this study, a particular ester derivative, an ethanolamino ester (**2**), was found to have the greatest CNS penetration with minimized peripheral exposure of the parent drug. Here we report a new series of prodrugs that feature improved CNS distribution compared to the originally reported ethanolamino ester and, in the process, it was discovered that these ester promoieties undergo an intramolecular rearrangement to form the corresponding amides, which were found to be the pharmacologically active forms of the prodrugs.

2. Results and Discussion

2.1 Chemistry

In line with the recently reported successful application of an ethanolamine-based ester prodrug of sobetirome¹⁸ and ethanolamine-based ester prodrugs of dexibuprofen,^{19, 20} a drug with structural similarities to sobetirome, a new series of ethanolamine-derived prodrugs of sobetirome were synthesized in an effort to expand upon these findings and improve their pharmacokinetic properties regarding CNS distribution (Scheme 1). Derivatization of the ethanolamine moiety within the series explores varying aspects of steric and electronic parameters with subtle differences in lipophilicity. Branching at the alpha carbon adjacent to the ester group was examined in an effort to impede hydrolysis via steric hindrance of the ester carbonyl (**3–12, 14–15**). Electron withdrawing trifluoromethyl groups were incorporated to weaken the associated ester bonds (**14–15**), and alkylation of the amino group of the promoiety (**7–8, 12–13, 15**) was implemented to modulate the amino group's pKa and deter potential interactions with monoamine oxidase (MAO).²¹ Additionally, degrees of freedom about the promoiety were altered by chain elongation (**9**) and incorporation of a heterocycle (**15**).

Modular synthesis of the series of ethanolamine-based prodrugs was accomplished in two steps starting with coupling of a benzyl-protected sobetirome fragment to an *N*-Cbz or *N*-(di)benzyl protected amino alcohol, followed by parallel protecting group removal by hydrogenolysis to form the ester prodrugs in moderate-to-good yield (Scheme 1). To selectively form ester species of interest, the amino alcohol's amino group was protected with Cbz, which significantly alters the polarity of the promoiety and provides for an easy separation and isolation of the *N*-protected amino alcohol. For amino alcohols that are not commercially available, zinc(II) perchlorate catalyzed nucleophilic attack of substituted epoxides by benzylated amines was employed according to a literature procedure.²² Generation of the acid chloride of phenol-benzylated sobetirome (Scheme 1, left) is accomplished by treating phenol-benzylated sobetirome with oxalyl chloride in the presence of a catalytic amount of DMF.^{18, 23} Coupling of the phenol-protected sobetirome acid chloride with *N*-protected amino alcohols occurs readily in the presence of DMAP in heated THF. Global deprotection of benzyl ether, *N*-Cbz, and *N*-benzyl protected fragments follows with treatment of the protected precursors with 10% Pd/C and triethylsilane in THF/MeOH.²⁴ Multiple precipitations of the resulting product from hexanes yields the desired ester derivatives in excellent purity.

All compounds in the series were characterized by ¹H NMR spectroscopy and assessed for purity by HPLC. While all isolated final compounds were spectroscopically determined to

be the desired ester derivatives, it was discovered that by eluting some derivatives on silica led to the isolation of fractions that correspond to the promoiety-rearranged amide species (Scheme 2). Formally known as an O,N acyl migration, this intramolecular rearrangement proceeds with nucleophilic attack of the ester carbonyl by the promoiety's amino group lone pair leading to facile formation of a five-membered cyclic intermediate, followed by rearrangement to the thermodynamically favored amide. Intramolecular O,N acyl migrations have been well documented in organic synthesis, observed in a variety of natural products, and employed in medicinal chemistry as prodrug strategies.^{25, 26} Characterization within the series regarding ester versus amide species is easily accomplished by ¹H NMR spectroscopy, where esters and amides can be distinguished on the basis of chemical shifts (see Supporting Information). Specifically, the proton attached to the oxygen-containing carbon within the promoiety displays a chemical shift around 5 ppm for esters, whereas this proton chemical shift is observed approximately 1 ppm upfield for the corresponding amide. Amide products also feature typical amide N-H peaks with chemical shifts occurring at 7.2 ppm in CD₃CN or CD₃OD within this series. Classically, this rearrangement is pH-controlled, favoring amide formation at neutral and alkaline pH (Scheme 2). The majority of deliberately prepared ester derivatives in this series readily rearrange to their amide counterparts in polar, protic solvents. HPLC analysis confirms the presence of both esters and amides, which can be distinguished on the basis of retention time and peak shape in each chromatogram. Both HPLC run conditions and sample preparation involve aqueous solutions, from which the majority of derivatives were shown to display only the amide product upon analysis by HPLC. Quick preparation and injection of ester derivative **3** (**3a**, Scheme 2) led to peaks in the chromatogram associated with both ester derivative and amide derivative by comparison to the chromatogram of the deliberately synthesized amide of **3** (**3b**, Scheme 2) under identical run conditions (see Supporting Information). Within 5 minutes of running approximately 25% of the ester had converted to the amide, and sampling the same solution in which ester **3a** was prepared for this run approximately 30 minutes later showed only the amide **3b** in the chromatogram. The only derivatives in this series which did not display rearrangement to the amide product were the derivatives which contained isopropyl groups next to the ester motif (**11** and **12**) and cyclic derivative **15**. Apparently, isopropyl groups represent the steric limit about the ester motif for which this rearrangement is inhibited. Interestingly, *N*-alkylated derivatives **7**, **8**, and **13** were observed to freely rearrange to their corresponding tertiary amides.

2.2 Biological Evaluation

To assess CNS penetration, a biodistribution study in mice was performed on each prodrug to determine brain concentrations and brain/serum ratios following systemic (i.p.) administration. Samples for this *in vivo* study and every subsequent *in vivo* study (i.p., p.o., and i.v.) were prepared in a vehicle consisting of 50% DMSO in saline, a solvent combination in which the prodrugs were found to rearrange to their amide conformation. Therefore, *all prodrugs tested were administered as their amide-rearranged isomers with the exception of esters 11, 12, and 15, which do not rearrange to amides*. Mouse cohorts received an equimolar dose (1.5 μmol / kg) of prodrug and one cohort received the same dose of sobetirome as a control. Whole brain and blood was collected 1 h after administration and the concentration of the parent drug sobetirome derived from each

