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Selective Chemical Rescue of a Thyroid Hormone Receptor Mutant, TRβ(H435Y), Identified in Pituitary Carcinoma and Resistance to Thyroid Hormone

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Layout 1:

Thyroid hormone receptor (TR) plays a critical role in development and homeostasis. The mutant TRβ(H435Y) has been identified in both cancer and resistance to thyroid hormone (RTH). The designed synthetic ligand QH13 selectively recovers cellular reporter gene activity of TRβ(H435Y) with a 5,850-fold improvement of mutant selectivity compared the natural hormone T3. This is perhaps the first example of chemical rescue of a mutant protein identified in multiple disease states.

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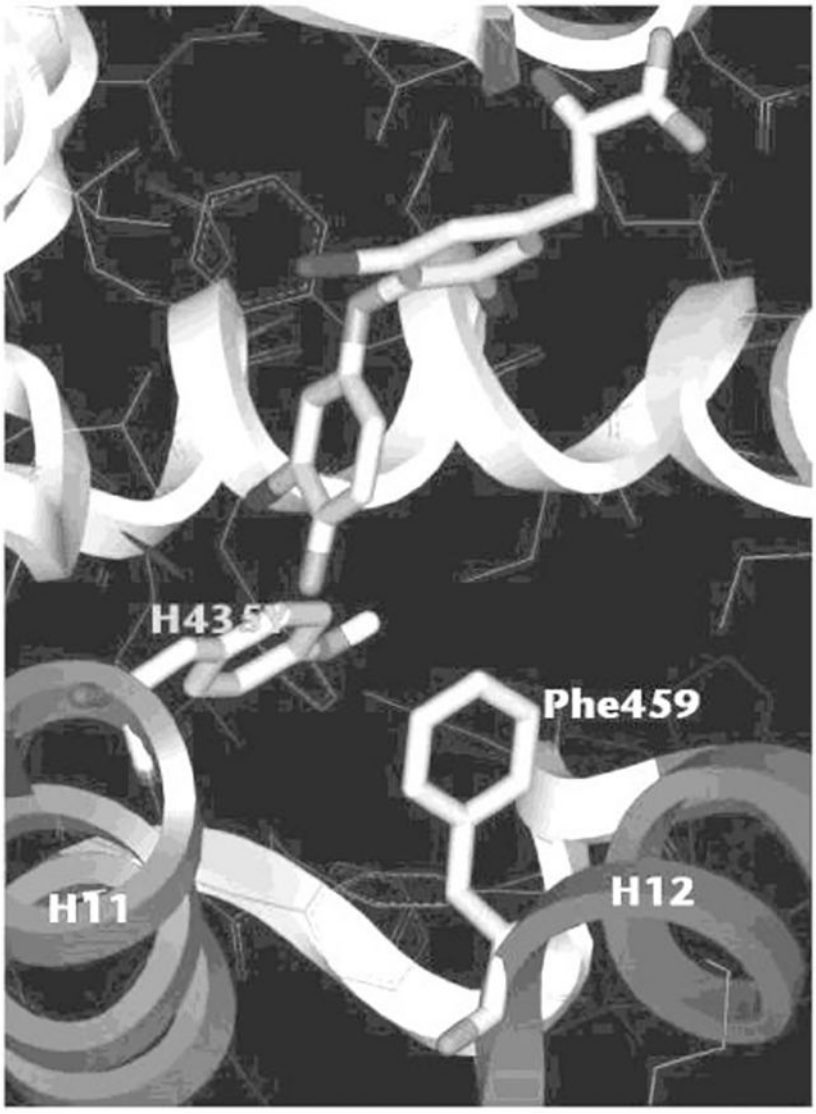
Experimental Section

See supplemental.



Keywords

Thyroid hormone receptor; resistance to thyroid hormone

The thyroid hormone receptors (TRs) are ligand-dependent transcriptional regulators that control critical genes in development and homeostasis in response to triiodothyronine (T3).[1] As an important regulator of differentiation, TRβ has been shown to be mutated in a high percentage of certain cancer types including kidney, pituitary, liver and thyroid cancer.[2, 3] These spontaneous TRβ mutations cause the reduction or loss of TR function similar to germline TRβ mutants associated with the inheritable genetic disease resistance to thyroid hormone (RTH).[4] Paradoxically, RTH patients do not appear to be predisposed to these same forms of cancer although in a few cases, the identical TRβ mutants have been identified in cancer and RTH.

