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Thyroid receptor ligands. Part 2: Thyromimetics with improved selectivity for the thyroid hormone receptor beta

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Abstract—A set of thyromimetics having improved selectivity for TR-β1 were prepared by replacing the 3′-isopropyl group of **2** and **3** with substituents having increased steric bulk. From this limited SAR study, the most potent and selective compounds identified were derived from **2** and contained a 3′-phenyl moiety bearing small hydrophobic groups *meta* to the biphenyl link. X-ray crystal data of **15c** complexed with TR-β1 LBD shows methionine 442 to be displaced by the bulky R3′ phenyl ethyl amide side chain. Movement of this amino acid side chain provides an expanded pocket for the bulky side chain while the ligand–receptor complex retains full agonist activity.

3,5,3′-Triiodo-L-thyronine (T3; **1** in Fig. 1) influences multiple physiological endpoints through binding to its cognate receptor, thyroid hormone receptor (TR).[1] Super-physiological amounts of T3 result in weight loss, bone and muscle wasting, cardiac hypertrophy, and tachycardia. Sub-physiological amounts of T3 produce the opposite effect resulting in weight gain and bradycardia. The cardiac side effects associated with doses of T3 sufficient for weight loss in obese patients prevent its use as an anti-obesity agent.

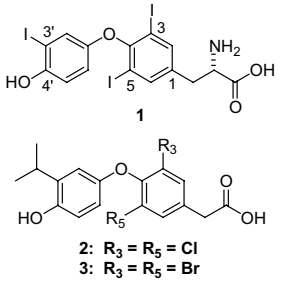

Figure 1. 3,5,3′-Triiodo-L-thyronine (**1**), KB-141 (**2**), and KB-131092 (**3**).

TR is encoded by two separate genes, TR-α and TR-β. Each produces multiple splice variants. The predominant ones are TR-α1 and TR-β1. Tissue distribution data[1b,2] and mouse knockout studies[3] have shown that TR-α1 is the predominant receptor in cardiac tissue. In light of the apparent central role TR-α1 plays in cardiac response to T3 levels, it appeared possible to separate cardiac side effects from metabolic rate increase and cholesterol lowering by designing agents selective for TR-β1.[4,5]

Initial efforts to identify TR-β1 selective thyromimetics resulted in the discovery of KB-141 (**2**), which was 14-fold selective for TR-β1.[5] The SAR derived from this study showed that substitution of the R3 and R5 iodines of T3 by chlorine and truncation of the amino acid side chain at R1 to acetic acid were fundamental alterations leading to increased selectivity for TR-β1. In the present

