Recent Advances in the Development of Agonists Selective for β_1–Type Thyroid Hormone Receptor

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Abstract: This mini-review will provide an overview on the recent design principles and structure-activity-relationship of β-selective thyroid hormone receptor (TR) agonists. The prospects for the treatment of metabolic diseases as dyslipidemia with TRβ-selective ligands are considerable enough so as to avoid cardiovascular acceleration mediated through TRα.

Key Words: Nuclear hormone receptors, thyroid hormones receptors, thyroid hormones agonists, X-ray, metabolic rate, cholesterol, heart rate, structure-activity-relationship (SAR), beta-selective.

BIOLOGICAL ROLE OF THYROID HORMONE RECEPTORS AND THYROID HORMONES

The thyroid hormone receptor (TR) belongs to the family of nuclear receptors that comprise a class of intracellular, mostly ligand-regulated transcription factors (48 human members) [1-3]. Fundamental genes in intestinal, skeletal and cardiac muscles, the liver and the central nervous system are regulated by thyroid hormones (THs), and influence metabolic rate, lipid levels, heart rate, and mood [4,5]. THs are currently used as replacement therapy for patients with hypothyroidism, characterized by low circulating levels of endogenous hormone. The most active endogenous thyroid receptor hormone 3,5,3'-triiodo-L-thyronine (**1**, L-T_3) (Fig. (**1**)) exert profound effects on growth, development and homeostasis in vertebrates. Although of less intrinsic potency than **1**, 3,5,3',5'-tetraiodo-L-thyronine (**2**, L-T_4) (Fig. (**1**)) is the most commonly prescribed of the natural hormones. 2-[4-[4-Hydroxy-3-iodophenoxy]-3,5-diiodophenyl]acetic acid (**3**, TRIAC) (Fig. (**1**)) is another known metabolite of L-T_3 (**1**) that exhibits good *in vitro* potency and has been investigated for potential applications to hyperlipidemia and osteoporosis [6]. Exciting prospects exist, however, for medicinal intervention that significantly surpass the current limited medicinal use of these endogenous compounds.

There are two major subtypes of the thyroid hormone receptors, α (TRα) and β (TRβ), expressed from two different genes. Differential ribonucleic acid (RNA) processing results in the formation of several isoforms from each gene. The $TR\alpha_1$, $TR\beta_1$ and $TR\beta_2$ isoforms bind thyroid hormones and act as ligand-regulated transcription factors. The $TR\beta_1$ isoform is prevalent especially in the liver and to a lower degree in the heart. The $TR\beta_1$ splice variant is the predominant systemic form, while the pituitary form is predominantly $TR\beta_2$. The $TR\alpha_1$ isoform is also widely distributed, although its levels are generally lower than those of the $TR\beta_1$ isoform. The literature suggests that most of the effects of thyroid hormones on heart, particularly on heart rate and rhythm, are mediated through activation of the $TR\alpha_1$ isoform, while most of the actions of the hormones on the liver (e.g. lipid-lowering) and other tissues are mediated through activation of the $TR\beta_1$ isoform [7-10]. Consequently, thyroid hormone analogues, either tissue selective or isoform specific, could potentially be used to treat a large number of conditions associated with the expression of L-T_3 (**1**) regulated genes without cardiovascular acceleration and toxicity.

STRUCTURE OF THYROID HORMONE RECEPTORS

Members of the family of nuclear receptors are identified by the presence of a highly conserved deoxyribonucleic acid (DNA) binding domain and a structurally conserved ligand binding domain of about 250 residues. The receptors also have an unconserved N-terminal domain of variable length. Several nuclear hormone receptors, including peroxisome-proliferator-activated receptors (PPARs), retinoic acid receptor (RAR), TRs and liver X receptors (LXRs) are also transcriptionally active as heterodimers with the retinoid X receptor (RXR) [11,12]. TRs function as homodimers or heterodimers and modulate transcription activity (repression or activation of transcription) by interacting with co-repressor and co-activators when bound to a thyroid response element (TRE). Unlike steroid receptors, the TR/RXR heterodimer binds to DNA in the absence of ligand (apo-form) that represses transcription (Fig. (**2**)). These intracellular events are reviewed in detail in reference [13].

