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Selective Thyromimetics Using Receptor and Tissue Selectivity Approaches: Prospects for Dyslipidemia

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INTRODUCTION

Thyroid hormones (THs) have diverse effects on metabolism and development. Consequently, significant metabolic changes are seen with variations in thyroid status in humans.[1] Early proof of the critical role of TH came in 1888 with a report indicating that destruction of thyroid glands is related to cretinism and adult hypothyroidism.[1] The efforts were then concentrated on extracting thyroid hormones from animal sources for the treatment of hypothyroidism and other related disorders. Emil Kocher, working on pioneering research on pathology and surgery of thyroid glands, was awarded the Nobel Prize in Medicine in 1909.[1] The first successful isolation of T_4 (3,3′,5,5′-tetraiodo-L-thyronine) from thyroid extracts was performed by Kendall in 1914,[2] which was later followed by synthesis of T_3 (3,3′,5-triodo-L-thyronine) and demonstration of its ability to cure experimentally induced goiter in rats.[3] In the next few decades, the research revolved around the pathophysiological role of TH. The involvement of TH in regulating transcription of target genes was suggested by rapid RNA synthesis in livers of T_3 treated hypothyroid rats, resulting in new protein formation and enhanced mitochondrial oxidation.[4] The evidence of thyroid hormone receptors (TRs) was established using radioactive T_3 binding to thyroid sensitive tissues.[5] Further transcription studies revealed that TRs behaved similarly to steroid hormone receptors with respect to nuclear site of action, recognition of specific DNA sequences, and ligand-dependent regulation of transcription using basal transcriptional machinery.[6] In addition, crystal structures of the TR ligand-binding domain (LBD) aided the understanding of the molecular mechanisms of THs. The understanding of the role of TR in physiological and pathological states has received a boost with the development of knockout and transgenic animal models.[7,8] Four isoforms of thyroid hormone receptors, namely, TRα1, TRα2, TRβ1, and TRβ2, have been successfully ablated in mice, individually, or in combination.[9−12] Data from TRα knockout mice showed that T_3-induced changes in the cardiovascular system are mediated by TRα1, deletion of which resulted in slow pulse rate, and administration of T_3 could not reverse this effect.[13,14] Conversely, TRβ1 is the major mediator of T_3 actions on cholesterol and lipoprotein metabolism in mice.[1,8] These findings raised the interest in the development of TH analogues that can selectively modulate the isoform of interest, mostly for their beneficial effects on cholesterol metabolism.[1,15]

BIOSYNTHESIS AND REGULATION OF THYROID HORMONES

Thyroxine (3,3′,5, 5′-tetraiodo-L-thyronine, T_4, **B** in Figure 1) and triiodothyronine (3,3′,5-triodo-L-thyronine, T_3, **A** in Figure 1) are synthesized and released from the thyroid gland.[1] T_4 is the major

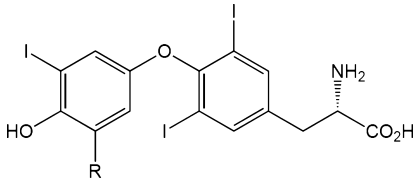

Figure 1. Thyroid hormones (L-T_3 (**A**) and L-T_4 (**B**)).

form of thyroid hormone secreted in humans. It is enzymatically deiodinated in peripheral tissues to more active T_3.[16]

More than 99% of the thyroid hormones in circulation stay bound to carrier proteins such as thyroxine binding globulin (TBG), albumin, and transthyretin. The free TH enters target cells and generates a biological response.[1] The local and systemic availability of T_3 relies on its generation by 5′ deiodination of the outer ring of T_4, a reaction catalyzed by selenoproteins known as deiodinases.[1,16] The production and secretion of thyroid hormones are regulated by a negative feedback mechanism in humans, which involves the hypothalamus, pituitary, and thyroid gland [hypothalamic−pituitary−thyroid (HPT) axis].[1] Thyrotropin releasing hormone (TRH) is a tripeptide (PyroGlu-His-Pro) synthesized by neurons of the paraventricular nucleus (PVN) of the hypothalamus.[17] TRH binds to TRH receptors in the pituitary that secrete thyroid-stimulating hormone (TSH). TRH stimulation leads to TSH synthesis in thyrotropes. TSH is a primary regulator of TH release and secretion. TSH binds to G-protein-coupled TSH receptor (TSHr), stimulating a number of proteins, including Na^+/I^- symporter (NIS), thyroglobulin (Tg), and thyroidperoxidase (TPO) that promote the synthesis of TH. Iodide is actively transported and concentrated in the thyroid by NIS. The intrapituitary conversion of circulating T_4 to T_3 greatly influences the negative regulation of TSH (Figure 2). In addition, catecholamines and somatostatin released from the hypothalamus decrease the secretion of TSH.

TH biosynthesis starts by activation of the Tg gene in the follicular epithelial cells of the thyroid gland. The gene product, Tg, undergoes iodination and tyrosine condensation. The iodination is facilitated by active accumulation of iodide into the follicular cells. Iodination of specific tyrosines located on Tg yield monoiodinated and diiodinated residues (MIT, monoiodotyrosine; DIT, diiodotyrosine), which, on further coupling, produce T_4 and T_3. The internalized Tg is incorporated in phagolysosomes and undergoes proteolytic digestion, recaptures

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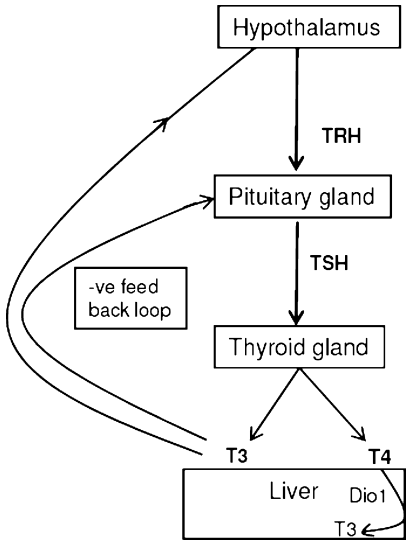

Figure 2. Hypothalamus–pituitary–thyroid (HPT) axis.

MIT and DIT, and releases T_4 and T_3 into the circulation via the basal surface.[18]

Deiodinases convert TH to active or inactive forms depending on the type of deiodinases catalyzing the enzymatic reaction. Type I deioidinase is responsible for the conversion of the majority of T_4 to T_3 in circulation, whereas type II deiodinases (Dio2) primarily convert T_4 to T_3 for intracellular use.[19] Type I deioidinase (Dio1) is located in liver and kidney, while type II deiodinase is found in brain, pituitary, and brown adipose tissue. 5′-Deiodination by type I deiodinases and type III deiodinases, located primarily in placenta, brain, and skin, leads to the generation of reverse T_3 (rT_3), which serves as a key step for the inactivation of TH. TH is excreted in bile or taken back into circulation by a reuptake mechanism. rT_3 and T_3 are further deiodinated in the liver and are sulfo- and glucuronide-conjugated before excretion in the bile. The deconjugation of TH during enterohepatic circulation by intestinal flora stimulates thyroid hormone reuptake.

Thyroid hormones are carried by serum proteins to the target organ. These proteins serve as a circulating depot for thyroid hormones, which would be necessary for a consistent and homogeneous supply to the tissues. Active pathways involving membrane bound transporters are the major effectors of TH transport across the cell membranes. Amino acid transporters (systems L and T), classical multispecific organic anion/cation transporters, and multidrug resistance (MDR) pumps are the major systems involved in the TH transport.[20] There are high-affinity, saturable TH-binding sites on plasma membranes of different cell types, which modulate cellular TH uptake.[21] Inside the cytoplasm, TH binds to its chaperones, cytoplasmic TH-binding proteins (CTHBPs), for translocation to the nucleus. CTHBPs also regulate few of the nongenomic (extranuclear) actions of TH.[22] The biological effects of TH are mainly mediated through TRs, located in the nucleus, where its binding to TR, associated with nuclear chromatin, regulates gene transcription.[6] The actions of TH are thus regulated by the process of cellular uptake, transport of TH to the nucleus, and TH metabolism within target tissues/cells, such as the conversion of T_4 to T_3 by 5′-deiodinases or TH inactivation by 5-deiodinases and other enzymes.

METABOLIC EFFECTS OF THYROID HORMONES

Thyroid hormones stimulate fat mobilization and oxidation of fatty acids in major tissues.[23] Plasma cholesterol is reduced by thyroid hormones primarily through its action on the TRβ receptors.[15,24] Thyroid hormones increase elimination of neutral sterols and bile acids, decrease intestinal cholesterol absorption, and increase removal of low density cholesterol (LDL) by enhancing hepatic LDL uptake.[15] Hepatic LDL receptors (LDLRs) are up-regulated by thyroid hormones, causing increased LDL cholesterol uptake by the liver. Four functional thyroid hormone response elements (TREs) exist in the promoter region of the LDL receptor gene, and sterol regulatory element binding protein 2 (SREBP-2), a potent activator of LDLR transcription, is activated by thyroid hormones.[25] In addition, it influences the human acetyl-CoA carboxylase (ACC) gene, which results in reduction of circulating LDL levels.[26,27]

Reverse cholesterol transport is a multistep process resulting in the net movement of cholesterol from peripheral tissues back to the liver via the plasma. Thyroid hormones increases the expression of ApoA1 and scavenger receptor class B type I (SRBI) high density lipid (HDL) receptors that results in enhanced HDL cholesterol and activated "reverse" cholesterol transport.[28] Though cholesterol synthesis in liver is increased by thyroid hormones, more significant increase in cholesterol degradation and biliary excretion via cholesterol α-hydroxylase (CYP7α1) ultimately results in reduced circulating cholesterol.[29] Cyp7α1 expression in human hepatocytes is highly sensitive to T_3, and treatment with T_3 reduces Cyp7α1 mRNA and cholic and chenodeoxycholic acid synthesis.[30] Conversely, bile acids, through increase in Dio2 activity, stimulate conversion of T_4 to T_3, promoting energy expenditure and cyclic adenosine monophosphate (AMP) production. Increasing T_3 concentration inhibits bile acid synthesis and reduces cyclic AMP production and cyclic AMP-dependent Dio2 activity owing to a negative feedback mechanism.[31]

Thyroid hormones increase basal metabolic rate through activation of TRs. It is primarily mediated through increased ATP turnover (Na^+/K^+ ATPase activity as well as content).[31] The interaction of TRs with catecholamines is also important for stimulation of T_3 synthesis and expression of uncoupling protein 1 (UCP-1) required for adaptive thermogenesis.[32] The two TR isoforms TRα and TRβ are pivotal for adaptive thermogenesis, each having a specific role in brown adipose tissue (BAT) and white adipose tissue (WAT).[33] TRα regulates potentiation of lipolytic actions of catecholamines in WAT[34] and BAT, whereas both isoforms are required for adaptive thermogenesis in BAT.[32] TRβ isoforms predominantly stimulates UCP1 expression in BAT during thermogenesis.[33] However, though UCP-1 levels are regulated by TRβ1, action of TR on sympathetic stimulation is regulated by TRα.

It is well established that food intake as well as the thermic effect of food is generally decreased in hypothyroidism. In contrast, a hyperthyroid state is associated with the increased amount of food eaten and increasing thermic effect of food. The mechanisms by which thyroid hormones stimulate food intake remain undefined. Several studies have tried to elucidate a possible relationship between the anorexigenic hormone leptin and thyroid, especially in thyroid dysfunction, but no clear conclusions have been drawn so far.[35] TSH production by the pituitary is under hypothalamic control via release of thyrotropin-releasing hormone. TRH and TSH are regulated by the negative feedback mechanism activated by T_4 and T_3 concentrations.[17] Apart from the feedback regulation by thyroid hormones, TRH synthesis and release are modulated by the central nervous system circuitry. There exist anatomical

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connections of neurons in the NPY-leptin circuit with the hypophysiotropic TRH neurons, as well as regulatory sites in the TRH gene.[35] These projections play a key role in the leptin mediated restitution of the thyroid axis during food deprivation, resulting in reduced TRH mRNA expression in the PVN, which contributes to lower serum concentrations of thyroid hormone.[17,35] Leptin reduction results in lowering of TRH set point, which is being modulated by T_3. Such modulation subsequently lowers TRH, TSH and thereby reduces thyroid hormones. Orexigenic NPY inhibits the transcription of TRH, whereas anorexigenic CART stimulates synthesis and release of TRH. Catecholamines promote higher levels of thyroid hormones by increasing the sensitivity of TRH to T_3.[32]

Consistent with its thermic effects, glucose (and carbohydrate) utilization by nonhepatic tissue is significantly increased by thyroid hormones.[23,32] Thyroid hormones increase peripheral glucose uptake primarily through increased expression of glucose transporters such as glucose transporter type 4 (GLUT4).[36] On the other hand, thyroid hormones increase the glucose production in liver via gluconeogenesis.[23,32]

Heart is a major target organ for thyroid hormone action. Most of the cardiac effects of thyroid hormones are due to stimulation of TRα1.[37] Thyroid hormones cause dose-dependent increases in heart rate (positive chronotropic effect) as well as effects on cardiac function (positive inotropic and lusitropic effects).[37] On the other hand, hypothyroidism is characterized by a low cardiac index, decreased stroke volume, decreased vascular volume, and increased systemic vascular resistance. Total blood volume is decreased in hypothyroidism and varies directly as a function of basal metabolism rate.[37,38]

Thyroid hormones can also act through nongenomic pathways independent of their actions on nuclear TRs. There also can be rapid onset of action (typically seconds to minutes) and utilization of membrane-signaling pathways, typically involving kinases or calmodulin, that have not been implicated in direct TR function.[39,40] Some major potential targets of T_3 in the plasma membrane include Ca^{2+}-ATPase, adenylate cyclase, and glucose transporters.[6] Thyroid hormones can also modulate cell structure proteins by nongenomic mechanisms.[6,22]

CLINICAL MANIFESTATIONS OF THYROID HORMONE EXCESS AND DEFICIENCY

Thyroid hormones exert profound effects on the lipid metabolism, cardiovascular system, and glucose homeostasis. Hence, any disturbance in thyroid hormone homeostasis may destabilize vital physiological functions.[1,6,35,37,38] Hyperthyroidism is characterized by elevated levels of circulating T_3 or T_4. The typical symptoms of thyrotoxicosis, i.e., severe hyperthyroidism, are weight loss, hypermetabolism, lowering of serum cholesterol levels, cardiac arrhythmias, heart failure, muscle weakness, bone loss in postmenopausal women, and anxiety.[41] Hyperthyroidism increases LDLR expression in liver, resulting in higher cholesterol uptake in liver. Hyperthyroidism also causes an increased cholesterol excretion into bile.[28] Taken together, these effects modulate the net decrease in serum LDL despite higher cholesterol biosynthesis. Cholesteryl ester transfer protein (CETP), which activates the exchange of core lipid triglycerides and cholesteryl esters, is regulated by thyroid status.[42] Thyroid hormones also regulate hepatic lipase (HL), which catalyzes lipolytic enzyme for the conversion of IDL to LDL and HDL_2 to HDL_3.[41] Hyperthyroidism increases the activity of HL, resulting in decreased or normal plasma HDL levels.[41]

In contrast to hyperthyroidism, hypothyroidism occurs either through reduced thyroid production of thyroid hormones (primary or thyroidal hypothyroidism) or through diminished pituitary TSH release by TRH (central hypothyroidism). As against hyperthyroidism, which primarily manifests itself in decreased plasma cholesterol levels, hypothyroidism is a major cause of secondary dyslipidemia because of decreased cholesterol excretion. Hypothyroidism also causes a marked increase in apo B lipoproteins due to reduced number of LDL receptors on the liver cell surface.[27,43] Increase in total and LDL cholesterol levels in hypothyroidism exacerbates the risk of atherogenic events. In addition, the low levels of HDL also contribute to the atherogenic lipid profile, which may result in increased incidences of coronary heart diseases.

