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Increasing Thyromimetic Potency through Halogen Substitution

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Sobetirome is one of the most studied thyroid hormone receptor β (TRβ)-selective thyromimetics in the field due to its excellent selectivity and potency. A small structural change—replacing the 3,5-dimethyl groups of sobetirome with either chlorine or bromine—produces significantly more potent compounds, both in vitro and in vivo. These halogenated compounds induce transactivation of a TRβ-mediated cell-based reporter with an EC_{50} value comparable to that of T3, access the central nervous system (CNS) at levels similar to their parent, and activate an endogenous TR-regulated gene in the brain with an EC_{50} value roughly five-fold lower than that of sobetirome. Previous studies suggest that this apparent increase in affinity can be explained by halogen bonding between the ligand and a backbone carbonyl group in the receptor. This makes the new analogues potential candidates for treating CNS disorders that may respond favorably to thyroid-hormone-stimulated pathways.

Introduction

Thyroid hormone (TH) in the form of 3,3′,5-triiodothyronine (T3) derived from deiodination of the pro-hormone tetraiodothyronine (T4) (Figure 1) is a key signal for oligodendrocyte differentiation and myelin formation during development and also stimulates remyelination in adult models of multiple sclerosis (MS).[1] However, TH is not an acceptable long-term therapy due to the lack of a therapeutic window in which remyelination can be induced while avoiding the cardiotoxicity and bone demineralization associated with chronic hyperthyroidism. A more promising approach comes from thyroid hormone analogues that can activate TH-responsive genes while avoiding the associated downsides of TH by exploiting molecular and physiological properties of thyroid hormone receptors (TRs).[2] These receptors are expressed in two major forms with heterogeneous tissue distribution and overlapping but distinct sets of target genes.[3] TRα is enriched in the heart, brain, and bone, whereas TRβ is enriched in the liver.[4] Developing selective thyromimetics has been challenging due to the high sequence similarity of TR subtypes; only one amino acid residue on the internal surface of the ligand binding domain cavity varies between the α_1 and β_1 forms. Sobetirome (Figure 1) was one of the first potent analogues that demonstrated significant TRβ selectivity in vitro[5,6] and in vivo.[7–9] While sobetirome was designed as a cardiac-sparing drug for treating hypercholesterolemia by activating TRβ in the liver,[10] it is unique within the thyromimetic field in possessing significant distribution in the brain.[7,11] Recent studies highlight the potential advantage of this property by demonstrating its activity in in vitro models of human CNS demyelination diseases such as MS[12] and X-linked adrenoleukodystrophy (X-ALD).[13]

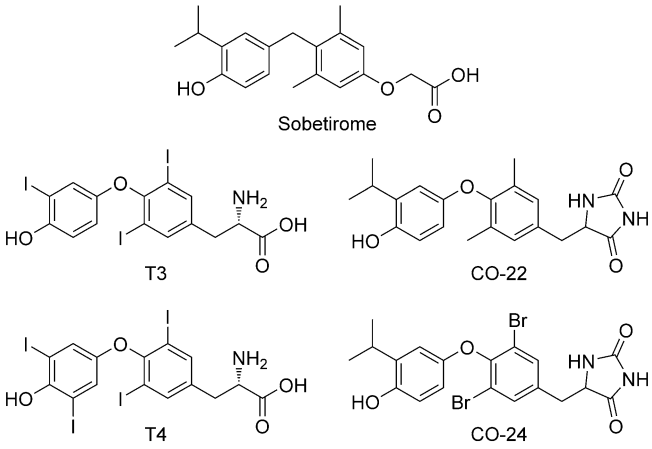

Figure 1. Structures of selected TR agonists.

Although sobetirome is a very promising drug candidate for central nervous system (CNS) indications, its potency is lower than that of T3 (Figure 1). A more potent analogue would evoke the same effects at a lower dose or greater stimulation of the target at the same dose. While many of the structural features of sobetirome are critical for its binding affinity and receptor selectivity,[6,12] the 3,5-dimethyl constituents have not been investigated. There is a large body of computational and biological structure–activity relationship (SAR) data demonstrating that thyromimetics with 3,5-dimethyl substitutions have significantly lower activity than structurally similar ana-