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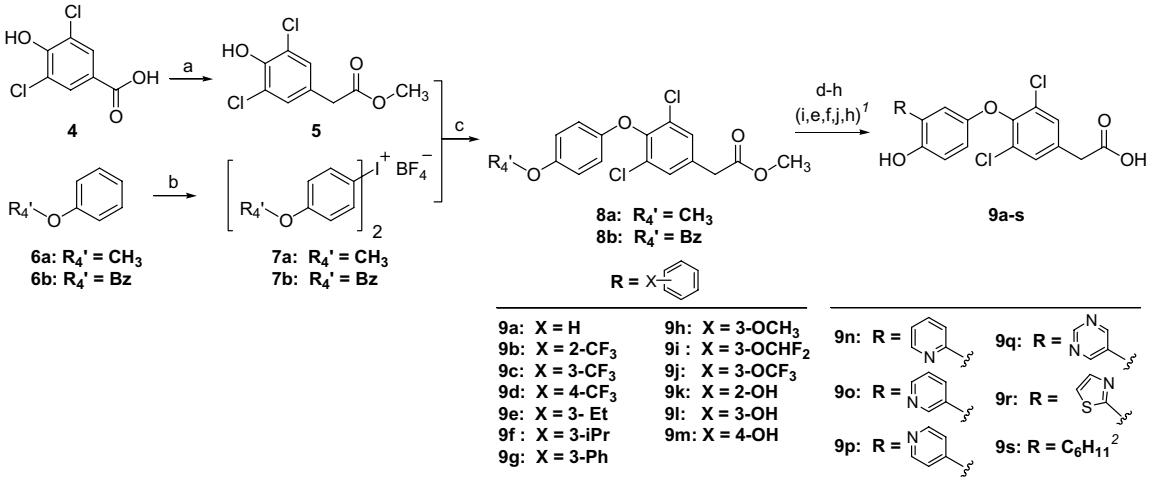
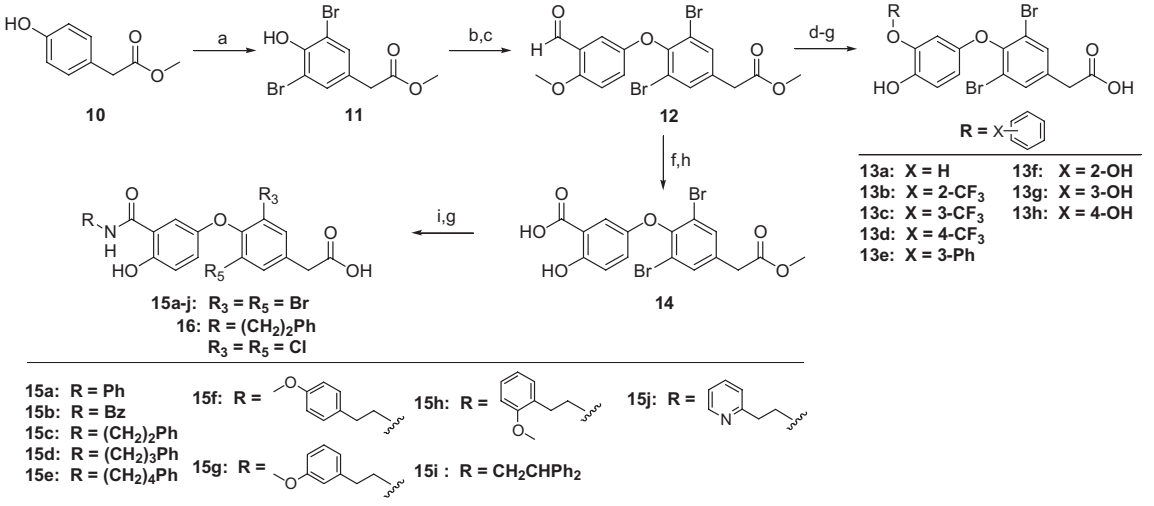
study, we report our efforts to improve selectivity for TR-β1 through modulation of the steric bulk and physiochemical properties of the substituents located at R3′ of KB-141. At the outset of this study, such modifications were known to give thyromimetics with good binding affinity and full agonist activity but with unknown isoform selectivity.[6a] Recent disclosures from investigators at Pfizer[6b] and UCSF[6c] describe work carried out in parallel with this study. Both show that replacing the 3′-isopropyl group with more sterically demanding side chains is a general strategy for gaining improved selectivity for TR-β. We expand on these initial disclosures with a third chemotype, which gave improved selectivity for TR-β through the same strategy.

Three structural motifs were surveyed: 3′-phenyls and related heterocycles (synthesis in Scheme 1), and 3′-phenoxys, and 3′-amides (synthesis in Scheme 2). The latter two series were derived from the 3,5-dibromophenyl acetic acid analog of KB-141 (**3**) in order to provide a higher affinity starting point albeit with slightly lower selectivity (Table 1).

Replacement of the 3′-isopropyl group of **2** with the more sterically demanding phenyl group (**9a**) caused a greater loss of affinity for TR-α1 than for TR-β1, resulting in improved selectivity for TR-β1 (Table 1). Placement of a trifluoromethyl group in the *meta* position of the 3′-phenyl ring (**9c**) showed that lipophilic groups provide increased binding affinity for both TR isoforms and maintained selectivity for TR-β1. The corresponding *ortho*- and *para*-substituted analogs (**9b** and **9d**, respectively) were relatively unselective. This initial observation was expanded upon with a series of