Clinical studies on patients with spontaneous hypothyroidism and hyperthyroidism indicate a predominant effect of thyroid hormones on the heart and cardiovascular system.[37] Thyrotoxicosis decreases peripheral vascular resistance, while the cardiac output and cardiac contractility are increased. On the other hand, hypothyroidism causes increased peripheral vascular resistance and decreased cardiac output and cardiac contractility.[44] Thyroid hormones enhance total protein synthesis in the heart and result in cardiac hypertrophy in the hyperthyroid state.[45] Hyperthyroidism is also associated with tachycardia, and rapid, forceful palpitations may occur. The characteristic hyperthyroidism-induced heart failure displays decreased cardiac contractility, abnormal cardiac compliance, and pulmonary congestion.[46]

Conversely, bradycardia and diastolic hypertension are the most common clinical signs of hypothyroid conditions.[46] The association of hypercholesterolemia and diastolic hypertension increases the incidences of atherosclerosis and coronary heart diseases in hypothyroidism.[38] However, the reduced expression of α and β myosin heavy chain (MHC) mRNA and phospholamban contributes to the impaired cardiac contractility.[47,48] In conclusion, thyroid hormone dysfunction directly (nuclear mediated transcription effects) and indirectly (cardiac response to increased cardiac work) affects the cardiovascular system, resulting in various atherosclerotic complications.

Hyperthyroidism has been recognized as a reversible cause of hyperglycemia, which is initiated by an unopposed activation of gluconeogenesis attributed to excessive availability of substrate (alanine) resulting from the thyrotoxicosis-associated catabolic state.[49] Thyrotoxicosis is also associated with a decreased insulin half-life, indicating an accelerated degradation.[36]

Resistance to thyroid hormone (RTH) is a syndrome characterized by reduced sensitivity of tissues to the action of thyroid hormone.[50] This condition is characterized by elevated levels of circulating thyroid hormones associated with normal or high levels of serum thyroid stimulating hormone. Some of the clinical features that have been reported include goiter, short stature, decreased weight, tachycardia, hearing loss, attention deficit–hyperactivity disorder, decreased IQ, and dyslexia.

THYROID HORMONE RECEPTORS

TRs belong to the superfamily of nuclear hormone receptors. The two major TR isoforms, designated as TRα and TRβ, are encoded on separate genes on human chromosomes 17 and 3, respectively. Both TR isoforms bind T_3 (dissociation constants between 10^{-9} and 10^{-10} M) and mediate TH-regulated gene expression.[6] Alternative splicing of the initial RNA transcript of the TRα gene generates two mature mRNAs that each encodes

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two proteins: TRα-1 and *c-erbAα-2*. The *c-erbAα-2* is unable to bind T_3 because of its 122-amino acid carboxy terminus, which replaces a specific region in TRα1 that is critical for facilitating TH binding; however, *c-erbAα-2* binds TREs weakly and is unable to transactivate TH-responsive genes. This led to the conclusion that TRα1 is an authenticated TR and not *c-erbAα-2*.[51] Similarly, TRβ gene derives two important isoforms, namely TRβ1 and TRβ2, along with two additional variants, TRβ3 and TR$\Delta\beta$3, of unknown physiological significance.[52] The TR isoforms found in human, rat, and mice are highly homologous with respect to their amino acid sequence.[53] The expression of the isoforms is highly tissue specific; TRα1 mRNA has highest expression in skeletal muscle, heart, and brown fat, whereas TRβ1 mRNA has the highest expression in brain, liver, and kidney. TRβ2 has the highest expression in the anterior pituitary gland and specific areas of the hypothalamus along with the developing brain and regions of the inner ear.[53] The up- and down-regulation of gene expression in target tissues by TH involves functional domains, thyroid response elements, and thyroid receptor complexes, which ultimately leads to activation or repression of transcription by TRs upon TH binding.

TRs share a similar domain organization with other family members comprising a N-terminal transactivation domain (AF-1), the DNA binding domain (DBD), and the carboxy-terminal ligand-binding domain (LBD).[54,55] The DNA-binding domain (domain C) is a central domain located in the amino half of the protein. It contains two "zinc fingers" (DBD) and is the most highly conserved domain among members of the superfamily. The ligand-binding domain (domain E) is in the carboxyl half of the protein and is conserved among TRs across the species. A hinge region containing the nuclear localization signal is situated between DBD and LBD. The N-terminal A/B domain is highly variable in sequence and length. Its role in the TR function in vivo is unclear; however, this domain contains a transactivation function at least for TRs in some tissues. TRs show remarkable similarity among rats and human beings. The rat and human TRs are 97% and 99% identical in their DBDs and LBDs, respectively, but only 85% identical in their amino-terminal domains.[56]

The integrity of the zinc fingers in DNA binding domain is essential for DNA binding and transcriptional activity.[57−59] In addition to sequence-specific recognition, it also contacts nucleic acids and phosphate groups within the major groove of the thyroid response elements (TRE).[57,60] TRs can bind to DNA as monomers, homodimers, and heterodimers formed with other members of the thyroid−retinoid receptor subfamily (Figure 4). For example, TRs can heterodimerize with RXRs and can bind to TREs and these TR/RXR heterodimers bind to TREs with a 5′ to 3′ polarity with TR in the downstream position.[61,62]

LBD consists of a major hydrophobic pocket, which has inwardly turned core region serving as a binding site for the thyroid hormone ligand. LBD is necessary for TH binding and also plays a critical role for dimerization, transactivation, and basal repression by unliganded TR.[63] The hydrophobic surface of ligand binding cavity is contributed by the most carboxy-terminal region (helix 12) of LBD. The interactions of TR with coactivators may be determined and regulated by the relative positions of the helix 12 and the helices bordering helix 12.[64]

The hinge region is located between DBD and LBD, which contains a nuclear localization signal and influences both DNA binding and transactivation.[59] It also facilitates the interactions with corepressors once the receptors are translocated to the nucleus.[65−67]

MOLECULAR MECHANISM OF THYROID RECEPTOR ACTIONS

TRs act by binding to specific DNA sequences, thyroid response elements, in the promoters of their target genes, thereby stimulating or repressing transcription. TRs can bind to TREs as monomers, homodimers, and heterodimers.[68] Most of the TREs are located upstream from the minimal promoter; however, in certain cases, they may be located in 3′-flanking sequences downstream from the coding region.[68,69] TREs may vary in primary nucleotide sequence as well as in the number, spacing, and orientation of their half-sites.[70] The importance of the TREs can be gauged from the report stating that flanking and spacing sequences in TREs can affect DNA-binding and transcriptional activation, by either making contacts with TR or local DNA bending.[71] The formation of homo- or heterodimers marks the important step in the hormone-regulated transcription. TRs could bind to synthetic and natural TREs as monomers, homodimers, and heterodimers. TR auxiliary proteins (TRAPs) mobilize the heterodimerization and the binding to TREs. TR/TRAP heterodimers, especially with retinoid X receptors (RXR), have better binding ability to TRE than TR homodimers.[71]

The regulation of transcription by TH involves various regulatory proteins that bind the receptor−DNA complexes. In general, those proteins that promote transcriptional activation by binding the ligand-bound form of the receptor are defined as coactivators and those involved in repression of transcription by binding the ligand-free receptor are the corepressors. Unliganded TRs can activate transcription of certain negatively regulated promoters such as TSH. This is possible because of receptor phosphorylation by cell-specific kinases or phosphatases or because of cell-specific expression of certain membrane-signaling receptors.[72] Corepressors [e.g., silencing mediator for retinoid and TH receptors (SMRT) and nuclear receptor corepressor (NCoR)] form multimeric complexes containing histone deacetylases (HDACs), while many coactivators (e.g., steroid receptor coactivator-1 (SRC-1), CBP/p300) are histone acetyltransferases (histone acetyltransferases (HAT) or histone acetylases).[62] Both corepressors and coactivators appear to be present in multimeric complexes alongside other proteins and RNA which is present at least in the case of an SRC-1 complex.[59,62] NCoR mediates basal repression exclusively by TR and retinoic acid receptor (RAR), as well as orphan members of the nuclear hormone receptor family. However, NCoR also has strong interaction with transcription factors TFIIB, TAFII32, TAFII70 and represses transcription, suggesting that part of its transcription repression ability is attributed to its ability to interact with the basal transcriptional machinery.[73,74] On the other hand, SMRT has similar repression and nuclear receptor interaction domains as NCoR. Transcription repression involves the interaction between corepressors and deacetylase. Additionally, the local histone deacetylation may play a critical role in basal repression brought about by nuclear hormone receptors. It is likely that histone deacetylation by unliganded TR/corepressor complex may help to maintain local chromatin structure in a state that shuts down basal transcription. In addition to histone deacetylation, methylation of DNA is a vital process for repression activities, whereas the TR activated transcription in

the absence of ligand involves TR recruitment of corepressors.[75]

The coactivators are the vital link between the liganded TR complexes with the components of basal transcription machinery and enhance their ligand dependent transcription. The important coactivators are CREB binding protein (CBP), which interacts with CREB,[76,77] and SRC-1,[64] which enhances transcriptional activation for steroid hormone receptors. The AF-2 region located in helix 12 of the LBD is the focal point for the interaction of these coactivators. These coactivators have many common features among themselves ranging from the interaction sites to the dimerization interfaces. The binding of coactivators to nuclear hormone receptors is determined by the amino acid sequence (LXXLL) of the interaction site.[78] Similarly, the coactivators may bind to DNA as a basic helix–loop–helix (bHLH) motif which exists in the amino-terminal region just next to DBD. Per-Arnt-Sim (PAS) domain is also located in this region, and the role of this domain is to facilitate the dimerization and allow the interactions among the coactivators or transcription factors. This PAS region is also seen in several transcription factors that regulate the circadian rhythm and in the heterodimer partner of the dioxin receptor. These coactivators have multiple interactions among themselves as well as the proteins aligned to the coactivators.[39,70]

One of the basic differences in liganded and unliganded transcription activities are the changes in the chromatin assembly. The liganded TR/RXR disrupts chromatin structure, releases the corepressors complex, and recruits coactivators such as CBP and PCAF with HAT activity, whereas unliganded TR/RXR heterodimer results in transcriptional repression without affecting chromatin structure. In addition, TRs may recruit DRIP/TRAP complex, which in turn may recruit or stabilize RNA Pol II holoenzyme by virtue of shared subunits, though DRIP/TRAP (vitamin D receptor interacting proteins (DRIPS)) complex lacks the HAT activity. Thus, at least two coactivator complexes are involved in transcription of the thyroid hormone responses. It is possible that the p160/SRC complex may initiate transcriptional activity by recruiting cofactors with HAT activity to ligand-bound nuclear hormone receptors,[79,80] and DRIP/TRAP complex may then bind to nuclear receptors that can then recruit RNA Pol II holoenzyme to promote transcription of target genes. There are many other classes of coactivators influencing TR-mediated transcription other than p160/SRC and DRIP/TRAP, although the functional role of these coactivators is not known.[81]

SELECTIVITY IN THYROID HORMONE ACTIONS

TRs are present in almost all tissues, but the relative expression of TR isoforms varies among tissues (Table 1). TRα and TRβ isoforms exhibit numerous differences including expression in development,[7,82,83] tissue distribution,[84] ligand affinity for thyroid hormone analogues, isoform-specific gene regulation, and isoform-specific antagonism by mutant TRs.[85] The expression pattern of TR isoforms is consistent across the species. In the early stages of life, rats showed predominant expression of TRα, while in adult life TRβ expression achieves predominance.[84,86] The metabolic effects of TH are principally regulated by TRβ1 mRNA expression in liver, while TRβ2 mRNA expression was shown to be restricted to the pituitary and certain areas of the brain.[51,87] The cardiovascular effects of TH are mediated via TRα expressed in heart and smooth muscles.[44]

TRβ is critical in controlling hepatic cholesterol metabolism and TSH suppression, owing to high expression of TRβ in liver and pituitary.[86,88] Liver is a key regulator of cholesterol metabolism, which balances hepatic cholesterol synthesis and hepatic uptake of plasma lipoproteins from the circulation against the excretion of hepatic cholesterol and acids in the bile.[28,41] Hence, it was rightly contemplated that selective agonists of TRβ isoform can produce a full thyromimetic effect on plasma cholesterol levels.[89] Data from various TR knockout mice studies suggest that TRα1 mediates the effects of thyroid hormones on heart rate, whereas TRβ1 is important in mediating the cholesterol lowering and TSH suppressant effects of T_3. Deletion of the TRβ gene has been associated with the syndrome of resistance to thyroid hormone.[50,90] The mutated TRβ gene products lead to impaired feedback suppression of TSH gene expression. This leads to increased levels of TSH and consequently elevation of T_3 and T_4, goiter, stippled epiphyses, and deafness. The elevated thyroid hormone levels tends to result in a mostly euthyroid state by normalizing the defect. However, this may vary with the activities that are predominately mediated by TRα, resulting in tachycardia. The increased contractility in heart of the RTH patients could be attributed to the relatively low amount of dominant negative TRβ in the context of elevated circulating TH levels and to the sensitivity toward TH.[91] TRβ is necessary for T_3 actions on cholesterol metabolism, and the overexpression of TRα1 may not substitute the role of TRβ in lipid metabolism.[8]

The simultaneous inactivation of both TRβ1 and TRβ2 isoforms in mice did not show any apparent compensation of increased expression from the TRα gene in any of the tissues.[7,92] Lack of TRβ expression in these animals elevated serum thyroid hormone concentration, while TSH levels were higher than in the wild type animals. These phenotypes had distinctive similarities with that of the RTH kindred phenotype. According to the established TR isoforms distribution pattern, TRα is expressed in both the vestibular structures and cochlea but TRβ is expressed only in the cochlea. The TRβ gene knockout animals displayed loss of auditory ability due the functional cochlear defect similar to RTH patients which

Table 1. Tissue Specific Expression of Thyroid Hormone Receptor Subtypes[a]

	TRα1		TRβ1		TRβ2	
tissue	mRNA	% protein	mRNA	% protein	mRNA	% protein
brain	very high	61	moderate	29	nil	10
pituitary	moderate	ND	low	ND	moderate	ND
kidney	moderate	45	high	40	nd	15
heart	high	41	high	41	nil	18
liver	low	13	high	70	nil	17

[a]ND: not done. mRNA levels are represented in order of expression. The table is adopted from tissue distribution of TR isoforms in rats.[94]

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signifies the critical role of TRβ gene in inner ear development.[9] The role of TRβ gene in neuronal development seems to be minimal.

