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Thyroid receptor ligands. Part 5: Novel bicyclic agonist ligands selective for the thyroid hormone receptor β

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Abstract—Based on the examination of the crystal structure of rat TRβ complexed with 3,5,3′-triiodo-L-thyronine (**2**) a novel TRβ-selective indole derivative **6b** was prepared and tested in vitro. This compound was found to be 14 times selective for TRβ over TRα in binding and its β-selectivity could be rationalized through the comparison of the X-ray crystallographic structures of **6b** complexed with TRα and TRβ.

Endogenous thyroid receptor hormones 3,5,3′,5′-tetraiodo-L-thyronine (T_4, **1**) and 3,5,3′-triiodo-L-thyronine (T_3, **2**) exert profound effects on growth, development, and homeostasis in mammals (Fig. 1).[1] Thyroid hormone receptor subtypes (TRα and TRβ) mediate differential functions, suggesting the possibility of developing selective thyromimetics that cause therapeutic increases in metabolic rate (anti-obesity effect) and lipid lowering without deleterious effects on the heart.[2] Our hypothesis is that selective TRβ activation can give such a profile.[3]

TRα and TRβ differ in a single amino acid residue in the hormone binding pocket (Ser-277 vs Asn-331, respectively). It has been proposed that this amino acid difference accounts for most of the observed selectivity for TRβ-binding in a series of phenyl acetic acid thyromimetics.[4] This amino acid residue as well as the α-alanine side chain of **2** is located in a highly flexible region of the receptor.[5] We reasoned that rigidification of the ligand carboxylic acid side chain through ring formation may lead to increased opportunities for subtype selectivity. In addition, the fused ring provides a rigid scaffold on which substituents could be introduced to more directly exploit this amino acid difference. We also decided to

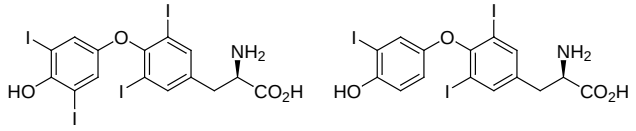

Figure 1. Chemical structures of L-T_4 and L-T_3.

replace the iodine atoms with alternative substituents since iodine is highly susceptible to reductive deiodination and furthermore hinders biaryl ether formation due to its large steric bulk. With regard to achieving a reasonable duration of action in vivo, replacing the iodine atoms with alternative substituents eliminates a potential route of metabolic deactivation via enzymatic deiodination.

An examination of the crystal structure[5] of rat $TR\alpha_1$ complexed with **2** (Fig. 2) reveals a significant unoccupied space in the binding cavity next to position-2 of the ligand. This suggests that a ring fusion between the benzylic carbon atom of the position-1 substituent and the position-2 of the aromatic ring would be sterically tolerated by the receptor.

Based on the observations above, we prepared a number of TR-analogues in which an additional ring has been fused to the inner ring of the TR ligand. As outlined below (Scheme 1), the first synthetic step involved