Human $TR\alpha_1$ and $TR\beta_1$ is 410 and 462 residues long respectively. The difference in length is mainly due to the larger N-terminal domain of $TR\beta_1$, which results in the different sequence numbering in $TR\alpha_1$ and $TR\beta_1$. Several TR-structures of both isoforms have up to this date been determined and can be found in the literature [14-21]. Like most nuclear hormone receptors the ligand binding domain (LBD) of TR is characterized by a 3-layer antiparallel α-helical sandwich formed by 11 α helices. This helical arrangement forms a wedge-shaped molecule with the ligand binding pocket located in the narrower part of the domain. The remaining secondary element consists of a 2 stranded, anti-parallel β-sheet and the C-terminal helix 12 (H12) that are located on opposite sides of the ligand binding pocket (Fig. (**3**)).

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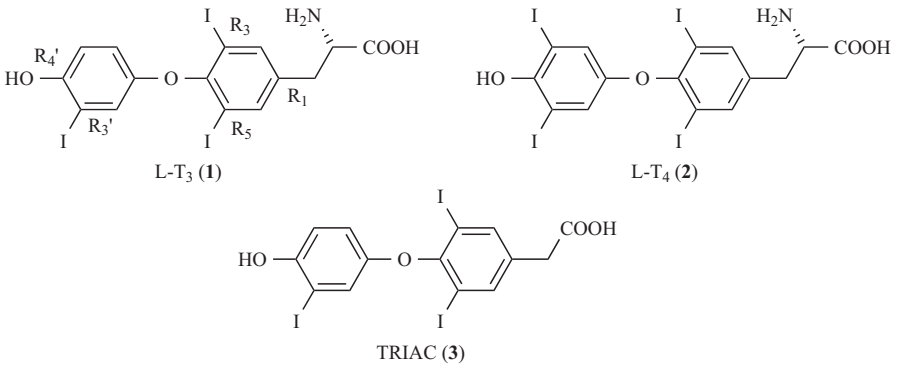


Fig. (1). Chemical structures of L-T_3 (**1**), L-T_4 (**2**) and TRIAC (**3**), including ring-numbering of L-T_3 (**1**).

H12 serves as a molecular switch by allowing the receptor to interact with co-activator proteins when an agonist is bound, and with co-repressors when antagonist or apo-bound. Structural studies of the RXR apoform estrogen receptor (ER) antagonist raloxifen compared to agonist bound forms of several receptors led to the "mouse trap hypothesis". In this hypothesis H12 acts as a lid to the ligand binding pocket of the LBD, which has to be closed to allow coactivators to bind and is open in the apoform [15,22,23].

The ligand binding pocket has a volume of approximately 440 $Å^3$. The ligand binding pocket has a hydrogen bond acceptor constituted by His435 (TRβ_1 numbering) close to H12 at one end of the receptor and a hydrophilic positively charged area at the other end. The positive charge is generated by three flexible arginine (282, 316, 320) residues on the β-hairpin tip of the β-sheet. The cavity between the arginines and His435 is mainly covered by hydrophobic residues. TRα_1 and β_1 are highly homologous proteins and the only sequencial difference in the direct vicinity of the ligand binding pocket is Ser277 and Asn331 in TRα_1 and β_1, respectively. That sequencial difference is located on the tip of the β-hairpin loop of the β-sheet. At first glance, this perceptibly generates a significant challenge when considering rational design of isoform selective ligands, but as will be outlined below there are several other options available for design of TRβ_1-selective ligands.

EARLY STRUCTURE ACTIVITY RELATIONSHIP OF THYROID HORMONE RECEPTOR LIGANDS

Prior to the discovery of TR subtypes and their purification and structural elucidation, the historical *in vitro* structure activity relationship (SAR) of thyromimetics was driven by data generated using competition binding assays with unpurified TRs obtained from sources such as rat livers cells. In addition, the *in vivo* activity of early thyroid hormone analogues was measured using an antigoiter assay in the rat. Excellent reviews are available that describe these assays and summarize this large body of work [24-28]. These findings have been remarkably well validated by the general conclusions from the x-ray picture of ligand binding discussed earlier. The section below summarizes the essential *in vitro* SAR findings of thyroid hormone analogs developed prior to 1990. For ring-position numbering see Fig. (**1**).

1) A hydrogen bond between the R_4'-phenolic hydroxyl group and His435 in the receptor is important for binding as well as functional activity.