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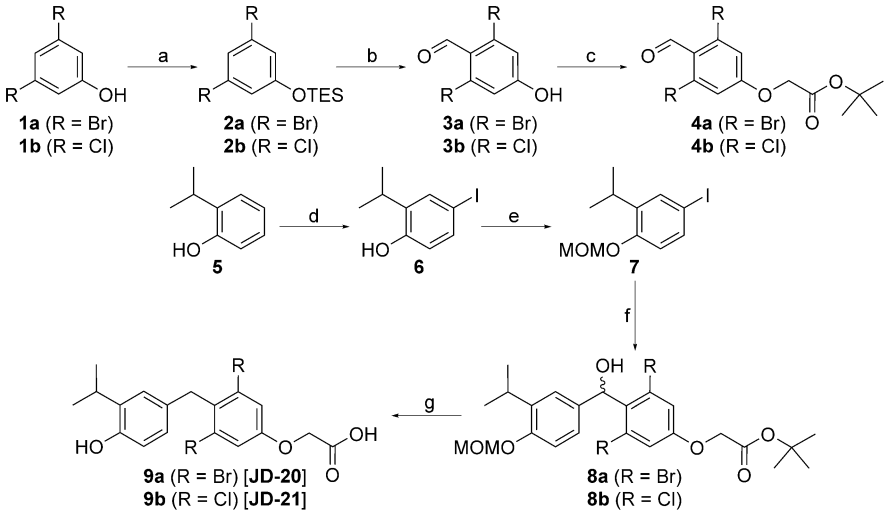
logues with 3,5-position halogen substitutions. In the TRα-selective compounds CO-22 and CO-24 (Figure 1) replacement of 3,5-dimethyl groups with bromines was found to improve binding affinity 15-fold.[14,15] A computational SAR study of thyroid hormone analogues suggested a potential mechanism for these findings: halogen atoms at the 3,5-position can form a dipole–dipole interaction with a backbone carbonyl group in the TR ligand binding domain, which influences binding affinity and selectivity.[16] Halogen bonding interactions are strongly influenced by the geometry of the donor and halogen, as the dipoles must be close enough to interact and oriented so that the lone pair electrons of the donor and the partial positive charge on the halogen face each other.[17] The interaction is generally stronger with increasing halogen atom size due to the increasing polarizability of larger halogens producing a greater partial positive charge on the atom. These data suggest that replacing the 3,5-dimethyl groups of sobetirome with halogens may result in thyromimetics with higher affinity and potency at the thyroid hormone receptor.

Results and Discussion

3,5-position halogen analogues of sobetirome required a new synthetic approach (Scheme 1). Work by Dabrowski et al.[18] provided a template for producing the necessary 4-hydroxy-2,6-dihalobenzaldehyde intermediates by selectively deprotonating the 4-position of silyl-protected 3,5-dihalophenols with lithium amide reagents. The method was improved by replacing the methyl ether and trimethylsilyl ether protecting groups used by Dabrowski et al. with the sterically bulkier triethylsilyl ether protecting group, which significantly improved the selectivity of the deprotonation. These intermediates were used in a slightly altered version of the recently reported improved sobetirome synthesis.[19] The 4-hydroxy-2,6-dihalobenzaldehyde intermediates could not be alkylated with *tert*-butyl chloroacetate using the standard cesium carbonate/DMF conditions due to the halogen substitutions decreasing the nucleophilicity of the phenol. However, the reaction went to completion and in good yield after converting the chloroacetate into an iodoacetate via an in situ Finklestein reaction. After forming the *tert*-butyl oxyacetate intermediate, the carbon–carbon bond formation proceeded in the same fashion as with sobetirome by forming an arylmagnesium with **7** that attacked the benzaldehyde to form a carbinol intermediate. The arylmagnesium nucleophile was critical to the success of the synthesis, as it does not exchange with aryl chlorides or bromides at cryogenic temperatures and is compatible with the *tert*-butyl ester protecting group.[20] Reduction of the carbinol and deprotection of the *tert*-butyl ester and methoxymethyl ether protecting groups proceeded simultaneously with trifluoroacetic acid and triethylsilane in dichloromethane. The dibromo analogue JD-20 was synthesized in 27% overall yield, and the dichloro analogue JD-21 was synthesized in 17% overall yield, both in five steps.

A electroporation-based transfection system with U2OS or HeLa cells was previously used for measuring the potency of TR-mediated transactivation.[5,15] The electroporation method was limited by poor transfection efficiencies and decreased dynamic range that delivered inconsistent EC_{50} values for T3 at each receptor subtype. Previous reports suggested that lipofectamine reagents sequestered lipophilic compounds such as sobetirome.[21] The electroporation-based assay was modified and optimized using Lipofectamine 2000 and HEK293 cells. In comparison with the previous method, the new version has significantly higher transfection efficiencies and a greater dynamic range while being operationally simpler and delivering similar EC_{50} values for T3 at both subtypes.

Transactivation assays showed that JD-20 and JD-21 have greater potency than their parent compound sobetirome. Increases in potency at TRα were significant, with more modest improvements at TRβ, boosting the analogues to match the EC_{50} value of T3 (Figure 2, Table 1).



Scheme 1. Synthesis of JD-20 **9a** and JD-21 **9b**. *Reagents and conditions*: a) triethylsilyl chloride, imidazole, CH_2Cl_2, 0 °C, 95%; b) 1. *n*BuLi, DIA or TMP, THF, −78 °C, 2. DMF, 56–67%; c) *tert*-butylchloroacetate, NaI, Cs_2CO_3, acetone, 60–65 °C, 84–88%; d) NaI, NaOH, NaOCl, MeOH, H_2O, 87%; e) MOMCl, TBAI, NaOH, CH_2Cl_2, H_2O, 81%; f) 1. *i*PrMgCl, THF, 0 °C→RT, 2. **4a** or **4b**, −78 °C, 54–79%; g) TFA, triethylsilane, CH_2Cl_2, 0 °C→RT, 58–69%.