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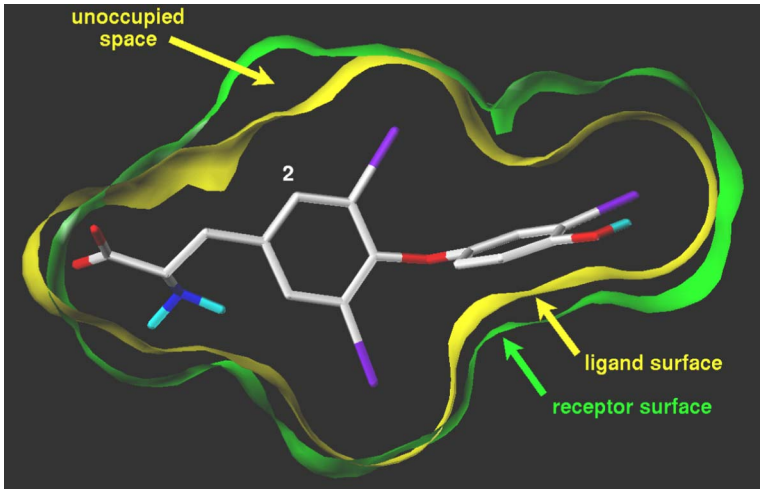
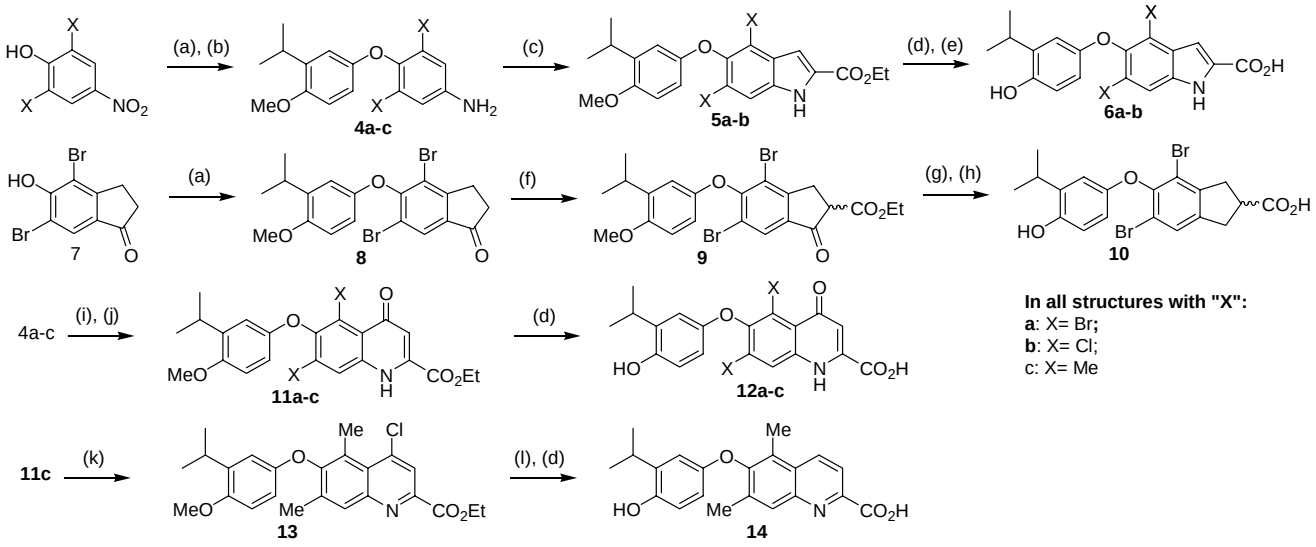


Figure 2. Comparison of the solvent accessible surface areas of thyroxine (**2**; yellow surface; ligand atom coloring scheme: white = carbon, blue = nitrogen, red = oxygen, purple = iodine, and light blue = hydrogen atoms) and the interior binding cavity of TRα (green surface) derived from the crystallographic structure of rat TRα complexed with thyroxine.[5] The solvent accessible surfaces are Z-clipped for the sake of clarity. The unoccupied space adjacent to position-2 of the ligand suggests that a ring fusion between the benzylic carbon atom of the position-1 substituent and the position-2 of the aromatic ring would be sterically tolerated by the receptor.



Scheme 1. Synthetic route for the preparation of the **6a**,**b**, **10**, **12a**–**c** and **14**. Reagents and conditions: (a) bis(3-isopropyl-4-methoxyphenyl)iodonium tetrafluoroborate, Et_3N, Cu; (b) $SnCl_2$; (c) (i) HCl; (ii) $NaNO_2$, $SnCl_2$, HCl; (iii) ethyl pyruvate, H_2SO_4, AcOH; (iv) PPA; (d) BBr_3, CH_2Cl_2; (e) NaOH, THF; (f) diethylcarbonate, NaH, C_6H_6; (g) triethylsilane, TFA; (h) BBr_3, CH_2Cl_2, −78 °C; (i) EtO_2CCCCO_2Et, MeOH; (j) PPA; (k) $POCl_3$; (l) H_2–Pd–C, KOH.

