Ligand Design

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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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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 in a similar way to germline TRβ mutants associated with inheritable genetic disease resistance to thyroid hormone (RTH).[4] Paradoxically, RTH patients do not appear to be predisposed to these forms of cancer, although, in a few cases, 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 the TRβ "His-Phe switch" motif, which mediates ligand-dependent transactivation response, can dramatically impair receptor function.[5] Herein, we describe 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, our results serve perhaps as 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 the repositioning of helix 12 to form a coactivator-binding interface (Figure 1a).[6] For most nuclear receptors, the hormone does not make direct contact with helix 12, but rather interacts with residues on helix 11. These residues make contacts with helix 12 through a His-Trp or a His-Phe switch, which transduces ligand binding into a transcription response.[5,7] Mutations to the His-Phe switch of TRβ have been associated with dramatic (320–>5000-fold) reductions in ligand potency.[2,8,9]

The high-resolution crystal structures of T3-bound TRβ and TRα suggest that His435 forms a hydrogen bond with the 4′-OH group of T3 and participates simultaneously in aryl–aryl interactions with the Phe459 residue of helix 12 (Figure 2, left). We demonstrated previously that 4′-alkoxy derivatives of the thyroid-hormone-receptor agonist GC-1 have greater potency and efficacy with TRβ(H435A) than the natural hormone T3; however, these analogues are ineffective in rescuing the activity of the His435 mutants TRβ(H435Y) and TRβ(H435L), which are known to be associated with RTH and cancer.[5] Whereas TRβ(His435L) is inactive at all T3 concentrations tested ($\leq 5\ \mu M$), T3 is a full agonist (100% efficacy) for TRβ(H435Y), although it is 390 times less potent with this mutant than with wild-type (wt) TRβ. These results suggest that TRβ(H435Y) retains its intrinsic ability to mediate ligand-dependent transcription response but requires extreme supraphysiological concentrations of T3 that would not be tolerated in vivo owing to the overstimulation of wild-type TRs. As in other studies in which the thyroid hormone receptor was targeted, 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 TRβ(H435Y) suggested that the Tyr435 side chain is still able to engage Phe459 through aryl–aryl interactions (Figure 2, right). Although the phenol hydroxy group 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 analogues may be able to rescue potency to TRβ(H435Y) selectively by restoring hydrogen-bonding/aryl–aryl interactions of the His-Phe switch through the creation of a novel Tyr-Phe switch. This strategy presented a unique challenge, as the side chain of tyrosine is considerably longer than that of histidine; therefore, it was necessary to introduce a hydrogen-bonding group 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 (Figure 1b, right). For ease of synthesis and product stability, we chose to make analogues of the halogen-free thyromimetic GC-1[13] rather than analogues of T3 itself. We could then vary the alkyl substituent at the 3′-position with the aim of optimizing the hydrogen-bond geometry and hydrophobic contacts of the 3′ substituent (Scheme 1). As a control, we also synthesized a "phenyl" analogue of GC-1, QH9, in which the phenol hydroxy group has been replaced by a hydrogen atom. Pyridyl analogues were derived from the corresponding 2-substituted 4-cyanopyridines by the nucleophilic addition to 4-cyanopyridine of alkyl radicals generated by silver-promoted radical decarboxylation of the corresponding carboxylic acids. This method provided efficient access to the 2-alkyl pyridine series of ligands (see Scheme S1 in the Supporting Information).[14]