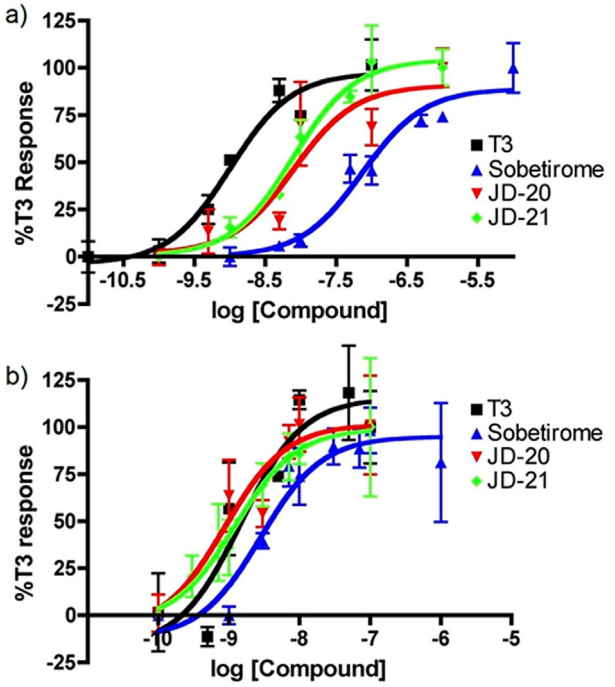


Figure 2. TRE-driven dual luciferase transactivation assays with calculated sigmoidal dose–response curves against a) hTRα_1 and b) hTRβ_1 in transiently transfected HEK293 cells. Plots show data normalized to T3 response as mean values of triplicates with standard error.

Table 1. Subtype selectivity measured by EC_{50} values from TRE-driven dual luciferase transactivation assays.

Compound	EC_{50} [nM][a]		TRα/TRβ
	TRα	TRβ	
T3	1.0 ± 0.4	1.5 ± 1.6	0.7
Sobetirome	74.7 ± 28.9	2.8 ± 1.8	26.5
JD-20	8.0 ± 7.0	0.9 ± 1.1	9.0
JD-21	7.8 ± 3.6	1.2 ± 1.3	6.3

[a] Data are the mean ± SEM of $n = 1$ experiment performed in triplicate.

A distribution study was carried out in C57BL/6J mice to determine the concentrations in brain and serum after systemic (i.p.) administration. Mice were given single 9.14 μmol kg^{-1} doses of sobetirome, JD-20, or JD-21. Tissue and blood were collected 1 h post-injection, and the concentration of the drugs was determined by LC–MS/MS analysis (Figure 3). JD-20 and JD-21 showed brain uptake similar to that of sobetirome, while the serum levels of JD-20 and JD-21 were both significantly ($p < 0.05$) lower than sobetirome. The brain/serum ratio of JD-21 trended higher than sobetirome ($p = 0.055$), while JD-20 had a brain/serum ratio similar to sobetirome.

Induction of the TR-regulated gene *Hairless* (*Hr*) in the brain was determined by qPCR and normalized to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) mRNA (Figure 4). Vehicle (1:1 saline/DMSO) was used as a negative control, and saturating doses of T3 (0.305 μmol kg^{-1}) and sobetirome (9.14 μmol kg^{-1}) were used as positive controls. JD-20 and JD-21 at 0.914 μmol kg^{-1} had similar *Hr* induction to a 10-fold higher dose of sobetirome.

The EC_{50} values of *Hr* mRNA induction in the brain normalized to *GAPDH* mRNA expression by sobetirome, JD-20, and JD-21 (Figure 4) were determined using the same experimental protocol. The respective EC_{50} values for sobetirome, JD-20, and JD-21 were 8.20 ± 12.65, 1.49 ± 1.08, and 1.21 ± 1.75 μmol kg^{-1}, making the halogenated analogues roughly six-fold more potent than sobetirome at inducing *Hr* mRNA expression in the brain.

Conclusions

While sobetirome has become one of the standard TRβ-selective thyromimetics in the field, this study makes clear that a simple structural change—replacing the 3,5-dimethyl groups with bromine or chlorine—produces more potent compounds that largely maintain TR-isoform selectivity and brain uptake properties of the parent compound. This improvement is potentially due to a halogen bonding interaction between the ligand and a backbone carbonyl group in the TR ligand binding domain. These results suggest that JD-20 and JD-21 may be useful molecules for studying and treating CNS demyelination diseases such as X-ALD and MS.

The improved potency of JD-20 and JD-21 is consistent with numerous thyromimetic SAR studies that have found superior activity for analogues with halogen atoms at the 3,5-position

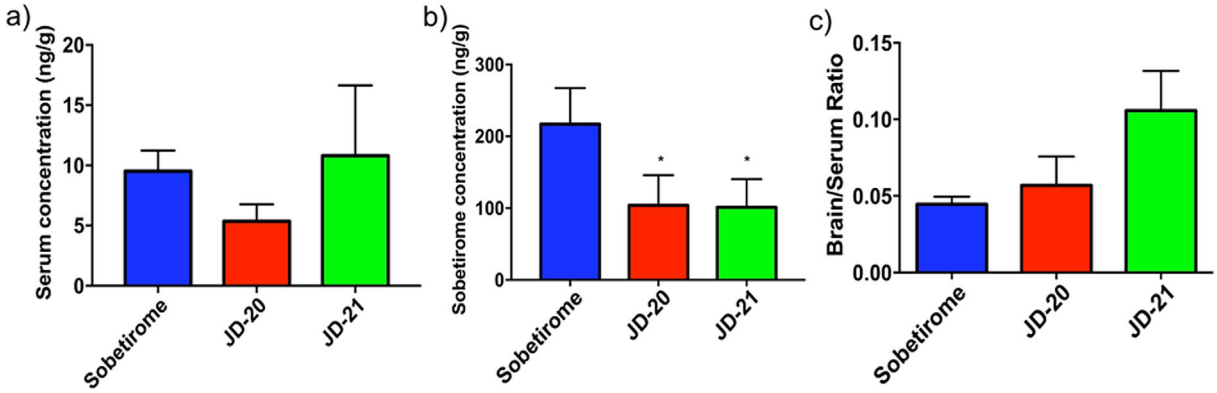


Figure 3. In vivo concentrations of GC-1, JD-20, and JD-21 in C57BL/6 mouse tissues 1 h after systemic administration (9.14 μmol kg^{-1} i.p.), measured by LC–MS/MS in a) brain and b) serum; c) the ratio of brain/serum drug concentration; *$p < 0.05$ relative to sobetirome.