2) Substituents at the R_3, R_5, and R_3'-positions interact with hydrophobic regions of the receptor and the absence of hydrophobic groups in these positions will result in significant loss of affinity.

3) A perpendicular orthogonal relationship between the two aromatic rings of the diphenyl ether scaffold of the ligand, important for the active conformation, is enforced by substitution at R_3 and R_5.

4) Receptor structural demands at the R_1-position are low as arginine residues are highly flexible. Consequently, this position can be varied without significant loss of affinity for TR but should advantageously be substituted by an acidic group.

5) At R_1 the L-amino acid moiety found in **1** is not critical for activity, and the amino group can be dispensed with entirely.

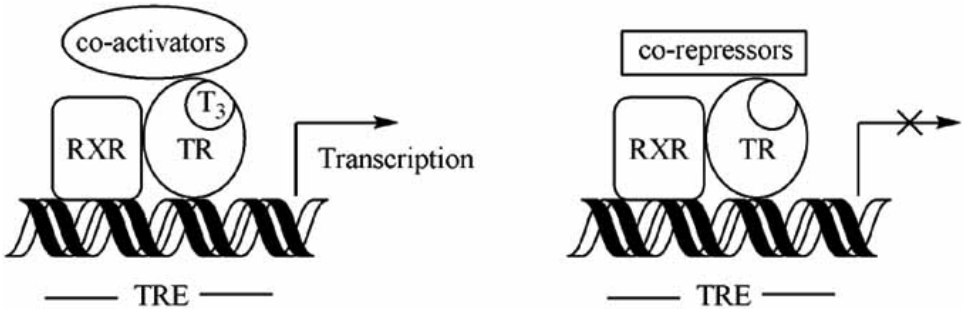


Fig. (2). RXR/TR heterodimer and role of co-repressor and co-activators in transcriptional regulation of nuclear hormone receptors.

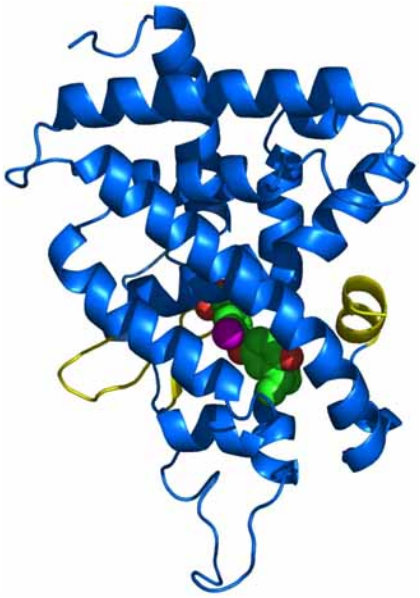


Fig. (3). The three dimensional structure of the ligand binding domain of TRβ_1 in complex with KB141 (1nax.pdb). The ligand (green) is surrounded by α helices (blue). H12 (yellow) covers the right entrance of the pocket while a β-sheet (yellow) blocks the opposite side.

After 1990 it became clear that the classical SAR conclusions based on L-T_3 (**1**) may not hold for compounds with alternative groups at R_1. For example, if the R_1 group was an oxamic acid, the analogs would bound to rat liver nuclear TRs better than L-T_3 (**1**). Ethyl 2-[4-[3-[[4-fluorophenyl][hydroxy]methyl]-4-hydroxyphenoxy]-3,5-dimethylphenylamino]-2-oxoacetate (**4**, Axitirome, CGS-26214) and N-[3,5-dimethyl-4-(4-hydroxy-3-isopropylphenoxy)-phenyl]oxamic acid (CGS-23425, **5**) was one of the most important members of a new extensively studied class of R_1-position oxamic acid thyromimetics [29-32] (Fig. (**4**)). The structure of Axitirome (**4**) consist of a large hydrophobic group at the R_3'-position (α-OH-p-F-benzyl group) and alternative substituents at R_3 and R_5 (bromines), a pattern of substitution somewhat reminiscent of that found in the earlier 2-amino-3-(3,5-dibromo-4-[4-hydroxy-3-(6-oxo-1,6-dihydro-pyridazin-3-ylmethyl)phenoxyl]phenyl)propionic acid (SKF-94901, **6**) [26,33-35] (Fig. (**4**)). Since Axitirome (**4**) exhibited a 100-fold preference for binding to nuclei of HepG2 cells over cardiac myocytes [36], selective tissue uptake was proposed to explain the compound's selectivity, as demonstrated for the first-generation selective thyromimetics such as SKF-94901 (**6**) [37]. In pre-clinical animal models, Axitirome (**4**) demonstrated potent cholesterol lowering activity free of T_3-like cardiovascular and thermogenic effects [38]. In transient transfection assays in human fetal hepatoma cells using CAT reporters with rat apoA1 promoters, CGS-23425 (**5**) increased transcription with an EC_{50} = 0.002 nM for TRβ_1 versus an EC_{50} = 1 nM for TRα_1. Citing the greater abundance of TRβ_1 in the liver versus TRα_1 in the heart [39,40] it was proposed that subtype selectivity also contributes to the hepatic selectivity and relative cardiac safety of CGS-23425 (**5**). Although Axitirome (**4**) progressed through Phase I clinical trials for cholesterol-lowering, its development, including that of CGS-23425 (**5**), was subsequently discontinued in 1998 for unknown reasons [41]. The SAR of this class of compounds has been reviewed in larger detail [28].