sample was quantified using LC-MS/MS. Most of the prodrugs produced increased brain sobetirome levels compared to the equimolar dose of sobetirome (Table 1). The highest sobetirome concentration in brain was delivered from prodrug **3**, which contains the (*S*) enantiomer of 1-amino-2-propanol as its promoity. Prodrug **3** delivers 3.6-fold more sobetirome to the brain than an equimolar dose of unmodified sobetirome, and its levels are 1.7-fold higher than the previously reported ethanolamine-derived prodrug of sobetirome **2** in a side-by-side comparison.¹⁸ In addition to BBB penetration, an ideal prodrug would be stable in blood and limit exposure of the parent drug in peripheral tissues. Most prodrugs in this series displayed higher brain/serum ratios than the control dose of sobetirome. While prodrug **6** provided the greatest brain/serum ratio of 1.2, it was attended by very low brain levels which likely suggests that **6** hydrolyzes too slowly to be effective as a prodrug for delivering sobetirome to the CNS. Following prodrug **6**, the next highest brain/serum ratio is that for prodrug **3** with a value of 0.5, which is ~13-fold higher than the brain/serum ratio of sobetirome, and ~7-fold higher than previously reported **2**.¹⁸ From this biodistribution study, it appears that the prodrugs which can undergo this intramolecular ester-to-amide rearrangement of their promoities display the desired PK-ADME properties for CNS distribution, suggesting that amide prodrugs of sobetirome are the pharmacologically active species of interest. This was confirmed by analyzing prodrug **3** synthesized as an ester (**3a**) side-by-side with the deliberately prepared amide of **3** (**3b**), which gave statistically identical brain levels and brain/serum ratios at equimolar doses (see Supporting Information). These findings suggest that a similar rearrangement may occur *in vivo* with the previously reported dexibuprofen ethanolamine prodrugs that inspired our efforts with sobetirome.^{19, 20} This previous work reported an ethanolamino ester, *N*-alkyl ethanolamino ester, and an *N,N*-dialkyl ethanolamino ester derivative, of which it seems likely that the former two could undergo a similar rearrangement to the corresponding amides. However, their results run counter to that which we observe, where the *bona fide* ester in their series (*N,N*-dialkyl ethanolamino derivative) displayed the best BBB permeability. Despite the structural similarities between the parent drugs, the CNS penetration mechanisms may be different; the dexibuprofen prodrugs may utilize a partial active transport process for derivatives containing promoities that were not found to be effective for CNS penetration with sobetirome.

Following the single time point study, an 8-hour time-course distribution study in mice was conducted to further understand the pharmacokinetic properties of prodrug **3** versus sobetirome. Analyzing for sobetirome concentration, pharmacokinetic time-course curves were generated, and area under the curve (AUC) values for brain, serum, liver, heart, and kidney were obtained (Figure 1, Table 2). Trends in the single point study are corroborated in the AUC analyses; prodrug **3** increases sobetirome exposure in brain (~2-fold) and significantly decreases sobetirome exposure in peripheral tissues compared to direct equimolar sobetirome administration. Although at earlier time points in this particular experiment the sobetirome brain levels generated from prodrug **3** are slightly lower than were found in the 1 h single time point study (*vide supra*), increased sobetirome brain levels are observed extending out to the 8 h time point. This is likely reflective of a slow hydrolysis rate for prodrug **3** once it has accessed the CNS. Prodrug **3** reduces peripheral exposure of the parent drug by ~3.5-fold in serum, ~2-fold in liver, ~3-fold in heart, and ~2-fold in

kidney. The $AUC_{\text{brain}}/AUC_{\text{serum}}$ ratio for sobetirome was found to be 0.02 consistent with our previous findings.¹⁸ The $AUC_{\text{brain}}/AUC_{\text{serum}}$ ratio for prodrug **3** was found to be 0.13 representing an approximate 3-fold improvement compared to the same previously reported ratio for prodrug **2**.¹⁸ While both values lie on the low end of the range for approved CNS drugs,^{27,28} prodrug **3** offers a significant improvement in CNS distribution. Clear C_{max} and T_{max} values were obscured by spread in the data, but occurred between the initial 15 and 30 min time points in all tissues analyzed for sobetirome and prodrug **3**, suggesting a rapid distribution phase for both in mice.

To further evaluate the PK-ADME properties of prodrug **3**, an oral bioavailability study was performed on **3** and the parent drug sobetirome for comparison. The pharmacokinetics for sobetirome described herein represent a re-evaluation of previously published data.¹⁸ Triiodothyronine (T3) is known to participate in enterohepatic circulation (EHC),^{29, 30} a process which recycles drugs or drug metabolites between the intestinal tract, portal circulation, and liver via the biliary tract and is characterized by multiple peaks in circulating drug concentration within the concentration-time profile from oral dosing, and often a long apparent drug half-life.³¹ As a synthetic analog of T3, we questioned whether sobetirome and related prodrugs may also be subject to EHC. To evaluate the potential involvement in this process and obtain more accurate pharmacokinetic data, the serum concentration-time profiles were extended from 8-hours to 24-hours. Mouse cohorts were treated with a single dose of sobetirome or prodrug **3** administered by intravenous injection (i.v., 3.05 $\mu\text{mol} / \text{kg}$) and oral gavage (p.o., 30.5 $\mu\text{mol} / \text{kg}$) and drug blood levels were measured over a 24-hour time-course. Serum samples from each cohort were quantified using an LC-MS/MS method analyzing for both prodrug **3** and sobetirome (Fig. 2). Analysis of the data from sobetirome administration revealed a circulating half-life ($t_{1/2}$) of approximately 7 h (Figure 2, D), a clearance of 8 mL / min / kg, and an oral bioavailability (%F) of 92%. In addition to its exceptional oral bioavailability, these data suggest that sobetirome has an unusually long half-life and correspondingly low clearance in mice.³² Furthermore, the sobetirome concentration-time profile from oral dosing clearly displays a secondary peak in circulating drug concentration at around 8 h (Figure 2, C) which is distinct from C_{max} and is indicative of recycling of the bolus oral dose of sobetirome by EHC operating on the fraction of sobetirome subject to first-pass metabolism. The presence of EHC indicates that hepatic metabolism of sobetirome involves a reversible conjugation reaction such as glucuronidation which is the case with the fraction of T3 that is subject to EHC.³⁰

Prodrug **3** was also found to have a relatively long circulating half-life ($t_{1/2}$) of approximately 7 h (Figure 2, D), a clearance of 28 mL / min / kg, and an oral bioavailability (%F) of 36% (Figure 2, A) in mice. Sobetirome concentration analyzed in these samples provided AUCs that correspond to the levels of parent drug liberated from **3** upon bolus intravenous or oral delivery into the mouse (Figure 2, B). The amount of sobetirome from cleavage of **3** in blood was found to be 2.4-fold greater from dosing **3** orally compared to intravenously. Total drug oral bioavailability (prodrug **3** + sobetirome **1**) can be estimated by adding the cleaved parent drug sobetirome AUCs (Figure 2, B) to the prodrug **3** AUCs (Figure 2, A) to give a value of %F = 56%. This %F corresponds to the calculated percent of

combined prodrug and parent drug that make it into systemic circulation following an oral dose. A possible explanation for the reduced oral bioavailability of prodrug **3** may lie in the pH-controlled Scheme 2 for ester and amide interconversion. Upon dosing orally, the prodrug is placed in a low pH environment within the gut, which should effectively favor rearrangement to the ester, which is hydrolytically less stable. Differences in how sobetirome and prodrug **3** are metabolized following oral administration may account for the remainder of the discrepancy. Additionally, the second peak in the oral concentration-time profile of **3** around 8 h, along with its long half-life of 7 h, implicate the prodrug's involvement in EHC akin to the parent drug sobetirome. The recycling of prodrug **3** by virtue of EHC increases its time in and exposure to the gastrointestinal track likely increasing the fraction of prodrug cleavage to sobetirome before the prodrug reaches systemic circulation from an oral dose.