As part of our studies exploring applications of chemical rescue by small-molecule complementation, we previously examined how mutations to TRβ's "His-Phe switch" motif, which mediates ligand dependent transactivation response, can dramatically impair receptor function.[5] Here we explore a new strategy to rescue a naturally occurring TRβ mutant, His435→Tyr, by reorienting hydrogen-bonding interactions at the ligand-receptor interface. As TRβ(H435Y) has been found in both RTH and pituitary carcinoma, this represents perhaps the first example of chemical rescue that targets a mutant protein involved in multiple disease states.

Upon ligand binding, TR undergoes a conformational change, that involves repositioning helix-12 to form a co-activator binding interface (Scheme 1A).[6] For most nuclear receptors, the hormone typically does not make direct contact with helix-12, but rather interacts with residues on helix-11, that make contacts with helix-12 via a "His-Trp" or a "His-Phe switch" that transduce ligand binding into a transcription response[5, 7]. Mutations to the His-Phe switch of TRβ have been associated with dramatic reductions in ligand potency (320- to >5000-fold).[2, 8, 9]

The high-resolution crystal structures of T3 bound TRβ and TRα suggest that His435 simultaneously forms a hydrogen bond with the 4'-OH of T3 and aryl-aryl interactions with Phe459 of helix-12 (Fig 1A). In prior work, we demonstrated that 4'-alkoxy derivatives of GC-1 have greater potency and efficacy with TRβ(H435A) than the natural hormone T3, however, these analogs are ineffective in rescuing activity to His435 mutants, TRβ(H435Y) or TRβ(H435L), known to be associated with RTH and cancer. Whereas TRβ(His435L) is inactive at all T3 concentration tested (≤ 5 μM), T3 is a full agonist (100% efficacy) with the TRβ(H435Y), though it is 390-times less potent with this mutant than with TRβ(wt). This suggests that TRβ(H435Y) retains its intrinsic ability to mediate ligand-dependent transcription response but requires extreme supra-physiological concentrations of T3 that would not be tolerated in vivo due to over-stimulation of wild-type TRs. As with other studies targeting the thyroid hormone receptor, the delicate balance of TR activity within the hypothalamic-pituitary-thyroid axis emphasizes the need for a ligand with subtype selectivity.[4, 10–12]

Molecular modeling of TRβH435Y) suggests that the Tyr435 side chain is still capable of engaging Phe459 via aryl-aryl interactions (Fig 1B).

Although the phenol hydroxyl of tyrosine is capable of forming a hydrogen bond, it is not appropriately positioned to interact with receptor-bound T3. We reasoned that appropriately designed hormone analogs may be able to selectively rescue potency to TRβ(H435Y) by restoring hydrogen bonding/aryl-aryl interactions of the His-Phe switch by creating a novel "Tyr-Phe" switch. This presents a unique challenge because the side chain of tyrosine is considerably longer than that of histidine, implying that a hydrogen-bonding group needs to be introduced while making the overall ligand structure smaller. As an initial approach, we reasoned that the outer phenyl ring of T3 could be replaced by a pyridyl ring (Scheme 1B, right panel). For ease of synthesis and stability of the products, we chose to make analogs of the halogen free-thyromimetic GC-1[13] rather than T3 itself, which allowed us to vary the 3'-alky substituent as a potential means to optimize the hydrogen bond geometry

and hydrophobic contacts of the 3'-substitutent (Fig 2). As a control we also synthesized a "phenyl" analog of GC-1, QH9, where the phenol hydroxyl is replaced by hydrogen. Pyridyl analogs were derived from the corresponding 2-substituted-4-cyanopyridines by nucleophilic radical addition to 4-cyanopyridine by alkyl radicals generated by silver-promoted radical decarboxylation of corresponding carboxylic acids providing an efficient entry into the 2-alkylpyridine series (Scheme S1, See supplemental).[14]

