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Thyroid receptor ligands. Part 4: 4′-amido bioisosteric ligands selective for the thyroid hormone receptor beta

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Abstract—Based on the examination of the X-ray crystallographic structures of the LBD of TRα and TRβ in complex with KB-141 (**2**), a number of novel 4′-hydroxy bioisosteric thyromimetics were prepared. Optimal affinity and β-selectivity (33 times), was found with a medium-sized alkyl-substituted amido group; *iso*-butyl (**12c**). It can be concluded that bioisosteric replacements of the 4′-hydroxy position represent a new promising class of TRβ-selective synthetic thyromimetics.

Hyperlipidemia is a predisposing risk factor for development of coronary heart disease that is the leading cause of morbidity and mortality in the developed world.[1] The active endogenous thyroid hormone (L-T_3, **1**) (Fig. 1) is a potent lipid-lowering agent but cannot be used therapeutically in patients with hyperlipidemia, mainly due to the side effect of tachycardia. There exist two subtypes of thyroid hormone receptors (TRs), α and β, unequally distributed in the body. TRα is most abundant in the heart and most effects of **1** on the heart are mediated through TRα, while the majority of actions in the liver are mediated through TRβ. Consequently, a viable strategy for the treatment of hyperlipidemia is to develop ligands that are selective for TRβ in order to avoid cardiotoxicity.[2]

We have previously shown that TRβ-selectivity is achieved both in vitro and in vivo when the R_1-amino acid side chain of **1** is truncated to acetic acid and the iodine atoms at the R^3- and R^5-positions are substituted with chlorine. This gave KB-141 (**2**), which was 14 times selective for TRβ.[3] When the $R^{3'}$-isopropyl moiety was substituted with larger hydrophobic groups, TRβ-selectivity was even further improved[4] (Fig. 1). The in vivo selectivity of this last series of compounds remains, however, to be demonstrated. In the present study, we wanted to explore the possibility of replacing the $R^{4'}$-hydroxy group with a bioisosteric amide group,[5] while keeping the R^1, R^3, and R^5-groups identical compared with **2**.

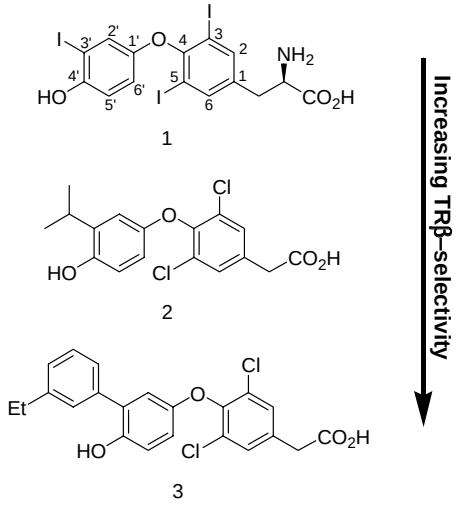

Figure 1. Chemical structures of L-T_3 (**1**), KB-141 (**2**) and **3**.

A comparison of the crystallographic structures of **2** complexed with either TRα or TRβ revealed that the temperature factors of residues of the receptor

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surrounding the outer ring (='prime-ring') of the ligand are higher in β compared with α (Fig. 2),[6] suggesting that the prime-ring binding pocket of TRβ is more flexible than in TRα. Consequently, bulky substituents attached to the prime ring might be better accommodated in TRβ, thus providing ligands with increased β-selectivity compared with **2**. Replacement of the 4′-hydroxy group of **2** with a bioisosteric amide group might provide means to explore this strategy.