Having shown that prodrug **3** delivers more sobetirome to the CNS from a systemic dose compared to unmodified sobetirome, we next evaluated whether this translated into increased potency of target engagement in the brain. *Hairless (Hr)* is a gene that is positively regulated by thyroid hormone in the CNS, and is suggested to play a role in influencing the expression of downstream thyroid hormone-responsive genes.^{33, 34} Upregulation of *Hr* was examined and dose-response data was collected and compared for prodrug **3**, sobetirome, and endogenous thyroid hormone (T3). Brains were collected from cohorts of mice (n = 3) that received six different systemically administered doses of **3**, sobetirome, or T3 (i.p., once-daily, 7 days) and *Hr* expression was analyzed by qPCR (Fig. 3). ED₅₀ values obtained follow an expected trend in potency where T3 (0.09 $\mu\text{mol} / \text{kg}$) > **3** (1.005 $\mu\text{mol} / \text{kg}$) > sobetirome (1.8 $\mu\text{mol} / \text{kg}$). The data are in agreement with previously reported *Hr* activation by T3 in primary mouse cerebrocortical cell cultures,³⁵ and the observed ~20-fold difference in ED₅₀ values reported here between T3 and sobetirome parallels the approximate 20-fold difference in thyroid-stimulating hormone (TSH) levels in cholesterol-fed rats treated with T3 and sobetirome,³⁶ which is another direct readout of receptor activation in the CNS following peripheral dosing.³⁷ An approximate 2-fold increase in potency is observed for **3** compared to sobetirome, which can be fully accounted for by the increase in sobetirome brain exposure delivered by prodrug **3** (*vide supra*). Thus, prodrug **3** significantly improves the delivery and distribution of sobetirome in the CNS resulting in a formal increase in the CNS potency of sobetirome mediated TR activation with a concomitant decrease in peripheral sobetirome exposure.

3. Conclusion

A series of sobetirome ester prodrugs were synthesized that feature promoieties based on the ethanolamine motif. The majority of these derivatives were found to readily undergo intramolecular O,N acyl migration, isomerizing the esters to the corresponding amides, which were found to be the pharmacologically active species *in vivo*. Most derivatives analyzed delivered increased CNS exposure of sobetirome with prodrug **3** demonstrating superior properties including increased brain penetration and minimized peripheral exposure of the parent drug. Pharmacokinetic analysis revealed the likely involvement of both sobetirome and prodrug **3** in enterohepatic circulation. Systemic dosing of prodrug **3** was

found to stimulate transcription of the TR target gene *Hr* in the brain with greater potency than unmodified sobetirome. Together, these results indicate that prodrug **3**, and more generally amide-based prodrugs of sobetirome, may offer advantages for thyromimetic targeting of the CNS.

4. Materials and methods

4.1 Animal studies

Experimental protocols were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Oregon Health & Science University Institutional Animal Care & Use Committee. Wild type male C57Bl/6 mice, aged 8–10 weeks, were housed in a climate-controlled room with a 12-hour light-dark cycle with ad libitum access to food and water. Mice were injected once intraperitoneally (ip) with sobetirome and prodrugs at 1.5 $\mu\text{mol} / \text{kg}$. Oral gavage was performed with the use of plastic feeding tubes (20 ga $\times$ 38 mm, Instech Laboratories Inc., PA, USA) connected to 500 μL insulin syringes (Covidien LLC MA, USA). Euthanasia was performed on three mice per time point at the following times: 0.25 h, 0.5 h, 1 h, 2 h, 4 h, and 8 h (except where noted otherwise) and the tissues and blood were harvested. Tissues were immediately frozen and blood was kept on ice for a minimum of 30 minutes and then spun down at $7,500 \times G$ for 15 minutes. Serum (100 μL) was collected and was stored with tissues at -80°C until samples were processed.

4.2 Serum processing

The serum samples were warmed to rt and 10 μL of 2.99 μM internal standard (d_6 -sobetirome³⁸) was added to them. Acetonitrile (500 μL) was added and the sample was vortexed for 20 seconds. The sample was then centrifuged at $10,000 \times G$ for 15 minutes at 4°C . Next, 90% of the upper supernatant was transferred to a glass test tube and concentrated using a speedvac for 1.5 hours at 45°C . The dried sample was then dissolved in 400 μL of 50:50 ACN:H₂O and vortexed for 20 seconds. The resulting mixture was transferred to an eppendorf and centrifuged at $10,000 \times G$ for 15 minutes. The supernatant was filtered with 0.22 μm centrifugal filters and submitted for LC-MS /MS analysis. The standard curve was made with 100 μL of serum from an 8–10 week old mouse not injected with sobetirome or prodrug. The processing was performed exactly the same except after filtering the sample was split amongst 6 vials. To 5 out of the 6 vials was added sobetirome to make final concentrations in matrix of (0.1 pg/ μL , 1 pg/ μL , 10 pg/ μL , 100 pg/ μL , and 1000 pg/ μL).

4.3 Brain processing

The brain samples were warmed to r.t. and transferred to a homogenizer tube with 3 GoldSpec 1/8 chrome steel balls (Applied Industrial Technologies). The resulting tube was weighed and then 1 mL of H₂O was added, followed by 10 μL of 2.99 μM internal standard (d_6 -sobetirome³⁸). The tube was homogenized with a Bead Bug for 30 seconds and then transferred to a falcon tube containing 3 mL of ACN. ACN (1 mL) was used to wash homogenizer tube and the solution was transferred back to the falcon tube. The sample was

then processed using the same method for the serum processing except the sample was concentrated in a glass tube using a speed vac for 4 hours at 45 °C.

4.4 Liver processing

The liver samples were warmed to rt and transferred to a homogenizer tube with 3 GoldSpec 1/8 chrome steel balls (Applied Industrial Technologies). The resulting tube was weighed and then 1 mL of H₂O was added, followed by 10 µL of 2.99 µM internal standard (*d*₆-sobetirome). The tube was then homogenized with a Bead Bug for 30 s. A small sample (100 µL) was then taken from the homogenized mixture and processed. This was done because the liver levels found in some samples were too high for the LC—MS/MS instrument. The samples were then processed using the serum processing method.

4.5 Heart and kidney processing

Heart and kidney samples were warmed to r.t. and transferred to a homogenizer tube (2 mL) with 3 GoldSpec 1/8 chrome steel balls (Applied Industrial Technologies). The resulting tube was weighed and then 400 µL of H₂O was added, followed by 10 µL of 2.99 µM internal standard (*d*₆-sobetirome³⁸). The tube was homogenized with a Bead Bug for 120 s and then 1.2 mL acetonitrile was added and the tube vortexed for 20 s. The samples were then processed using the serum processing method.

4.6 LC-MS/MS analysis for sobetirome and prodrugs

Sobetirome and *d*₆-sobetirome internal standard were analyzed using a QTRAP 4000 hybrid/triple quadrupole linear ion trap mass spectrometer (Applied biosystems) with electrospray ionization (ESI) in negative mode. The mass spectrometer was interfaced to a Shimadzu (Columbia, MD) SIL-20AC XR auto-sampler followed by 2 LC-20AD XR LC pumps and analysis on an Applied Biosystems/SCIEX QTRAP 4000 instrument (Foster City, CA). The instrument was operated with the following settings: source voltage –4500 kV, GS1 50, GS2 60, CUR 15, TEM 650, and CAD MEDIUM. The scheduled multiple-reaction-monitoring (MRM) transitions are based on the precursor ion *m/z* and their corresponding diagnostic product ions. Compounds were infused individually and instrument parameters optimized for each MRM transition. MRM parameters are shown in the supporting information. The gradient mobile phase was delivered at a flow rate of 0.5 mL/min, and consisted of two solvents, A: 10 mM ammonium formate in water and B: 10 mM ammonium formate in 90% acetonitrile, 10% water. An Imtakt Scherzo SS-C18 50 × 2mm 3 µm (prod# SS022) was used with an Imtakt Guard cartridge Scherzo SS-C18 5×2mm 3µm precolumn (prod# GCSS0S) and kept at 40 °C, and the autosampler was kept at 30 °C. Gradient was as follows, initial concentration of B was 10%, held for 0.5 min, followed by an increase to 98% B over 4.5 min, held for 0.9 min, dropping back to 10% B over 0.1 min, and held at 10% B for 2 min for a total run time of 8 min. Data were acquired using SCIEX Analyst 1.6.2 software (Framingham, MA, USA) and analyzed using Multiquant 3.0.2.