RECENT DEVELOPMENT OF THYROID HORMONE RECEPTOR AGONISTS

Due to the increased interest of selective thyroid hormone agonists, parts of the recent achievements have already been covered in the literature [42,43]. The continuation of this overview will, however, emphasize strategies to achieve TRβ_1 selectivity in the more recent literature. For the sake of clarity and reference, a compilation of all ligand TR-binding data discussed henceforth is shown at the end of this section in Table **1**.

As mentioned above, receptor structural demands on the R_1-position are low as its moiety involves a number of highly flexible hydrophilic amino acids. Consequently, this position can be varied without significant loss of affinity for TR. The basic SAR involving variation of the R_1-position by substitution of simple alkyl carboxylic acids has been published [18] and also reviewed in larger detail [42]. Within

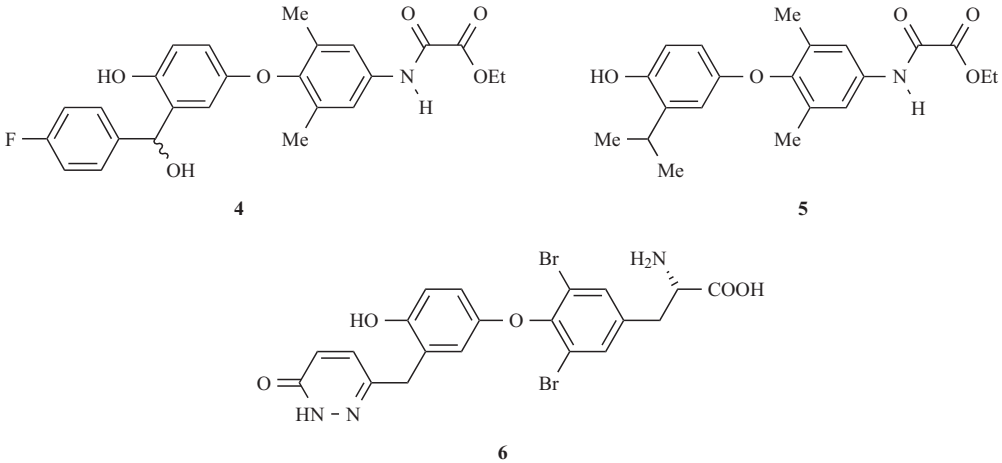


Fig. (4). Chemical structures of Axitirome (**4**, CGS-26214), CGS-23425 (**5**) and SKF-94901 (**6**).

Table 1. Thyroid hormone receptor binding affinities for L-T_3 (1) and synthetic thyromimetics 7-15[a]

Compound	TRα_1	TRβ_1	α/β [b]	Reference
L-T_3 **(1)**	0.4	0.3	0.8	[44]
KB141 **(7)**	25	1.1	13	[44]
GC-1 **(8)**	1.8	0.1	18 [c]	[48]
9	13	0.20	38	[19]
GC-24 (**10**)	13	0.33	41	[56]
11	[d]	0.59	105 [e]	[17]
12	[d]	4	10	[57]
13	240	4.3	33	[58]
14	14	0.3	25	[59]
15	1.4	0.060	14	[60]