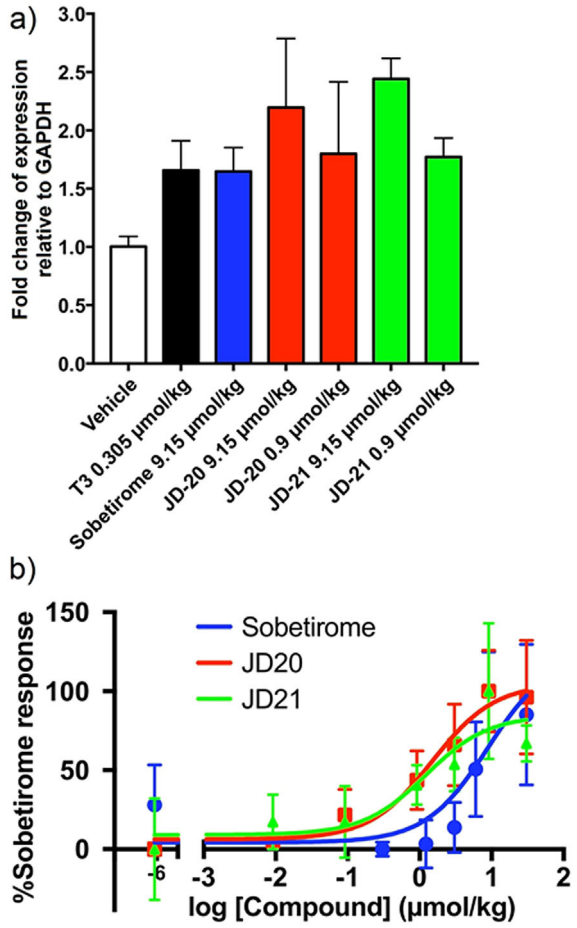


Figure 4. a) Expression of TR-regulated gene *Hairless* (*Hr*) mRNA normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA measured by qPCR in C57BL/6mouse brain (three mice per dose) 2 h after systemic administration (i.p.) of saturating doses of T3 (0.305 μmol kg^{-1}) or sobetirome (9.15 μmol kg^{-1}) plus escalating doses of JD-20 and JD-21 (0.9 and 9.15 μmol kg^{-1}). b) Expression of TR-regulated gene *Hairless* (*Hr*) mRNA normalized to GAPDH mRNA measured by qPCR in C57BL/6 mouse brain (three mice per dose) 2 h after systemic administration (i.p.) of GC-1, JD-20, and JD-21.

compared with similar analogues with methyl groups at those positions. It is surprising in this instance that by most measures JD-20 and JD-21 appear to have very similar properties. The previous thyromimetic SAR studies found that changing the halogen substitution frequently produced dramatic shifts in both potency and selectivity.[16] However, the in vitro and in vivo potencies of JD-20 and JD-21 are roughly the same within error. This is different from most known thyromimetics and suggests something unique about the chemical context of the sobetirome scaffold. Additionally, except for the improvements in potency, the only major difference between these novel compounds and sobetirome is the decreased serum concentrations. This hints that these compounds may be distributed by different mechanisms, such as being actively transported across the blood–brain barrier rather than by passive diffusion, where halogen bonding or steric effects might alter the relative uptake rates through a putative transporter. It will be important to determine whether uptake of sobetirome and these analogues into the CNS occurs primarily through an active transport mechanism.

Experimental Section

Biological assays

Transactivation assay: Human epithelial kidney (HEK293) cells were grown to 80% confluency in high-glucose (4.5 g L^{-1}) Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 50 U mL^{-1} penicillin, and 50 μg mL^{-1} streptomycin. Cells were trypsinized with 0.25% trypsin, then diluted to 5×10^5 cells per mL with high-glucose DMEM. Cells were added to Costar 3917 96-well plates at 5×10^4 cells per well, then incubated at 37 °C for 24 h. TR expression vector (1.5 μg; full-length TRα–CMV or TRβ–CMV), reporter plasmid (1.5 μg) containing a DR4 thyroid hormone response element (TRE) direct repeat spaced by four nucleotides (AGGTCAcaggAGGTCA) cloned upstream of a minimal thymidine kinase promoter linked to a firefly luciferase coding sequence, and a pRL–SV40 constitutive *Renilla* luciferase reporter plasmid (0.75 μg) were diluted into OptiMEM (540 μL). Lipofectamine 2000 reagent (27 μL) was diluted into 540 μL OptiMEM. The plasmid and lipofectamine dilutions were combined, then incubated at room temperature for 10 min. The mixture was then diluted into 4.29 mL OptiMEM. Plates were washed with 100 μL phosphate-buffered saline (PBS) at pH 7.2 without magnesium or calcium chloride per well. Transfection mixtures were added at 50 μL per well, then incubated at 37 °C for 4 h. Modified DME/F-12 Ham's medium without phenol red containing 15 mM HEPES and bicarbonate, 5 mM L-glutamine, charcoal-stripped FBS, 50 U mL^{-1} penicillin, and 50 μg mL^{-1} streptomycin was added at 50 μL per well, then the plates were incubated at 37 °C for 20 h. Drug stocks were made at 10 mM in DMSO, then serially diluted to 1× concentrations in DME/F-12 Ham's. Plates were washed with 100 μL PBS (pH 7.2) per well. Each drug stock (100 μL) was added to the wells in triplicate, then the plates were incubated at 37 °C for 24 h. Cells were assayed for luciferase activity using the Promega DualGlo kit. Luciferase Reagent (50 μL) was added to each well, the plate was rocked for 15 min at room temperature, and then read for firefly luciferase activity. Stop & Glo Reagent (50 μL) was added to each well, then the plate was read for *Renilla* luciferase activity. Data normalized to *Renilla* internal control were analyzed with GraphPad Prism v.4a using the sigmoidal dose–response model to generate EC_{50} values ± SEM. Experiments were performed in triplicate.

