Letters

Thyroid Receptor Ligands. 6. A High Affinity "Direct Antagonist" Selective for the Thyroid Hormone Receptor

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Abstract: A new high-affinity thyroid hormone antagonist **6** with druglike properties was designed and synthesized. The compound behaved as an antagonist in a cell transactivation assay, and in a first in vivo experiment in rats.

Nuclear hormone receptors comprise a class of intracellular, ligand activated transcription factors, which include receptors for thyroid hormones (THs[a]). Thyroid receptors (TRs) exert profound effects on development and homeostasis in mammals.[1] Endogenous THs, 3,5,3′,5′-tetraiodo-L-thyronine (T_4, **1a**), 3,5,3′-triiodo-L-thyronine (T_3, **1b**) (Figure 1), and its metabolites, regulate crucial genes throughout the organism and influence basal and adaptive metabolism, lipid levels, bone and muscle metabolism, heart rate, development, mood, and overall sense of well being.

When the body is exposed to elevated levels of circulating **1a** and **1b**, this might result in hyperthyroidism. Clinically, this state often manifests itself by weight loss, hypermetabolism, lowering of serum lipid levels, cardiac arrhythmias, heart failure, muscle weakness, bone loss in postmenopausal women, and anxiety. At present, treatment of hyperthyroidism is directed to reduce overproduction of THs by inhibiting their synthesis or release or by ablating thyroid tissue with surgery or radioiodine. TR antagonists may have significant clinical use such as for treating hormone excess states, as it might quickly restore the euthyroid state and consequently improve the clinical manifestations mentioned above.

Several crystallographic structures of the ligand binding domains (LBDs) have been determined for TR agonists, but none include a TR antagonist.[2−9] Therefore, certain assumptions have to be made in terms of the design of new TR antagonists.

In the literature, ligand design of TR antagonists has generally been based on the attachment of a large extension group at the 5′-position of **1b** or other close analogues. This extension is believed to distort the folding of the C-terminal helix (helix 12) to the body of the LBD and thus the formation of the coactivator site. We prefer to describe this class of compounds as "direct antagonists" in order to separate them from "indirect" or "passive" antagonists.[10] Examples of synthetic TR antagonists using this "extension theory" is depicted in Figure 1 by the extensively studied *O*-[4-hydroxy-3,5-diisopropylphenyl]-L-tyrosine[11−17] (**2**), 3,5-dibromo-4-(3,5-diisopropylphenoxy)benzoic acid[18] (**3**), {4-[4-hydroxy-3-isopropyl-5-(4-nitrophenyl)-benzyl]-3,5-dimethylphenoxy}acetic acid (**4**),[19] and {4-[4-hydroxy-3-isopropyl-5-(4-nitrophenylethynyl)benzyl]-3,5-dimethylphenoxy}acetic acid (**5**).[20−21]

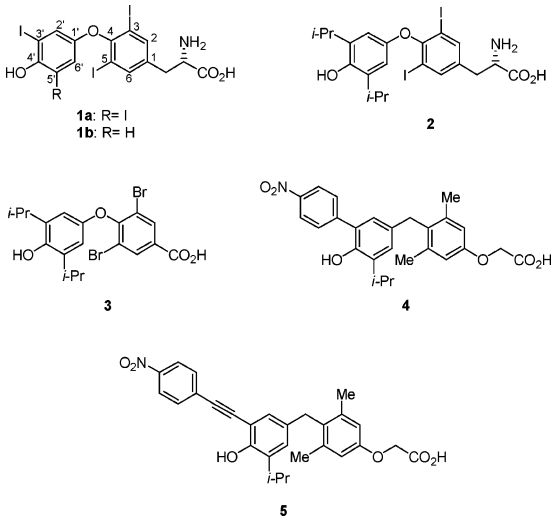

Figure 1. Chemical structures of tetraiodothyronine (**1a**), triiodothyronine (**1b**), including ring-numbering, and reported "direct" thyroid hormone antagonists ligands **2**−**5**.