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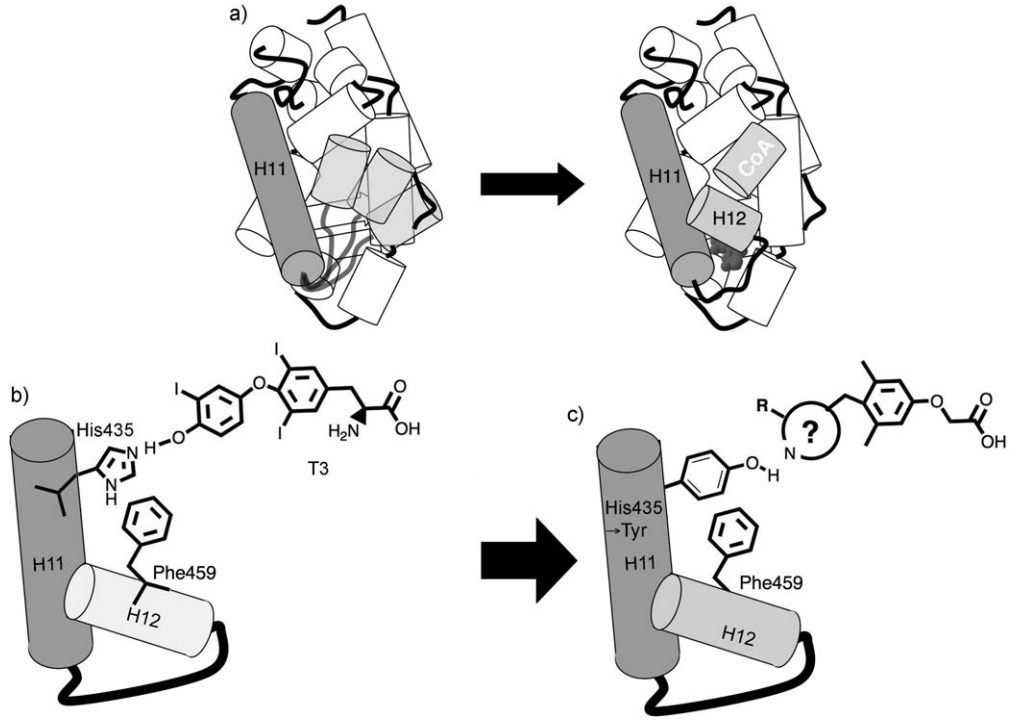


Figure 1. a) The conformational change to helix 12 (H12) upon ligand binding leads to the recruitment of transcriptional coactivators (CoA). b) The His-Phe switch involves residues on helix 11 (H11) and H12. c) The His435→Tyr mutation disrupts the normal His-Phe switch. The new residue on H11 may be complemented by appropriate heterocyclic ligands.

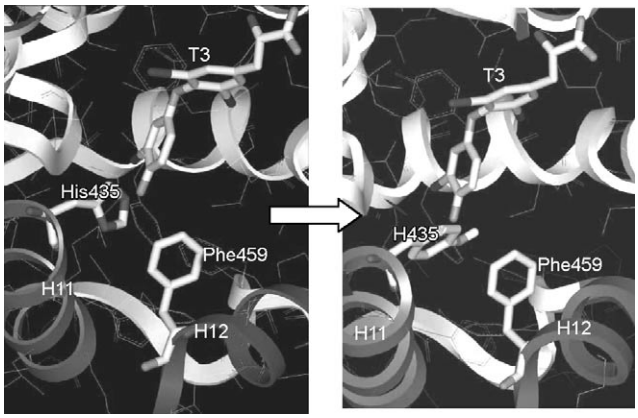
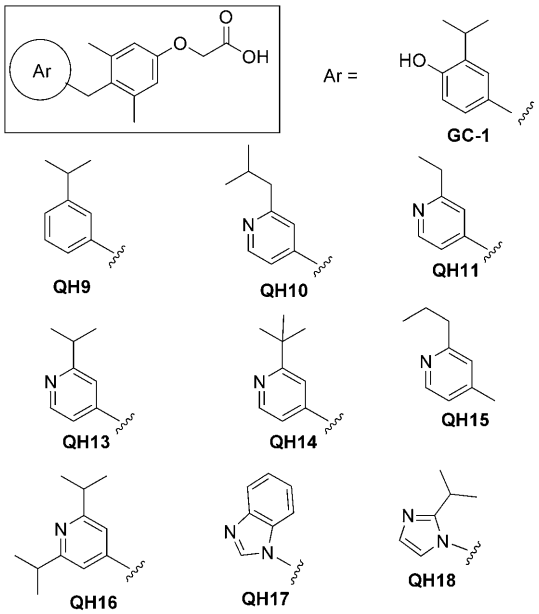


Figure 2. Left: TRβ(wt)/T3 cocrystal structure (PDBID: 1BSX). Right: Modeled structure of T3 in TRβ(H435Y).



Scheme 1. Structure of GC-1 and QH9–QH18.

All monosubstituted pyridine analogues, except QH15, had potencies at or below 1 μM for the mutant TRβ(H435Y) (Figure 3). Although many factors influence potency, the pyridine analogues QH10–QH14 all show a preference for the mutant TRβ(H435Y) over TRβ(wt). In contrast, GC-1 and T3 are strongly biased (by a factor of 225 and 390, respectively) for the wild type. Thus, the substitution of the phenol ring for a pyridine moiety appeared to be a successful strategy to complement the His→Tyr mutation (Figure 3b). One analogue, QH13 ($EC_{50} = 151$ nM), is twice as potent as the parent compound GC-1 and more potent than T3 with the mutant TRβH435Y (Figure 3c).