Animal studies: Experimental protocols were in compliance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Oregon Health & Science University Institutional Animal Care & Use Committee. Wild-type male C57BL/6J mice, aged 8–10 weeks, were housed in a climate-controlled room with a 12-h light–dark cycle with ad libitum access to food and water.

Distribution studies: Mice were injected once intraperitoneally (i.p.) with drugs at 9.14 μmol kg^{-1}, and analogues at 0.914, 9.14, and 30.5 μmol kg^{-1}. Euthanasia was performed on three mice per dose at 1 h, and the tissues and blood were harvested. Tissues were immediately frozen, and blood was kept on ice for a minimum of 30 min and then centrifuged at 7500 *g* for 15 min. Serum (100 μL) was collected and was stored with tissues at −80 °C until samples were processed.

Serum processing: The serum samples were warmed to room temperature, and 10 μL of internal standard (2.99 μM [D_6]sobetirome) was added to them. Acetonitrile (500 μL) was

added, and the sample was vortexed for 20 s. The sample was then centrifuged at 10000 *g* for 15 min at 4 °C. Next, 90% of the upper supernatant was transferred to a glass test tube and concentrated by centrifugal evaporation (SpeedVac) for 1.5 h at 45 °C. The dried sample was then dissolved in 400 μL MeCN/H_2O (50:50) and vortexed for 20 s. The resulting mixture was transferred to an Eppendorf tube and centrifuged at 10000 *g* for 15 min. 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 serum from an 8- to 10-week-old mouse not injected with T3, sobetirome, or analogues. The processing was performed exactly the same, except after filtering the sample was split amongst six vials. To five out of the six vials was added sobetirome, JD-20, and JD-21 to make final concentrations of each compound in a matrix of: 0.1, 1, 10, 100, and 1000 pg μL^{-1}.

Brain processing: The brain samples were warmed to room temperature and transferred to a homogenizer tube with five Gold-Spec 1/8-inch (diameter) chrome steel balls (Applied Industrial Technologies). The resulting tube was weighed, and then H_2O (1 mL) was added, followed by 10 μL of 2.99 μM internal standard ([D_6]sobetirome). The tube was homogenized with a Bead Bug homogenizer (Benchmark Scientific, Edison, NJ (USA)) for 30 s and then transferred to a falcon tube containing 3 mL MeCN. MeCN (1 mL) was used to wash the 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 SpeedVac for 4 h at 45 °C.

Gene activation: Mice were injected once intraperitoneally (i.p.) with vehicle (1:1 saline/DMSO), T3 at 0.305 μmol kg^{-1}, sobetirome at 9.14 μmol kg^{-1}, and analogues at 0.914 and 9.14 μmol kg^{-1}. Euthanasia was performed on three mice per dose at 2 h, and the tissues were harvested. 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.[22] 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 for single-dose experiments was done using the comparative C_T method to monitor the relative differences in *Hr* gene expression. Data analysis for dose–response experiments was done using GraphPad Prism v.4a with the sigmoidal dose–response model to generate EC_{50} values ± SEM. Experiments were performed in triplicate.

Chemistry

General: ^{1}H NMR spectra were taken on a Bruker 400 instrument. All ^{1}H and ^{13}C NMR data were calibrated to the NMR solvent reference peak ([D_6]acetone, $CDCl_3$). High-resolution mass spectrometry (HRMS) with electrospray ionization was performed by the Bioanalytical MS Facility at Portland State University. Anhydrous tetrahydrofuran (THF) and *N*,*N*-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 instrument with an Agilent Eclipse Plus C_{18} 5 μM column (4.6×250 mm) with a gradient of 10–95% MeCN (0.1% TFA) over 15 min.

(3,5-dibromophenoxy)triethylsilane (**2a**). Compound **1a** (5.04 g, 20 mmol) and imidazole (4.09 g, 60 mmol) were dissolved in 80 mL of CH_2Cl_2. The solution was cooled to 0 °C, then triethylsilyl chloride (5.03 mL, 30 mmol) was added, then the reaction was stirred at 0 °C for 30 min. The reaction was diluted with 160 mL Et_2O, washed with H_2O (2×50 mL) and brine (2×50 mL), then dried with $MgSO_4$, filtered, and concentrated to give **2a** in quantitative yield, which was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (t, 1H, J = 1.6 Hz), 7.10 (d, 2H, J = 1.6 Hz), 1.02 (t, 9H, J = 7.9 Hz), 0.82 ppm (q, 6H, J = 7.9 Hz).