In order to optimize drug feasibility by increasing water solubility, we decided to substitute the R_5'-position with a pyridyl vinyl group. Furthermore, the alkynyl part in **5** can be problematic because it potentially can ring-close with the R_4'-hydroxy to form a benzofuran ring at elevated pH,[22] perceptibly not possible with an alkene group. We have also previously showed that affinity for TR increases in the order formic, acetic, and propionic acids and that accompanying optimal substitution patterns for affinity and stability involve bromine groups at the R_3 and R_5 positions and an isopropyl group at the R_3'-position.[6]

Structure-based design work intended for the displacement of helix-12 (H12) revealed that a viable substitution of the R_5'-position indeed could be accomplished through the use of a pyridylvinyl group. The alkene part extends precisely through a well-defined hole between helix-3 and helix-11, and the terminal pyridyl group is directed into H12, thus potentially distorting the folding of H12 to the body of the LBD (Figure 2).[23]

The designed ligand, 3-{3,5-dibromo-4-[4-hydroxy-3-isopropyl-5-((*E*)-2-pyridin-4-ylvinyl)phenoxy]phenyl}propionic acid (**6**), was prepared as outlined in Scheme 1. The starting material, methyl 3-[3,5-dibromo-4-(3-isopropyl-4-methoxyphenoxy)phenyl]propionate (**7**),[24] was regioselectively nitrated at the R_5'-position, the nitro group reduced, diazotizated, and substituted with potassium iodide to give the intermediate methyl 3-[3,5-

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[a] Abbreviations: THs, thyroid hormones; TRs, thyroid receptors; LBDs, ligand binding domains; H12, helix-12; hTRa1, human TRα1; CHO K1 cells, Chinese hamster ovary K1 cells; alkaline phosphate, ALP; TRAFα1, CHO K1 cells stably transfected with hTRα1 and an ALP reporter gene downstream the thyroid response element; LDL-C, low-density lipoprotein cholesterol; SARs, structure−activity relationships; 3D, three-dimensional.

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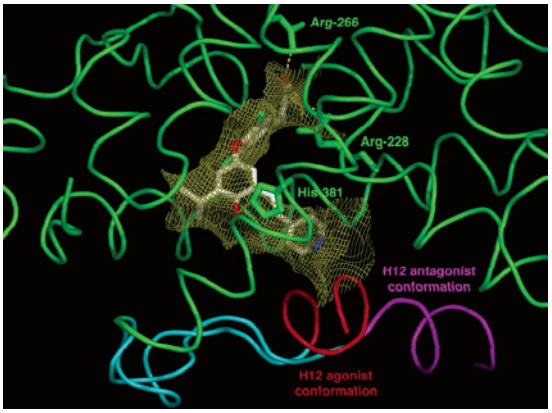
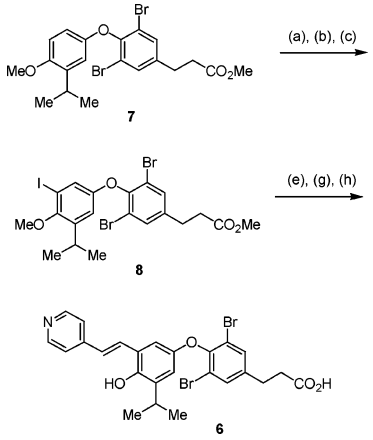


Figure 2. Depiction of the designed antagonist (**6**) docked into the ligand binding domain of TRβ. The backbone of the protein is displayed as a green tube (except the loop between H11 and H12, which is colored cyan, and antagonist conformation of H12 as magenta, and the agonist conformation of H12 as red). The side chains of residues Arg228, Arg266, and His381 are displayed as green capped sticks, and the ligand is displayed as capped sticks (white = carbon, red = oxygen, blue = nitrogen, green = bromine). Hydrogen bonds between the ligand and protein are represented by dashed yellow lines, and the surface of the ligand binding cavity is represented as a yellow mesh. There is a severe steric clash between the 5′-substituent of **6** and the agonist conformation of H12, which forces H12 into the antagonist conformation. This figure was created using the PyMol molecular graphics system.[25]

Scheme 1[a]



[a] Reagents and conditions: (a) HNO_3, C_6H_6, room temp; (b) $Na_2S_2O_4$, EtOH, 70 °C; (c) $NaNO_2$, HCl, KI; (d) BF_3·Me_2S, CH_2Cl_2; (e) 4-vinylpyridine, $Pd(OAc)_2$, TEA, DMF, 100 °C; (f) LiOH, THF, room temp.

dibromo-4-(3-iodo-5-isopropyl-4-methoxyphenoxy)phenyl]propionate (**8**) in moderate yield. After deprotection, the final product **6** was obtained in good yields via a regioselective cross-coupling between the R_5'-iodo and 4-pyridylvinyl. Total yields from the starting material **7**, using six synthetic steps, were 15% for **6**.