[a] Data for L-T_3 **(1)**, KB141 **(7)**, **9**, **11**, **13**, **14** and **15** are expressed as IC_{50}'s, and GC-1 **(8)** and GC-24 (**10**) as K_D's. Data for **12** are provided as EC_{50}'s derived from TR-transfected HUH-2 cells. All values are given in nM. [b] "Normalized" selectivity was determined for compounds **1**, **7**, **9**, **13**, **14** and **15** (IC_{50} hTRα_1 / (IC_{50} hTRβ_1 x 1.7). For an explanation see reference [18]. [c] In reference [47] this selectivity ratio was only 7 times. [d] Data not given in the corresponding reference. [e] This ratio was calculated as the ratio of TRβ_1K_i / TRβ_1K_i and appears therefore to be uncorrected.

this series 3,5-dichloro-4-[(4-hydroxy-3-isopropyl-phenoxy) phenyl]acetic acid (**7**, KB-141) (Fig. (**5**)) was found to reveal the most promising *in vitro* data in terms of TRβ_1-selectivity.

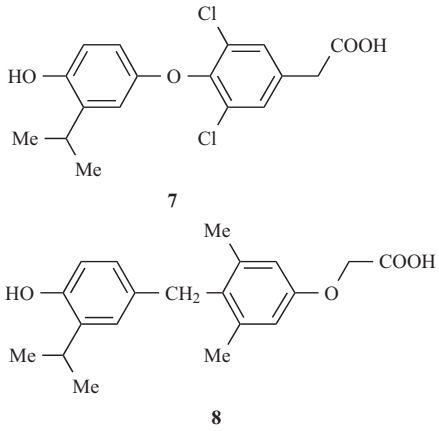


Fig. (5). Chemical structure of KB141 (**7**) and GC-1 (**8**).

In a radioligand binding assay for the human TRα_1 and TRβ_1, KB141 (**7**) bound with IC_{50}'s of 25 and 1.1 nM, respectively, and displayed full agonism in a transactivation assay. The reason for its TRβ_1 selectivity is most probable the difference in the single amino acid in the ligand binding pocket (Ser277 in TRα_1 or Asn331 in TRβ_1). This produces a conformational difference between Arg228α and 282β that positions the terminal guanidino N-ω and -ω' atoms of Arg282β closer to the ligand carboxylate atom. The net result is an extra bifurcated salt bridge between Arg282β and the ligand carboxylate that is not present in TRα_1. This bifurcated salt bridge probably accounts for the majority of the increased selectivity of KB141 (**7**) for TRβ_1. The importance of the interaction between Ser277α/Asn331β and Arg228α/282β regarding TRα_1 and TRβ_1 in their binding to TRIAC has been proposed previously [16]. The most important molecular interactions of KB141 (**7**) (as well as for other similar compounds) are shown in Fig. (**6**).

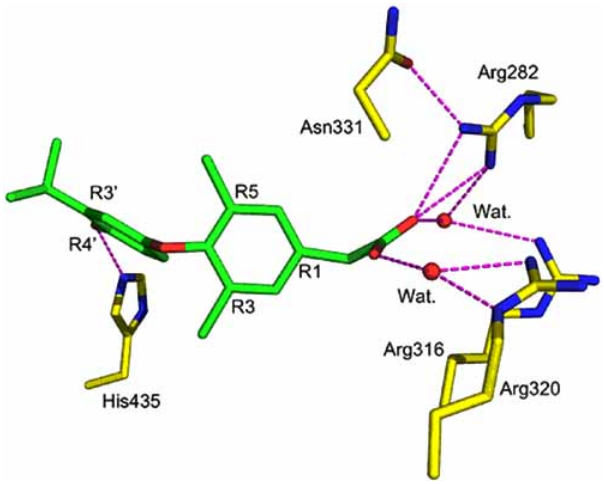


Fig. (6). The binding of KB141 (green) in complex with TRβ_1 (yellow) (from the pdb coordinates of 1nax.pdb at http://www.rcsb.org) oxygen atoms in red and nitrogen atoms in blue. The red spheres are defined water molecules. The ligand binding pocket is built up by several hydrophobic residues defining the shape of the pocket (not shown). At the left side is His435 coordinating the R_4' hydroxyl and at the left do three arginines take care of the negatively charged carboxyl group of the ligand. Asn331 is the only residue in the ligand binding pocket that differs between TRα_1 and α_1 (The corresponding residue in TRα_1 is Ser277).

