Bacterial biosensors for screening isoform-selective ligands for human thyroid receptors α-1 and β-1

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ABSTRACT

Subtype-selective thymimetics have potential as new pharmaceuticals for the prevention or treatment of heart disease, high LDL cholesterol and obesity, but there are only a few methods that can detect agonistic behavior of TR-active compounds. Among these are the rat pituitary GH3 cell assay and transcriptional activation assays in engineered yeast and mammalian cells. We report the construction and validation of a newly designed TRα-1 bacterial biosensor, which indicates the presence of thyroid active compounds through their impacts on the growth of an engineered Escherichia coli strain in a simple defined medium. This biosensor couples the configuration of a hormone receptor ligand-binding domain to the activity of a thymidylate synthase reporter enzyme through an engineered allosteric fusion protein. The result is a hormone-dependent growth phenotype in the expressing E. coli cells. This sensor can be combined with our previously published TRβ-1 biosensor to detect potentially therapeutic subtype-selective compounds such as GC-1 and KB-141. To demonstrate this capability, we determined the half-maximal effective concentration (EC50) for the compounds T3, Triac, GC-1 and KB-141 using our biosensors, and determined their relative potency in each biosensor strain. Our results are similar to those reported by mammalian cell reporter gene assays, confirming the utility of our assay in identifying TR subtype-selective therapeutics. This biosensor thus provides a high-throughput, receptor-specific, and economical method (less than US$ 0.10 per well at laboratory scale) for identifying important therapeutics against these targets.

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1. Introduction

Thyroid hormones play an essential role in the physiological regulation of different tissues, as well as overall metabolic rate, cholesterol level and heart rate. The targets of thyroid hormones are the thyroid receptors (TRs), which belong to the nuclear receptor (NR) superfamily. Two major classes of TR receptors are known, TRα and TRβ, each of which is expressed in multiple isoforms (TRα-1, TRα-2, TRβ-1, TRβ-2). The thyroid receptors TRα-1 and TRβ-1 each contain six domains (A–F), similar to estrogen receptors α and β and other NRs. The DNA binding (C), hinge (D) and ligand binding (E) domains in the TRα-1 and TRβ-1 isoforms are respectively 88%, 71% and 86% identical, while no homology has been observed in activation function-1 domain (A/B; AF1), which are isoform specific. The domains E/F effect transcription activation upon ligand binding and receptor dimerization, while the E domain contains activation function-2 (AF-2). The TR isoforms are expressed at different levels in different tissues. For example, the TRα-1 isoform is dominant in heart (70%), while the TRβ-1 isoform is dominant in the liver (80%), suggesting that these receptors may be important targets for subtype-selective thyroid hormone receptor modulator (STRM) therapeutics [1–3].

Thyroid hormone receptors are essential for proper infant central nervous system (CNS) development, and their production is regulated by the hypothalamic-pituitary-thyroid feedback system. Among non-isoform selective TR-binding compounds, T3 is the native hormone in the human body, and is produced by follicular cells of the thyroid gland [1,4]. These cells accumulate iodide from plasma through their membranes and use it for the production of secreted human thyroid hormones. A deficiency or excess of these hormones, referred to as hypothyroidism or hyperthyroidism, may lead to myxedema coma, cretinism, and other serious disorders.

Database: Human TRα-1 and TRα-2 sequence data are available in the GenBank database under the accession numbers BC008851.2 and NM_003250, respectively.

Abbreviations: pMIT::TR, plasmid Maltose Binding Protein-Interin-Thymidylate Synthase, with inserted TR LBD; MBP, maltose-binding protein; N-Mtu, the first 110 amino acid residues of the Mtu RecA intein; C-Mtu, the last 58 amino acid residues of the Mtu RecA intein; TS, T4 thymidylate synthase enzyme