The results of a binding assay for the human TRα_1 and TRβ_1 for **3**−**6** and the indirect antagonist 3-(3,5-dibromo-4-cyclohexylmethoxyphenyl)propionic acid (**9**)[10] are summarized in Table 1. Compound **6** displayed high affinity for both TRα_1 and TRβ_1 and represents with respect to TRα_1-binding an improvement of affinity compared with the previously known high-affinity ligands **4** and **5**. Furthermore, **6**, in contrast to **4** and **5**, does not appear to be selective for either TR isoform, which is likely to be an advantage when treating the hyperthyroid state. Also, with the present binding data at hand for the high-affinity "direct antagonists" **4**−**6**, the value of the design approach of indirect TR antagonists appears at least in this context as somewhat less valuable. The viability of this approach

Table 1. Thyroid Hormone Receptor Binding Affinities of Synthetic Thyromimetics **3**−**6** and **9**[a]

	hTRα	hTRβ	hTRα/hTRβ[b]
3	1600	910	1.0
4	200 ± 60	35 ± 12	5.7
5	93 ± 29	20 ± 7	4.6
6	36 ± 3	22 ± 3	0.96
9	460	190	1.4

[a] All values are expressed as nM. The value for **6** is expressed as the mean IC_{50} ± SE. Data for **3** and **9** have been published before and are average mean IC_{50} values of two runs. Data for **4** and **5** were taken from ref 20 and are expressed as mean K_D ± SE. [b] Selectivity was "normalized" for **3**, **6**, and **9**: $1.7 \times [IC_{50}(hTR\alpha_1)/(IC_{50}(hTR\beta_1)]$. For an explanation, see ref 6.

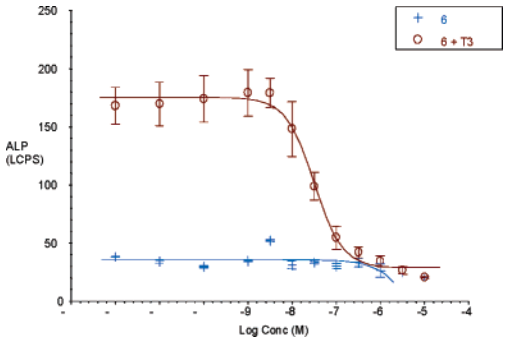


Figure 3. Transcriptional effects of **6** + L-T_3 (red curve) and **6** only (blue curve) on CHO K1 cells, stably transfected with hTRα_1 and with an ALP reporter gene downstream to a TRAFα_1.[26] The *y*-axis is expressed as light units emitted from ALP and the *x*-axis as log of the concentration of added ligand. The concentration of **6** required for 50% inhibition of L-T_3 is 32 nM. The response value for each concentration of ligand is the mean of triplicate determinations with ±SD for each value indicated.

warrants, however, further investigation and continued structure−activity relationship work.

The result from a reporter cell assay employing CHO K1 cells (Chinese hamster ovary K1 cells) stably transfected with hTRα_1 and an alkaline phosphate reporter gene downstream the thyroid response element (TRAFα_1), is shown in Figure 3. Clearly **6** acts as a full antagonist in the TRAFα cell assay and the IC_{50} for TRAFβ was similar to that for TRAFα. The IC_{50} values were 32 nM for both TRAFα_1 and TRAFβ_1.

In order to confirm the antagonism of **6** in an in vivo experiment setting, we utilized our "standard screen" for TR agonists:[27] the cholesterol fed rat model. In this model the animals confirmed the TR antagonism of **6** by a lowering of the heart rate (−10 ± 2.5% versus vehicle treated animals, $p < 0.05$) and a possible trend toward an increase of low-density lipoprotein cholesterol (LDL-C) (+13 ± 13%). This experiment is, however, highly preliminary, and we need to further confirm the antagonism in vivo.

We have shown that a potentially druglike high-affinity direct antagonist can be designed from available crystal structures and synthesized in reasonable overall yield. Although substantial additional work on structure−activity relationships (SARs), synthetic procedures, 3D crystal structural work, and in vivo experiments is required to ensure its relevance, this approach represents a promising approach to novel and high-affinity TR antagonists.

Supporting Information Available: Experimental procedures and characterization data of **6** and methods of characterization in vivo and in vitro. This material is available free of charge via the Internet at http://pubs.acs.org.

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