The effect of the KB141 (**7**) in a cholesterol-fed rat model using p.o. administration gave 40-fold selectivity for cholesterol lowering (ED_{50}) and 12-fold selectivity for in-

crease of metabolic rate (MVO_2, ED_5) versus tachycardia (ED_{15}). In the same model, L-T_3 (**1**) caused significant tachycardia at doses causing cholesterol reduction. In primates, KB141 (**7**) caused significant cholesterol, Lp(a) and body weight reduction with no tachycardia [44]. The biological properties and design of KB141 (**7**) have been reviewed in greater detail [45,46].

Another example of a novel variation of the R_1-group as well as a replacement of the biaryl ether linkage with a methylene linkage is 3,5-dimethyl-4-(4-hydroy-3-isopropyl-benzyl)-phenoxy acetic acid GC-1 (**8**) (Fig. (**5**)). This is a ligand where the R_1-position substituted with an oxyacetic acid group and the bridging oxygen is replaced with methylene. It exhibited TRβ_1-selectivity in the range of 7-18 times [47,48] and a cellular transcription assay of the compounds was also performed that showed more than 10-fold preference for TRβ_1 in transactivation [47]. In hypercholesterolemic rats, GC-1 (**8**) lowered serum cholesterol at doses that did not affect heart rate [49]. More importantly, a 10-fold separation between therapeutic increases in metabolic rate and tachycardia was found for this compound in this study, suggesting the possibility for anti-obesity therapy [50]. While being 10-fold more selective for TRβ_1, GC-1 (**8**) also appears to penetrate the heart 30-fold less than L-T_3 (**1**) and to control cofactor association differentially in the heart [51]. With these combinations of activities, it was not surprising that GC-1 (**8**) was significantly less prone to modulate the cardiac pacemaker, hyperpolarization-activated cation channel 2 (HCN2) [49]. It has also been recently shown for GC-1 (**8**) that the TRβ-ligand more strongly associates with co-regulators such as SCR2 and SCR3 while the TRβ-ligand associates with SRC1 and SCR2, suggesting that SRC2 and 3 are important in cholesterol regulation [52]. In cardiac ventricular myocytes, GC-1 (**8**) is less effective in upregulating sarco(endo)plasmic reticulum Ca(2+)-ATPase (SERCA) and shifts in myosin heavy chain isoforms compared to L-T_3 (**1**), are consistent with the effects observed in animals [53]. As done with KB141 (**7**), this compound has also been reviewed in larger detail [54,55].

The data mentioned above suggest that even moderate TRβ_1-selective agonists can be useful in the development of anti-obesity and lipid-lowering drugs with heart sparing properties. The question is, however, if the pharmacological ratios are large enough, how these ratios would translate to therapeutic windows for humans.

Lately, significant efforts have been directed toward the synthesis of highly TRβ-selective compounds (Fig. (**7**)). A set of compounds was prepared in which the basic structural features of KB141 (**7**) was kept, but where the R_3'-isopropyl group was replaced with substituents of larger sterical bulk [19]. The majority of compounds had agonist activity, but displayed increased TRβ_1-selectivity compared with KB141 (**7**). The most TRβ_1 selective ligand found from this study was [3,5-dichloro-4-(3-ethyl-6-hydroxybiphenyl-3-yloxy)phenyl]acetic acid (**9**) that bound 0.20 nM to TRβ_1 and was 38 times selective for TRβ_1 over TRα_1. This careful SAR study suggests the portion of the TR receptor binding pocket interacting with the R_3'-moiety to be flexible in such a way that the bulky R_3'-substituents move away from Met442β, which then enables the receptor to accommodate the substituent with retained agonist conformation. The influence that this conformational change has on the isoform selectivity is not completely certain owing to the lack of a complementary x-ray crystal structure with the TRα_1-LBD. These findings are, however, in general agreement with the studies outlined below.