Although QH13 is a weaker agonist with TRβ(H435Y) than T3 is with TRβ(wt), QH13 shows 15-fold selectivity for the mutant. This result corresponds to a 5850-fold improvement in selectivity for the mutant relative to that of T3 (Table 1). Furthermore, QH13 shows very weak activity towards TRα(wt) ($EC_{50} > 10$ μM, data not shown). QH10, which has a 3′-isobutyl substituent, was also selective (by a

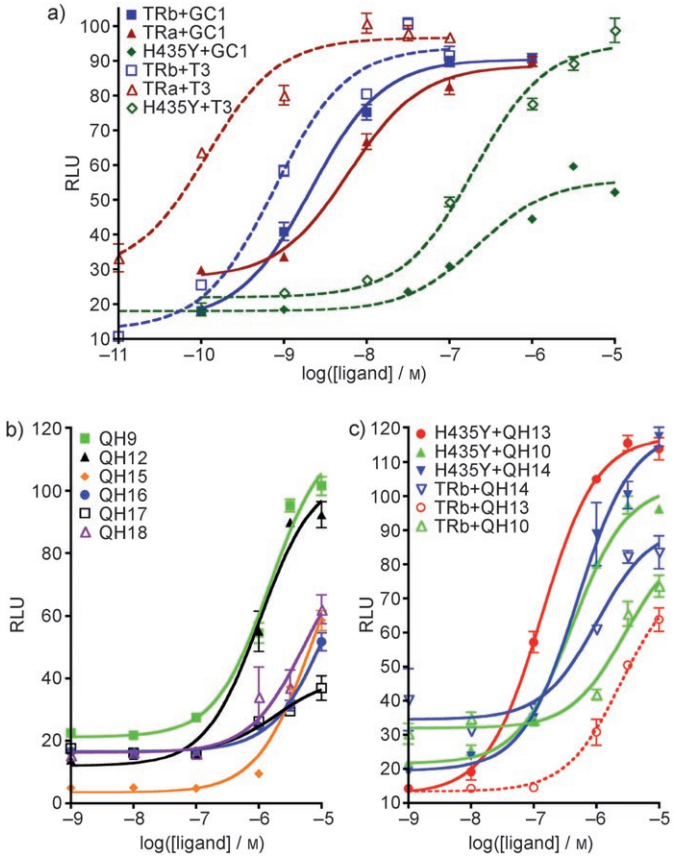


Figure 3. Cellular reporter-gene response to designed analogues. RLU = relative light units.

Table 1: Potencies and efficacies of natural and synthetic ligands for TRβ and TRβ(H435Y) on the direct repeat 4 (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)[a]	–
QH16	1860 (35)	n.d. (50)[a]	–
QH17	n.d. (50)[a]	n.d. (40)[a]	–
QH18	n.d. (35)[a]	n.d. (60)[a]	–

[a] Efficacy at a concentration of 10 μM (n.d. = not determined).

factor of 6.8), but was slightly less potent (EC_{50} = 421 nM; Figure 3c). Although nearly isosteric with the pyridyl analogue QH13, the phenyl analogue QH9 is six times less potent and shows almost no (1.5-fold) selectivity for the mutant over the wild type. Thus, the hydrogen-bonding group appears to be critical for potency and selectivity. The nature of the substituent at the 3′-position of the pyridine analogues has a significant effect on their selectivity and efficacy, whereby the most potent and efficacious analogue QH13, like GC-1, has an isopropyl group.

Compounds QH18 and QH17 were prepared in an attempt to create more room within the binding site for the larger Tyr side chain of the mutant. QH18 is related to GC-1 through the exchange of the phenol ring for an imidazole ring to complement the corresponding replacement of the imidazole ring of histidine with a phenol moiety in the His→Tyr mutation. Unfortunately, these compounds have poor potencies, and accurate EC_{50} values could not be determined; however, they did show partial activity at the highest concentrations tested. The high-affinity binding of T3 and GC-1 is 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 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 those of the natural ligand T3 or GC-1. With the caveat of having tested only 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 ligand to accommodate a smaller binding site leads invariably to a diminishment in the ligand–receptor interactions needed to maintain high potency.

The role of TR mutations in cancer and other diseases 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 or cancer progression, or as a potential therapeutic target. We are currently investigating QH13 and related analogues for their ability to rescue the tumor-suppressor properties of TR in in vitro cancer models.

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