iodonium salt coupling[6] with the appropriate starting phenols **3a**–**c** and **7**, to obtain the corresponding biaryl ethers **4a**–**c** and **8** in good yield. Synthesis of the final target ligands 4,6-dibromo-**6a** and 4,6-dichloroindole **6b**[7] was accomplished via a modification of the Fisher Indole synthesis starting from the anilines **4a**,**b**. The indane-2-carboxylic acid **10** was prepared via direct 2-position carboxylation of the indanone **8**.[8] The 5,7-dibromo (**12a**), 5,7-dichloro- (**12b**), and 5,7-dimethyl-6-tetrahydroquinoline-2-carboxylic acids (**12c**) were all prepared from **4a**–**c** by reaction with ethynyldicarboxylic acid ethyl ester. The intermediate Schiff base was treated with PPA to give ring closure to intermediates **11a**–**c**. The 4-oxo substituent could be removed through treatment of **11c** with phosphorus chloride followed by reduction to give the quinoline-2-carboxylic acid **14**.

The results of a radioligand binding assay for the human $TR\alpha_1$ and $TR\beta_1$, as well as a reporter cell assay employing CHOK1-cells (Chinese hamster ovary cells) stably transfected with $hTR\alpha_1$ or $hTR\beta_1$ and an alkaline phosphatase reporter gene linked to a thyroid response element ($TRAF\alpha_1$ and $TRAF\beta_1$), are summarized in Table 1.[9] The endogenous hormone, **2**, binds to $TR\alpha_1$ and β_1 with an IC_{50} of 0.24 and 0.26 nM, respectively. Compared with **2**, the indole derivative **6a** binds with equal affinity for β_1, but 10 times lower for α_1, thus resulting in a normalized

Table 1. Thyroid hormone receptor binding affinities (IC_{50}s), reporter cell line potency efficacies (EC_{50}s), and efficacies of synthetic thyromimetics **2**–**14**

ID	TRα, IC_{50}[a], nM	TRβ, IC_{50}[a], nM	α/β[b]	TRAFα, EC_{50}, nM (% agonism)[a]	TRAFβ, EC_{50}, nM (% agonism)[a]
2	0.24	0.26	0.54	1.3 (100)	3.5 (100)
6a	2.5	0.28	5.2	10 (85)	4.2 (96)
6b	1.4	0.060	14	10 (80)	4.7 (83)
10	38	8.5	2.6	4.1 (95)	4.3 (97)
12a	250	51	2.9	Nt[c]	Nt
12b	3500	870	2.4	Nt	Nt
12c	620	240	1.5	Nt	Nt
14	200	15	7.8	83 (95)	49 (96)

[a] Values are means of two experiments. The variability was on average 25%.
[b] For an explanation for the calculation of selectivity ratios, see Ref. 3a.
[c] Nt = not tested.

selectivity ratio of 5.2. The halogen substitution at the R_4 and R_6-positions affects affinity for both TRα_1 and TRβ_1. When the bromine atoms of **6a** are replaced with chlorines (**6b**), binding affinity increase for both isoforms. However, the binding increased is more pronounced for β_1, which resulted in an improved selectivity ratio of 14. It can be observed that **6b** binds approximately four times better than the endogenous hormone **2** for TRβ_1. A reverse structure–activity relationship was observed when the affinities of **12a**–**c** were compared, in view of the fact that the bromo analogue **12a** is the best binder as well as the most β_1-selective ligand in the particular sub-series. Unmistakably the 4-oxo group is detrimental for affinity, most likely through unfavorable desolvation energies, since **14** gained almost 20 times the affinity for TRβ_1 when compared with **12c**. The binding data described above suggest that 5-membered rings fused to the inner ring are better accommodated in the receptor than the 6-membered rings. This trend is even more striking when the binding data of **10** are considered. Structural demands on the ligand appear to be significant as **10** drops both in affinity and β_1-selectivity, when compared with **6a**,**b**. This might be due to a non-optimal orientation of the carboxylate group in **10**. In addition, as shown below, the indole NH of **6b** is hydrogen bonded to the backbone carbonyl group of Met-313, whereas this hydrogen bonding interaction is eliminated in **10**. The assay results of **6a**,**b** and **14** in TRAFα_1 and β_1 reporter cells reflect to some degree similar trends as the binding assay, but **10** displayed the highest potency, which might be a manifestation of its more hydrophobic nature. The general discrepancy for the other ligands in potency of TR-binding and TRAF might be due to the lack of active transporters in the cultivated transactivation assay. This discrepancy was also noted in the KB-141 series.[3a] The ligands display similar efficacy as **2** for the TRAFβ_1 assay.