4.7 Pharmacokinetics

To determine the oral bioavailability of **3**, orally (p.o.)- and intravenously (i.v.)-administered 8 h serum AUCs were obtained and compared as follows. An 8 h i.v. AUC was obtained by

administering equimolar doses of **3** (3.05 $\mu\text{mol} / \text{kg}$) by tail vein injection to cohorts ($n = 3$) of age-matched c57bl/6 mice and collecting blood samples at spaced intervals post-injection (5 min, 10 min, 20 min, 1 h, 2 h, 4 h, 8 h). The blood was stored on ice for a minimum of 30 minutes and then spun down at $7,500 \times G$ for 15 minutes. Serum (100 μL) was collected and stored at -80°C until samples were processed. Serum samples were processed according to the method described in the experimental of this paper (vida supra). An 8 h oral AUC was obtained by administering equimolar doses of **3** (30.5 $\mu\text{mol} / \text{kg}$) via oral gavage to cohorts ($n = 3$) of age-matched c57bl/6 mice and harvesting blood samples at spaced intervals post-gavage (10 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h). The blood was stored on ice for a minimum of 30 minutes and then spun down at $7,500 \times G$ for 15 minutes. Serum (100 μL) was collected and stored at -80°C until samples were processed. An identical standard curve as described for serum processing was generated for prodrug **3**. All serum samples were processed according to the method described in the experimental of this paper and the concentrations of both **3** and sobetirome (**1**) were quantified in each sample using the LC-MS/MS method also described in this paper's experimental section (vida supra). AUCs for both the i.v.-dosed and orally-dosed experiments were generated and oral bioavailability was calculated using the equation: % oral bioavailability (%F) = $(\text{AUC}_{\text{p.o.}} / \text{AUC}_{\text{i.v.}}) \cdot (\text{dose}_{\text{i.v.}} / \text{dose}_{\text{p.o.}}) \cdot 100$. The half-life of sobetirome and prodrug **3** was determined from the i.v.-administered serum AUCs described above. Data from the five latest time points, which approximate the elimination phase of the pharmacokinetic profile, was plotted as log (concentration in ng / g) vs time (h). The $t_{1/2}$ values for sobetirome and **3** were calculated from the slopes of these plots. Clearance values were calculated from the AUC of the drug concentration vs. time plots.

4.8 Quantitative PCR

Mice were injected once intraperitoneally (i.p.) with vehicle (1:1 saline/DMSO), sobetirome (6 doses; 30.5, 9.14, 6, 3.05, 1.22, and 0.305 $\mu\text{mol} / \text{kg}$), **3** (6 doses; 9.14, 3.05, 1.22, 0.305, 0.0914, and 0.00914 $\mu\text{mol} / \text{kg}$), and T3 (6 doses; 3.05, 0.305, 0.20, 0.12, 0.0914, and 0.00914 $\mu\text{mol} / \text{kg}$). Cohorts of 3 mice ($n = 3$) for each dose were used and tissues were collected 2 h post-injection. The brain tissues collected for qPCR analysis were processed according to a protocol for RNA extraction using Trizol reagent and the PureLink RNA mini kit, using a Qiagen RNase-free DNase kit during the optional DNase treatment step. Extracted RNA (1 μg) was used to synthesize cDNA via a reverse transcription (RT) reaction using the Qiagen QuantiTect Reverse Transcription kit. DNA contamination was controlled for by duplicating one sample without the addition of RT. Expression of the *Hairless* (*Hr*) gene was measured by qPCR using the QuantiTect SYBR green PCR kit from Qiagen. The primer sequences for hairless (Fwd: CCA AGT CTG GGC CAA GTT TG; Rev: TGT CCT TGG TCC GAT TGG AA) were previously described by Barca-Mayo et al.³⁹ The template cDNA was diluted two-fold to minimize the interference of RT reagents in the qPCR reaction. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was the housekeeping gene used for normalizing between samples. Data analysis was performed using the comparative C_T method to monitor the relative differences in *Hr* gene expression, then a sigmoidal dose—response model to generate ED_{50} values $\pm$ SEM was followed using GraphPad Prism v.4a. Experiments were performed in triplicate.

4.9 Statistical analysis

Data are expressed as the mean $\pm$ the standard error of the mean (SEM). Statistical analyses were done using Microsoft Excel and GraphPad Prism®. Differences between means were determined using multiple t-tests, and considered to be statistically significant $p < 0.05$.

4.10 Experimental section

4.10.1 General Chemistry— ^1H NMR were recorded on a Bruker Avance 400 MHz nuclear magnetic resonance spectrometer. All ^1H NMR spectra were calibrated to the NMR solvent reference peak (δ , $\text{d}_6\text{-DMSO}$, CDCl_3 , CD_3OD) and are reported in parts per million (ppm). High-resolution mass spectrometry (HRMS) with electrospray ionization was performed by the Bioanalytical MS Facility at Portland State University. Inert atmosphere reactions were performed under argon gas passed through a small column of drierite and were conducted in flame-dried round-bottom flasks. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), and dimethylformamide (DMF) were obtained from a Seca Solvent System. All other solvents used were purchased from Sigma-Aldrich or Fisher. Purity analysis of final compounds was determined to be $>95\%$ by HPLC. HPLC analysis was performed on a Varian ProStar HPLC with an Agilent Eclipse Plus C18 $5\ \mu\text{m}$ column ($4.6 \times 250\ \text{mm}$) with a gradient of 10% to 95% acetonitrile (0.1% TFA) over 30 minutes.

4.10.2 Chemistry materials—Sobetirome (**1**), 2-(4-(4-(benzyloxy)-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetic acid (benzyl protected (phenol) sobetirome, 2-(2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetoxylethyl)-1-amine (**2**) and the in situ generated acid chloride of benzyl protected sobetirome were synthesized as previously reported.^{18, 38} 1-(dibenzylamino)-3-methylbutan-2-ol and 1-(benzyl(methyl)amino)-3-methylbutan-2-ol were synthesized according to a published procedure,²² and 1-(isopropylamino)propan-2-ol was synthesized following a literature protocol.⁴⁰ Amino alcohols were N-Cbz protected according to standard procedures.⁴¹

4.10.3 Representative procedure for preparation of acid chloride of benzyl protected sobetirome—A solution of oxalyl chloride (200 μL , 2.33 mmol) in 2 mL of DCM was slowly added to a 0°C solution of benzyl protected sobetirome (209 mg, 0.5 mmol) and DCM (4mL). DMF (2 μL) was then added and the reaction mixture was stirred at room temperature for 3 hours. The solution was then concentrated under reduced pressure. DCM (4mL) was added to the residue and the solution was concentrated again, this process was repeated once more. The crude residue was of sufficient purity and was used immediately in the subsequent ester couplings.