4-hydroxy-2,6-dibromobenzaldehyde (**3a**). A flask was loaded with molecular sieves, then flame-dried under vacuum. After cooling under argon, **2a** (5.49 g, 15 mmol) was loaded, then the flask was sealed, evacuated, and flushed with argon. Dry THF (30 mL) was added and degassed, then the solution was cooled to −78 °C. A second flask was loaded with molecular sieves, then flame-dried under vacuum. After cooling under argon, diisopropylamine (4.6 mL, 33 mmol) was added, followed by 60 mL dry THF, then the solution was degassed and cooled to −78 °C. *n*-Butyllithium (2.5 M solution in hexanes; 12 mL, 30 mmol) was added, then the solution was stirred for 1 h at −78 °C. The lithium diisopropylamide solution was transferred dropwise via cannula to the **2a** solution, then the deprotonation was stirred for 1 h at −78 °C. Dry DMF (5.8 mL, 75 mmol) was added, then the reaction was stirred for 1 h at −78 °C. The reaction was decanted into 50 mL 1 N aqueous HCl. The aqueous layer was extracted with Et_2O (3×90 mL). The organic fractions were combined, washed with brine (2×50 mL), then dried with $MgSO_4$, filtered, and concentrated to give the crude product, which was precipitated from hexanes at −78 °C to give 2.8 g of **3a** (67% yield). ^{1}H NMR (400 MHz, [D_6]acetone): δ = 10.16 (s, 1H), 7.27 ppm (s, 2H).

***tert*-butyl 2-(3,5-dibromo-4-formylphenoxy)acetate** (**4a**). Compound **3a** (2.8 g, 10 mmol), sodium iodide (3 g, 20 mmol), and cesium carbonate (3.24 g, 10 mmol) were dissolved in 40 mL acetone. *tert*-Butyl chloroacetate (2.86 mL, 20 mmol) was added, then the reaction was held at reflux at 65 °C for 2 h. The reaction was diluted with 80 mL of Et_2O, washed with water (2×30 mL) and brine (2×30 mL), then dried with $MgSO_4$, filtered, and concentrated. The product was precipitated from hexanes and collected by filtration, then dried under vacuum to give 3.49 g of **4a** (88% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.23 (s, 1H), 7.19 (s, 2H), 4.59 (s, 2H), 1.52 ppm (s, 9H).

(3,5-dichlorophenoxy)triethylsilane (**2b**). Compound **1b** (6.54 g, 40 mmol) and imidazole (8.18 g, 120 mmol) were dissolved in 160 mL of CH_2Cl_2. The solution was cooled to 0 °C, then triethylsilyl chloride (10 mL, 60 mmol) was added, then the reaction was stirred at 0 °C for 30 min. The reaction was diluted with 320 mL of Et_2O, washed with H_2O (2×100 mL) and brine (2×75 mL), then dried with $MgSO_4$, filtered, and concentrated to give **2b**, which was used without purification and weighed 10.58 g after drying (95% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98 (t, 1H, J = 1.8 Hz), 6.76 (d, 2H, J = 1.8 Hz), 1.02 (t, 9H, J = 7.9 Hz), 0.77 ppm (q, 6H, J = 7.9 Hz).

4-hydroxy-2,6-dichlorobenzaldehyde (**3b**). A flask was loaded with molecular sieves, then flame-dried under vacuum. After cooling under argon, **2b** (3.6 g, 13 mmol) was loaded, then the flask

was sealed, evacuated, and flushed with argon. Dry THF (13 mL) was added and degassed, then the solution was cooled to −78 °C. A second flask was loaded with molecular sieves, then flame-dried under vacuum. After cooling under argon, 2,2,6,6-tetramethylpiperdidine (1.84 g, 13 mmol) was added, followed by 13 mL of dry THF, then the solution was degassed and cooled to −78 °C. *n*-Butyllithium (2.5 M solution in hexanes; 5.2 mL, 13 mmol) was added, then the solution was stirred for 20 min at 0 °C. The lithium TMP solution was transferred dropwise via cannula to the **2b** solution, then the deprotonation was stirred for 30 min at −78 °C. Dry DMF (5 mL, 65 mmol) was added, then the reaction was stirred for 30 min at −78 °C. The reaction was decanted into 15 mL 1 N aqueous HCl. The aqueous layer was extracted with EtOAc (3×15 mL). The organic fractions were combined, washed with brine (2× 15 mL), then dried with $MgSO_4$, filtered, and concentrated to give the crude product, which was recrystallized from hexanes at −20 °C to give 1.39 g of **3b** (56% yield). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 10.37 (s, 1H), 7.01 ppm (s, 2H).