Albeit promising in vitro, the effect of the compounds needs to be further evaluated in relevant animal models of the dysmetabolic syndrome prior to establishing its therapeutic potential.

In order to understand the β_1-isoform selectivity of **6b**, the crystal structures of the ligand and the TR-isoforms (TRα_1/**6b** and TRβ_1/**6b**) were obtained to resolutions of 2.55 and 2.30 Å, respectively.[10] Data collection and refinement statistics are given in Table 2. As previously observed in a pair of crystallographic structures of TRα and TRβ complexed with a phenyl acetic acid thyromimetic,[3] Arg-228 forms two hydrogen bonds with Ser-277 in TRα, while the corresponding Arg-282 in TRβ forms a strong bifurcated salt bridge with one of the carboxylate oxygen atoms of **6b**. The salt bridge, which is present in the TRβ but not in TRα probably, accounts for the majority of the observed selectivity of **6b** for TRβ. An additional interesting observation is that backbone Leu-276 to Glu-279 of TRβ_1/**6b** is differently oriented compared with Leu-330 to Glu-333 in TRα_1/**6b** (Fig. 3). This places the relatively acidic C-α methane hydrogen atom of Asn-331 in TRβ within 2.3 Å of the carboxylic oxygen atom of **6b**, while the corresponding distance from Ser-277 in TRα to the ligand carboxylic oxygen atom is 3.2 Å. This favorable electrostatic interaction in the β-isoform complex may also contribute to the selectivity of **6b** for TRβ.

In summary, we have shown that when the inner ring of a thyromimetic ligand is fused with its R_1 side-chain, thus forming a bicyclic structure, this promotes TRβ-selectivity. The in vitro properties of this class of bicyclic thyromimetics are highly dependent on the size and shape of the ring as well as the resulting orientation of its terminal carboxylate group. Selectivity for the β_1-isoform also depends on nature of the substituents at the R_3- and R_5-positions of the ligand. The X-ray crystallographic structures of the LBD of TRα_1 and TRβ_1 in complex with **6b** reveal that one source of β_1-selectivity is due to a difference in conformation of β turn loop. Considering the proximity of the backbones to the

Table 2. Data collection and refinement statistics of complexes human TRα_1LBD + **6b** and TRβ_1LBD + **6b**

	TRα_1LBD + **6b**	TRβ_1LBD + **6b**
Space group	$P6_522$	$C222_1$
Unit cell	$a = b = 109.8$, $c = 136.8$	$a = 77.5$, $b = 107.5$, $c = 67.4$
Resolution	2.1 Å (2.1–2.21 Å)	2.25 Å (2.25–2.33 Å)
Redundancy	11.7	4.0
Completeness (%)	99.7 (99.6)	98.6 (96.8)
$\langle I\rangle/\langle\sigma(I)\rangle$	20.2 (3.0)	10.0 (2.4)
R_{sym} (%)	6.9 (39.4)	7.3 (26.7)
Refinement	2.55 Å (2.55–2.64 Å)	2.3 Å (2.3–2.44 Å)
R_{cryst}	23.5 (34.5)	20.2 (24.9)
R_{free}	26.3 (36.0)	23.4 (26.7)
Number of atoms	2106	2081
Average *B*-factor	52.6	39.7

Values in parentheses refer to the highest resolution shells. $R_{sym} = \sum(|I - \langle I\rangle|)/\sum(I)$, where I is the integrated intensity of a given reflection and $\langle I\rangle$ is the average intensity over symmetry equivalents. $R_{cryst} = \sum(|F_o - F_c|)/|\sum(F_o)|$, where F_o and F_c are the observed and calculated amplitudes, respectively. R_{free} is calculated similarly using a test set of reflections.