3,5-Dimethyl-4-(4-hydroxy-3-benzyl)benzylphenoxyacetic acid (GC-24, **10**) (Fig. (**7**)) belongs to a series of analogs that were designed from the GC-1 scaffold. GC-24 (**10**) was also designed to test the environments of the LBD for accommodating a larger R_3'-substituent. Competition binding studies with radio-labeled L-T_3 (**1**) revealed that GC-24 (**10**) had both a high affinity and strong selectivity for the β_1-subtype. GC-24 (**10**) bound TRβ_1 with a K_D that was slightly weaker than that of L-T_3 (**1**) but showed an average affinity for TRβ_1 of 40-fold. Thus, addition of a bulky phenyl extension to the R_3'-position improves the specificity of binding to TRβ_1 with no reduction in binding affinity [56].

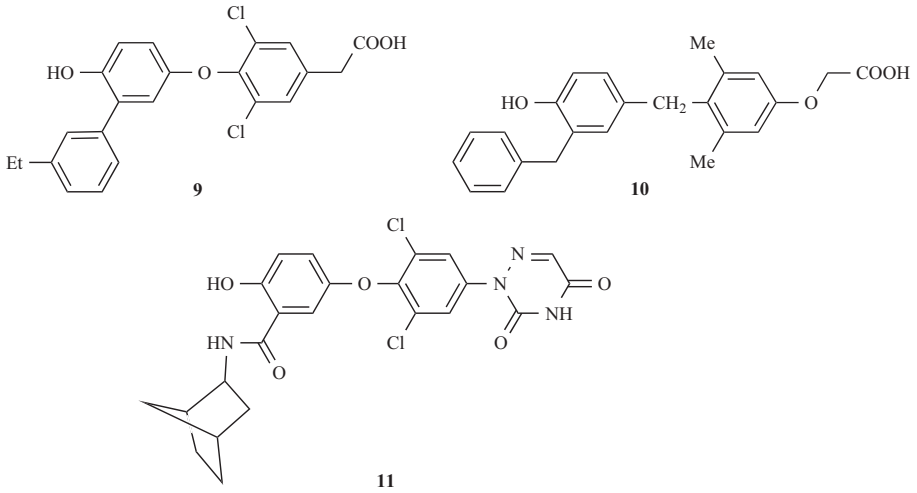


Fig. (7). Chemical structure of **9**, GC-24 (**10**) and **11**.

Specific substitution with heterocyclic rings at the R_1-position was accomplished through 6-azauracil-based thyromimetics [17] (Fig. (**7**)). SAR-work on the R_3'-position provided compounds with highly enhanced TRβ_1 affinity and selectivity. Significant increases in TRβ_1-selectivity were seen when the R_3'-position was substituted with various carboxamides and sulfonamides respectively. Most selective from the two series was *N*-(1R,2S,4S)-bicyclo[2.2.1]hept-2-yl-5-[2,6-dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2 (3H)yl)-phenoxy]-2-hydroxybenzamide (**11**) that was binding 0.59 to TRβ_1 and was 105 times TRβ_1-selective (ratio from TRβ_1K_i / TRβ_1K_i).

The studies above both suggest the portion of the TRβ_1 receptor binding pocket interacting with the R_3'-moiety to be more flexible than is the case for TRα_1, while still permitting the receptor to adopt an agonist conformation. Due to the large TRβ_1-selectivity of compounds like **9**, GC-24 (**10**) and **11**, these selective agents are potents agents for increasing metabolic rate and lowering lipids, while minimizing cardiovascular side effects; however, as no *in vivo* data is published, this remains to be seen.