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Several therapeutic strategies have been devised to treat thyroid-related disorders. For example, thiourelenes can be used in hyper-thyroidism to inhibit thyroid hormone production, as well as the conversion of the less active 3,5,3′,5′-tetradiodo-L-thyronine (thyroxine; T₄) to more active 3,3′,5′-triiodo-L-thyronine (triiodothyronine; T₃). Direct administration of T₃ is also used to treat hypothyroidism and associated obesity. Unfortunately, the use of T₃ is limited by its agonist activity against both TR isoforms, and resulting cardiovascular side effects such as tachycardia. The presence of additional tissue-specific side effects, arising from varying TR isoform levels in differing tissues, suggests that it may be desirable to develop subtype-selective TR modulators (STRMs). Work in this area led to the finding that a single amino acid residue difference (Ser277→Asn331) in the ligand-binding pockets of TRα and TRβ have a direct effect on the binding selectivity of potential STRMs [5,6]. Triiodothyroacetic acid (Triac) has been found to be TRβ selective as well [7], but the exact mechanism of its selectivity is not well understood. Martinez et al. suggested that the observed 2- to 3-fold selectivity of Triac for TRβ is connected to conformational changes in Triac itself, possibly caused by the high flexibility of its carboxylate group [8].

These studies have facilitated the recent development of several potentially therapeutic isoform-selective TRβ agonists, which include Sobetreotide (GC-1), which lowers LDL cholesterol level with no effect on the cardiovascular system, as well as Eprotirome (KB-2115) and MB07811 for dsipilidism [9–11]. In addition to these, several TR antagonists have been developed for potential therapeutic uses, such as 1–850, DIBRT (low potency), NH-3 (high potency), and the partial agonists have been developed for potential therapeutic uses, such as Axitirome and MB07811 for dislipidemia [9–11]. In addition to these, several TR antagonists have been developed for potential therapeutic uses, such as Axitirome and MB07811 for dislipidemia [9–11].

Several additional strategies for detecting and identifying hormone-like compounds rely on fusions between the hormone receptor ligand-binding domain (LBD) and various other functional proteins in yeast and mammalian cells [23]. One group of these includes direct end-to-end or insertional fusions of LBDs to functional enzymes, where the binding of a ligand by the LBD will directly activate the fused reporter protein [24–26]. A second group involves fusion of the NR LBD to a GAL4 DNA-binding domain to generate a highly sensitive transcriptional assay for ligand function [27,28]. Both of these assay types are highly effective and allow generation of new assays by simple LBD swapping. Strengths of these assays include their ability to function in yeast and mammalian cells, high sensitivity to ligands, and the lack of requirement for NR-specific cofactors or coactivators. These strengths have led a few of these assays to be commercialized, including the HEK 293T cell-based GeneBLAzer beta-Lactamase reporter system (Invitrogen, Carlsbad, CA) for detection of agonists and antagonists against a variety of available NRs. One drawback of these assays is the potential for misclassification of the tested ligands. This can arise from the cellular context of a given assay (e.g., yeast versus human tissue), which may exhibit differences in coactivator levels, membrane transport characteristics, and genetic background [29–32]. Additional artifacts can arise from the use of isolated NR LBDs in fusion to the non-native reporter protein domains, which can misreport the relative activities of antagonists and agonists. A final, yet significant drawback is the cost of some of these assays, which can approach one thousand U.S. dollars per 384-well plate in the case of the GeneBLAzer reporter system mentioned above.

The biosensor assay presented here is an Escherichia coli (E. coli) growth-based technique. In this assay, the conformation of the TR LBD is linked to the activity of a thymidylate synthase (TS) reporter enzyme through an engineered allosteric biosensor protein. The engineered sensor protein consists of the TS reporter enzyme linked to an intein splicing domain and maltose binding protein. In previous work, we have shown that the activity of the TS reporter is repressed when it is in fusion to the intein splicing domain, likely due to steric blockage of TS dimerization [33]. In the engineered biosensor protein, the NHR LBD is inserted into the splicing domain, which appears to stabilize the correct fold of the LBD while simultaneously blocking TS dimerization. Our hypothesis is that the repositioning of helix 12 of the TR LBD upon ligand binding induces a conformational change in the intein domain, which leads to dose-dependent activation of the TS domain [34].