The tissue and receptor specific effects of thyroid hormones are also correlated with the coactivators. For example, the cell response to TH is mediated by the autoregulation of TR isoforms and NCoAs with tissue specificity.[10] Similarly, the transgenic mice with targeted mutation in the TRα gene (TRα1PV mouse) or TRβ gene (TRβPV mouse) indicated that the regulation of genes that are critical for lipid metabolism by TRs in liver is isoform-dependent.[93]

Complete deletion of TRα gene (TR$\alpha^{-/-}$) (mice in which both TRα-1 and *c-erbA*α-2 have been deleted) resulted in severe phenotype which was unable to survive beyond weaning period. The animals had low T_3, abnormal TSH levels, growth retardation, and underdeveloped intestine. However, these animals retained T_3 responsiveness indicated by better survival rate of the animals after T_3 administration to pups in the early stages of weaning.[11] The transgenic animals (TR$\alpha1^{-/-}$) with genotype exhibiting the lack of TRα1 had generated a milder phenotype with decreased body temperature and prolonged QT interval on electrocardiogram indicating the vital role of TRα1 in cardiac functions. The occurrence of Ca^{2+} sparks in TR$\alpha1^{+/m}$ cells raises the possibility of enhanced arrhythmic susceptibility in stressed hearts of the larger animals exhibiting point mutation in TRα1.[12] On the other hand, a novel role of TRα1 for regulation of adaptation of cardiac activity by the autonomic nervous system could be of great value in managing the patients exhibiting a deficit in cardiac adaptation to stress or physical activity and displaying increased sensitivity to β-adrenergic blockers.[13] TR$\alpha1^{-/-}$ mice show that increased oxygen consumption in response to T_3 is mediated by both TRα and TRβ and is independent of heart rate alteration in TR$\alpha1^{-/-}$ mice, suggesting that the development of TRβ-selective agonists could yield potential antiobesity and lipid-lowering therapeutics without the cardiac effects of endogenous thyroid hormone. Similarly, by use of the evidence for myosin isoform-specific regulation by TRs from transgenic knockout experiments, the development of TRα1 selective agents for treatment of congestive heart failure (CHF) was envisaged.[14]

EFFECT OF THYROID HORMONE ON HPT AXIS

The levels of free TH in blood are controlled by the HPT axis: TRH is synthesized in the hypothalamus and stimulates TSH production in the anterior pituitary, the secretion of which induces TH synthesis by the thyroid gland. Increased levels of TH in blood suppress TRH and TSH expression by a negative feedback mechanism. This suppression of TRH and TSH can be induced by thyromimetics to enhance the thyroid activity in target organs (liver in the case of TRβ selective compounds) and suppress thyroid activity in brain. A number of TRβ selective agonists suppressed functions of HPT axis, thereby confirming their TRβ specific activity.[95] Studies on mutant mice (TR$\alpha^{o/o}$) expressing no TRα isoforms showed higher TRH expression and hypersensitivity to TH in terms of TRH regulation than in wild-type control (hyperthyroid) animals. This hypersensitivity to TH could be related to overexpression of hypothalamic TRβ isoforms in TR$\alpha^{o/o}$ mutants or the possible repressive effect of TRα1. The expression of pituitary gene coding for the TSHβ subunit is also modulated by TRβ isoforms.[95] Thus, the ligand mediated regulation of the HPT axis depends on the mechanism eliciting two vital gene expressions in the CNS. These mechanisms could rely on tissue-specific factors, such as the relative levels of synthesis of TR isoforms or comodulators, and on different response elements in the promoters of these genes. From an understanding of the role of TR isoforms, the divergent effects exhibited by TRβ selective agonists on various tissues would be dependent on their ability to influence the transcription mechanisms of vital genes in the respective tissues. By exclusion of this variable, the therapeutic activity of a thyromimetics in the target tissue would be proportional to the tissue specific distribution of the compound of interest. For example, a liver activated prodrug from Metabasis, which is selectively activated in liver (as against heart or brain), has a safety advantage over other TRβ selective agonists with respect to regulation of the HPT axis as well as cardiac hypertrophy, at least in the rodent studies.[88]

STRUCTURAL ARRANGEMENT OF TR BINDING DOMAIN

An extensive mutational analysis has resulted in elucidation of the conformational and structural properties of the thyroid hormone receptors.[53,96,97] TRs that bind the native thyroid hormones (or exogenous thyromimetics) have a similar domain organization as that found in all nuclear hormone receptors and consists of an amino-terminal A/B domain, a DBD, a hinge region containing the nuclear localization signal, and the carboxy-terminal LBD, as shown in Figure 3. LBD is the most

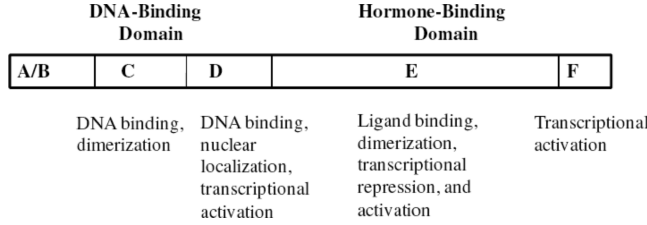

Figure 3. Domain structure of thyroid hormone receptor.

important part when it comes to designing putative thyromimetics. The LBD of TRs is compact with the ligand buried deep within a hydrophobic pocket of the receptor. The LBD of TRs is made by a three-layer antiparallel α-helical sandwich formed by 12 α-helices. This helical arrangement creates a "wedge-shaped" molecular scaffold that maintains a sizable ligand-binding cavity at the narrower end of the domain into which the hormone binds. This arrangement of the ligand binding pockets indicates the role of the TR agonist in protein stabilization. A two-stranded antiparallel β-sheet along with the C-terminal helix 12 (H12) is located on the opposite side of the ligand binding pocket. H12 acts as a key regulating the interaction of the receptor with coactivators or corepressors upon binding with agonist or antagonist, respectively. This is a simplification of the structural rearrangement, since reports indicate the expansion or contraction of the ligand-binding pocket depending on the nature of the binding ligand.[98]

The earliest efforts for the development of the selective TR agonists were carried out with comparisons of the X-ray crystallographic structures of human (hTR) and rat (rTR) TR complexed to 3,5,3′-triiodithyroacetic acid as a ligand.[99] As already discussed, the TRβ as well as TRα LBD consists of 12 α-helices and 4 β-strands organized in 3 layers. The LBDs of the α and β TRs are markedly conserved in residues that contact ligand and show only one amino acid residue difference

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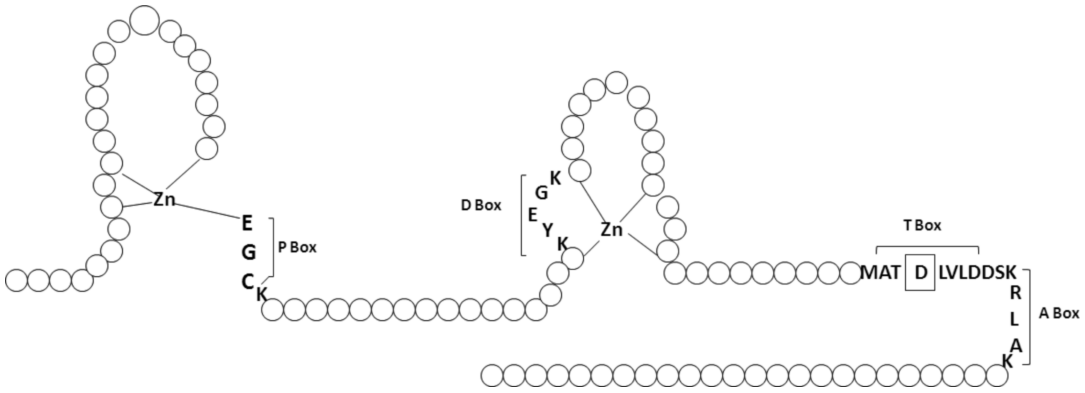

Figure 4. DNA-binding domains of human TRβ receptor.

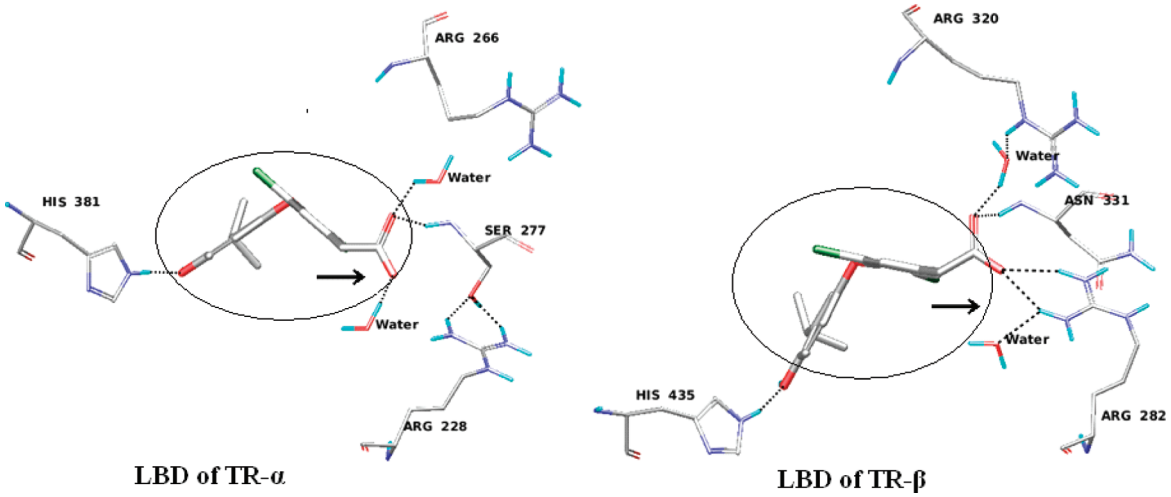

Figure 5. T_3 bound into the ligand-binding pocket.

around the hormone-binding pocket, namely, Asn 331 (TRβ), which is Ser 277 in TRα (Figure 5). This is in contrast to the case with the different subtypes of the RARs or the PPARs and other nuclear receptor gene families. The binding cavity in the LBD is divided into two parts: a hydrophobic portion that contacts the iodinated inner and outer rings of the hormone, and a hydrophilic portion that interacts with the charged, polar substituent at position 1 of the inner ring. The hydrogen bond acceptor consists of His435 at the site of H11, which is relatively closer to H12, while the positive charge is generated by three highly flexible arginine residues, namely, 282, 316, 320.[100] TRs have a dynamic but compact LBD in which H12 can assume both corepressor-favorable and coactivator-favorable conformations in the presence or in the absence of ligand but with different populations in each case. Hence, though the single sequence difference in the ligand-binding pocket mentioned above might generate a significant challenge when considering the rational design of isoform selective ligands, a number of options are available for the design isoform selective ligand.

■ LESSONS FROM EARLY THYROMIMETICS

The early modifications to modulate the structure−activity relationship (SAR) started with T_4 (**B** in Figure 1).[101] This study utilized in vivo rat antigoiter activities and in vitro binding affinities to intact rat hepatic nuclei as a measure of efficacy. These studies provided the first systematic QSAR examination of drug−receptor interactions and of the dependence of in vivo activity on such interactions and concluded that binding to nuclear receptors is the first step in initiating the events that lead to subsequent hormonal expression. This work also suggested factors that need to be considered in designing new analogues.[101]

T_4 is a biaryl ether derivative that is substituted at the R^1-position with an α-alanine group, at positions R^3, R^5, $R^{3\prime}$, and $R^{5\prime}$ with iodines, and at $R^{4\prime}$ with a hydroxyl group. The benzene ring with $R^{5\prime}$ is considered as "outer ring" while the ring on its right side is called "inner ring", as shown in Figure 6.

A hydrogen bond between the $R^{4\prime}$-phenolic hydroxyl group and a histidine in the receptor is important for binding as well as functional activity. Substituents at the R^3, R^5, $R^{3\prime}$, and $R^{5\prime}$ positions interact with hydrophobic regions of the receptor, and the absence of hydrophobic groups in these positions will result in loss of affinity. The R^1-position points into a polar pocket that contains several arginine residues.[102] Receptor structural demands at the R^1-position are low, as arginine residues are highly flexible. Consequently, this position of T_3 analogues can be varied without significant loss of affinity for TR but should optimally be substituted by acidic functions such as the α-amino acid group of T_3. A perpendicular orthogonal relationship between the two aromatic rings of the biphenyl ether scaffold of the ligand, important for the active conformation, is enforced by substitution at R^3 and R^5.[103,104] Hence, the bridging group X is an important determinant of potency and

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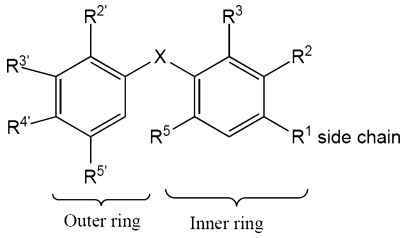

Figure 6. General thyromimetic scaffold.

affinity in thyromimetic design.[105] The $R^{3'}$ substitution with iodine gives the most potent in vitro activity, while the replacement of iodine with isopropyl retains the binding affinity of T_3 and imparts resistance to deactivation by $R^{3'}$ deiodinase activity and thereby improves the potent antigoiter efficacy,[106−108] whereas polar group substitution at $R^{3'}$ reduces binding, resulting in weak TR activity. The presence of a hydrogen bond between the $R^{4'}$-hydroxyl group and His435 in the receptor is important for binding and functional activity. The simultaneous substitution at $R^{4'}$ and $R^{5'}$ is detrimental to pharmacological activity and may lead to the antagonism of the thyroid hormone action.[109,110]

T_4 is mostly converted in peripheral tissues to T_3 by removal of the 5′ iodine group, and the binding affinity of T_3 to TRs is higher than that of T_4.[1,16] The bulkier T_4 ligand gets accommodated by expansion of a niche within the pocket that precisely fits the 5′ iodine group, created by repositioning of the loop between H11 and H12 and rearrangements of amino acid side chains in the pocket relative to the T_3-liganded structure.[81,98,99] The TR−T_4 complex was less stable than the TR−T_3 complex, and hence it was concluded that the preferred ligand binding pocket is that occupied by T_3 and that expansion of the pocket in the presence of T_4 is accompanied by reduced stability of H12.[81,98,99]

The early attempts toward the therapeutic use of the thyroid hormone activity were started with the use of T_3 and T_4. The endogenous hormones L-T_3 and L-T_4 were indicated for hypothyroidism. However, neither of these was useful in lipid lowering or obesity because of adverse events like tachycardia.[37,46]

Since the basis of selective thyroid action was not clear, it was thought that a less potent analogue of thyroid hormone, dextrothyroxine, **1** (D-T_4, Figure 7), might lower plasma cholesterol, sparing the cardiac effects.[111,112] A large trial was conducted to evaluate the lipid lowering activity of compound **1**.[111,112] Although the trial had to be terminated because of sudden mortalities in the T_4 treated group, presumably due to L-thyroxine contamination of pharmaceutical D-thyroxine, it gave a very important signal for development of selective thyroid analogues.[113]

The next stage in the development of selective thyromimetics can be exemplified by the synthesis of **2** (SKF-94901, Figure 7).[114] In compound **2**, bromines replaced the deiodinase susceptible iodines at R^3 and R^5 positions of the native hormones. The substitution of the iodine at the $R^{3'}$ position with a methylenepyridazinone group reduced the liver binding while retaining its lipid lowering activity. Thyromimetic activity of compound **2** was indicated by enhanced metabolic rate and decreased plasma TSH, despite little or no effects on the heart.[114−116] The half-life of the compound was increased by substitution at R^3 and R^5 positions with a bromine group, which blocks the deactivation by deiodinases.[117] It was suggested that the

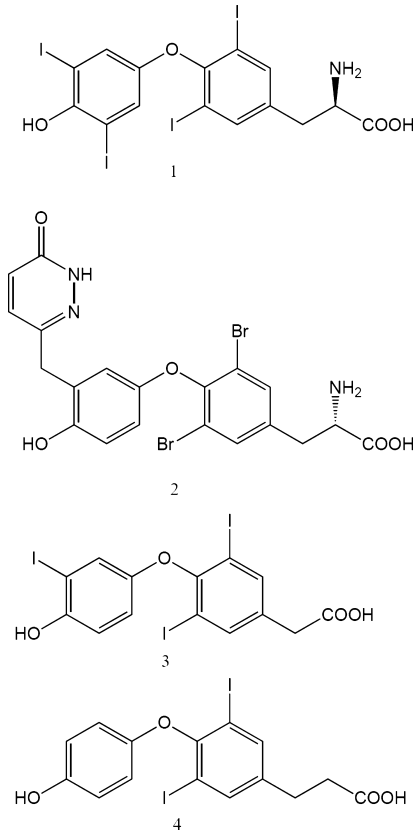

Figure 7. Chemical structures of compounds **1**, **2**, **3**, and **4**.

pharmacological selectivity of compound **2** was attributed to its tissue selectivity elicited by the difference in the tissue transport. The lipid lowering potency with minimal cardiac effect was thus explained by the relatively poor uptake of **2** in heart compared to liver.[118,119] Nevertheless, development of compound **2** failed because of toxicities in kidneys and bones during the preclinical studies.[119]

Another thyroid analogue, 3,5,3′-triiodothyroacetic acid, **3** (TRIAC, Figure 7), was designed empirically, where the R^1-position side chain of T_3 is replaced with an acetic acid side chain.[120] Compound **3** exhibited liver specificity over peripheral tissues with greater affinity for TRβ1.[120,121] It showed potent lipid lowering activity in euthyroid patients at the equipotent doses of T_4 (for TSH suppression), suggesting a liver selective action.[122] Treatment with compound **3** also increased the urinary calcium excretion along with the serum osteocalcin level, which was the concern for accelerating bone demineralization. This concern limited the therapeutic use of compound **3** as a lipid-lowering agent, though it was found effective in the treatment of RTH.[120]