Scheme 1. Synthesis of the 3′-phenyl series. Reagents and conditions: (a) (i)[8] oxalyl chloride, cat. DMF, CH_2Cl_2; (ii) CH_2N_2, Et_2O; (iii) $PhCO_2Ag$, triethylamine, CH_3OH; (b)[9] (i) I_2, HNO_3, Ac_2O, TFA; (ii) aq $NaBF_4$; (c)[9] Cu^0, triethylamine, CH_2Cl_2; (d) Br_2, DCM; (e)[10] bis(pinacolato)diborane, $PdCl_2$dppf, KOAc, DMSO, 85 °C; (f) aryl bromide, Na_2CO_3, $Pd(PPh_3)_4$, dimethoxyethane/H_2O, 80 °C; (g) BBr_3, CH_2Cl_2, −78 to 0 °C; (h) aq NaOH, CH_3OH; (i) Br_2, HOAc, NaOAc; (j) H_2 (1 atm), PtO_2, ethanol.[1] Alternate reaction sequence for **6h**–**j** for which $R_{4'} = Bz$.[2] Trifluoromethanesulfonic acid cyclohex-1-enyl ester used in place of aryl bromide in step (f) followed sequentially by steps (j), (g), and (h).



Scheme 2. Synthesis of the 3′-phenoxy and 3′-amide series. Reagents and conditions: (a) Br_2, H_2O, rt; (b)[9] **7a**, Cu^0, triethylamine, CH_2Cl_2; (c) Cl_2CHOCH_3, $SnCl_4$, CH_2Cl_2, 0 °C; (d) H_2O_2, H_2SO_4, CH_3OH; (e) aryl boronic acid, $Cu(OAc)_2$, triethylamine, CH_2Cl_2; (f) BBr_3, CH_2Cl_2, −78 to 0 °C; (g) aq NaOH, CH_3OH; (h) NaClO, $NaHSO_3$, THF/H_2O; (i) diverse amines, EDCI, HOAt, triethylamine, CH_2Cl_2/DMF.

Table 1. IC_{50} and selectivity data for compounds **1**–**3**, **9a**–**s**, **13a**–**h**, **15a**–**k**, and **16**

Compounds	IC_{50} (nM)[a,b]		Sel[c]	Compounds	IC_{50} (nM)[a,b]		Sel[c]
	TR-α	TR-β			TR-α	TR-β	
1	0.24	0.26	0.9	**13a**	123	5.97	12
2	25	1.1	14	**13b**	244	7.63	19
3	1.4	0.1	9	**13c**	50	1.1	27
9a	152	2.9	31	**13d**	326	9.5	20
9b	18	3.4	3	**13e**	499	60	5
9c	40	0.94	25	**13f**	1475	83	10
9d	515	67	4	**13g**	549	47	7
9e	13	0.20	38	**13h**	237	6.0	23
9f	124	4.0	18				
9g	454	21	13	**15a**	424	35	7
9h	297	11	16	**15b**	84	4.2	12
9i	30	0.77	23	**15c**	15	0.62	14
9j	100	3.4	17	**15d**	203	6.8	18
9k	1052	50	13	**15e**	281	18	9
9l	1160	75	9	**15f**	228	37	4
9m	847	87	6	**15g**	93	4.8	11
9n	1525	139	6	**15h**	96	8.5	7
9o	819	41	12	**15i**	18	0.47	23
9p	145	124	1	**15j**	1133	67	10
9q	269	67	2				
9r	558	28	12	**16**	127	3.5	21
9s	258	7.7	20				

[a] Competitive binding affinities versus radioiodinated T3 using full length cloned hTR-α1 and hTR-β1.[5,11]
[b] IC_{50} values determined from a minimum of duplicate data. Values have an average variability of ±25%.
[c] Selectivity = (IC_{50} hTR-α1)/(IC_{50} hTR-β1 × 1.7).[5,11]

analogs (**9e**–**g**) designed to probe the effects of more sterically demanding substituents in the *meta* position. The rank order of binding affinity for this series was found to be $Et > CF_3 > i\text{-}Pr > Ph$, with selectivity decreasing for TR-β1 as the *meta* substituents increased in size. Isosteric replacement of ethyl with methoxy (**9h**), difluoromethoxy (**9i**), and trifluoromethoxy (**9j**) lowered both binding affinity and selectivity for TR-β relative to **9e**. Introduction of hydrophilic groups onto the 3′-phenyl ring, such as hydroxy (**9k**–**m**), resulted in concomitant loss of binding affinity and selectivity for TR-β1 regardless of its position on the 3′-phenyl ring. In an attempt to decrease the overall hydrophobicity of the 3′-phenyl series, several analogs containing heterocyclic isosteres of the 3′-phenyl ring (**9n**–**r**) were synthesized. Without exception, binding affinity and selectivity for TR-β1 were decreased. The fully saturated analog of **9a**, compound **9s** (3′-cyclohexyl) had similar binding affinity and somewhat less selectivity for TR-β1. In all of the aforementioned analogs, full agonist activity was maintained (>80% induction gene transcription normalized to T3)[7] with one exception; the pyridin-3-yl analog (**9o**) had only partial agonist activity (60% induction)[7] for TR-α1 but was a full agonist with TR-β1.