4.10.4 (*S*)-1-aminopropan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (3a**)**—To a 0°C solution of benzyl (*S*)-(2-hydroxypropyl)carbamate (262 mg, 1.25 mmol), DMAP (183 mg, 1.5 mmol), and THF (8 mL) was slowly added a solution of the acid chloride generated from benzyl protected sobetirome (vide supra, 0.5 mmol) in 4 mL THF. The reaction mixture was allowed to warm to room temperature, then heated to 50°C overnight with stirring. Filtration and evaporation of the resulting filtrate gave a light-yellow oil which was purified using flash chromatography (silica, 10% to 30% ethyl acetate/hexanes). The resulting ester (101 mg,

0.166 mmol, 33% yield) was dissolved in 5 mL of dry methanol with 1 mL THF and 10% Pd/C (80 mg) was added to generate a suspension. The reaction mixture was subjected to vacuum for approximately 1 min, then placed under argon for approximately 1 min. This process was repeated three times to ensure the mixture was properly degassed. Triethylsilane (0.82 mL, 5.15 mmol) was then added dropwise to the suspension and the reaction mixture was stirred for 4 h at room temperature. Filtration over a pad of celite with methanol and concentration *in vacuo* gave an oily residue which was precipitated with cold hexanes and washed with hexanes to give the desired product as a white solid (59 mg, 0.153 mmol, 90% yield, 30% overall yield). ¹H NMR (400 MHz, CD₃OD): δ 6.77 (s, 1H), 6.61 (s, 2H), 6.54 (d, *J* = 8 Hz, 1H), 6.48 (d, *J* = 8.3 Hz, 1H), 5.19 (m, *J* = 3 Hz 1H), 4.70 (d, *J* = 4 Hz, 2H), 3.84 (s, 2H), 3.19 (m, 2H), 3.16 (sept, *J* = 6.8 Hz, 1H), 2.16 (s, 6H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.09 (d, *J* = 6.9 Hz 6H). HRMS exact mass calcd for C₂₃H₃₂N₁O₄ [M+H]⁺: m/z 386.23258. Found m/z 386.23287.

4.10.5 (*S*)-2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)-*N*-(2-hydroxypropyl)acetamide (3b)—To a solution of sobetirome (**1**, 203 mg, 0.618 mmol) in 3 mL dry methanol was added 1 drop of concentrated sulfuric acid and the mixture was heated to 65° C in a sealed reaction tube with stirring for 1 h. After cooling to r.t., TLC confirmed complete conversion to the sobetirome methyl ester. (*S*)-(+)-1-amino-2-propanol (279 mg, 3.71 mmol) in 2 mL methanol was added to the solution of sobetirome methyl ester and the reaction mixture was again heated to 65° C with stirring in a sealed reaction tube for 1h. The reaction mixture was then cooled to r.t., diluted with 50 mL of DCM, and added to 100 mL of 0.5 N aqueous NaOH. The product was extracted with 3 × 50 mL DCM, the organic layers were combined, dried with MgSO₄, and evaporated to a crude product that was further purified on silica (10% MeOH in DCM) to give 185 mg of a colorless sticky solid (78% yield). ¹H NMR (400 MHz, CD₃OD): δ 7.24 (br, 1H), 6.97 (s, 1H), 6.92 (s, 1H), 6.71 (s, 2H), 6.65 (d, *J* = 8.2 Hz, 1H), 6.56 (dd, *J* = 8.2, 2.3 Hz, 1H), 4.46 (s, 2H), 3.89 (s, 2H), 3.83–3.85 (m, 1H), 3.30–3.38 (m, 1H), 3.13–3.21 (m, 1H, overlapping), 2.21 (s, 6H), 1.15 (d, *J* = 6.9 Hz, 6H), 1.10 (d, *J* = 6.4 Hz, 3H). ¹³C NMR (101 MHz, CD₃CN): δ 169.02, 155.54, 151.92, 138.48, 134.49, 131.19, 125.92, 125.30, 117.38, 114.91, 114.05, 66.96, 66.20, 46.07, 33.19, 26.72, 21.93, 20.11, 19.65.

4.10.6 (*R*)-1-aminopropan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (4)—To a 0° C solution of benzyl (*R*)-(2-hydroxypropyl)carbamate (262 mg, 1.25 mmol), DMAP (183 mg, 1.5 mmol), and THF (8 mL) was slowly added a solution of the acid chloride generated from benzyl protected sobetirome (0.5 mmol) in 4 mL THF. The reaction mixture was allowed to warm to room temperature, then heated to 50° C overnight with stirring. Filtration and evaporation of the resulting filtrate gave a light-yellow oil which was purified using flash chromatography (silica, 10% to 30% ethyl acetate/hexanes). The resulting ester (50 mg, 0.082 mmol, 17% yield) was dissolved in 5 mL of dry methanol with 1 mL THF and 10% Pd/C (40 mg) was added to generate a suspension. The reaction mixture was subjected to vacuum for approximately 1 min, then placed under argon for approximately 1 min. This process was repeated three times to ensure the mixture was properly degassed. Triethylsilane (0.4 mL, 2.51 mmol) was then added dropwise to the suspension and the reaction mixture was stirred

for 4 h at room temperature. Filtration over a pad of celite with methanol and concentration *in vacuo* gave an oily residue which was precipitated with cold hexanes and washed with hexanes to give the desired product as a white solid (22 mg, 0.057 mmol, 68% yield, 12% overall yield). ¹H NMR (400 MHz, CD₃OD): δ 6.77 (s, 1H), 6.62 (s, 2H), 6.55 (d, *J* = 8.1 Hz, 1H), 6.45 (d, *J* = 8.2 Hz, 1H), 5.20 (m, *J* = 3.0 Hz, 1H), 4.71 (d, *J* = 8.2 Hz, 2H), 3.84 (s, 2H), 3.21 (sept, *J* = 6.8 Hz, 1H), 3.18 (m, 2H), 2.16 (s, 6H), 1.31 (d, *J* = 6.5 Hz, 3H), 1.09 (d, *J* = 6.9 Hz, 6H). HRMS exact mass calcd for C₂₃H₃₂N₁O₄ [M+H⁺]⁺: m/z 386.23258. Found m/z 386.23349.

4.10.7 1-aminopropan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (5)—This compound was synthesized followed the procedure described for the synthesis of (4) except with *N*-Cbz-1-amino-2-propanol and yielded an oil (2.2 mg, 0.0052 mmol, 2.1%): ¹H NMR (400 MHz, CD₃OD) δ 6.75 (s, 1H), 6.54 (m, 3H), 6.47 (dd, *J* = 2.0, 6.0 Hz, 1H), 5.18 (m, 1H), 4.69 (d, *J* = 4.8 Hz, 2H), 3.84 (s, 2H), 3.15 (m, 3H), 2.15 (s, 6H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.07 (d, *J* = 6.8 Hz, 6H). HRMS exact mass calcd for C₂₃H₃₂N₁O₄ [M+H⁺]⁺: m/z 386.23258. Found m/z 386.23308.