Owing to the clinical findings that the decline in serum T_3 is proportional to the severity of heart failure, it was contemplated that the cardiac effects of TH could be therapeutically beneficial for the treatment of congestive heart failure. A thyromimetic, 3,5-diiodothyropropionic acid, **4** (DITPA, Figure 7), with a propionic acid side chain at the R^1-position and lacking iodides on the outer ring, exhibited cardiac specific actions.[123] It improved cardiac function by increasing α-MHC and reducing the demand for oxygen.[123−125] Treatment with compound **4** also improved cardiac output and shortened diastolic relaxations in patients with heart failure.[126] Treatment with compound **4** resulted in the reduction of total serum

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cholesterol and triglycerides; however, the narrow therapeutic window indicated the search of better analogues.[126]

STRUCTURE–ACTIVITY RELATIONSHIP OF SELECTIVE THYROMIMETICS

Since thyroid hormones have some actions that might be therapeutically useful but others that are deleterious, a number of attempts at selective modulation of thyroid hormone action have been made. Several agents have been shown to have improved β-selective, hepatic selective, and/or cardiac sparring thyromimetic activities. The development of SAR in this area also helped in the understanding of mechanisms for the specificity of action.[35] The SAR was mainly derived using the advances of mice knockout studies that indicated the beneficial effect of an isoform specific hormone action. The transactivation assays and the studies on gene regulation downstream from thyroid hormone receptors were the most important tools for confirming the intricate actions of novel thyromimetics. In addition, X-ray crystallography was vital in deciphering the interactions of novel thyromimetics with the TRs. Apart from the isoform selectivity, tissue specific distribution of thyromimetic was an important parameter that surfaced during the evolution of SAR. The cellular transport and uptake of the thyroid hormones are transporter and energy dependent processes necessitated by the variable intracellular concentrations of thyroid hormones in tissues.[20] Cellular transporters such as Na/taurocholate cotransporting polypeptide (NTCP), organic anion transporting polypeptides (OATPs), and amino acid transporters such as large neutral amino acid transporter (LAT) are the major transporters for thyroid hormones. NTPC, owing to its high density in hepatocytes, is crucial for the liver targeting thyromimetics. The limited exposure to brain would be an important character of HPT-sparing thyromimetic. OATPC1 displays high affinity and allows both import and efflux for T_4 but not for T_3,[127] which suggests that OATPC1 might be an important transporter of T_4 across the blood–brain barrier (BBB). Monocarboxylate transporter 8 (MCT8) was characterized as a highly specific TH transporter, particularly for T_3 in humans, while it has affinity for both T_3 and T_4 in rodents.[128] The expression of MCT8 is most prominent in liver. The TH transport by MCT8 can be compensated by alternative TH transporting pathways barring the CNS in the case of mutations in SLC16A2, the gene encoding MCT8. This mutation leads to severe psychomotor retardations in individuals carrying the mutated gene,[129,130] which is more common than anticipated earlier. Developing a thyromimetic with MCT8 independent transport mechanism across the BBB could be a potential treatment for such patients without eliciting the adverse effects. A novel approach, although present in its nascent stages for TH, to permit the selective expression of desirable gene by targeting the molecular mechanisms of receptor is envisaged.[119,131] The ligand development that locks the receptor to certain conformations might involve an altered balance of coactivator or corepressor recruitment.[39]

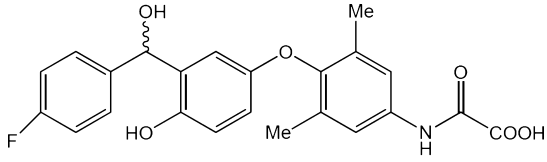
The strategy to modulate the isoform specific thyromimetics is centered on the distribution pattern of α and β TR isoforms. As discussed earlier, the lipid lowering ability of thyromimetics is fundamentally based on TRβ1 expression in the liver while the cardiac effects are mediated by TRα1 expression in the heart.[100] In order to develop a TRβ1 selective ligand for the treatment of hypercholesterolemia, the suppression of its cardiac related effects is of primary importance.[100,132,133]

SUBSTITUTIONS AT R^1-POSITION

The substitutions at the R^1-position in the thyromimetic scaffold do not sacrifice the affinity for TRs. However, increasing chain length was found to have a profound effect on affinity and selectivity in a radioreceptor binding assay for the human TRα1 and TRβ1 receptors. Similar effects were observed in a functional reporter cell assay. The affinity for TRs increased in the order of formic, acetic, and propionic acid, where the β-selectivity was highest when the R^1-position was substituted with acetic acid. The β-selectivity can be further increased with substitution of chlorine at R^3 and R^5 positions compared to bromine, but this variation causes severe loss (approximately 10 times) of affinity for TR.[134]

OXAMIC ACID DERIVATIVES WITH SUBSTITUTION AT R^1-POSITION

Compound **5** (axitirome, Figure 8) has an oxamic acid group substituted at R^1-position of the thyromimetic scaffold.[106,135]



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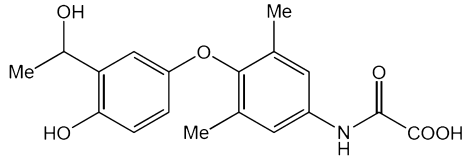

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Figure 8. Chemical structures of compounds **5** and **6**.

The substitution of the oxamic acid group at R^1-position markedly changes the order of affinity for R^3 and R^5 positions, which is reported to increase with Me ~ I > Cl > Br in thyroid hormones. Methyl substitution at R^3 and R^5 positions of oxamic acid derivatives results in compounds with potency similar to that of the natural thyroid hormones.[100] R^3 and R^5 positions of compounds **5** and **6** (CGS-23425, Figure 8) carried a methyl group.[135] In addition to this substitution, compound **5** also featured a large hydrophobic group at the $R^{3'}$-position (α-OH-*p*-F-benzyl group). Compound **5** showed high affinity to liver (HepG2) cells compared with cardiac myocytes, which indicated a selective uptake of the compound in liver.[135] However, the further development of these compounds beyond phase I clinical trials was not reported.

The subsequent work highlighted the significance of the oxamic acid scaffold. Pfizer used the efficacy end points of whole-body oxygen consumption and activity at liver mitochondrial α-glycerophosphate dehydrogenase, instead of cholesterol lowering, to develop these analogues. A combination with statins was chosen as a strategy to achieve efficacy to avoid side effects at the chosen doses.[136]

OXYACETIC ACID GROUP SUBSTITUTION AT R^1-POSITION

The compound 3,5-dimethyl- 4-(4′-hydroxy-3′-isopropylbenzyl)phenoxy acetic acid, **7** (sobetirome or GC-1, Figure 9), is the most widely studied thyromimetic, at least in preclinical

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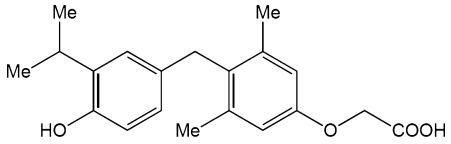

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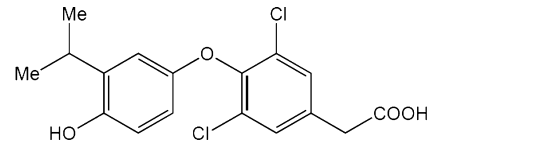
Figure 9. Chemical structure of compound **7**.

studies.[137,138] It has the R^1-position substituted with an oxyacetic acid group, and the bridging oxygen is replaced with a methylene group. In addition, methyl substitutions are carried out at the R^3 and R^5 positions.[137] Compound **7** binds to the hTRβ with several-fold the affinity of the hTRα, exhibiting K_d values of 2.5 and 0.2 nM for binding to TRα1 and TRβ1, respectively.[138] It has lower molecular weight (due to differences in methyl vs iodine moieties), while the presence of the oxyacetic acid side chain provides opportunities for hydrogen bonding in the polar part of the ligand-binding pocket where TRα–TRβ differences are apparent.[138] The selective tissue uptake of **7** was evident with 30-fold less penetration in heart as compared to T_3 where it differentially controls cofactor association[139] and was significantly less prone to modulate the cardiac pacemaker, HCN2.[89] The optimum cholesterol lowering effects of compound **7** were devoid of any cardiac alterations in hypercholesterolemic rats. Additionally, the compound showed marked increase in metabolic rate with a wide therapeutic window between metabolic rate and tachycardia, suggesting a potential antiobesity activity.[140] Lack of cardiac effects in compound **7** was attributed to less prominent up-regulation of SERCA and shifts in myosin heavy-chain isoforms in cardiac ventricular myocytes compared to T_3.[45,141] In clinical development, compound **7** showed a reduction of up to 41% in LDL cholesterol at doses up to 100 μg per day, though a further clinical development report is awaited.[15,142]

■ ACETIC ACID SUBSTITUTION AT R^1-POSITION

The development of 3,5-dichloro-4-[(4-hydroxy-3-isopropyl-phenoxy)phenyl]acetic acid, **8** (KB-141, Figure 10), has been



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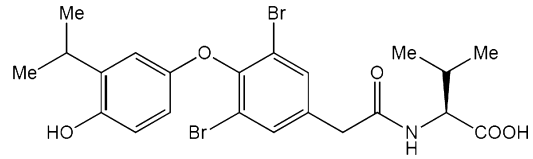

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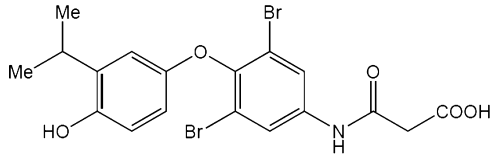

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Figure 10. Chemical structures of compounds **8**, **9**, and **10**.

facilitated by determination of the three-dimensional structures of the ligand binding domains of the α and β isoforms of the thyroid hormone receptor.[143] It is one among a series of compounds developed with the different carboxylic acid substitutions at the R^1 position. The selectivity for the TRβ1 isoform was highest when the R^1-position was substituted with acetic acid. Compound **8** shows potent in vitro affinity and selectivity for TRs, with IC_{50} values of 24 and 1.1 nM for TRα1 and TRβ1, respectively.[110] It showed excellent lipid lowering activity with greater safety margin. Compound **8** has approximately a 10-fold therapeutic window between cardiac side effects and cholesterol lowering, reflecting its 10-fold selectivity for TRβ1 in cholesterol fed rats after subcutaneous (sc) administration. Upon oral administration to cholesterol fed rats, it showed 27-fold selectivity for cholesterol lowering and 10-fold selectivity for metabolic rate versus tachycardia while in primates it showed reduction in lipoprotein-a (Lp(a)) and body weight reduction along with the cholesterol lowering without causing tachycardia. It was also reported to improve insulin sensitivity and has potent antidiabetic and antiobesity activity in various animal models of metabolic syndrome.[143] These antiobesity and antihypercholesterolemic effects are most likely mediated by the selective affinity for the TRβ isoform.[144] Compound **8** failed to reach the clinic owing to its relatively low therapeutic window (with respect to tachycardia), which was considered too tight for human beings. Additional N-acylated α-amino acid derivatives were tested for TRβ1 selectivity and affinity, of which one with R^1-position *N*-acylmethylbutanoic acid substitution, **9** (Figure 10), showed equipotent or improved efficacy in the in vivo and in vitro assays.

■ MALONIC ACID AND HETEROCYCLIC RING SUBSTITUTION AT R^1-POSITION

With further optimization in the thyromimetic series, Karo Bio developed (3-[[3,5-dibromo-4-[4-hydroxy-3-(1-methylethyl)-phenoxy]phenyl]amino]-3-oxopropanoic acid, **10** (eprotirome, Figure 10), which has malonic acid at R^1 and bromine substitutions at R^3 and R^5.[24] In moderately overweight and hypercholesterolemic subjects, compound **10** was found to be safe and well tolerated and elicited up to 40% lowering of total and LDL cholesterol after 2 weeks of treatment. Bile acid synthesis was stimulated without evidence of increased cholesterol production, indicating that the net effect of compound **10** was reduction in circulating cholesterol. The effects of could be mediated by its actions on the LDL or HDL pathway or both and on expression of cholesterol α-hydroxylase, the rate-limiting enzyme in cholesterol breakdown to bile acids.[24] However, because of the lack of preclinical data, it is hard to decipher the exact mechanism for the selective action of compound **10**. It did not produce detectable effects on the heart (increase heart rate, provoked QTc) or serum T_3 in humans, whereas serum total and free T_4 levels were modestly decreased with compound **10** therapy. An explanation for the thyroxine-lowering effect of **10** was increased activity of type I iodothyronine monodeiodinase in the liver, an expected consequence of the increased hepatic action of thyroid hormones. Compound **10** was initially evaluated for the treatment of high risk patients with the hereditable condition heterozygous familial hypercholesterolemia (HeFH) in the EU.

Different heterocyclic ring substitutions were tried at the R^1 position to develop the selective TRβ agonists. Various hydrogen bond acceptors were found to have specific activity for TRβ. R^1-Substituted thiazolidinedione derivative, **11** (Figure 11), exhibited as potent a TRα1 agonist activity as T_3, using recombinant COS-1 cells,[145] although TRβ1 selectivity of these derivatives is not known. By use of the initial information on

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the affinity of these compounds, few TRα1 selective compounds were synthesized. The imidazolidine-2,4-dione derivative, **12** (CO-23, Figure 11), showed high affinity binding for

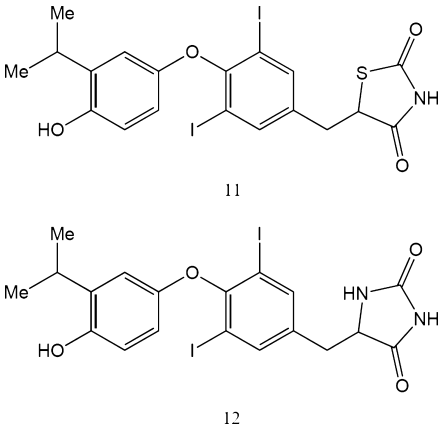

Figure 11. Structures of compounds **11** and **12**.

TRα1 in a DR4-driven dual-luciferase reporter assay using U2OS cells, as well as in transactivation in HeLa cells. Similarly, compound **12** showed potent TRα1 selective actions in *Xenopus laevis* metamorphosis as model system in vivo and was considered as a reliable probe to evaluate TRα1 selective action in vivo.