The SAR of the 3′-phenoxy series diverged somewhat from that of the 3′-phenyl series. The parent compound in the 3′-phenoxy series (**13a**) was approximately equipotent with **9a** and less selective for TR-β1. Systematic placement of trifluoromethyl groups around the 3′-phenoxy ring (**13b**–**d**) showed a preference for hydrophobic groups in the *meta* position, consistent with the trend observed in the 3′-phenyl series. The steric limitations for the *meta* position in the 3′-phenoxy series were probed using the *meta* phenyl analog (**13e**). Consistent with the result obtained in the 3′-phenyl series, this analog lost both binding affinity and TR-β1 selectivity. However, in contrast to the 3′-phenyl series where hydroxy groups universally caused the loss of both affinity and selectivity, a hydroxy group positioned *para* to the biphenyl ether linkage yielded a compound (**13h**) that was approximately equipotent with **13a** and slightly more selective. The other two positional isomers, **13f** and **13g**, were significantly less potent and less selective.

Amide formation at C-3′ provided ready entry into more diverse structures through simple amide bond coupling with carboxylic acid **14** (Scheme 2). Binding affinity for a homologous series of phenyl alkyl amides (**15a**–**e**) peaked with the 2-phenyl ethyl amide **15c**, while selectivity for TR-β1 was approximately the same for **15c** and its homolog **15d**. However, the increase in selectivity over that of **3** was modest (1.5-fold). Another trend clearly established by this series was the gradual decrease in agonist activity for both isoforms as the aliphatic chain length increased reaching a low of 37% and 57% induction of gene transcription[7] for TR-α1 and TR-β1, respectively, for compound **15e**. Among the more interesting analogs examined in this series was the 2,2-diphenyl ethyl amide **15i**, which was equipotent with **15c** and as selective, despite challenging the receptor binding pocket with a second terminal phenyl ring. As with the 3′-phenyl series, introduction of a basic pyridine onto the amide side chain (**15j**) caused a loss of binding affinity and selectivity for TR-β1.

Insight into the mechanism by which the receptor accommodates sterically demanding 3′-side chains was obtained by comparison of X-ray crystal data of the

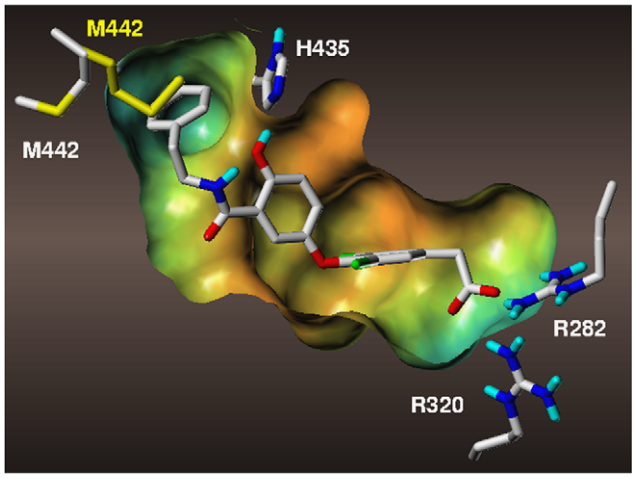


Figure 2. X-ray crystal structure of **15c**/TR-β1 LBD complex[12] illustrating the movement of M442 (shown in yellow for the X-ray structure of **2**/TR-β1 LBD[5]).