Since TS activity is required for E. coli cell growth, the configuration of the TR LBD is reflected in the TS phenotype of the cells expressing the biosensor protein. The TS phenotype can be observed and quantified using positive selection in a defined liquid growth medium that lacks thymine (-Thy medium), or negative selection using -Thy medium supplemented with thymine and trimethoprim (TTM medium) [35]. Thus, an important aspect of the screen is the ability to confirm the effects of a given ligand on LBD-dependent TS activity through the mirror image phenotypes observed with -Thy and TTM media. In this case, a general growth effect (e.g., nutritional effect or toxicity) may produce a positive growth phenotype in one medium, but would fail to produce the mirror image phenotype in the alternate growth medium. Generation of dose-response curves in -Thy and TTM liquid media permits an estimate of the relative binding affinities of test compounds for the TR LBD targets, thus providing a rapid means for detecting and characterizing isofrom-selective ligands. Because this assay relies on simple E. coli growth in liquid medium, it is nonradioactive, economical and simple to use. Further, only the LBD of the desired NR is cloned into the E. coli cells, which greatly simplifies the generation of specific NR biosensors. In this work, we demonstrate the capability of the system to readily detect several TR ligands (Fig. 1), and to identify subtype-selective thyromimetic ligands.

2. Materials and methods

2.1. Ligands

Compounds were acquired from the following sources: E₂ (17β-estradiol) and Triac (3,3′,5′-triiodothyroacetic acid, 95%) from Sigma (Saint Louis, MO), as well as T₃ (3,3′,5′-triiodo-L-thyronine sodium salt hydrate, 95%) from Aldrich Chemical (Milwaukee, WI). The selective TRβ agonists, GC-1 (3,5-dimethyl-4- (4′-hydroxy-3′-isopropylbenzyl)-phenoxacyc acid) was synthesized by Thomas S. Scanlan’s Laboratory (Oregon Health and Sciences University, Portland, OR, USA). The selective TRβ agonists, GC-1 (3,5-dichloro-4- (4′-hydroxy-3′-isopropylphenyl)-phenolacyc acid) were provided as a gift from Dr. Gary Grover (UMDNJ, NJ). The structures of the compounds are included in Fig. 1.
2.2. Construction of the fused chimeric protein containing human thyroid receptors

A TRα-2 gene fragment, comprising bases 3030–3697 of the plasmid pCMV6-XL5 hTRα-2 (GenBank:NM_003250, GI:40806158; OriGene Technologies; Rockville, MD), was fused to an additional 110 bases of the TRα-1 ligand-binding domain to form the putative TRα-1 LBD. The putative TRα-1 LBD contains bases 1091–1867 (corresponding to TRα-1 ORF amino acids E149 to D407) of the full-length TRα cDNA (GenBank:BC008851.2, GI:39644841) [36]. The constructed TRα-1 was inserted into the Δ mini-intein, comprised of the first 110 amino acid residues of the Mtuberculosis recA intein, the last 58 amino acid residues of the Mtuberculosis recA intein, TRα-1 — human thyroid hormone receptor α-1 ligand-binding domain; TRβ-1 — human thyroid hormone receptor β-1 ligand-binding domain; TS — thymidylate synthase enzyme. The boundaries of the TR LBDs sequence are indicated by arrows above each diagram.

2.3. Phenotype determination

The TS-deficient E. coli strain D1210ΔthyA:Kan6 [F−Δ(gpt-proA)62 leuB6 supE44 ara-14 galK2 lacY1 Δ(mcr-c-mrr) rpsL20 (Strr) xyl-5 metIF recA13 lacP1] was transformed with pMIT::TRα-1 and pMIT::TRβ-1 for growth phenotype determinations. Fresh transformant colonies were used to inoculate 5 ml cultures of Luria-Bertani (LB) medium supplemented with 100 μg/ml ampicillin and 50 μg/ml thymine. These cultures were then incubated in a shaking water bath at 37 °C until the OD600 of the culture reached 1.3–1.7. These cultures were then diluted 1:200 into 50 ml of -Thy medium (per liter: 10 ml of 10% casamino acid, 10 ml of 20% glucose, 200 μl 1% thiamine HCl, 200 ml of 5xMinimal Davis Broth (MDB; 35 g dipotassium phosphate, 10 g monopotassium phosphate, 2.5 g sodium citrate, 0.5 g magnesium sulfate and 5 g ammonium sulfate), 10 ml of Thy Pool (2 mg/ml of each of following amino acids, L-Arg, L-His, L-Leu, L-Met, L-Pro and L-Thr), 1 ml of 0.1 M CaCl2, pH 7.0) supplemented with 100 μg/ml ampicillin. The diluted cells where then transferred to 96 well plates at 198 μL/well, and each well was supplemented with 2 μL of each compound diluted in DMSO at the desired concentration. Importantly, the DMSO concentration in each well was kept constant at 1% throughout each experiment, regardless of the final ligand concentration. The 96 well plates were then incubated at 34 °C, 150 rpm agitation and 80% humidity to assure equal volumes across the wells. Over time, the growth of the E. coli cells in each 96 well plate was measured by optical absorbance at a wavelength of 600 nm (OD600) using a Biorekt Synergy 2 spectrophotometer.