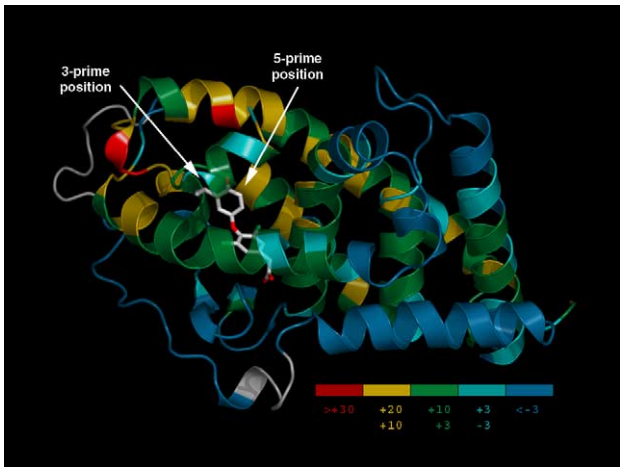
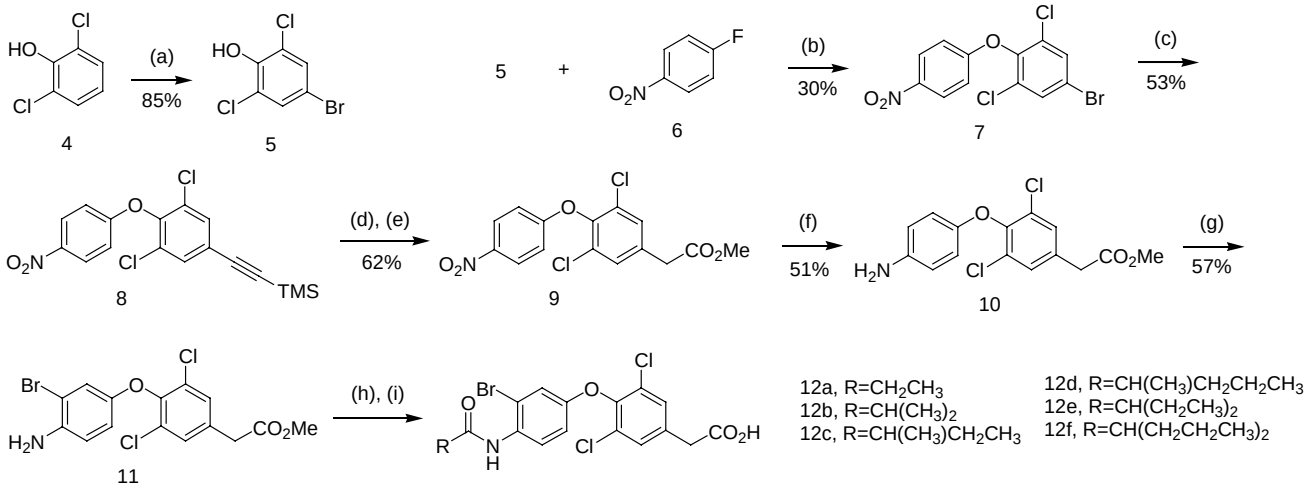


Figure 2. Depiction of the relative flexibility[6] of the ligand-binding domains of TRα versus TRβ. The protein structure shown is of TRβ (ribbons = alpha helices, arrows = beta sheets, and tubes = loops) complexed with KB141 (tube diagram, white = carbon, red = oxygen, and chlorine = green). The protein is colored according to the relative temperature factors of structurally conserved residues between the TRα and β complexes of KB141. Positive relative temperature factors (in order of decreasing magnitude: red, yellow, and green) represent regions of greater relative flexibility in the TRβ structure, while negative values (blue) represent a greater flexibility in TRα. Regions of similar flexibility are depicted as cyan, while residues that are not structurally conserved are colored white. This figure was produced using the PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA, USA (http://www.pymol.org/). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

A series of thyromimetics, all varying the length and branch of the alkyl substitution of a $R^{4\prime}$-amido group, was prepared as outlined in Scheme 1. We also decided to introduce a bromo substituent at the $R^{3\prime}$-position as this would be a synthetically convenient way to introduce the required hydrophobic group at $R^{3\prime}$. Commercially available phenol **4** was brominated at the *para*-position to give **5**, which was coupled with commercially available 4-fluoronitrobenzene **6** in the presence of potassium carbonate and copper. Application of the Sonagashira coupling of the resulting bromo derivative **7** with trimethylsilylacetylene gave **8**, which after desilylation and subsequent oxidation, employing hydrogen peroxide in the presence of sodium hydroxide, gave the phenylacetic acid, which after standard re-esterfication gave methyl ester **9**. This intermediate was reduced by hydrogenation at 1-2 atmospheres, which gave the aniline product **10** in good yield. Other combinations of catalysts, solvent, and hydrogen pressure, or alternatively transfer hydrogenation, may be employed for this step. However, with more active catalysts such as palladium on graphite, higher pressures of hydrogen, and/or higher temperatures, there is an increased risk of dehalogenation during catalytic hydrogenation or transfer hydrogenation.[7] Regioselective *ortho*-bromination at the $R^{3\prime}$-position with 2,4,4,6-tetrabromo-2,5-cyclohexadienone gave **11** in good yield. The aniline intermediate **11** was acylated to provide the $R^{3\prime}$-bromo substituted phenoxyamides **12a–f** after standard saponification of the ester function.