A more potent series of agonists for TRβ were reported by Pfizer with novel 6-azauracil R^1-substituted ligands. SAR-guided modifications on the R^3, R^5, and $R^{3'}$ positions resulted in compounds with good affinity and improved selectivity for TRβ1.[146] The substitutions on R^3 and R^5 positions increased the potency and selectivity in the order Me, Me < Me, Cl < Cl, Cl, while substitution of $R^{3'}$-position with various relatively large carboxamide and sulfonamide groups imparted TRβ1 selectivity. Compound **13** (Figure 12) of this series possessed

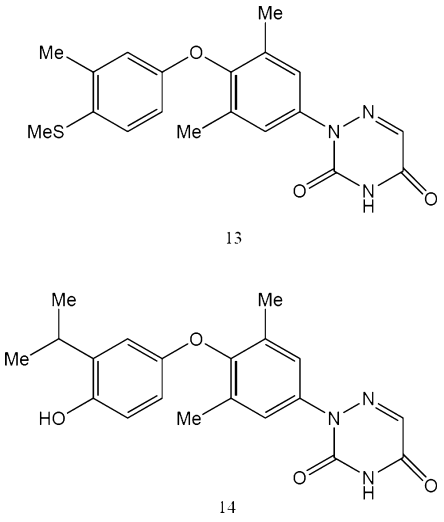

Figure 12. Structures of compounds **13** and **14**.

good TRβ1 selectivity even with the absence of H-donor at the $R^{4'}$-position (H-donator at $R^{4'}$-position considered vital for TRβ1 selectivity and affinity in classical SAR); however, the functional agonism for TRs was lost.[146] Further research on this series presented other molecules with $R^{4'}$-hydroxyl and $R^{3'}$-isopropyl functionalities exhibiting better TRβ1 affinity and selectivity. It was concluded that the 6-uracil moiety was responsible for the TRβ1 selectivity of compounds **13** and **14** (Figure 12).[106]

PHOSPHONIC ACID DERIVATIVES WITH A PRODRUG APPROACH

Because beneficial effects on plasma cholesterol are mediated primarily by activation of the TRβ isoform in liver and deleterious effects on cardiac function are mediated by TRα in heart, the primary approach to improve the therapeutic index of TR agonists has focused on TRβ-selective agents. However, it was contemplated that this strategy does not circumvent other potentially deleterious effects of TRβ activation in extrahepatic tissues, among which are thyroid hormone axis suppression, muscle wasting, and bone loss. The design of a synthetic TR agonist that specifically targets the liver, the major site of cholesterol metabolism, and reduces or eliminates the deleterious cardiac and other extrahepatic side effects should in theory result in an effective therapeutic agent. With this approach, Metabasis developed the liver-activated prodrug, (2*R*,4*S*)-4-(3-chlorophenyl)-2-[(3,5-dimethyl-4-(4′-hydroxy-3′-isopropylbenzyl)phenoxy)methyl]-2-oxido[1,3,2]dioxaphosphonane, **15** (MB07811, Figure 13).[147] The design was based on the nature

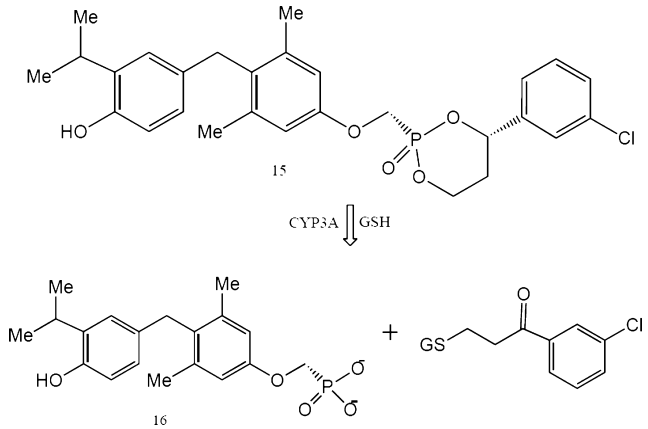

Figure 13. Chemical structures of compounds **15** and **16** and glutathione conjugate (lower right).

of negatively charged phosphonic acids that often differs from that of the corresponding carboxylic acid analogues in their interactions with proteins and their tissue distribution properties at physiological pH. In vivo, phosphonates exhibit a lower volume of distribution[147,148] presumably because of decreased cellular penetration arising from their greater negative charge or reduced recognition by the cellular transporters used to transport carboxylates. Pharmacokinetic studies in rats demonstrated that compound **15** undergoes first-pass hepatic extraction and subsequent cleavage by cytochrome P450, which generates the free methylphosphonic acid, 3,5-dimethyl-4-(4′-hydroxy-3′-isopropylbenzyl)phenoxy)methylphosphonic acid, **16** (MB07344, Figure 13), which is not distributed to extrahepatic tissues and is rapidly eliminated in the bile.[147] In vitro analysis showed that compound **16** has relatively less binding affinity to TR and modest TR selectivity in comparison to compound **8**. The K_i values for **16** were 35.2 nM for TRα1 and 2.17 nM for TRβ1 and the selectivity ratio for TRβ1 was 15.8-fold, whereas compound **8** showed K_i values of 7.18 nM for TRα1 and 0.37 nM for TRβ1 with a

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selectivity ratio of 19.4 for TRβ1 in the same study. On the other hand, HepDirect prodrug compound **15** was selected based on its low affinity for TRs (TRβ K_i = 0.6 ± 0.5 μM; TRα K_i = 12.5 ± 0.6 μM). This compound exhibited excellent cholesterol lowering activity in cholesterol fed rats with decent oral bioavailability (ED_{50} for cholesterol lowering was found to be 0.4 mg kg^{-1} day^{-1}).[88] In normal rats, liver targeting of compound **15** was demonstrated (inducing maximal mitochondrial glycerol 3-phosphate dehydrogenase activity) with improved cardiac sparing. In diet induced obese mice, compound **15** reduced serum cholesterol and triglycerides without any effect on glucoregulation and body weight.[88] As expected, the effects on the HPT axis were minimal at lower doses. The strategy to target a TR agonist to the liver provides an opportunity to achieve the end points of the lipid lowering therapy without adverse effects on extrahepatic tissues and relatively larger therapeutic margin. However, the proof of efficacy of this concept in the clinic is yet awaited.

■ R^2-POSITION SUBSTITUTIONS

The quest for the development of TRβ selective compounds led to the modification of the inner ring structure, consequently opening up the possibility of enhancing TRβ selectivity. The crystal structure of T_3-rat TRα1 complex revealed that a significant unoccupied space exists in the binding cavity next to the R^2-position of the ligand.[149] This observation prompted the synthesis of a series of compounds where the inner ring was fused to produce indoles, quinolines, and indanes.[150] This led to the concept that increasing steric hindrance by fusion of the R^1-position and R^2-position can improve binding affinity. The indole compound, having the inner ring as a 2-carboxylindole and substituted with chlorine at R^3 and R^5 positions, exhibited highest selectivity for TRβ1 (14-fold) and good TR binding (0.06 nM to TRβ1). The activities of bicyclic thyromimetics were highly dependent on the size and shape of the ring, as well as the orientation of the terminal carboxylate group. Arg228 forms two hydrogen bonds with Ser277 in TRα1, while the corresponding Arg282 in TRβ1 also forms a strong bifurcated salt bridge with one of the carboxylate oxygen atoms of the indole derivatives. This bonding was considered important for the TRβ1 selectivity of the indole derivatives, demonstrated by analogue **17** (Figure 14).[150] Similar to the indoles, the quinoline derivatives exhibited excellent binding affinity to TRβ1. The removal of the 4-oxo group from 5,7-dimethyl-6-tetrahyroquinolin-2-carboxylic acid led to synthesis of the more potent quinoline derivative **18** (Figure 14), which improved the binding affinity for TRβ1 by 20-fold.[150] In addition to this, thyromimetics carrying carboxy-substituted benzofurans as replacements for the amino acid side chain of T_3 were synthesized (**19**, Figure 14).[107] The incorporation of a methylene spacer between the benzofuran and the carboxylic acid led to highly potent TR agonists; however, these derivatives lacked isoform selectivity. Treatment of mice with compound **19** for 7 days caused dose dependent reduction in plasma cholesterol (about 32−41%) beginning at 0.1 mg/kg dose. However, this treatment resulted in increased heart weight, indicating that this compound lacked selectivity for TRβ1.[107]

■ REPLACEMENT OF BRIDGING GROUP X

The active form of a thyromimetic needs a perpendicular orthogonal relationship between the two phenyl rings, which is

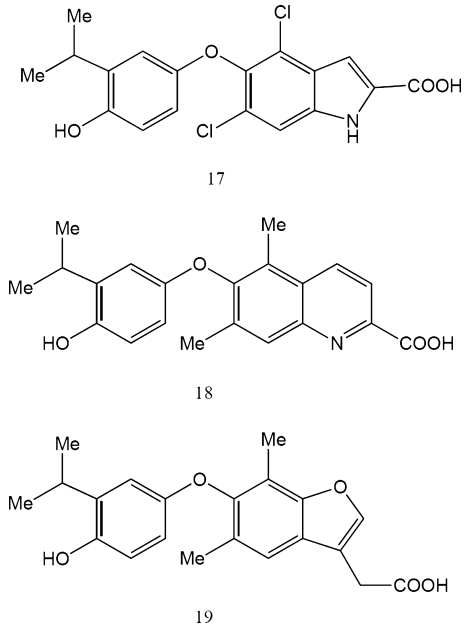

Figure 14. Structures of compounds **17**, **18**, and **19**.

potentiated by substitution at the R^3 and R^5 positions. Hence, any change in the oxygen or methylene as the bridging group "X" has a negative impact on the TR binding affinity.

An analogue of **7** having a long alkylamide extension similar to functional estrogen receptor antagonist **20** (ICI-164384, Figure 15)[151] was synthesized to capitalize the similarity in the LBDs of estrogen receptor α and TRα1. Compound **20** with its long alkylamide chain projecting from the ligand binding pocket results in structural perturbations on the surface of the LBD that interact with coregulatory factors and impart functional ER antagonism on binding to the receptors.[151] The substitution on the linker carbon in **7** actually resulted in a competitive antagonist **21** (Figure 15) of TRs. Further modifications indicated that the larger the bulk of the substituent, the greater is the loss of affinity. Compound **22** (Figure 15) demonstrated consistent TR antagonistic activity.[152] Optimization of the length of the hydrophobic chain is necessary to have potent agonistic activity, which might be due to efficient interactions with coactivators. The potency of the ligands decreases because of space constraints at the site of substitution, which was observed with the 2,4-dimethoxyphenyl derivative **23** (Figure 15).[152]

Replacement of the bridging group with the nitrogen atom also resulted in generation of antagonists. A thiazolidinedione group, a bioisostere for carboxylic acid, was placed at the terminal polar group, leading to synthesis of series of diphenylamine derivatives with very weak partial agonistic activity (**24**, Figure 16). Some derivatives of the series with bulkier substitution of the *N*-alkyl group show potent antagonistic activity indicating that combinations of linker atom and terminal polar group may yield selective TR agonists and antagonists.[152]

A new variation in the bridge -X- was the direct joining of the biphenyl rings, which resulted in novel and potent phenyl-naphthyl analogues.[103] The isopropyl-substituted ligand **25** (Figure 17) was found to bind to TRβ1 with an IC_{50} of 0.3 nM and exhibited 25 times more selectivity for TRβ1 over TRα1.[104] However, the exchange of bromine for the isopropyl (**26**, Figure 17) has led to a major loss of β selectivity.[104] The reason

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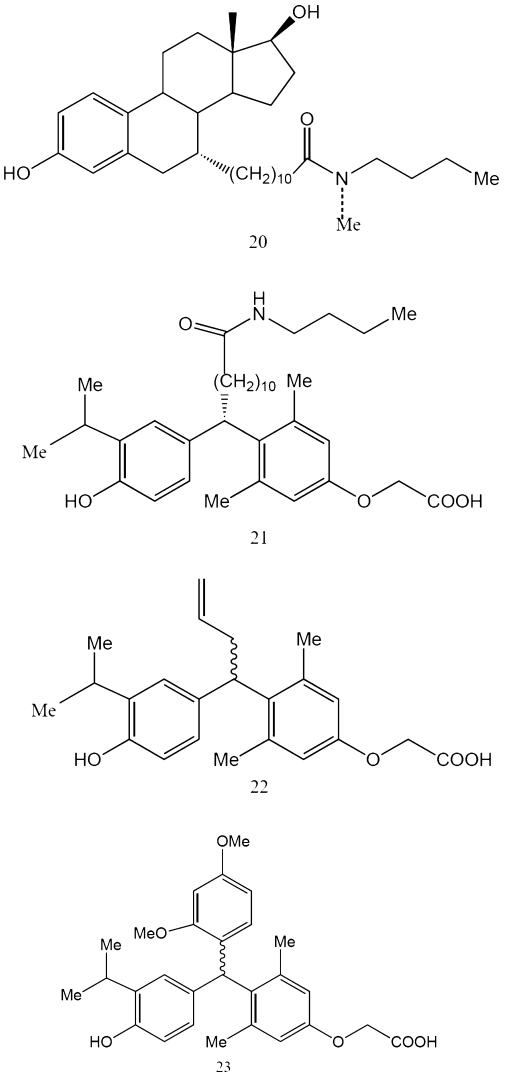

Figure 15. Chemical structures of compounds **20**, **21**, **22**, and **23**.

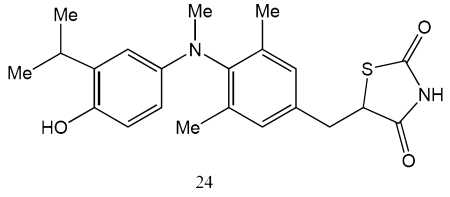

Figure 16. Structure of diphenylamine derivative.

for the loss of TRβ selectivity with the bromine substitution is not very clear. Besides this, the acetic acid substitution at R^1 position (as in compound **8**) in this series of compounds failed to exhibit the expected binding affinity (**27**, Figure 17).[104] The SAR of these compounds significantly differs from those of the classical thyromimetics, which contain a biphenyl ether core.

■ SUBSTITUTIONS AT $R^{3'}$-POSITION

The substitution at the $R^{3'}$-position influences the TR isoform selectivity of the compounds that translated in the improvement of safety in the in vivo effects. The bulk substitution at the $R^{3'}$-position improved the potency and selectivity for TRβ over TRα.[114] 3,5-Dimethyl-4-(4-hydroxy-3-benzyl)benzyl-phenoxyacetic acid, **28** (GC-24, Figure 18), was developed

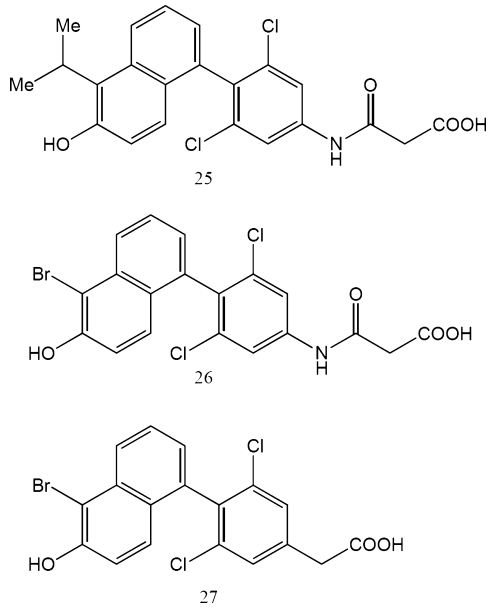

Figure 17. Phenylnapthyl-based ligands **25**, **26**, and **27**.