All of the monosubstituted pyridine analogs except QH15 had potencies at or below 1 μM for the mutant TRβ(H435Y) (Fig 3). Whereas there are many factors that influence potency, significantly, the pyridine analogs QH10-QH14 all show a preference for the mutant TRβ(H435Y) over TRβ(wt). In contrast, GC-1 and T3 are strongly biased (225- and 390-fold) for the wild type suggesting that the phenol→pyridine substitution was a successful strategy to complement the His→Tyr mutation (Fig 3B). One analog, QH13 (EC_{50}= 151 nM), is twice as potent as the parent compound GC-1 and more potent than T3 with the mutant TRβH435Y (Fig 3C).

Although QH13 is weaker agonist with TRβ(H435Y) than T3 is with TRβ(wt), QH13 is 15-fold selective for the mutant, representing a 5,850-fold improvement in selectivity relative to T3 (Table 1). Additionally QH13 has very weak activity towards TRα(wt) (EC_{50}>10 μM, data not shown). QH10, having a 3'-isobutyl substituent, was also selective (6.8-fold) but was slightly less potent (EC_{50}=421 nM) (Fig 2C). Though nearly isosteric with the pyridyl analog QH13, the phenyl analog, QH9, is 6-times less potent and has almost no mutant-to-wild-type selectivity (1.5-fold) suggesting that the hydrogen bonding group is critical for potency and selectivity. The nature of the 3'-substitutent of the pyridine analogs has a significant effect on their selectivity and efficacy with the most potent and efficacious analog QH13 having an isopropyl group similar to GC-1.

Compounds QH18 and QH17 were made in an attempt to create more room within the binding site for the larger Tyr side-chain of the mutant. QH18 represents the metathetical exchange of the phenol ring of GC-1 for an imidazole to complement the corresponding conversion the imidazole of histidine to a phenol associated with the His→Tyr mutation. Unfortunately these compounds have poor potencies and accurate EC_{50} values could not be obtained, however, they did show partial activity at the highest concentrations tested. The high affinity binding of T3 and GC-1 are due in part to the extensive hydrophobic contacts of the receptor with the outer phenol ring of the ligand. Therefore, it is not clear if the lower potency is due to a geometric misalignment of hydrogen bonding partners or due to the weaker hydrophobic contacts of the receptor with the smaller and more polar imidazole ring.

Overall, chemical complementation of the His435→Tyr mutant by changing the presentation of hydrogen bonding groups on the ligand is a successful strategy for rescuing TRβ(H435Y) function with a modest change in ligand potency and a dramatic change in receptor selectivity relative to the natural ligand T3 or GC-1. With the caveat of having only tested a limited set of ligands, we speculate that potency and efficacy are intrinsically more difficult to rescue from receptor mutations that encroach into the ligand binding pocket, as the strategy of making a smaller ligands to accommodate a smaller binding site invariably leads to a reduction in ligand-receptor interactions needed to maintain high potency.

The role of TR mutations in cancer and disease remains poorly understood. Whereas the dominant negative actions of TRβ mutants in RTH have been well documented, the identification of identical somatic mutations in cancer presents a unique paradox that may ultimately help reveal a role for TR in carcinogenesis, cancer progression or as a potential therapeutic target. QH13 and related analogs are currently being investigated for their ability to rescue the tumor suppressor properties of TR in *in vitro* cancer models.

Acknowledgements:

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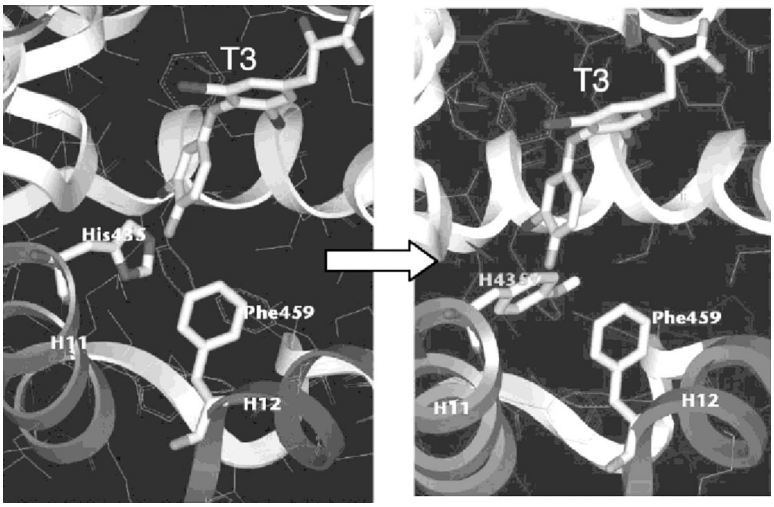


Figure 1.

A. TRβ(wt)/T3 co-crystal structure (PDBID: 1BSX). B. Modeled structure of T3 in TRβ(H435Y).

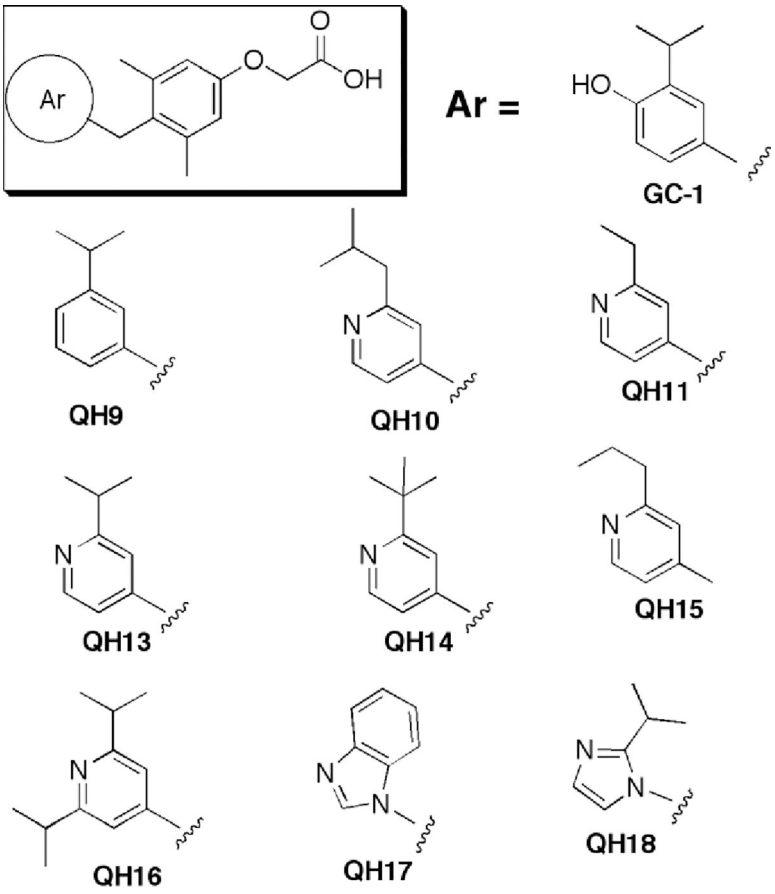


Figure. 2.
Structure of GC-1 and QH9-QH18.