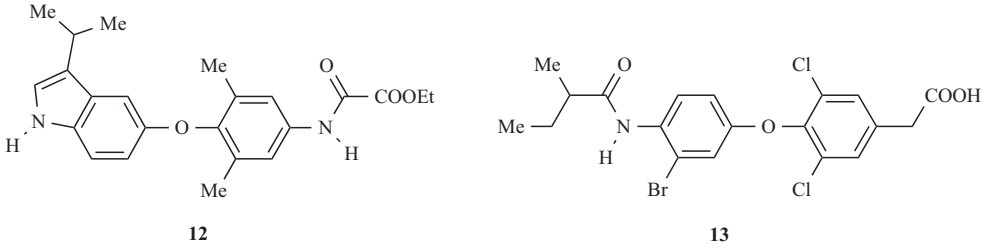
Two recent papers have shown that the "obligatory" R_4'-hydroxy group can be replaced by an alternative H-donator; i.e. NH, without loss of functional activity (Fig. (**8**)). High affinity, but moderate TRβ_1-selectivity was observed in heterocycle-fused thyromimetics carrying indoles or indazoles instead of a phenolic group, even though highly potent agonists were identified in both series. For instance, N-[4-(3-isopropyl-1H-indol-5-yloxy)-3,5-dimethylphenyl]oxalamic acid ethyl ester (**12**) was 10 times TRβ_1-selective and had an EC_{50} of 4 nM for TRβ [57]. Based on the examination of the X-ray crystallographic structures of the LBD of TRα_1 and TRβ_1 in complex with KB141 (**7**), a number of novel R_4'-hydroxy "non-classical" bioisosteric thyromimetics were prepared. Again, the design of these ligands was based upon the fact that bulk-substitution is better accommodated by TRβ_1 than TRα_1 in the moiety of the prime ring. Optimal affinity and maximum β_1-selectivity (33 times) were found with a medium-sized alkyl-substituted amido group (isobutyl); {4-[3-bromo-4-(2-methylbutanoyl-amino)-phenoxy]-3,5-dichlorophenyl}acetic acid (**13**) (Fig. (**8**)).

It can therefore, be concluded that bioisosteric replacements of the 4'-hydroxy position represent a new highly promising class of TRβ_1-selective synthetic thyromimetics [58].

A highly novel variation on the "linker theme" was recently described through directly joined phenylnaphthyl analogs as TR agonists [59]. The orthogonal relationship between the two aromatic ring systems mimics the relationship similar to that of the two aryl rings in the diphenyl ether scaffold of the natural hormones. In this series N-[3, 5-dichloro-4-(7-hydroxy-6-isopropylnaphtalen-1-yl)phenyl] malonamic acid (**14**) (Fig. (**9**)) was found to bind to TRβ_1 with an IC_{50} of 0.3 nM and to be 25 times selective for TRβ_1 over TRα_1. The binding data in this series of compounds indicate that the SAR diverges significantly from thyromimetics containing a diphenyl ether core. When the inner ring of a thyromimetic ligand is fused with its R_1 side-chain, thus forming a bicyclic structure, TRβ_1-selectivity is also promoted. The *in vitro* properties of this class of bicyclic thyromimetics described are, however, highly dependent on the size and the shape of the ring as well as the resulting orientation of its terminal carboxylate group. Selectivity for the β_1-isoform was highest when the inner ring was a 2-carboxylindole and especially when substituted with chlorines at R_3 and R_5: 4,6-dichloro-5-(4-hydroxy-3-isopropylphenoxy)-1*H*-indole-2-carboxylic acid (**15**) (Fig. (**9**)) [60].

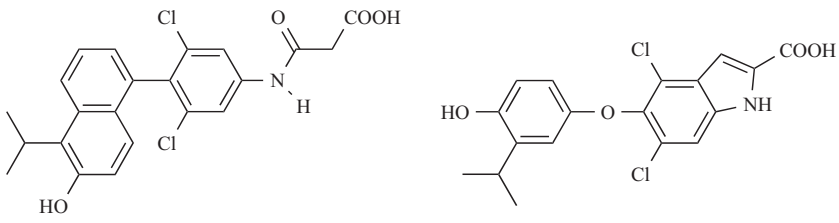
CONCLUSIONS

Several of the studies above suggest the "prime-ring" moiety in the TR receptor binding pocket to be more flexible in TRβ_1 than in TRα_1, while still permitting the receptor to



12 **13**

Fig. (8). Chemical structures of **12** and **13**.



14 **15**

Fig. (9). Chemical structure of **14** and **15**.

adopt an agonist conformation. Due to the high $TR\beta_1$-selectivity of compounds like **9-11** and **13-14**, there is potential for these selective agents to display improved therapeutic windows compared with KB141 (**7**) and GC-1 (**8**). However, as no *in vivo* data was published in these works, the hypothesis that increased $TR\beta_1$-selectivity results in a larger therapeutic window still remains unproven. The data mentioned above also suggest that even moderate $TR\beta_1$-selective agonists can be useful in the development of anti-obesity and lipid-lowering drugs with heart sparing properties. Furthermore, as evident also from the older literature, tissue selectivity can contribute to selectivity. Ideally, a combination of both $TR\beta_1$- and tissue-selectivity would give the largest safety margin toward cardiac toxicity, while retaining the lipid lowering effects.

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