To confirm the results of the -Thy medium test, the cells were also grown in -Thy medium supplemented with 10 μg/ml trimethoprim and 50 μg/ml thymine (TTM medium) and incubated at 37 °C. The TTM medium reverses the phenotype of the -Thy medium, providing direct evidence of a specific effect of the ligand on TS activity, as opposed to a more general ligand effect on cell growth.

2.4. Statistical analysis

The statistical significance of our results was verified by calculating the Z factor for each test as described by Zhang et al. [38]. Additionally, the signal-to-noise (S/N), and signal-to-background (S/B), ratios were analyzed to determine the significance of the observed signal [39].

3. Results

3.1. Detection of agonism

The reported TR agonists Triac, T3, GC-1 and KB-141, and the negative control (E2), were each tested at a concentration of 10 μM in the presence of the pMIT::TRα-1 and pMIT::TRβ-1 biosensor strains (Figs. 2A, 3A and B). Each well contained cells growing in -Thy medium with a constant final concentration of either 1% (v/v) DMSO or 1% (v/v) DMSO with dissolved ligand. Control experiments indicate that this concentration of DMSO does not impact cell growth in -Thy medium, suggesting that the use of DMSO as a delivery vehicle has minimal impact on E. coli cell viability (see Fig. 52). The time-dependent growth of cells harboring either pMIT::TRα-1 or pMIT::TRβ-1, incubated at 34 °C in the presence of 10 μM T3 (in 1% DMSO) or solvent only (1% DMSO) in -Thy medium, is presented in Supplemental Material for the time period 15–24 h (see Figs. S1A and S1B). In all cases, the growth rate of the biosensor cells increased in the presence of reported agonists (Table 1). This result is presumably due to an increase in TS activity upon ligand binding, and is consistent with the behavior of our previously reported NR biosensors exposed to known agonists (e.g., ERα and ERβ strains exposed to estrogen). A specific ligand-LBD interaction is further supported by the observed decrease in growth sequences).
but had no agonistic effect on the ERβ biosensor. Some toxicity was observed at high concentrations for non-interacting ligands (e.g., 100 μM T3 with pMIT::ERβ; Fig. 4A). Under these conditions, cell viability is compromised due to thymine starvation, and very high ligand concentrations can lead to further decreases in cell growth. This effect is generally not observed in cases where the ligand stimulates healthy cell growth and high viability. Thus, to detect specific cytotoxicity of ligands, cells are grown under non-selective conditions (in the presence of thymine) in the presence of high ligand concentrations. In these tests, none of the tested compounds showed significant toxicity against our bacterial sensor strains (data not shown).

3.2. Potency and selectivity of ligands

The relative potencies of the ligands were based on dose response determinations, where the ligand concentrations were varied by serial dilution from a high concentration of 100 μM (final concentration in the growth medium) to a low concentration where no growth effect was observed (typically below 1 nM) (Fig. 5). To determine EC50 values, growth rates at various ligand concentrations were normalized and fitted to a standard sigmoidal dose-response equation using nonlinear regression with variable slope (Prism ver. 5.01, GraphPad software, San Diego, CA). Once the EC50 values had been determined for each ligand/sensor combination, the subtype-selective binding of the tested ligands was defined as follows:

\[
\text{Selectivity} = \frac{E_{50}^{\text{Ligand}(TRx)}}{E_{50}^{\text{Ligand}(TR\beta)}}
\]

In all cases, the results generated by our system qualitatively matched those reported by other investigators (Table 1). For example, in our system, the native ligand T3 showed similar EC50 values of 0.52 and 0.58 μM for TRx-1 and TRβ-1, respectively (Tables 2 and S2). The most potent ligands for TRx-1 were Triac and T3, while KB-141 and GC-1 were less potent and exhibited similar binding to each other. Triac was also the most potent ligand for TRβ-1, while the GC-1 and KB-141 potencies were 2-fold lower and T3 exhibited 8-fold lower potency than Triac. The dose-response curves used to calculate the EC50 values for each compound indicated that the detection limits, which we define as the lowest concentration of a test compound that generates an unambiguous growth signal by visual inspection, of our TRx and TRβ biosensors for T3, KB-141 and GC-1 is approximately 100 nM, and the detection limit for T3 is approximately 10 nM (Fig. 5).