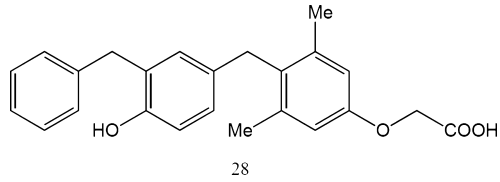

Figure 18. Chemical structure of compound **28**.

using the compound **7** scaffold, in which the bulky isopropyl group at $R^{3'}$ was replaced by the phenyl extension.[108] Compound **28** moves two framework helices in the moiety of the outer ring outside the T_3 binding pocket (water channel) to create additional space, providing novel opportunities for specific agonist receptor interaction, whereas in compound **7** or **8**, binding is dependent on amino acid sequence composition of the water channel in the ligand binding pocket.[108] Compound **28** had potent binding affinity and selectivity for TRβ1, suggesting that the phenyl extension at $R^{3'}$-position improves specificity of binding to TRβ1. It was also confirmed that increased steric bulk alone at the $R^{3'}$-position does not lead to TR antagonism, an assumption based on compound **2**.[116] One of the reasons for the TRβ1 selectivity of compound **28** might be enhanced flexibility in the portion of the TRβ1 receptor binding pocket interacting with the $R^{3'}$-moiety compared with that of the TRα1. The differential stability of the TR isoforms of the TR/RXR heterodimer may also add to the TRβ1 selectivity of compound **28**.[108,114,153]

The SAR derived from compound **8** showed that substitution of the R^3 and R^5 iodine of T_3 by chlorine and truncation of the amino acid side chain at R^1 to acetic acid were the fundamental alterations leading to increased selectivity for TRβ1. The replacement of the $R^{3'}$-isopropyl group with more sterically demanding side chains is a general strategy for gaining improved selectivity for TRβ.[103] The scaffolds studied were $R^{3'}$-phenyls and related heterocycles, $R^{3'}$-phenoxys, $R^{3'}$-amides, and 3,5-dibromophenylacetic acid analogue of compound **8**. The replacement of the $R^{3'}$-isopropyl group of compound **8** with the more sterically demanding phenyl group led to synthesis of **29** (Figure 19).[103] This replacement of the

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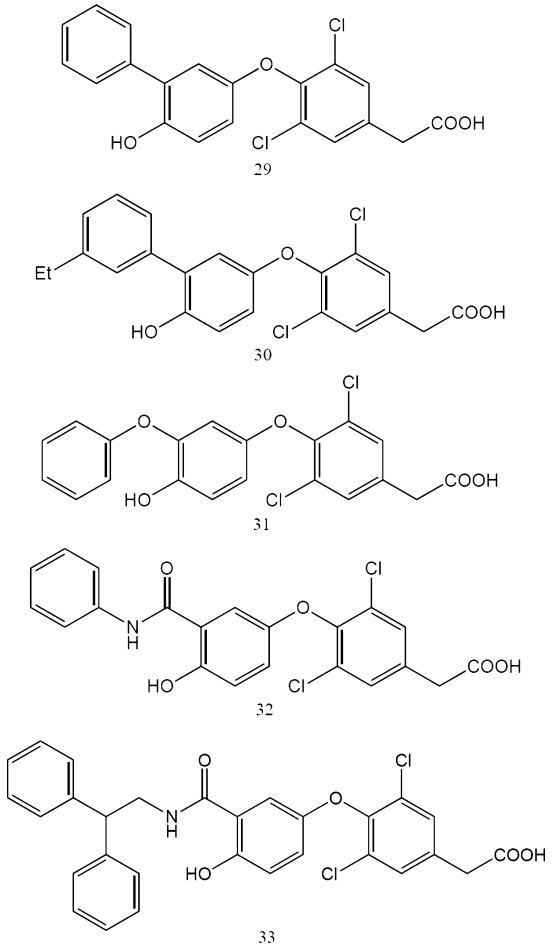

Figure 19. Structures of $R^{3'}$-substituted derivatives, compounds **29**, **30**, **31**, **32**, and **33**.

$R^{3'}$-isopropyl group caused a greater loss of affinity for TRα1, while this led to 30-fold increased selectivity for TRβ1 (TRα1 EC_{50} = 152 nM, TRβ1 EC_{50} = 2.9 nM) in competitive binding assays. Subsequent SAR modifications indicated that substitution with a lipophilic trifluoromethyl group in the meta-position of the $R^{3'}$-phenyl ring could increase binding affinity for both TR isoforms while maintaining TRβ1 selectivity. The rank order of binding affinity for this series was found to be Et > CF_3 > *i*-Pr > Ph; however, the TRβ1 selectivity decreases as the size of the substituents at the meta position increases.[103] The ortho- and para-substituted TR analogues were relatively less selective, while substitution of the phenyl ring with meta-ethyl exhibited high binding affinity and selectivity for TRβ1 over TRα1 (**30**, Figure 19).[103] The study of SAR of other analogues showed that introduction of hydrophilic groups onto the $R^{3'}$-phenyl ring, such as hydroxy, resulted in concomitant loss of binding affinity and selectivity for TRβ1 regardless of its position on the $R^{3'}$-phenyl ring. Systematic placement of groups around the $R^{3'}$-phenyl ring showed a general preference for hydrophobic groups in the meta-position to influence the affinity and selectivity of the ligands.[103]

In addition to this, the simple amide bond coupling with the carboxylic acid of the $R^{3'}$-phenoxy analogue resulted in phenylamide derivative **32** (Figure 19), which has significantly reduced binding affinity and selectivity compared to the phenoxy compound **31** (Figure 19).[103] The homologous series of phenylalkylamides was synthesized and explored for better affinity and selectivity. Among these compounds, **33** (Figure 19) displayed good potency and selectivity, having potent TRβ binding (EC_{50} of 18 and 0.47 nM for TRα) despite challenging the receptor binding pocket with a second terminal phenyl ring.[103] It also indicated that the binding region of the TR is highly flexible and can be explored for further improvement in selectivity. This region of the TR binding pocket sterically interacted with the $R^{3'}$-position moiety to be flexible in such a way that the $R^{3'}$-phenylethyl substituent moved away from Met442β, which then enabled the receptor to accommodate the substituent with retained agonist conformation. This report also suggested that in addition to variation of steric bulk of $R^{3'}$ hydrophobic groups to have selectivity and potency, the alteration in the trajectories and steric bulk of groups located at the $R^{3'}$-position of thyromimetics may assist in designing a partial agonist or an antagonist.[103]

SUBSTITUTIONS AT $R^{3'}$ AND $R^{4'}$ POSITIONS: FUSED HETEROCYCLIC RING THYROMIMETICS

Thyromimetics carrying indoles or indazoles fused to one of the phenyl rings in the biphenyl core led to the synthesis of analogues having moderate TRβ selectivity. Indoles, indazole, benzimidazole, and carbazoles were prepared using this approach. Out of these, benzimidazoles and carbazoles did not show TR agonist activity, whereas indole series showed 10-fold TRβ1 selectivity.[106] An isopropyl group pointing into the 3′-pocket of the receptor is important for affinity and selectivity for TRβ1 isoform. The synthesis of isopropylindole analogue **34** (Figure 20) showed 10-fold TRβ1 selectivity, while phenyl substitution at the β-position of the indole reduced potency by almost 30-fold (**35**, Figure 20).[106] The substitution in the outer ring also exerts an influence on the potency. More lipophilic groups result in higher potency (vinyl < Me, CF_3, Cl < Br), while TRβ1 selectivity was reduced (**36**, Figure 20).[106] However, the oxamic acid (at $R^{3'}$-position) analogues indicated that the substitution at R^3 and R^5 positions with methyl group seem to be an optimal substitution to achieve potency and selectivity. This report suggested that structural modifications in the core region of the TR ligand as well as in the distant carboxy group can be tolerated and may result in potent and selective TRβ selective analogues.[106,107]

SUBSTITUTIONS AT $R^{4'}$-POSITION

It was observed that the TRβ1 binding pocket can accommodate the bulk substitution on the outer ring.[109] For example, the TRβ1 selectivity and affinity were very high with medium-sized alkyl-substituted amido group (isobutyl) analogue **37** (Figure 21).[110] However, the lack of affinity and selectivity observed with 2-methylpropanoylamino derivatives **38** (Figure 21) and **39** (Figure 21) indicated that it was difficult to develop SAR in this series.[110]

SUBSTITUTIONS AT OUTER RING THAT GENERATED TR ANTAGONISTS

Substitutions at $R^{3'}$ and $R^{4'}$ positions on the outer ring has greater implications on functional activity of the analogues. The displacement of the C-terminal helix (helix 12) from a coactivator binding conformation to one that promotes corepressor binding, by the attachment of a large extension group at the outer ring, can change the activity of the ligand from agonist to antagonist. Hence, the approach to design antagonists could be steric displacement of helix 12 or direct blocking of the coactivator site of the receptor. This

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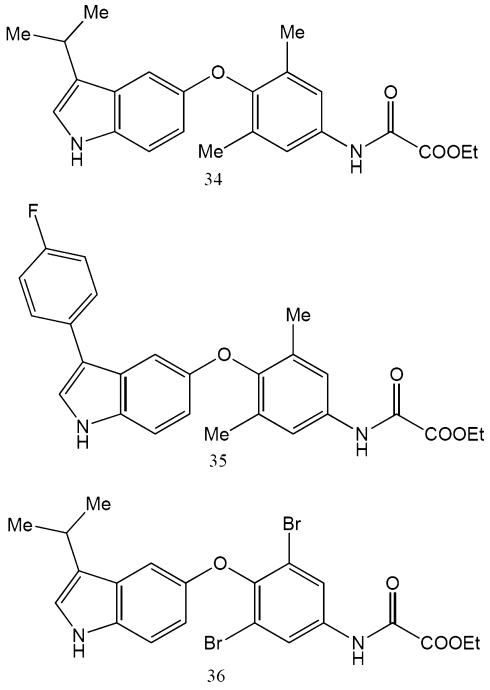

Figure 20. Indole derivatives **34**, **35**, and **36**.

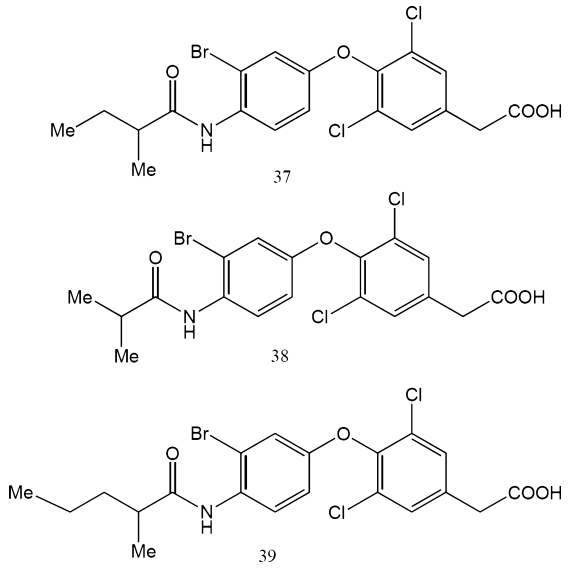

Figure 21. $R^{4'}$-Amido derivatives **37**, **38**, and **39**.

transformation of an agonist into antagonist by introducing a large extension group at the $R^{5'}$-position of T_3 or closely related thyromimetic is termed as direct antagonism approach.[154,155] A classical example for this substitution is 3,5-dibromo-4-(3′,5′-diisopropyl-4′-hydroxyphenoxy)benzoic acid, **41** (DIBRT, Figure 22), which is derived from the thyromimetic 3,5-dibromo-4-(3′-isopropyl-4′-hydroxyphenoxy)benzoic acid **40** (MIBRT, Figure 22).[156] The isopropyl group substitution of compound **40** at the $R^{5'}$-position abolished the TR agonistic activity, and the resultant **41** displayed antagonism in functional assays employing both positive and negative TREs. Compound **41** also restricted the TR binding of T_3 and blocked the formation of the coactivator binding surface.[154,155]

The valuable information obtained by the SAR studies of compound **7** and its X-ray crystallographic structure assisted in

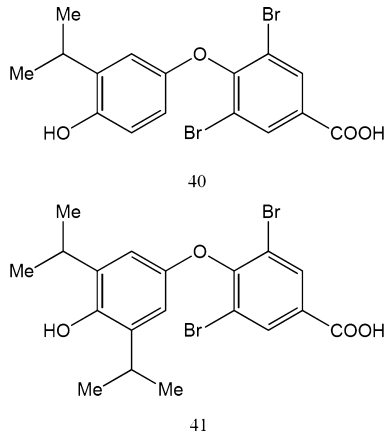

Figure 22. Chemical structures of compounds **40** and **41**.

designing of the TR antagonists.[138] The X-ray structure of compound **7** bound to the TRβ1 LBD revealed that the $R^{5'}$-position is close to the loop between H11 and H12 and that the substitution at this position is not accommodated by the ligand binding pocket.[149] Hence, a variety of $R^{5'}$-substituted phenyl derivatives based on the scaffold of compound **7** were designed to achieve the synthesis of a potent antagonist. The substitutions were tried at both $R^{3'}$ and $R^{5'}$ positions. Only the $R^{5'}$-nitrophenyl derivative [4-(6-hydroxy-5-isopropyl-4′-nitrobiphenyl-3-ylmethyl)-3,5-dimethylphenoxy]acetic acid, **42** (GC-14, Figure 23), was able to demonstrate potent antagonistic activity (K_D values of 200 and 35 nM for TRα1 and TRβ1, respectively), while other derivatives retained their agonistic properties.[138] It was concluded that the position, chemical nature, and electronic properties of the group affect the binding affinity. The size of the substituent at the $R^{3'}$-position had little effect on the TR antagonistic properties, demonstrated by compound **43** (Figure 23) that carries a significantly larger phenyl substituent as compared to compound **42** and still exhibits potent agonist activity.[94] Similar nitro-group substitutions on the scaffold of compound **7** yielded a better antagonist, $R^{5'}$-nitrophenylethynyl-substituted analogue **44** (NH-3, Figure 23).[156,157] However, the functional cell-based assays demonstrated a partial agonist and antagonistic activity of **44**. In addition, compound **44** blocked the thyroid hormone-dependent development in *Xenopus laevis* tadpole metamorphosis assay by restricting TH induced morphological changes in this whole animal assay.[79] Compound **44** is the first thyroid hormone antagonist to demonstrate potent inhibition of thyroid hormone action in both cell culture- and animal-based assays. In mammals, **44** showed both partial agonistic and antagonistic activity at different dose levels. Lower doses of **44** showed antagonist activity on heart rate, cholesterol, and TSH, whereas at higher dose it displayed partial agonist activity in rats.[158] This indicates that **44** demonstrates potent inhibition of thyroid hormone action presumably by displacing H12 in whole animal-based assays, although in mammals the effect is not dose dependent. The SAR of related derivatives showed that creation of large hydrophobic clusters with the ligand and protein component imparts increased affinity with the thyroid hormone receptors.[108,159]

The pyridylvinyl group substitution of the $R^{5'}$-position with its alkene part extends precisely through a well-defined hole between H3 and 11, and the terminal pyridyl group directed

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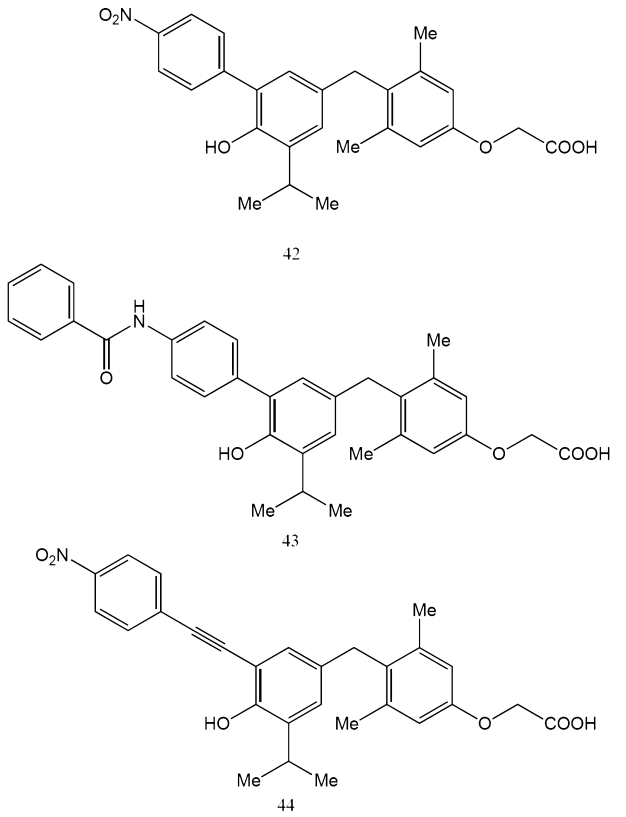

Figure 23. Structures of compounds **42**, **43**, **44**.

into H12 achieves the displacement of H12. This rearrangement by pyridylvinyl-substituted ligand **45** (Figure 24)

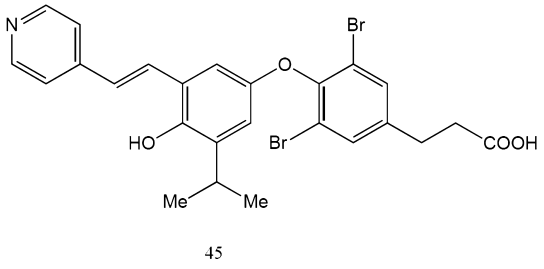

Figure 24. Structure of $R^{3'}$-pyridylvinyl derivative **45**.

provided incremental improvements of affinity and antagonism to TR compared with compound **44**.[160]