4.10.8 1-amino-2-methylpropan-2-yl 2-(4-{4-hydroxy-3-(propan-2-yl)phenyl}methyl)-3,5-dimethylphenoxy)acetate (6)—To a 0 °C solution of *N*-Cbz-1-amino-2-methylpropan-2-ol (223 mg, 1 mmol), DMAP (92 mg, 0.75 mmol), and THF (3 mL) was added a solution of acid chloride of benzyl protected sobetirome (0.25 mmol) and THF (2 mL). The reaction mixture was allowed stir at 45 °C overnight. The reaction mixture was then concentrated, redissolved in a minimal amount of DCM, and purified using flash chromatography (silica, 20% to 50% ethyl acetate / hexanes) to yield the coupled *N*-Cbz ester (37 mg, 0.059 mmol). The resulting ester was dissolved in 2 mL MeOH and 2 mL of THF and purged with argon. 10% Pd/C (50mg) was added followed by the dropwise addition of triethylsilane (283 μL, 1.77 mmol). The reaction mixture was stirred at room temperature for 3 h and then filtered over a pad of celite with methanol. The solution was then concentrated under reduced pressure and precipitated with hexanes and ether to yield the product as an oily residue (5.6 mg, 5.6 % overall). ¹H NMR (400 MHz, CDCl₃): δ 7.04 (m, 1H), 6.94 (d, *J* = 2 Hz, 1H), 6.66 (s, 2H), 6.62 (d, *J* = 8.08 Hz, 1H), 6.54 (dd, *J* = 8.08 Hz, 2.02 Hz, 1H), 4.55 (s, 2H), 3.92 (s, 2H), 3.38 (d, *J* = 6.32 Hz, 2H), 3.19 (sept, *J* = 6.82 Hz, 1H), 2.23 (s, 6H), 2.20 (br, 1H), 1.25 (s, 6H), 1.23 (d, *J* = 6.82 Hz, 6H). HRMS exact mass calcd for C₂₄H₃₄N₁O₄ [M+H⁺]⁺: m/z 400.24824. Found m/z 400.24765.

4.10.9 1-(methylamino) propan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3, 5-dimethylphenoxy) acetate (7)—This compound was synthesized followed the procedure described for the synthesis of (4) except with benzyl (2-hydroxypropyl)(methyl)carbamate to yield a white solid in 49 % yield. ¹H NMR (400 MHz, CDCl₃): δ 6.92 (s, 1H), 6.64 (s, 2H), 6.60 (d, *J* = 8.1 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 4.70 (d, *J* = 8.2 Hz, 2H), 4.08 (m, 1H), 3.88 (s, 2H), 3.54 (m, 1H), 3.30 (m, 1H), 3.18 (s, 3H), 3.02 (s, 1H), 2.19 (s, 6H), 1.22 (d, *J* = 6.9 Hz, 6H), 1.18 (d, *J* = 6.4 Hz, 3H). HRMS exact mass calcd for C₂₄H₃₂N₁O₄ [M-H⁺]⁻: m/z 398.23258. Found m/z 398.23336.

4.10.10 2-(isopropylamino) ethyl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (8)—This compound was synthesized followed the procedure described for the synthesis of (4) except with benzyl (2-hydroxypropyl)(isopropyl)carbamate to give a sticky residue in 24 % yield. ¹H NMR (400 MHz, CDCl₃): δ 6.93 (s, 1H), 6.68 (s, 2H), 6.53 (d, *J* = 8.0 Hz, 1H), 6.49 (d, *J* = 8.0 Hz, 1H), 4.74 (s, 2H), 4.60 (m, 1H), 4.23 (sep, *J* = 6.8 Hz, 1H), 3.91 (s, 2H), 3.56 (dd, *J* = 14.6, 8.4 Hz, 1H), 3.15 (sep, *J* = 7.0 Hz, 1H), 3.09 (m, 1H), 2.22 (s, 6H), 1.21 (d, *J* = 6.8 Hz, 6H), 1.15 (d, *J* = 6.9 Hz, 6H), 1.08 (d, *J* = 6.5 Hz, 3H). HRMS exact mass calcd for C₂₆H₃₆N₁O₄ [M-H⁺]⁺: *m/z* 426.26389. Found *m/z* 426.26464.

4.10.11 4-aminobutan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3, 5-dimethylphenoxy)acetate (9)—This compound was synthesized followed the procedure described for the synthesis of (4) except with benzyl (3-hydroxybutyl)(isopropyl)carbamate to yield the product as a white solid in 45% yield. The ¹H NMR spectrum corresponds to the amide rearranged product. ¹H NMR (400 MHz, CD₃CN): δ 7.31 (m, 1H), 6.92 (s, 1H), 6.70 (s, 2H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.57 (dd, *J* = 8.1 Hz, 2.04 Hz, 1H), 4.43 (s, 2H), 3.90 (s, 2H), 3.70–3.76 (m, 1H), 3.49 (m, 1H), 3.32–3.13 (m, 2H, overlapping), 2.22 (s, 6H), 1.61 (m, 1H), 1.46 (m, 1H), 1.13 (d, *J* = 7.0 Hz, 6H), 1.11 (d, *J* = 6.6 Hz, 3H). HRMS exact mass calcd for C₂₄H₃₂N₁O₄ [M-H⁺]⁺: *m/z* 398.23258. Found *m/z* 398.23335.

4.10.12 1-aminobutan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (10)—To a 0° C solution of benzyl (2-hydroxybutyl)carbamate (587 mg, 2.63 mmol), DMAP (321 mg, 2.63 mmol), and THF (8 mL) was slowly added a solution of the acid chloride generated from benzyl protected sobetirome (0.66 mmol) in 4 mL THF. The reaction mixture was allowed to warm to room temperature, then heated to reflux overnight with stirring. Filtration and evaporation of the resulting filtrate gave a light-yellow oil which was purified using flash chromatography (silica, 10% to 50% ethyl acetate/hexanes). The resulting ester (236 mg, 0.378 mmol, 58% yield) was dissolved in 5 mL of dry methanol with 1 mL THF and 10% Pd/C (100 mg) was added to generate a suspension. The reaction mixture was subjected to vacuum for approximately 1 min, then placed under argon for approximately 1 min. This process was repeated three times to ensure the mixture was properly degassed. Triethylsilane (1.8 mL, 11.3 mmol) was then added dropwise to the suspension and the reaction mixture was stirred for 4 h at room temperature. Filtration over a pad of celite with methanol and concentration *in vacuo* gave an oily residue which was precipitated with cold hexanes and washed with hexanes. The desired product was obtained as a white solid after drying under high vacuum (87 mg, 0.217 mmol, 58% yield, 34% overall yield). The ¹H NMR spectrum corresponds to the amide rearranged product. ¹H NMR (400 MHz, CD₃CN): δ 7.13 (m, 1H), 6.92 (s, 1H), 6.72 (s, 2H), 6.64 (d, *J* = 8.1 Hz, 1H), 6.57 (dd, *J* = 7.9, 2.1 Hz, 1H), 4.46 (s, 2H), 3.90 (s, 2H), 3.50–3.60 (m, 1H), 3.31–3.40 (m, 1H), 3.16–3.21 (m, 2H, overlapping), 2.22 (s, 6H), 1.30–1.49 (m, 2H), 1.15 (d, *J* = 7.1 Hz, 6H), 0.93 (t, *J* = 6.7 Hz, 3H). HRMS exact mass calcd for C₂₄H₃₂N₁O₄ [M-H⁺]⁺: *m/z* 398.23258. Found *m/z* 398.23331.