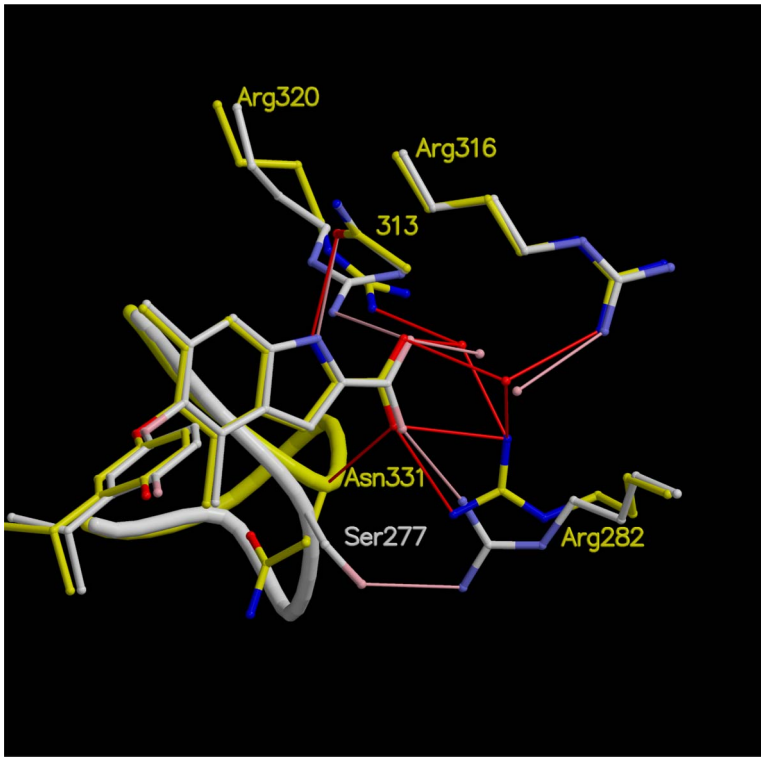


Figure 3. Superposition of the crystallographic structures of **6b** complexed with TRβLBD (yellow carbon, red oxygen, and blue nitrogen atoms) and with TRαLBD (white carbon, pink oxygen, and purple nitrogen atoms). The protein backbone of residues Leu-330 to Glu-333 of TRβLBD + **6b** is depicted as a yellow tube and residues Leu-276 to Glu-279 of TRαLBD + **6b** shown in white. Hydrogen bond bonding interactions in TRα and TRβ in pink and red, respectively.

ligand, this difference can provide further opportunities for the design of β_1-selective ligands.

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10. Protein expression, purification, and crystallization was done according to the description given in Ref. 3. X-ray data collection and structure determination: data of

$TR\alpha_1LBD + \mathbf{6b}$ and $TR\beta_1LBD + \mathbf{6b}$ were collected at beam line X13, DESY, Hamburg, using a MARCCD detector. Data were collected at 100 K using a wavelength of 0.8013 Å. Data processing was carried out with the HKL suite (Otwinowski, Z.; Minor, W. Processing X-ray Diffraction Data Collected in Oscillation Mode. In *Methods in Enzymology*; Academic Press: New York, 1997; p 307); Refinement: starting models for the refinement of the $TR\alpha_1$ and $TR\beta_1$ structures were TR complexes previously solved. In both cases, refinement was initiated with a round of rigid body refinement followed by simulated annealing starting at 2000 K. Well-defined electron density was observed in the ligand binding clefts in which **6b** was built. Refinement and map calculations were performed with CNX (Brünger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr., Sect. D* **1998**, *54*, 905); All model building was done with the program 'O' (Kjeldgaard. *Acta Crystallogr., Sect. A* **1991**, *47*, 110). The figure of the ligand binding pocket was produced with Molscript (Kraulis, P. *J. Appl. Crystallogr.* **1991**, *24*, 946,) and rendered with Raster3D (Merrit, E.; Murphy, M. *Acta Crystallogr., Sect. D* **1994**, *50*, 869).