An alternative approach to achieve antagonism on TRs was through ligands that are smaller than direct antagonists. This can be exemplified in the structures where the ring containing the phenol moiety in thyromimetics (outer ring) was replaced with straight, branched, or cyclic alkyl entities.[161,162] The antagonistic ligand stabilizes the nonproductive conformation of the key residues in the ligand-binding pocket, thus deviating the equilibrium from the agonist conformation of H12.[161,162]

Various substitutions in this series yielded a potent cyclohexylmethoxyphenyl derivative (**46**, Figure 25), whereas the straight hexyl chain derivative (**47**, Figure 25) displayed approximately 10-fold less potency.[100,132,161] The relative ease of introducing a variable set of functional groups at different positions encouraged the use of benzyl instead of methylcyclohexyl as the outer ring. This strategy resulted in a series of ligands where the outer ring of a thyromimetics was replaced with a substituted benzyl group. Out of these, the 3-bromobenzyloxy derivative (**48**, Figure 25) had optimal affinity and

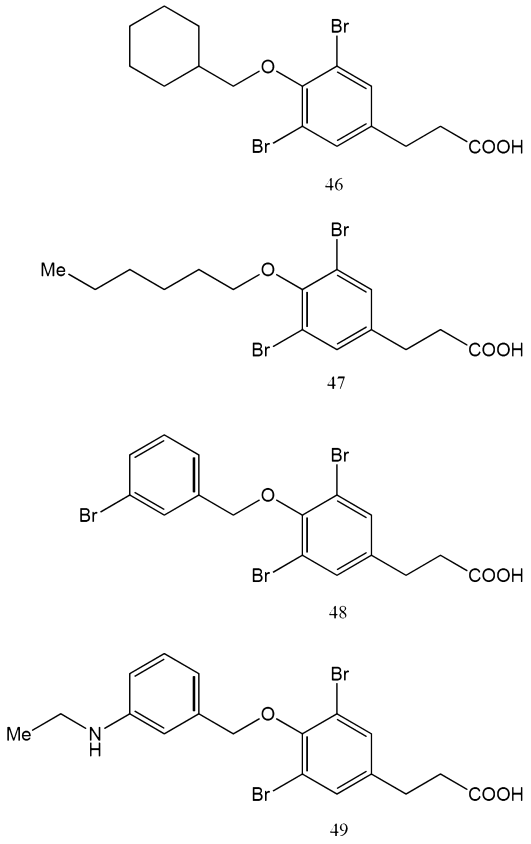

Figure 25. Indirect TR antagonists, compounds **46**, **47**, **48**, and **49**.

selectivity for the antagonistic action.[161] Moreover, the substitution with a secondary amine further improved TR affinity, even though the subtype selectivity with this compound (**49**, Figure 25) somewhat leaned toward TRβ1.[161] This ligand (secondary amine derivative) behaved like an agonist in the transactivation assay. This change might have occurred because of the H-binding capacity of the ligand to His435, which can rearrange the ligand-binding pocket into an agonist binding conformation. There is a limitation to the size of groups that can be accommodated within this region by the ligand-binding pocket of TR. Besides this, the exact orientation of the group regulates the affinity, selectivity, and antagonist activity of the ligand. This strategy showed incremental improvement for affinity.[161,162]

DETERMINANT OF SAR IN THYROMIMETIC CHEMISTRY: KEY TO ACHIEVE SELECTIVE MODULATION

The continued efforts that span more than half of the century have led to the development of a class of selective thyromimetic compounds. Drugs of this type may be useful in treating patients with hypercholesterolemia and various symptoms that occur with perturbations of thyroid hormone excess or deficiencies. These agents will be particularly useful in treating patients who do not respond to conventional lipid lowering therapies (e.g., statins) or those who could not reach the treatment goals with the existing therapies. They also may be used as an add-on treatment for metabolic syndrome, which

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may comprise obesity, hepatic steatosis, congestive heart failure, and type 2 diabetes. The selective binding of the ligand to TR isoforms has vital implications on thyroid actions and its physiological functions. Therefore, it is vital to have a detailed understanding of the basics for generating TR-isoform selectivity. Thyroid hormones act on virtually every biological tissue by controlling the expression of different sets of genes. Typically, thyroid hormones interact with two isoforms of the receptor, TRα and TRβ, located in the nucleus of the target cells, which turn gene transcription on or off and thus mediate the biological effects associated with thyroid hormone release.[86] The TRα (mainly TR α1) isoform has a major functional impact in brain, skeletal muscle, heart, and bone, whereas the TRβ (mainly TRβ1) pathways play a metabolic role in liver and adipose tissue. Studies in TRα and TRβ knockout mice suggested that the effect of native thyroid hormones on plasma cholesterol is mediated through TRβ1. These findings, coupled with the determination of TR structure, led to the design of isoform-specific, TRβ1-selective thyromimetics, such as compounds **7**, **8**, and **10**.[163]

The preceding discussion clearly demonstrates that several factors appear to contribute to the regulation of the actions of various ligands for nuclear receptors including TR. The space available in the ligand-binding pocket of the receptor to accommodate the ligand is a critical factor in designing the agonist or antagonist for the thyroid hormone receptor.[100] Agonists that bind to nuclear receptors fall within a small range of size, since the native hormone occupies a large proportion of the hydrophobic core of the receptor.[164] The shape of the ligand-binding pocket is the dominant factor in the discrimination between retinoid isomers by the RAR and RXR retinoid receptors.[165] However, the similarity in the shape of the ligand-binding pocket of the receptor subtype often allows crosstalk between the two receptor subtypes or the other receptors. Subtle differences between specific receptor side chains can produce discrimination between closely related ligands at the level of a single atom.[166] In addition, adaptation of the nuclear hormone receptor to distinct molecular shapes to permit the ligand binding to the internal binding sites and the recruitment of the coactivators or corepressors is vital for the functional activities elicited by the ligand.[162]

Crystallographic studies have shown that only one amino acid residue (Ser277 in hTRα1, Asn331 in hTRβ1) is different in the ligand-binding cavity of the two receptors. This residue does not directly contact the ligand, but a comparison of the cocrystal structures of TRβ-LBD with compound **7** bound and with T_3 bound shows that Asn331 participates in a hydrogen bonding network with neighboring arginine residues that is arranged differently depending on the ligand present. This flexibility within LBDs can be exploited for designing the TR selective ligand. However, the ligand in complex with the LBD may achieve a rearrangement, which may elicit the desired conformation for the TH action. Some TR ligands with $R^{5'}$ extensions designed to perturb H12 act as antagonists, but other ligands have demonstrated agonist activity. Utilization of the areas of flexibility within LBDs that could accommodate extensions on the ligands may enhance the selectivity of agonist binding to a particular isoform.[98] However, the ligands that induce similar LBD structures may differ in their activities, and this poses one of the challenges to design a selective thyroid modulator. On the brighter side, if the areas of flexibility differ in closely related receptors, then placement of appropriately designed extensions could increase the specificity of ligand binding. The LBDs of TR are mobile, and dynamic structural alterations in LBD structure could play an important role in regulation of receptor activity. The vital information about the protein mobility in the favored configuration of receptor obtained by the X-ray crystallography is, however, limited.[98] More understanding of the dynamic structural alterations that accompany ligand binding and release or cofactor association with help of advance research tools perhaps by spectroscopic analysis of LBD conformation in solution and use of X-ray crystallographic structural models in molecular dynamics computer simulations may assist in the rational approach of designing the thyroid ligands. In addition to this, the ability of the ligand to permit the fixing of H12 in a position that would allow formation of the coactivator/corepressor-binding surface is vital for TR affinity. This ability of the ligand is another dominating factor in the discriminating functional activities of the ligand. For example, stabilization of the conformation is considered to impart TRβ selectivity to compound **3**.[167,168]

The primary SAR of the thyromimetics revolves around the modifications done in the basic thyromimetic scaffold, primarily to achieve TRβ selective analogues. Classically, the R^1 position demands less structural modification, since the neighboring arginine residues in the binding pockets of TR allow high flexibility. This position can be changed significantly without loss of efficacy, provided the side chain contains an H-acceptor, preferably acid. Compounds **3** and **4** are the primary examples of this approach.[122,123,169] Hence, the developments in the past decade indicate that the modifications at R^1 positions are the major determinant of the novelty as well as selectivity of the thyromimetics. Compound **5**, the first prominent example of this class, has an oxamic acid group substituted at the R^1-position of the thyromimetic scaffold.[100] Compounds **8** and **10** are the further developments in this class, indicating that improved selectivity can be achieved using these modifications.[140,170] A more significant modification in this domain was the 6-azauracil substitutions at R^1 positions.[146] Compound **7** has the R^1-position substituted with an oxyacetic acid group, and it differed from the previous compounds in the bridging oxygen, which is replaced with a methylene group. The design of compound **15**, a prodrug of the active moiety **16**, was based on replacing the carboxylic acid in compound **7** with negatively charged phosphonic acids.[147] On the other hand, ring closure by the fusion of R^1 and R^2 positions yielded nonselective thyromimetics.[150]

Structure−activity relationship work on the R^3 and R^5 positions provided compounds with good TRβ1 affinity and improved selectivity.[146] The alkyl substitutions on R^3 and R^5 positions resulted in loss of receptor affinity, while replacing the iodine with bromine was found to be suitable for retention of activity. The bridging between two phenyl rings is very important for thyromimetic activity. It is observed that any change in the oxygen or methylene as the bridging group "X" has negative impact on the TR binding affinity, resulting in antagonists or weak agonists of TRs.[152] The introduction of the phenylnapthyl linkage at the expense of the oxygen or methylene at "X" yielded promising results; however, the SAR was quite heterogeneous with the rest of the thyromimetic class for an effective conclusion.[106] The SAR derived from compound **8** showed that substitution of the R^3 and R^5 iodine of T_3 by chlorine and truncation of the amino acid side chain at R^1 to acetic acid were the fundamental alterations leading to increased selectivity for TRβ1. To further improve selectivity for TRβ1 through modulation of the steric bulk and

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physiochemical properties of the substituents located at $R^{3'}$ of compound **8**, various alterations were tried. The replacement of the $R^{3'}$-isopropyl group with more sterically demanding side chains is a general strategy for gaining improved selectivity for TRβ.[103]

The key findings of the SAR and the development of thyromimetics are summarized in Table 2.

FUTURE DIRECTIONS FOR EXPLORING AND UTILIZING THE PROMISE OF SELECTIVE THYROID MODULATIONS

Though thyromimetics have immense potential for therapeutic use, the potential for this class of drugs has been limited mainly for its use in cholesterol lowering and associated metabolic disorders.[170] The prospect for the treatment of metabolic diseases such as hypercholesterolemia with TRβ1-selective ligands was considered appropriate, since cardiovascular acceleration is mediated through TRα1. The capacity of existing tools for SAR development was, however, limited and partly misleading. On the other hand no information was available on the molecular interactions between compound **10** and TR isoforms.[24] Improved assay using fully characterized receptors, deeper insights of receptor−ligand interactions, and advanced understanding of the molecular biology of nuclear hormone receptors are required to develop new thyromimetics with selective and safer biological profiles. Because of the "druggability" approach in thyromimetic research, this information is still scarce. More studies with thyromimetic using knockout mice models would be required to decipher isoform-specific actions of thyromimetics. It would also be necessary to identify the mechanisms of action of thyromimetic that are mediated by TR monomers, homodimers, and heterodimers. A detailed understanding of the communication of the liganded TRs with the basal transcription factors and the regulation of this machinery by phosphorylation are still awaited. In theory, answers to these questions would be important to selectively modulate desirable genes using thyromimetics. Use of newer technologies that use phage display to detect different receptors or transporter conformation states in the liganded states will be useful in this approach.[171]

Some studies have characterized alternatively spliced TRβ isoforms, which are expressed widely. TRβ3 is a functional receptor, and TR$\Delta\beta$3 is a potent dominant negative antagonist that binds hormone, in addition to those described for TRα1.[52] A tissue specific and hormone-regulated variation in the relative concentration of all the TR variants would modulate target organ responsiveness to thyromimetics. Such a mechanism would be useful for application of suitable thyromimetics in different pathological states.

Despite a lack of the complete understanding of the mechanism, most of the TRβ selective thyromimetics were demonstrated in preclinical animal studies to markedly lower plasma cholesterol without deleterious effects on the heart at the effective dose.[43,142,143] This effect is mainly due to increased LDL-cholesterol plasma clearance through increased LDLR expression in liver.[91,92,146]

In addition, thyromimetic also reduced plasma triglycerides, which are parts of LDL and very low density lipoproteins (VLDL), mainly through their actions on SREBP1.[26,88,89,143] This effect appears to translate to higher species, including monkey and human, as both compounds **10** and **7** reportedly lower LDL cholesterol by 40% and significantly reduce plasma triglyceride, along with Lp(a).[24,142,170,172] The primary care Lipid Treatment Assessment Project (L-TAP) survey results showed that only 40% of patients with two or more risk factors receiving lipid lowering drug therapies were meeting the NCEP ATP II LDL-C goal, and only 18% patients with CHD were meeting the NCEP LDL-C goal.[173] The translation of the thyromimetic effect on cholesterol in the clinic and the need for a large patient population indicate that selective thyromimetics are severely needed. Excess unesterified cholesterol is toxic to cells, especially macrophages, and initiates local inflammation. Sequentially, hyperlipidemia leads to migration of microphages containing cholesterol into the arterial wall, which represents a crucial step of atherosclerotic development. Peripheral (circulating) cholesterol is returned to the liver via HDL for excretion in the bile and ultimately the feces by reverse cholesterol transport (RCT). Novel therapeutic strategies aim to promote reverse transport of cholesterol from such atherogenic macrophages back to liver and/or to promote hepatobiliary flux of excess cholesterol to disturb the vicious cycle taking place in the vasculature.[28] Selective thyromimetics, compound **7** and T-0681 (structure not disclosed), were reported to stimulate the expression of key players of RCT in mice and rabbits.[43,89,100] Both compounds markedly induced the expression of the HDL-receptor SRBI in the liver, stimulated the activity of CYP7α1, the rate-limiting enzyme of bile acid synthesis, and induced the expression of hepatic ABCG5 and ABCG8 (ABCG5/G8), which promote biliary cholesterol secretion. Similarly, thyromimetic compound **10** increases plasma 7α-hydroxy-4-cholesten-3-one, a surrogate marker of the bile acid synthesis, in humans.[24] Extrapolation of the mechanistic data from animal studies to humans indicates that thyromimetics promote RCT in humans, with up-regulation of hepatic SRBI and LDLR directing excessive cholesterol from the periphery to the liver and with activation of hepatic CYP7α1 and ABCG5/G8 directing the cholesterol from liver to the feces. More studies using mechanism-based markers like CETP would further confirm the protective roles of thyromimetics in the atherosclerotic conditions.[42] Statins are the current standards in the LDL lowering therapies. Hence, any new therapy shows work in addition to statins. Indeed, compound **10**, in a dose dependent manner, significantly lowered LDL cholesterol and triglycerides when added to statins.[24] In addition, compound **15** and atorvastatin (structure not disclosed) had additive effects on plasma cholesterol.[172] It was observed that the maximal dose of TRβ selective agonist is more efficacious in cholesterol lowering than statins in primates.[172]

Despite these encouraging advances, drug discovery in thyromimetic area remains a difficult task, since it is difficult to mimic the native hormones for selective actions. The primary untoward effect of any thyromimetic could be tachycardia and cardiac hypertrophy.[44,46] TRβ1-selective thyromimetics were reported to achieve a significant safety margin for the cardiac side effect. By use of the ratio ED_{15}(heart rate)/ED_{50}(cholesterol) as an index of selectivity, compound **8** is 27-fold more selective for cholesterol lowering versus tachycardia as compared to T_3. Similarly, compound **7** was reported to have relative selectivity for cholesterol lowering versus tachycardia of 18-fold, when compared to T_3.[100] Though tachycardia was observed at very high dose, it is expected because of limitations of the pharmacokinetics and other factors involved in the in vivo situation. This safety margin was translated in the clinic, as with efficacy, since both compounds