4.10.13 1-amino-3-methylbutan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (11)—To a 0° C solution of 1-(dibenzylamino)-3-

methylbutan-2-ol (745 mg, 2.63 mmol), DMAP (321 mg, 2.63 mmol), and THF (8 mL) was slowly added a solution of the acid chloride generated from benzyl protected sobetirome (0.66 mmol) in 4 mL THF. The reaction mixture was allowed to warm to room temperature, then heated to reflux overnight with stirring. Filtration and evaporation of the resulting filtrate gave a light-yellow oil which was purified using flash chromatography (silica, 10% to 50% ethyl acetate/hexanes). The resulting ester (128 mg, 0.187 mmol, 28% yield) was dissolved in 5 mL of dry methanol with 1 mL THF and 10% Pd/C (100 mg) was added to generate a suspension. The reaction mixture was subjected to vacuum for approximately 1 min, then placed under argon for approximately 1 min. This process was repeated three times to ensure the mixture was properly degassed. Triethylsilane (1.35 mL, 8.47 mmol) was then added dropwise to the suspension and the reaction mixture was stirred for 4 h at room temperature. Filtration over a pad of celite with methanol and concentration *in vacuo* gave an oily residue which was precipitated with cold hexanes and washed with hexanes. The desired product was obtained as a white solid after drying under high vacuum (69 mg, 0.167 mmol, 89% yield, 25% overall yield). ¹H NMR (400 MHz, CD₃CN): δ 6.72 (s, 1H), 6.69 (s, 2 H), 6.66 (d, *J* = 8.5 Hz, 1H), 6.53 (dd, *J* = 8.1 Hz, *J* = 2.5 Hz, 1H), 5.12 (m, 1H), 5.03 (d, *J* = 16.3 Hz, 1H), 4.75 (d, *J* = 16.5 Hz, 1H), 3.87 (s, 2H), 3.18 (m, 3H), 2.20 (s, 6H), 1.15 (d, *J* = 6.8 Hz, 6H), 0.92 (dd, *J* = 6.8, 1.7 Hz, 6H). HRMS exact mass calcd for C₂₅H₃₄N₁O₄ [M-H]⁺: *m/z* 412.24824. Found *m/z* 412.24907.

4.10.14 3-methyl-1-(methylamino)butan-2-yl 2-(4-(4-hydroxy-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetate (12)—To a 0° C solution of 1-(benzyl(methyl)amino)-3-methylbutan-2-ol (530 mg, 2.56 mmol), DMAP (312 mg, 2.55 mmol), and THF (8 mL) was slowly added a solution of the acid chloride generated from benzyl protected sobetirome (0.66 mmol) in 4 mL THF. The reaction mixture was allowed to warm to room temperature, then heated to reflux overnight with stirring. Filtration and evaporation of the resulting filtrate gave a light-yellow oil which was purified using flash chromatography (silica, 10% to 50% ethyl acetate/hexanes). The resulting ester (200 mg, 0.329 mmol, 50% yield) was dissolved in 5 mL of dry methanol with 1 mL THF and 10% Pd/C (100 mg) was added to generate a suspension. The reaction mixture was subjected to vacuum for approximately 1 min, then placed under argon for approximately 1 min. This process was repeated three times to ensure the mixture was properly degassed. Triethylsilane (1.6 mL, 10.04 mmol) was then added dropwise to the suspension and the reaction mixture was stirred for 4 h at room temperature. Filtration over a pad of celite with methanol and concentration *in vacuo* gave an oily residue which was precipitated with cold hexanes and washed with hexanes. The desired product was obtained as a white solid after drying under high vacuum (101 mg, 0.236 mmol, 72% yield, 36% overall yield). ¹H NMR (400 MHz, CD₃CN): δ 6.93 (s, 1H), 6.71 (s, 2H), 6.69 (d, *J* = 8.6 Hz, 1H), 6.54 (dd, *J* = 8.3, 2.2 Hz, 1H), 5.22 (m, 1H), 5.12 (d, *J* = 16.5 Hz, 1H), 4.77 (d, *J* = 16.5 Hz, 1H), 3.88 (s, 2H), 3.18 (m, 3H), 2.62 (s, 3H), 2.20 (s, 6H), 1.15 (d, *J* = 6.8 Hz, 6H), 0.93 (dd, *J* = 7.0, 2.1 Hz, 6H). HRMS exact mass calcd for C₂₆H₃₆N₁O₄ [M-H]⁺: *m/z* 426.26389. Found *m/z* 426.26456.

4.10.15 2-(methylamino)ethyl 2-(4-(4-hydroxy-3-methylbenzyl)-3,5-dimethylphenoxy)acetate (13)—To a 0° C solution of benzyl (2-hydroxyethyl) (methyl)carbamate (314 mg, 1.5 mmol), DMAP (183 mg, 1.5 mmol), and THF (8 mL) was

slowly added a solution of the acid chloride generated from benzyl protected sobetirome (0.5 mmol) in 4 mL THF. The reaction mixture was allowed to warm to room temperature, then heated to 50° C overnight with stirring. Filtration and evaporation of the resulting filtrate gave a light-yellow oil which was purified using flash chromatography (silica, 10% to 30% ethyl acetate/hexanes). The resulting ester (146 mg, 0.239 mmol, 48% yield) was dissolved in 5 mL of dry methanol with 1 mL THF and 10% Pd/C (100 mg) was added to generate a suspension. The reaction mixture was subjected to vacuum for approximately 1 min, then placed under argon for approximately 1 min. This process was repeated three times to ensure the mixture was properly degassed. Triethylsilane (1.2 mL, 7.53 mmol) was then added dropwise to the suspension and the reaction mixture was stirred for 4 h at room temperature. Filtration over a pad of celite with methanol and concentration *in vacuo* gave an oily residue which was precipitated with cold hexanes and washed with hexanes. The resulting residue was dissolved in 3 mL of ethyl acetate and 1 mL of 1 N HCl (ethyl acetate) was added and stirred 3 h. Evaporation of the solvent, followed by washing with hexanes gave the desired product as a white solid (37 mg, 0.088 mmol, 37% yield, 18% overall yield). ¹H NMR (400 MHz, CD₃CN): δ 6.91 (s, 1H), 6.68 (s, 2H), 6.64 (d, *J* = 8.1 Hz, 1H), 6.53 (d, *J* = 7.8 Hz, 1H), 4.79 (s, 2H), 4.48 (m, 2H), 3.86 (s, 2H), 3.20 (m, 2H), 3.17 (sept, *J* = 6.9 Hz, 1H), 2.60 (s, 3H), 2.19 (s, 6H), 1.13 (d, *J* = 7.0 Hz, 6H). HRMS exact mass calcd for C₂₃H₃₂N₁O₄ [M+H]⁺: m/z 386.23258. Found m/z 386.23259.

4.10.16 3-amino-1,1,1-trifluoropropan-2-yl 2-(4-(4-hydroxy-3-

isopropylbenzyl)-3,5-dimethylphenoxy)acetate (14)—To a 0° C solution of benzyl (3,3,3-trifluoro-2-hydroxypropyl)carbamate (207 mg, 0.786 mmol), DMAP (120 mg, 0.982 mmol), and chloroform (8 mL) was slowly added a solution of the acid chloride generated from benzyl protected sobetirome (0.392 mmol) in 4 mL chloroform. The reaction mixture was allowed to warm to room temperature, then heated to 50° C overnight with stirring. Evaporation of the product mixture gave a light-yellow oil which was purified using flash chromatography (silica, 10% to 30% ethyl acetate/hexanes). The resulting ester (79 mg, 0.119 mmol, 30% yield) was dissolved in 5 mL of dry methanol with 1 mL THF and 10% Pd/C (80 mg) was added to generate a suspension. The reaction mixture was subjected to vacuum for approximately 1 min, then placed under argon for approximately 1 min. This process was repeated three times to ensure the mixture was properly degassed. Triethylsilane (0.6 mL, 3.77 mmol) was then added dropwise to the suspension and the reaction mixture was stirred for 4 h at room temperature. Filtration over a pad of celite with methanol and concentration *in vacuo* gave an oily residue which was precipitated with cold hexanes and washed with hexanes to give the desired product as a white solid (39 mg, 0.089 mmol, 75% yield, 23% overall yield). ¹H NMR (400 MHz, CD₃OD): δ 6.78 (s, 1H), 6.59 (s, 2H), 6.55 (d, *J* = 8.1 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 1H), 4.64 (s, 2H), 4.28 (m, 1H), 3.84 (s, 2H), 3.23 (dd, *J* = 13.1, 3.2 Hz, 1H), 3.17 (sept, *J* = 6.9 Hz, 1H), 3.04 (dd, *J* = 13.2, 9.5 Hz, 1H), 2.15 (s, 6H), 1.09 (d, *J* = 7.0 Hz, 6H). HRMS exact mass calcd for C₂₃H₂₉F₃N₁O₄ [M+H]⁺: m/z 440.20487. Found m/z 440.20412.