***tert*-butyl 2-(3,5-dichloro-4-formylphenoxy)acetate (4b)**. Compound **3b** (1.15 g, 6 mmol), sodium iodide (1.8 g, 12 mmol), and cesium carbonate (1.94 g, 6 mmol) were dissolved in 24 mL of acetone. *tert*-Butyl chloroacetate (1.72 mL, 12 mmol) was added, then the reaction was held at reflux at 60 °C for 24 h. The reaction was diluted with Et_2O (30 mL), washed with water (2×10 mL) and brine (2×10 mL), then dried with $MgSO_4$, filtered, and concentrated. The crude oil was re-dissolved in a minimal amount of Et_2O then added dropwise to 100 mL of vigorously stirring hexanes at −78 °C. The precipitate was collected by filtration and dried under vacuum to give 1.545 g of **4b** (84% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.43 (s, 1H), 6.92 (s, 2H), 4.59 (s, 2H), 1.52 ppm (s, 9H).

4-iodo-2-isopropylphenol (6). Compound **5** (6.8 g, 50 mmol) and NaI (7.5 g, 50 mmol) were dissolved in 70 mL of MeOH. Aqueous NaOH (10 M; 5 mL, 50 mmol) was added, then the solution was cooled to 0 °C. Aqueous NaOCl (6.25% *w*/*v*; 62.5 mL, 50 mmol) was added dropwise over 24 h at 0 °C. The reaction was acidified to pH 7 with 12 N aqueous HCl, then quenched with 10 mL saturated aqueous $Na_2S_2O_3$. The aqueous layer was extracted with Et_2O (3× 100 mL). The organic fractions were combined, washed with brine (2×100 mL), then dried with $MgSO_4$, filtered, and concentrated to give the crude product, which was purified by flash chromatography (silica gel, hexane/EtOAc, 1–20%) to give 11.35 g of **6** (87% yield) as a reddish oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, 1H, *J* = 2.1 Hz), 7.36 (dd, 1H, *J* = 8.4 Hz, 2.2 Hz), 6.54 (d, 1H, *J* = 8.4 Hz), 3.16 (m, 1H, *J* = 6.9 Hz), 1.25 ppm (d, 6H, *J* = 6.9 Hz).

4-iodo-2-isopropyl-1-(methoxymethoxy)benzene (7): Compound **6** (2.62 g, 10 mmol) and tetrabutylammonium iodide (369 mg, 1 mmol) were dissolved in 100 mL of CH_2Cl_2. Aqueous NaOH (10 M, 10 mL) was added, followed by chloromethyl methyl ether (5 mL, 6 M in MeOAc). The reaction was stirred for 30 min at room temperature, then diluted with 200 mL of Et_2O. The organic layer was washed with H_2O (2×100 mL) and brine (2×100 mL), then dried with $MgSO_4$, filtered, and concentrated to give the crude product, which was purified by flash chromatography (silica gel, hexane/EtOAc, 1–20%) to give 2.48 g of **7** (81% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, 1H, *J* = 2.2 Hz), 7.42 (dd, 1H, *J* = 8.6 Hz, 2.2 Hz), 6.83 (d, 1H, *J* = 8.6 Hz), 5.18 (s, 2H), 3.47 (s, 3H), 3.27 (m, 1H, *J* = 6.9 Hz), 1.20 ppm (d, 6H, *J* = 7 Hz).

***tert*-butyl 2-(3,5-dibromo-4-(hydroxy(3-isopropyl-4-(methoxymethoxy)phenyl)methyl)phenoxy)acetate (8a)**. A flask was loaded with 4 Å molecular sieves and flame-dried under vacuum. Compound **7** (1.47 g, 4.8 mmol) was loaded, and the flask was sealed, evacuated, and flushed with argon. Dry THF (24 mL) was added and degassed, then the solution was cooled to 0 °C. Isopropylmagnesium chloride (2 M THF, 5.5 mL, 7.2 mmol) was added, then the reaction was stirred for 2 h at room temperature. A second flask was loaded with 4 Å molecular sieves and flame-dried under vacuum. Compound **4a** (946 mg, 2.4 mmol) was loaded and the flask was sealed, evacuated, and flushed with argon. Dry THF (12 mL) was added and degassed. The arylmagnesium solution was cooled to −78 °C, then the **4a** solution was added dropwise via cannula, and the reaction was stirred for 1 h at −78 °C. The reaction was quenched with 10 mL of 1 N aqueous HCl. The aqueous layer was extracted with EtOAc (3×10 mL). The organic fractions were combined and washed with brine (2×10 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated to give the crude product, which was purified by flash chromatography (silica gel, hexanes/EtOAc 4–40%) to give 1.089 g of **8a** (79% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24 (d, 1H, *J* = 1.9 Hz), 7.17 (s, 2H), 7.00 (d, 1H, *J* = 8.5 Hz), 6.90 (dd, 1H, *J* = 8.7 Hz, 1.9 Hz), 6.51 (d, 1H, *J* = 10.8 Hz), 5.21 (s, 2H), 4.53 (s, 2H), 3.49 (s, 3H), 3.36 (d, 1H, *J* = 10.8 Hz) 3.34 (m, 1H, *J* = 6.9 Hz), 1.52 (s, 9H), 1.21 ppm (t, 6H, *J* = 6.5 Hz).