The results of a radioligand binding assay for the human $TR\alpha_1$ and $TR\beta_1$ are summarized in Table 1. All new ligands had lower binding affinities (IC_{50}'s) when compared with the previously reported ligands **2** and **3**, however, in most cases the new ligands displayed improved TRβ-selectivity in comparison to **2**. Optimal affinity



Scheme 1. Reagents and conditions: (a) Br_2, AcCN; (b) K_2CO_3, Cu, DMF, Δ; (c) $PdCl_2(PPh_3)_2$, CuI, TEA, AcCN, TMS-acetylene, 60 °C, N_2; (d) cyclohexen, $B(OH)_4$/THF, 0 °C; (e) NaOH, H_2O_2, followed by MeOH and $SOCl_2$; (f) PtO_2, H_2 (1-2 atm), RT; (g) 2,4,4,6-tetrabromo-2,5-cyclohexadienone, CH_2Cl_2, −30 °C; (h) **12b**: RCOCl, TEA, CH_2Cl_2, RT; **12a**, **c–f**: RCO_2H, HBT, EDAC (CAS: 25952-53-8), CH_2Cl_2, Ar, 60 °C; (i) NaOH, MeOH; Yields for steps (h) and (i) : 10–40%.

Table 1. Thyroid hormone receptor binding affinities (IC_{50}) of **1**, and synthetic thyromimetics **2**, **3** and **12a–e**[a]

Compound	hTRα_1 IC_{50}	hTRβ_1 IC_{50}	α_1/β_1[b]
1	0.24	0.26	0.54
2	25	1.1	14
3	13	0.20	38
12a	3300	140	14
12b	630	18	21
12c	240	4.3	33
12d	1300	47	16
12e	610	16	22
12f	1200	45	16

[a] IC_{50} and EC_{50} values are expressed as nanomolar and are calculated means of duplicate runs for **12a–f**. Data for **1**, **2**, and **3** are taken from Ref. 3a,4a, respectively. These data are intended for comparison with the new ligands.

[b] Normalized selectivity: (IC_{50} hTRα_1/(IC_{50} hTR$\beta_1 \times 1.7$). For an explanation, see Ref. 3a.

and selectivity was found when the amido-function was substituted with an *iso*-butyl group (**12c**), whose TRβ-selectivity is within the same range as **3**. Both affinity and selectivity decreased with larger as well as with smaller groups.

In summary, based on a comparison of the X-ray crystallographic structures of TRα_1 and TRβ_1 in complex with **2**, we designed and prepared a number of novel 4′-hydroxy bioisosteric thyromimetics. Optimal affinity and selectivity was found with a medium size alkylsubstituted amido group; *iso*-butyl (**12c**). Most probably there is a limitation as to what size of groups can be accommodated in this region by TRβ, as well as a dependence on the exact orientation of branching. Bioisosteric replacements of the 4′-hydroxy position represent a new thyromimetic class of ligands that will be further examined in transactivation assay and in relevant animal models.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.11.002.

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5. This work was in part based on data disclosed in: PCT Int. Appl., 86 pp., 2001. Application: WO01/98256. Priority: GB00/15205. CAN 136:54021. While compiling this communication, investigators from Bayer AG disclosed a number of novel outer ring heterocycle-fused thyromimetics, carrying indoles or indazoles instead of the phenolic group in L-T_3 in: Haning, H.; Woltering, M.; Mueller, U.; Schmidt, G.; Schmeck, C.; Voehringer, V.; Kretschmer, A.; Pernerstorfer, *J. Bioorg. Med. Chem. Lett.* **2005,** 15, 1835. These ligands were, however, only moderately TRβ-selective, but clearly represent a highly novel approach.
6. The following procedure was used to calculate the relative difference in temperature factors for the TRα and TRβ structures (PDB accession codes 1NAV and 1NAX, respectively). First, the temperature factors for all atoms within all structurally conserved residues (residue numbers 157–180, 187–198, 207–406 and 211–235, 241–252, 261–461, for TRα and TRβ, respectively) were normalized to zero for each structure. Then for each structurally conserved residue in the TRβ structure, the average temperature factor for all the atoms within that residue was calculated and subtracted from the average temperature factor in the corresponding residue in TRα. Finally the temperature factors for all the atoms within the residue in the TRβ structure were set to this difference. These calculations were performed using a Python script (available from the authors upon request) using functions from the BioPython module Bio.PDB (Hamelryck, T., Manderick, B. (2003) PDB parser and structure class implemented in Python. *Bioinformatics,* 19, 2308–2310).
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