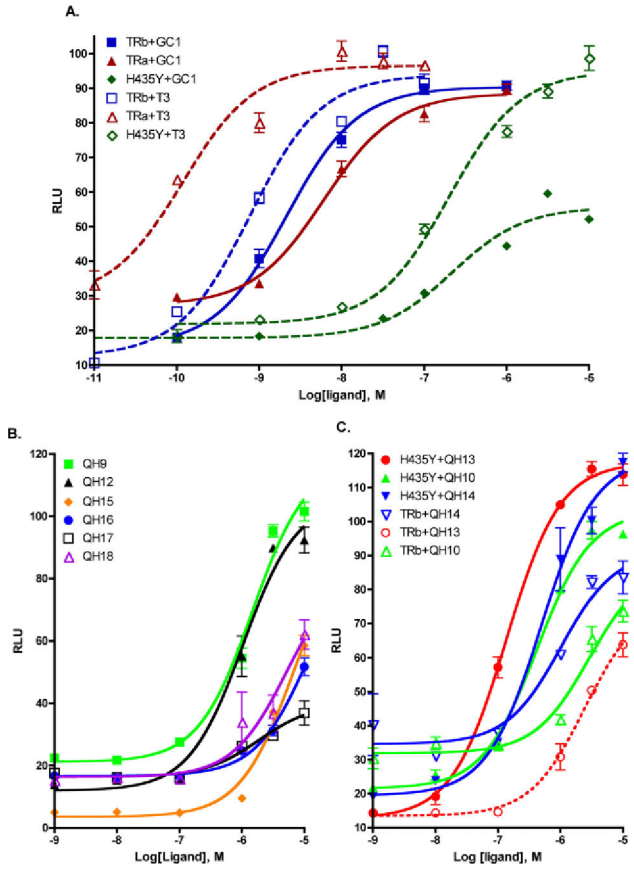
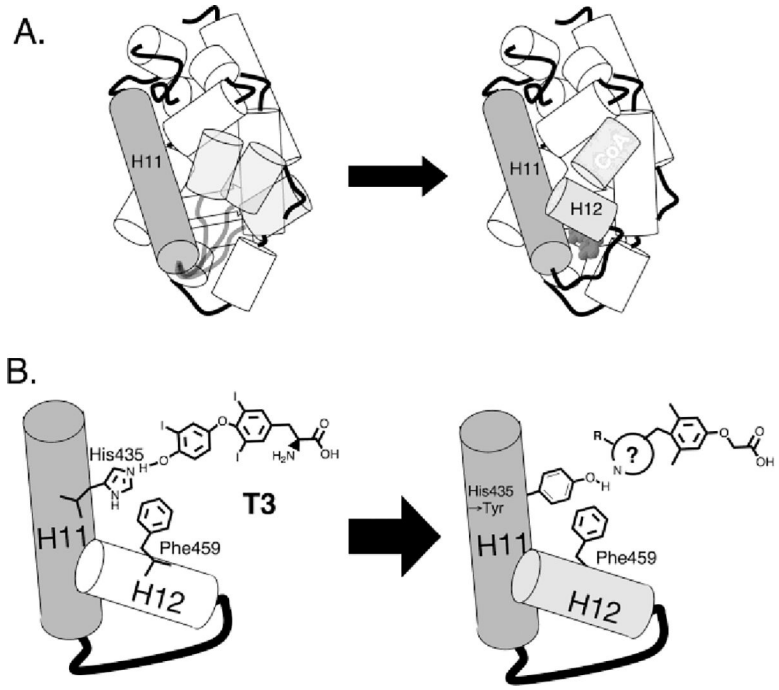


Figure. 3.
Cellular reporter gene response to designed analogs.



Scheme 1.

A. Ligand binding induces a conformational change to helix-12 (H12) that leads to recruitment of transcriptional co-activators (CoA). B. The His-Phe switch involves residues on helix 11 (H11) and H12. C. His435→Tyr mutation disrupts the normal His-Phe switch that may be complemented by appropriate heterocyclic ligands.

Table 1.

Potencies and efficacies of natural and synthetic analogues for TRβ and TRβ(H435Y) on DR4 promoter.

Compound	TRβ(wt) EC_{50} nM (% efficacy)	TRβ(H435Y) EC_{50} nM (% efficacy)	Selectivity wt/mutant
T3	0.51 (100)	199±84 (94)	1/390
GC-1	1.3 (93)	293±23 (56)	1/225
QH9	1400	920±43	1.5
QH10	2877 (75)	421±25 (100)	6.8
QH11	3194 (30)	780±33 (105)	4.1
QH13	2259 (65)	151±20 (115)	15
QH14	1018 (85)	501±32 (115)	2
QH15	1142 (105)	n.d. (60 *)	-
QH16	1860 (35)	n.d. (50 *)	--
QH17	n.d. (50 *)	n.d. (40 *)	--
QH18	n.d. (35 *)	n.d. (60 *)	--

n.d.=not determined

* efficacy@10 μM