TR-β1 LBD complexed separately to **2**[5] and **15c**[12] (Fig. 2). Comparison of key hydrogen bonding interactions between the LBD and the two ligands (e.g., arg282 and arg320 and the R1 acetic acid side chain; his435 and the R4′ phenol) showed the location and orientation of the R1 side chain and biaryl ether core of **15c** and **2** to be substantially the same. The key structural difference between the two complexes was movement of met442 (shown in yellow for the **2**/TR-β1 complex), resulting in significant enlargement of the 3′-binding pocket immediately adjacent to the 3′-phenyl ethyl amide of **15c** in a manner similar to that previously disclosed.[6c] The phenyl ethyl side chain of the ligand adopted a *gauche* conformation, complementing the shape of the enlarged binding pocket.

Investigation into the significance of these findings was carried out by docking compounds **15a**–**e** into a binding site model based on the **15c**/TR-β1 structure.[17] The computed energies (E_{ass} kJ/mol) for this series correlated well with their observed TR-β1 IC_{50} values ($r^2 = 0.93, n = 5$) suggesting that once the bulky 3′-substituents displaced met442, the differences in relative binding affinities were largely accounted for by steric interactions in the newly formed pocket. Movement of met442 may be involved in the binding of selective analogs from the 3′-phenyl and 3′-phenoxy series as well. The influence that this conformational change has on isoform selectivity is difficult to pinpoint owing to the lack of a complementary X-ray crystal structure with the TR-α1 LBD. In a separate study, a detailed analysis of binding data and crystallographic data for a novel TR-β selective thyromimetic, GC-25, and its progenitor GC-1, provided evidence that the 3′-binding pocket of TR-β is more flexible compared to that of TR-α and that this difference plays a dominant role in the improved selectivity of GC-25 for TR-β.[6c] It is reasonable to speculate, therefore, that a similar mechanism may be partly responsible for the increased TR-β selectivity observed for each of the three series describe in this work and for compounds disclosed by the group at Pfizer.[6b] Within the 3′-phenyl series, however, improved TR-β selectivity was not universally observed. For example, the SAR revealed by compounds **9a**–**j** shows that both good affinity and improved selectivity is obtained when the 3′-phenyl ring is either unsubstituted or substituted by a small, hydrophobic group in the *meta* position. In contrast, compound **9b**, which bears a small hydrophobic group in the *ortho* position of the 3′-phenyl ring, preferentially *gains* affinity for TR-α and hence has lower TR-β selectivity relative to compound **9a**. These SAR data point to the likelihood that *specific* contacts between the 3′-group of the ligand and the 3′-binding pocket of the LBD provide additional determinants for influencing selectivity for TR-β or for TR-α. The details of such interactions and their potential impact on the design of selective thyromimetics awaits further X-ray crystal analysis.

Improvements to the TR-β1 selectivity and potency of KB-141 have been achieved through SAR studies that vary the steric bulk of R3′ hydrophobic groups. This study, in agreement with others reported previously,[6b,c] showed that increasing the steric bulk of the 3′-group of thyromimetics is a general approach for improving selectivity for TR-β. These results further suggest that partial agonists and, perhaps, antagonists[18] can be designed by varying the trajectories and steric bulk of groups located at the 3′-position of thyromimetics in a way distinct from those needed to create selective agonists.

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12. The TR-β1 LBD was expressed and purified as described,[5] with the notable difference that the TR-β1 receptor started with residue 202 in the construct. The **15c**/TR-β1 complex was crystallized by the hanging drop vapor diffusion method (1 μL protein solution at 7 mg/mL concentration combined with 1 μL precipitant solution suspended above a reservoir composed of 1.5 M sodium acetate and 0.1 M sodium cacodylate at pH 7.2). The crystal was cryo-protected in crystallization solution containing glycerol. 3.0 Å data was collected at beam line BW7B, DESY, Hamburg using a MAR345 detector ($T = 100$ K, wavelength = 0.8337 Å). The data was indexed and reduced by Denzo and Scalepack[13] and belongs to space group $P3(1)21$ ($a = b$68.6 Å, $c = 129.2$ Å) with one molecule in the asymmetric unit. The structure was determined by molecular replacement with AMoRe.[14] All data (30–3.0 Å) was used in the refinement, but according to obsolete rejection criteria, the data is only complete and acceptable to 3.2 Å resolution. The structure was refined with Refmac using TLS[15] and the model was built using O.[16] The final structure has an Rfree of 34.7 and Rwork of 26.1. The final geometry is good (rmsd for distance is 0.015 Å and for angle is 1.5°). The coordinates have been deposited at www.rcsb.org (1r6g.pdb).
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