The calculated EC50 values also indicated some subtype-selective behavior in several of the compounds. Although the native T3 ligand showed no significant selectivity for either TR receptor, T3 showed higher potency when bound to TRβ (EC50 = 0.07 μM) vs. TRx (EC50 = 0.31 μM), corresponding to a selectivity ratio of 4.43 (Tables 2 and S2). Notably, KB-141 and GC-1 were designed to bind selectively to TRβ, and this behavior was confirmed by our biosensors. Specifically, the selectivity for TRβ over TRx was 4.04 for GC-1 and 4.66 for KB-141 (Tables 2 and S2). Further, GC-1 and KB-141 were both approximately 3-fold more potent than T3 when bound to the TRβ sensor.

Table 1

| Ligand | TR biosensors | Other methods | Refs. |
|--------|---------------|---------------|-------|
| T3     | Agonist       | Agonist       | [9,42]|
| Triac  | Agonist, TRβ | Agonist, TRβ | [2,8,41]|
| GC-1   | Agonist, TRβ | Agonist, TRβ | [6,11,44]|
| KB-141 | Agonist, TRβ | Agonist, TRβ | [15,16,45]|
| E2 (control) | No significant effect on TRx-1. | Agonist, ERα | [46]|
|        | Very weak | Agonist | of E2 to TRx or β has not been reported. | |
|        | agonist effect on TRβ-1 | β | | |

Table 2

| Ligand | pMIT::TRx-1 | pMIT::TRβ-1 |
|--------|-------------|-------------|
| EC50 [μM] | EC50 [μM] |
| T3     | 0.52        | 100.00      |
| Triac  | 0.31        | 167.74      |
| GC-1   | 0.56        | 89.66       |
| KB-141 | 0.71        | 32.34       |

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Fig. 4. The effect of 17-β-estradiol (E2; white circles) and 3,5,3′-triiodo-l-thyronine (T3; black squares) on E. coli D1210ΔthyA cells transformed with (A) pMIT::ERβ, (B) pMIT::TRα and (C) pMIT::TRβ. The experiments were performed in -Thy medium at 34°C. Experiments were carried out in triplicate, and optical densities at 600 nm were recorded after 24 h. Error bars represent one standard deviation from the mean for each ligand concentration.

Several aspects of the experimental design were examined for impacts, including growth media pH and plate edge effects. These tests indicate that the growth medium pH can lead to final OD variations of up to 25% over the range pH 6.9–7.1 (data not shown), and therefore great care was taken to adjust the growth media pH to precisely 7.0 during all experiments. Edge variations on the 96-well microtiter plates were as high as 10% in cases where cell growth levels are low, but were limited to approximately 4% at higher growth levels (data not shown). To minimize these systematic errors, each dose response series was carried out in triplicate on each plate, and results from three separate plates were used to calculate the final values for EC50.

To verify the ability of our sensor to report a statistically significant result for each test ligand, Z’ factors were calculated using the averages and standard deviations of the measured growth values at the highest and lowest concentrations of each ligand tested. This analysis yielded Z’ factors greater than 0.5 for all of our tests, indicating that the biosensor response to each of the tested compounds was unambiguously significant (Table 3). Further, all of the S/N ratios were above 66 for TRα and 16 for TRβ, while the S/B ratio was consistently above 3 for both biosensors.

4. Discussion

The first subtype-selective thyromimetics have appeared during the last 10 years [11]. In conventional transcriptional activation assays, the potency and selectivity (TRα/TRβ) of ligands can vary depending on the co-regulators present, which typically include SRC1–2, SRC3–2 and NCoR1–2 [19,40]. Other factors can also influence outcomes, such as the in vivo or in vitro method type, the physicochemical characteristics of the compounds, and the type of solvents.
used, which may enhance ligand penetration through cell membranes depending on the assay. Despite the large variety of assays available, there is a need for comparable qualitative and quantitative data for evaluating thyromimetic TR-subtype-selectivity [41].