2-(3,5-dibromo-4-((3-isopropyl-4-hydroxyphenyl)methyl)phenoxy)acetic acid (9a). Compound **8a** (1.089 g, 1.9 mmol) was dissolved in 19 mL of CH_2Cl_2 with 1.21 mL of triethylsilane (7.58 mmol). The solution was cooled to 0 °C, then TFA (4.35 mL, 56.9 mmol) was added, and the reaction was stirred for 30 min at 0 °C, then 2 h at room temperature. The solvent was removed under vacuum, then the product was precipitated by the addition of hexanes and collected by filtration. The solid was dried under vacuum to give 505 mg of JD-20 (**9a**) (58% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.19 (s, 2H), 7.10 (d, 1H, *J* = 1.9 Hz), 6.82 (dd, 1H, *J* = 8.7 Hz, 1.9 Hz), 6.64 (d, 1H, *J* = 10.8 Hz), 4.68 (s, 2H), 4.28 (s, 2H), 3.18 (m, 1H, *J* = 6.9 Hz), 1.24 ppm (d, 6H, *J* = 6.9 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.12, 156.17, 151.03, 134.18, 133.67, 130.33, 126.90, 126.08, 126.02, 118.98, 115.13, 64.87, 28.04, 27.04, 22.57 ppm; HRMS exact mass calculated for $C_{18}H_{17}Br_2O_4$ $[M+H]^+$: 459.94687, found: 459.94647.

***tert*-butyl 2-(3,5-dichloro-4-(hydroxy(3-isopropyl-4-(methoxymethoxy)phenyl)methyl)phenoxy)acetate (8b)**. A flask was loaded with 4 Å molecular sieves and flame-dried under vacuum. Compound **7** (459 mg, 1.5 mmol) was loaded, and the flask was sealed, evacuated, and flushed with argon. Dry THF (6 mL) was added and degassed, then the solution was cooled to 0 °C. Isopropylmagnesium chloride (2 M THF, 1.125 mL, 2.25 mmol) was added, then the reaction was stirred for 2 h at room temperature. A second flask was loaded with 4 Å molecular sieves and flame-dried under vacuum. Compound **4b** (305 mg, 1 mmol) was loaded and the flask was sealed, evacuated, and flushed with argon. Dry THF (4 mL) was added and degassed. The arylmagnesium solution was cooled to −78 °C, then the **4b** solution was added dropwise via cannula, and the reaction was stirred for 1 h at −78 °C. The reaction was quenched with 5 mL of 1 N aqueous HCl. The aqueous layer was extracted with EtOAc (3×5 mL). The organic fractions were combined and washed with brine (2×5 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated to give the crude product, which was purified by flash chromatography (silica gel, hexanes/EtOAc 2–20%) to give 260 mg of **8b** (54% yield). ^{1}H NMR (400 MHZ, $CDCl_3$): δ = 7.26 (d, 1H, *J* = 2.1 Hz), 6.99 (d, 1H, *J* = 8.5 Hz), 6.93 (dd, 1H, *J* = 8.2 Hz, 2.2 Hz), 6.92 (s, 2H), 6.50 (d, 1H, *J* = 10.8 Hz), 5.21 (s, 2H), 4.53 (s, 2H), 3.50 (s, 3H), 3.33 (m, 1H, *J* = 6.9 Hz), 3.23 (d, 1H, *J* = 10.8 Hz), 1.52 (s, 9H), 1.21 ppm (t, 6H, *J* = 6.8 Hz).

2-(3,5-dichloro-4-((3-isopropyl-4-hydroxyphenyl)methyl)phenoxy)acetic acid (**9b**). Compound **8b** (260 mg, 0.54 mmol) was dissolved in 5.4 mL of CH_2Cl_2 with 0.345 mL of triethylsilane (2.16 mmol). The solution was cooled to 0 °C, then TFA (1.24 mL, 16.2 mmol) was added, and the reaction was stirred for 30 min at 0 °C, then 2 h at room temperature. The solvent was removed under vacuum, then the product was precipitated by the addition of hexanes and collected by filtration. The solid was dried under vacuum to give 137 mg of JD-21 (**9b**) (69% yield). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.12$ (d, 1H, $J = 2$ Hz), 6.95 (s, 2H), 6.86 (dd, 1H, $J = 8.2$ Hz, 2.2 Hz), 6.64 (d, 1H, $J = 8.2$ Hz), 4.68 (s, 2H), 4.18 (s, 2H), 3.17 (m, 1H, $J = 6.9$ Hz), 1.24 ppm (d, 6H, $J = 6.9$ Hz); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 172.18$, 155.92, 151.09, 136.36, 134.15, 130.97, 130.67, 126.83, 121.46, 115.11, 115.04, 64.84, 27.06, 24.35, 22.53 ppm; HRMS exact mass calculated for $C_{18}H_{17}Cl_2O_4$ $[M+H]^+$: 367.04984, found: 367.05053.

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Keywords: brain · central nervous system · thyroid hormones · thyromimetics

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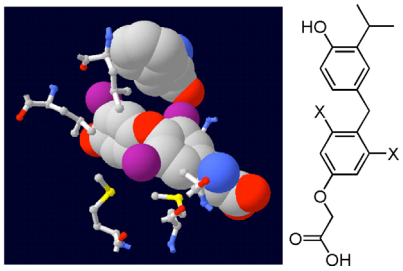
Increasing Thyromimetic Potency through Halogen Substitution



Modifying the structure of the potent TRβ-selective thyroid hormone analogue sobetirome by replacing the 3,5-dimethyl groups with chlorine or bromine to exploit halogen bonding interactions within the TR ligand binding domain gives compounds with significantly increased potency in vitro and in vivo while retaining distribution to the CNS. These improved characteristics make the new compounds attractive candidates for treating CNS demyelination diseases influenced by thyroid hormone stimulation.