4.10.17 3-(trifluoromethyl)azetidin-3-yl 2-(4-(4-hydroxy-3-(propan-2-yl)phenyl)methyl)-3,5-dimethylphenoxy)acetate (15)

—To a 0 °C solution of *N*-Cbz-1-amino-3-hydroxy-3-(trifluoromethyl)-azetidine HCl (250 mg, 1.41 mmol), DMAP

(122 mg, 1 mmol), and THF (3 mL) was added a solution of the acid chloride of benzyl protected sobetirome (0.25 mmol) and THF (2 mL). The reaction mixture was allowed stir at 45 °C overnight. The reaction mixture was then concentrated, redissolved in a minimal amount of DCM, and purified using flash chromatography (silica, 20% to 50% ethyl acetate / hexanes) to yield the coupled *N*-Cbz ester (75 mg, 0.121 mmol). The resulting ester was dissolved in 2 mL MeOH and 2 mL of THF and purged with argon. 10% Pd/C (50mg) was added followed by the dropwise addition of triethylsilane (484 μ L, 3.03 mmol). The reaction mixture was stirred at room temperature for 3 h and then filtered over a pad of celite with methanol. The solution was then concentrated under reduced pressure and precipitated with hexanes and ether to yield the product as an oily residue (6.8 mg, 6.0 % overall). ^1H NMR (400 MHz, CD_3OD): δ 6.82 (d, J = 2 Hz, 1H), 6.67 (s, 2H), 6.59 (d, J = 8.08 Hz, 1H), 6.52 (dd, J = 8.08, 2.02 Hz, 1H), 4.78 (br s, 4H), 3.90 (s, 2H), 3.62 (t, J = 5.86 Hz, 2H), 3.21 (sept, J = 7.02 Hz, 1H), 2.21 (s, 6H), 1.23 (d, J = 7.02 Hz, 6H). HRMS exact mass calcd for $\text{C}_{24}\text{H}_{27}\text{F}_3\text{N}_1\text{O}_4$ $[\text{M}-\text{H}^+]^-$: m/z 450.18924. Found m/z 450.18557.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations Used

ACN	acetonitrile
br	broad
Cbz	N-benzyloxycarbonyl
DMAP	4-dimethylaminopyridine
DMSO	dimethyl sulfoxide
ED ₅₀	median effective dose
HPLC	high performance liquid chromatography
LC-MS/MS	liquid chromatography tandem mass spectrometry
MeOH	methanol
MHz	megahertz
NMR	nuclear magnetic resonance spectroscopy

Pd/C	palladium on carbon
p.o	per os
r.t	room temperature
THF	tetrahydrofuran

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Highlights

- Prodrugs of thyromimetic sobetirome increase CNS exposure of parent drug.
- Ethanolamine-derived promoieties undergo O,N acyl migration.
- Pharmacokinetics of sobetirome and lead prodrug described.
- Lead prodrug found more potent than sobetirome at target engagement in the brain.

Figure 1.

Sobetirome (black trace) and prodrug **3** (red trace) AUCs measuring parent drug sobetirome levels in brain (A), serum (B), brain/serum (C), liver (D), heart (E), and kidney (F). Data are represented as the mean ($n = 3$ mice) with corresponding standard error of the mean at time points (0.25 h, 0.5 h, 1 h, 2 h, 4 h, and 8 h) over an 8 h period.

Figure 2.

A) Serum [**3**] vs. time dosing prodrug **3** in mice. Intravenous dosing (black trace, 3.05 $\mu\text{mol/kg}$) and oral dosing (red trace, 30.5 $\mu\text{mol/kg}$). B) Serum [**1**] vs. time dosing prodrug **3** in mice. Intravenous dosing (black trace) and oral dosing (blue trace). C) Serum [**1**] vs. time dosing sobetirome in mice. Intravenous dosing (black trace, 3.05 $\mu\text{mol/kg}$) and oral dosing (blue trace, 30.5 $\mu\text{mol/kg}$). D) Semilog plot of serum [**1**] (black symbols) and [**3**] (red symbols) vs. time.

Figure 3.

Dose — response curves for relative brain expression of *Hairless* (*Hr*) gene following systemic administration (i.p.) of 3,3',5-triiodo-L-thyronine (T3, blue •), sobetirome (black •), and prodrug 3 (red •).

Scheme 1.

Synthesis and structures of sobetirome and sobetirome prodrugs. Reagents and conditions:

(a) i) oxalyl chloride, DCM, DMF, (ii) *N*-Cbz amino alcohol or *N*-(di)benzyl amino alcohol, DMAP, THF (b) 10% Pd/C, Et₃SiH, MeOH/THF.

Scheme 2.

O,N and N,O acyl migration is an intramolecular rearrangement and is pH controlled (Top).

Ester-to-amide rearrangement of lead prodrug **3** (Bottom).

Table 1

Sobetirome concentrations (ng / g) in the brain and brain/serum ratios 1 h after administration of sobetirome (**1**, 1.5 μmol / kg, i.p.) or prodrugs **2–15** (1.5 μmol / kg, i.p.) in mice.^a

Compound	Brain		Brain / Serum	
	[1] (ng/g)	SEM	Ratio	SEM
1	2.32	0.32	0.037	0.0044
2	4.92 *	0.12	0.066	0.0011
3	8.26 **	0.28	0.47	0.15
4	8.12 **	0.31	0.19	0.062
5	5.92 **	0.18	0.21	0.0062
6	0.40 *	0.014	1.22	0.38
7	5.12	1.82	0.23	0.013
8	0.48	0.0031	0.23	0.0015
9	4.46	1.96	0.33	0.070
10	4.26	1.04	0.41	0.027
11	3.38	0.26	0.045	0.0027
12	1.79	0.71	0.024	0.0046
13	6.33 *	0.64	0.21	0.026
14	5.23 **	0.038	0.20	0.015
15	5.14 **	0.21	0.13	0.027

Significance compared to sobetirome (**1**):

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.005$.

^a Values are reported as the mean (n = 3 mice) with corresponding standard error of the mean (SEM).

Table 2

AUC values in ng/g*h for sobetirome and **3** by tissue.

Tissue	Sobetirome (1)	3
	AUC _{0→t} (ng/g*h)	AUC _{0→t} (ng/g*h)
Brain	9.9	17.2
Serum	472.6	136.5
Liver	2235	1017
Heart	180.3	57.2
Kidney	686.8	283.8