These biosensors provide an alternative method to mammalian cell reporter gene assays for characterizing potency and isomorph-selectivity of ligands. Although the results obtained from this biosensor method follow the qualitative trends observed in conventional in vitro studies, the sensitivity of the bacterial sensors is currently lower. For example, the native thyroid hormone T3 has been shown to bind both TRα and TRβ with similar affinity (Kd = 0.1 nM) and potency (EC50 = 2 nM), as determined via binding and transcriptional activation assays [21]. Our system reproduced the qualitative aspects of these results, indicating non-selective binding of T3 to the TR receptors, but yielded much lower apparent potencies in the context of the biosensor assay (potencies of 0.52 μM and 0.58 μM for TRα and TRβ, respectively).

In all cases, however, our biosensor system qualitatively reproduced important therapeutically relevant characteristics of the control ligands, including binding affinity relative to T3 and subtype-selective binding. For example, in one previous study, Triac was reported to have 6-fold higher potency than T3 when binding to TRβ [8], while in another, Triac was found to have approximately 3-fold higher affinity than T3 for TRβ and identical affinity to T3 for TRα [7]. Those subtype-selective differences are consistent with the relative potencies obtained in our work, where Triac was observed to be 8-fold more potent than T3 against TRβ, and 1.7 times more potent for TRα.

The in vitro affinity of GC-1 for the TR subtypes has also been studied previously, where it showed stronger binding to TRβ (Kd of 0.1 ± 0.02 nM) than TRα (Kd of 1.8 ± 0.2 nM) [4]. Chiellini et al. also reported EC50 values for GC-1 using a transcriptional activation assay, which again indicated preferential binding of GC-1 to TRβ (7 nM vs. 45 nM for TRα) [4]. These results are qualitatively similar to our results in terms of subtype-selectivity, although our actual EC50 values are 3-fold different (0.2 μM vs. 0.6 μM for TRβ and TRα, respectively). In additional previous work on KB-141, an in vitro radioactive displacement assay indicated a 10-fold TRβ binding selectivity for KB-141, while an in vivo transactivation assay confirmed the agonistic behavior of KB-141 and indicated an 8-fold greater binding affinity for TRβ when normalized to T3 [15]. In our study, GC-1 and KB-141 were respectively observed to be approximately 4- and 4.7-fold selective for TRβ over TRα.

Although our calculated EC50 values are substantially higher than those determined from previous in vitro binding and transactivation assays, our results are qualitatively consistent with these assays in terms of agonistic behaviors and relative potencies. The differences in EC50 between our and other assays likely arise from the non-transcriptional nature of the assay, and its reliance on membrane diffusion in bacterial cells. Further, the EC50 values exhibited by our system are reasonable for the detection of therapeutically relevant compounds (e.g., T3, GC-1 and KB-141). These compounds typically must have nanomolar binding affinities in order to exhibit a reasonable therapeutic index. Since our assay can tolerate concentrations several orders of magnitude above this, it can be used to detect the activity of these compounds up to their solubility limits. Since these limits are typically greater than 10 μM, we feel that the testable range of concentrations is adequate for initial library screening. The calculated EC50 values can then be benchmarked against the standard compounds described in this work. Importantly, our overall results were obtained with excellent reproducibility and robust statistical significance, with Z’ factors between 0.92 and 0.66. Finally, the signal-to-noise and background measurements were also excellent, and were indicative of very clear results (90%–5% N = 66 and 4.5%–SB > 3.5 for TRα and 46.5%–S/N = 16.5 and 4–S/B > 3.4 for TRβ).

In previous studies, compounds with low affinity were also characterized using similarly engineered ER β biosensors. The relative binding affinity (RBA = EC<sub>β</sub>estradiol/EC<sub>β</sub>estradiol × 100%) of bisphenol A for human ER β biosensor was reported as 1.15% (relative to 100% for 17-β estradiol), whereas for porcine ER β biosensor only 0.13% [39]. However, we have not determined the minimum detectable affinity for thyromimetics, and this is planned for future work with a greater variety of compounds.

Our thyroid hormone biosensors provide a means to identify TR agonists and determine relative EC50 values across a variety of ligands, which allows identification of subtype-selective compounds within large chemical libraries. Further, methods based on these biosensors are both simple and economical, and these approaches have shown utility in the discovery of subtype-selective compounds for ERα and ERβ [33,34,42].

It is therefore possible that these biosensors will become an important primary screen for TR-selective compounds that might be used to treat a wide range of metabolic disorders.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fob.2012.08.002.

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