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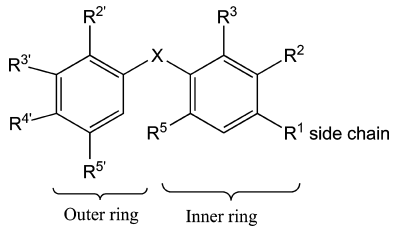
Table 2. Key Thyromimetics



compd	structural features	in vitro potency	SAR comments	receptor selectivity	tissue selectivity	therapeutic development
T_3[1]	$R^{5'}$ = H			nil		
T_4[1]	$R^{5'}$ = I	reduced	H-bond between $R^{4'}$ phenolic hydroxyl group and His345 in receptor is important.	nil		D-T_4 failed because of cardiac toxicity.[116,117]
2[121]	R^3 and R^5 = Br, $R^{3'}$ = methylenepyridazinone	reduced	Substitutions at $R^{5'}$ and $R^{3'}$ both lead to loss of activity. $R^{3'}$ position needs iodine or isopropyl but no polar groups.	nonselective	liver selective	preclinical toxicity in bone and kidneys
3[125,126]	R^1 = acetic acid	increased	R^1 position can be varied with side chain having terminal H-donor.	increased	liver selective	bone toxicity, has good potential in RTH
4[128,131]	R^1 = propionic acid, R^3 and R^5 = H	reduced		nonselective	nonselective	TSH suppression
5[140]	R^1 = oxamic acid, R^3 and R^5 = methyl, $R^{3'}$ = α-OH-*p*-F-benzyl	increased	$R^{3'}$ position can accommodate groups with larger bulk without loss of affinity.	increased	liver selective	This scaffold showed good effects on metabolic activation.
7[142]	R^1 = oxyacetic acid, X = methylene, R^3 and R^5 = methyl, $R^{3'}$ = isopropyl	equivalent	R^1 position is important for TRβ selectivity.	increased	liver selective	Phase I studies showed 41% reduction in LDL cholesterol.
8[143]	R^1 = acetic acid, R^3 and R^5 = chlorine, $R^{3'}$ = isopropyl	equivalent	R^1 position is important for TRβ selectivity.	increased	nonselective	The therapeutic window was not sufficient for translation of safety to clinic.
10[24]	R^1 = malonic acid, R^3 and R^5 = bromine, $R^{3'}$ = isopropyl	not known		not known	not known	Phase III clinical trials terminated because of cartilage toxicity observed after 1 year in dogs.
15 (prodrug that converts to the active moiety **16**)[152]	R^1-phosphonic acid side chain, X = methylene, R^3 and R^5 = methyl, $R^{3'}$ = isopropyl	decreased	R^1 position is important for TRβ selectivity and can be targeted for prodrug approach.	increased	highly liver selective	requires Cyp-dependent metabolism, improves therapeutic index, but reduces efficacy

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7 and 10 did not show any harmful effect on the cardiovascular system after 2 weeks of dosing in humans.[15,24]

Another untoward effect of thyromimetic therapy occurs because of thyroid receptors in tissues like pituitary, bone, and muscle. Primary effect of thyroid stimulation in the pituitary is the dysregulation of the HPT axis that may result in hypothyroidism. The production and secretion of thyroid hormones are regulated by a negative feedback mechanism in humans, which involves hypothalamus, pituitary, and thyroid gland (Figure 2),[1] in which TSH is a primary regulator of TH release and secretion. Increased plasma levels of T_3 and T_4 can reduce TSH release from the pituitary within hours. This rapid reduction in TSH release is followed by a continuing but slower rate of secretions as long as T_3 is still present. Suppression of TSH release is thought to be related to nuclear TR binding and therefore secondary to gene regulation. T_3 not only reduces production of TSH but also acts on the hypothalamus to reduce TRH release. A reduction in TSH and subsequently T_4 (and then T_3) may cause paradoxical hypothyroidism in some tissues. It is anticipated that thyromimetics would mimic T_3 and T_4 while acting on this feedback system,and result in relative and later overt hypothyroidism. However, clinical studies of such consequences were not yet carried out. The TRβ selectivity achieved by selective thyromimetics resulted in an improved therapeutic index for lipid lowering relative to cardiac effects such as heart rate, cardiac hypertrophy, and contractility.[100] However, comparatively lesser improvement was observed in the safety relative to HPT-axis effects, which are largely mediated by TRβ in the pituitary and result in TSH suppression. To achieve the widened therapeutic index with respect to HPT toxicity, a prodrug approach with enhanced liver targeting and subsequent fast biliary clearance allowed for the reduction in circulating plasma levels of the active compounds. Compound **15**, a prodrug, is activated within hepatocytes by enzymatic cleavage and is distributed poorly into other tissues. As a consequence, **15** reduces plasma cholesterol and triglyceride at doses with minimal effects on the HPT, although it still showed HPT axis perturbations at higher doses.[88,171] Further, since this technology requires enzymatic cleavage of the prodrug in liver, it increases hepatic metabolism load that may increase toxicity, more so since for achieving optimum efficacy, comparatively higher amounts of prodrug need to be administered. Tissue selectivity of thyromimetics is thought to be related to high rates of liver first-pass uptake, as well as differences in cellular uptake and retention mechanism.[1] The advanced clinical molecule, **7** is 10-fold more TRβ selective, it enters cardiac tissue 30-fold less efficiently than T_3, suggesting that selective tissue uptake may also explain cholesterol lowering action. Treatment with compound **10**, another clinical candidate, is reported to show effects on parameters of the HPT axis and thyroid hormone homeostasis, including discrete reduction in TSH and more evident reduction in free and total T_4.[170] These data indicate that compound **10** is recognized as a thyromimetic in the pituitary and that compound **10** acts together with the endogenous levels of T_3 and T_4 in the regulation of the HPT axis feedback control of the thyroid hormone homeostasis. Hence, long-term effects of compound **10** on the HPT axis need to be further evaluated in long-term studies. However, Karo Bio has terminated the development of compound **10** because of cartilage damage that was apparent only after 12 months of exposure in dogs treated with high doses in long term toxicological studies. The mechanism of this toxicity is not yet ascertained, although it indicates untoward activity of the compound in nonhepatic tissues.[174]

Several clinical strategies have been suggested to manage this HPT axis risk including dose titration, decreasing potency of the drugs, or increasing liver targeting with prodrug technologies; however, more fundamental changes to the design of thyromimetics are required to resolve this risk and maximize the therapeutic opportunity. Since the liver and pituitary forms of TRβ are similar, a hepatoselective tissue distribution strategy to achieve liver selective activation may have few untoward effects. Liver selective drug discovery approaches have been previously reported for various therapeutic classes including statins.[175] Since designing prodrugs that undergo liver specific metabolic activation has their own limitations, optimizing molecules for recognition and active uptake via liver specific transporters and synthesizing conjugates with liver targeting motifs such as bile acids or statins could be useful avenues. As already discussed, active transport mechanisms are involved in TH transport across the cell membranes.[20,21] A detailed understanding of the transporter systems could be vital in designing molecules that would be targeted to liver with the least systemic exposure. However, hepatotoxicity leading to adverse drug reactions can be a concern for this approach. Although liver injury has been associated with statins, the frequency of such toxicity is lower than that of the background population, and the value of biochemical monitoring remains unproved.[175] On the other hand, unlike statins that induce reduction in hepatic cholesterol synthesis, thyromimetics act through nuclear hormone receptors to increase LDL receptor levels. This may require efficient hepatic extraction but lesser liver residency time to induce a sustained reduction in circulating LDL levels. However, these hypotheses need to be tested in further developments in this area.

THs are among the most potent stimuli of the basic metabolic rate. Whereas hyperthyroid patients (e.g., in Graves–Basedow disease) and hyperthyroid animals show increased heart rate and body temperature, a decreased concentration of TH (e.g., in hypothyroidism, such as in Hashimoto's disease) is associated with reduced heart rate and reduced body temperature.[38] Adaptive thermogenesis is mediated by UCPs, which short-circuit the mitochondrial proton gradient. UCP1 is a major target for T_3 action and has been suggested to be the central mediator of increased heat production in hyperthyroid animals. Interestingly, knockout mice devoid of UCP1 displayed an increased Dio2 activity potentially central to body weight reduction. These and other data suggested that targeting the thyroid hormone–deiodinase system may be the route to effectively control body weight.[19,23,31,32] Overexpression of UCP1 in mice increased energy expenditure and made the mice resistant to diet-induced obesity.[33] Another classical example of massively T_3-regulated gene is the mitochondrial glycerol phosphate dehydrogenase (mGPD), the key enzyme of the glycerol phosphate shuttle. This shuttle transfers reducing equivalents from cytosolic NADH into the mitochondrion by converting cytosolic FADH2 molecule. The difference in energy between these two molecules is used to transport the reduction equivalents against the concentration gradient into the mitochondrion and is finally dissipated as heat. Triggering the glycerol phosphate shuttle therefore results in a net reduction of respiratory efficiency, which ultimately increases heat production. Major interest in this issue was renewed when diet-induced obesity was prevented, and even

reversed, by dietary supplementation with bile acids.[31] Remarkably, these effects do not appear to be regulated by nuclear receptors of bile acids but rather via G-protein-coupled receptor TGR5. In humans, other than in rodents, skeletal muscle is probably a major thermogenic tissue, although recent studies suggested that there is more BAT in adult humans than classically assumed.[35] Although more studies are required to decipher the mechanistic aspect of TGR5 modulation, this pathway holds great potential for therapeutic intervention in the case of obesity.

Apart from its putative antiobesity effects, thyroid hormones could have a beneficial role as an antidiabetic agent, since glucose (and carbohydrate) utilization by nonhepatic tissues is significantly increased by thyroid hormones.[22,23] Thyroid hormones increase peripheral glucose uptake primarily through increased expression of glucose transporters such as glucose transporter type 4 (GLUT-4).[36] On the other hand, thyroid hormone increases glucose production in liver via gluconeogenesis.[22,23]

Newer data suggest that TH effects on glucose uptake may be mediated by TRβ interacting with PI3 kinase, which may offer an additional means of selectively taking advantage of the salutary effects of TH on metabolism.[176] It is therefore difficult to assess the therapeutic potential of thyromimetics as antidiabetic agents. However, compounds **7**, **8**, and **15** are reported to possess antidiabetic and antiobesity activity in various animal models of metabolic syndrome.[88,142−144]

Pancreatic islet β-cell mass is controlled by a dynamic balance between cell proliferation and cell death. Diabetes occurs when this balance is disrupted by autoimmune-mediated cell destruction or by failure of the β-cell mass to compensate for metabolic demand. Interestingly, thyroid hormones increase the number of β-cells mainly through cell cycle progression and proliferation, restore insulin secretion, and improve glucose tolerance in diabetic mice.[177] This effect seems to be mediated by activation of the cyclin D1/CDK/Rb pathway in a TRα dependent manner,[177] whereas another report suggested that TRβ activation is a critical TR isoform in T_3-induced proliferation of hepatocytes and pancreatic acinar cells.[178] Taken together, these potential utilities of selected thyromimetic still make then attractive targets for antidiabetic therapy.

In addition to the promise of a potential lipid lowering agent, thyromimetics may be vital contributors to the development of an array of disorders that are directly or indirectly related to thyroid hormone modulations. One of these is Allan−Herndon−Dudley syndrome (AHDS) caused by mutations in the MCT8 gene, characterized by severe mental retardation and neurological dysfunction. AHDS patients may benefit from therapy utilizing thyromimetics with varied degree of dependence on transport proteins for entering tissues and the quantitative differences in T_4 and T_3 effects on liver compared with brain. It is noteworthy that the possible use of compound **4** for AHDS patients,[171] where compound **4** is effectively transported into the brain even if MCT8 is mutated, could be of immense therapeutic value for the treatment of AHDS patients using thyromimetics.

Mutation or deletion of the TRβ gene has been associated with the syndrome of RTH which is manifested by elevated TH levels as a result of impaired feedback suppression of TSH gene expression due to the TRβ gene.[90,91] The characteristic elevation of cardiac contractility in RTH patients could be attributed to the aporeceptor state of TRβ and reduced tissue sensitivity toward TH.[90,91] Hence, normalizing the sensitivity to TH using thyromimetics with TRβ-selective activity could be beneficial in the management of RTH without causing tachycardia.

The development of TRα1 selective agents for treatment of congestive heart failure is envisaged. The up-regulation of SERCA while exerting minimal effect on heart rate by thyromimetics with relative selectivity and moderate affinity for TRα1 could serve as a better therapeutic tool in CHF therapy.[14] Compound **4** was clinically investigated in humans and underwent phase II clinical trials for treatment of heart failure.[126]

CONCLUSION

Thyroid hormones profoundly influence both development and metabolic processes, and the primary actions of thyroid hormones are mediated by two receptor isoforms, namely, TRα and TRβ. Knockout animal models have represented the ideal tool for advancing the understanding of the functions of these receptors. The early thyromimetics were designed based on the classical QSAR techniques using structural information of LBD of TRs. However, the data derived from the earlier studies were not always conclusive, since transactivation assays, transporter mechanism, and cofactor regulation studies indicated that the thyroid hormone signaling is a more complex and tissue-specific process. However, the profiling of the selective thyromimetic initially in animal models and later in humans provided a remarkable stimulus to better understand the potential of the thyroid hormone system in the regulation of metabolism. Selective thyromimetics have immense potential as a new therapeutic approach for the treatment of dyslipidemia and prevention of atherosclerosis. Moreover, these agents can also be used in the treatment of obesity, diabetes, metabolic syndrome, cardiac failure, and a variety of thyroid disorders. Though mechanism-related side effects are associated with thyromimetic treatment, the future challenge lies in achieving a balanced combination of isoform specific and tissue selective action of thyromimetics using rational drug design.

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■ ABBREVIATIONS USED

ACC, acetyl-CoA carboxylase; AHDS, Allan–Herndon–Dudley syndrome; cAMP, cyclic adenosine monophosphate; BAT, brown adipose tissue; BBB, blood–brain barrier; bHLH, basic helix–loop–helix; CART, cocaine- and amphetamine-regulated transcript; CBP, CREB binding protein; CETP, cholesteryl ester transfer protein; CHF, congestive heart failure; ChREBP, carbohydrate response element binding protein; CTHBP, cytoplasmic TH-binding protein; CYP7α1, cholesterol 7-hydroxylase; DBD, DNA-binding domain; Dio1, type I deioidinase; Dio2, type II deiodinase; DIT, diiodotyrosine; H12, C-terminal helix 12; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDL, high density cholesterol; HL, hepatic lipase; Lp(a), lipoprotein-a; HPT, hypothalamus–pituitary–thyroid axis; LBD, ligand-binding domain; LDL, low density cholesterol; LDLR, low density cholesterol receptor; L-PK, liver-pyruvate kinase; MCT8, monocarboxylate transporter 8; MDR, multidrug resistance; MHC, myosin heavy chain; MIT, monoiodotyrosine; NcoR, nuclear receptor corepressor; NIS, Na^+/I^- symporter; NPY, neuropeptide Y; NTPC, Na/taurocholate cotransporting polypeptide; OAPT, organic anion transporting polypeptide; PLB, phospholamban; PVN, paraventricular nucleus; QSAR, quantitative structure–activity relationship; RAR, retinoic acid receptor; RCT, reverse cholesterol transport; RTH, resistance to thyroid hormone; rT_3, reverse T_3; RXR, retinoid X receptor; SAR, structure–activity relationship; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase; SMRT, silencing mediator for retinoid and TH receptors; SRBI, scavenger receptor class B type I; SRC-1, steroid receptor coactivator 1; SRE, sterol response element; SREBP-2, sterol regulatory element binding protein 2; TBG, thyroxine binding globulin; Tg, thyroglobulin; TH, thyroid hormone; TI, therapeutic index; TR, thyroid hormone receptor; TRAP, thyroid hormone receptor associated protein; TRAP, thyroid hormone receptor auxiliary protein; TRE, thyroid hormone response elements; TRH, thyrotropin releasing hormone; TSH, thyroid stimulating hormone; TSHr, thyroid stimulating hormone receptor; UCP-1, uncoupling protein 1; WAT, white adipose tissue

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