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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2018–2021

Thyroid receptor ligands. Part 7: Indirect antagonists of the thyroid hormone receptor with improved affinity

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Received 6 December 2006; revised 4 January 2007; accepted 7 January 2007
Available online 13 January 2007

Abstract—Based on the concept of 'indirect antagonism' of nuclear receptors, a series of thyroid hormone receptor (TR) antagonists were prepared with improved affinity compared with what was previously described. The results of a binding assay for the human TR and reporter cell assay revealed, within this series, that an *m*-bromobenzoyl substituent (**11f**) was optimal in terms of affinity and antagonist activity. Compared with already reported TR antagonists, their affinities are within the same range, thus potentially representing useful approach to novel and high-affinity TR-antagonists.

The thyroid hormone receptor (TR) belongs to the 'super-family' of nuclear hormone receptors (NRs), which are intracellular ligand activated transcription factors.[1] Thyroid hormones (THs) exert profound and central effects on development and homeostasis of the body and regulate a variety of important genes in the muscle, liver and central nervous system. Endogenous levels of TH control overall metabolic rate, lipid levels, heart rate and mood.[2] Endogenous thyroid hormones are currently used primarily as replacement therapy for patients with hypothyroidism. Treatment with 3,5,3′-tri-iodo-L-thyronine (L-T_3, **1**) and 3,5,3′,5′-tetra-iodo-L-thyronine (L-T_4, **2**) (Fig. 1) usually returns metabolic functions to the euthyroid state.

While the potential use of TR-agonists is well-established,[3] the clinical applications of TR-antagonists might be less obvious. A TR-antagonist may, however, have significant clinical use for treating the thyroid hormone excess states thyrotoxicosis and hyperthyroidism, which arise when tissues are exposed to elevated levels of circulating **1** and **2**.[4] Clinically, this state can manifest itself in weight loss, hypermetabolism, lowering of lipid levels, cardiac arrhythmias, heart failure, muscle weakness, bone loss in postmenopausal women and anxiety. Treatment of hyperthyroidism is currently directed towards reduction of the overproduction of THs by inhibiting their synthesis or release, or by ablating thyroid tissue with surgery or radioiodine.[5] However because of the long half-life of thyroid hormones, these therapies require several weeks before the euthyroid state is restored. A TR-antagonist would quickly restore the euthyroid state and consequently rapidly alleviate the clinical manifestations mentioned above. Therefore, a TR-antagonist could be used as a short-term supplemental therapy or even as a monotherapy if careful titration was applied. Ideally, such a ligand should be non-selective for either TRα or TRβ.

Rational ligand design of TR-antagonists has largely been based on the attachment of a large extension group at the $R^{5'}$-position of **1** or other close analogues. This extension is believed to displace the C-terminal helix (helix 12) from a conformation that promotes co-activator binding to a second conformation which promotes co-repressor binding (direct antagonism). A number of examples of synthetic TR-antagonists using this strategy are known in the literature and is exemplified in Figure 2

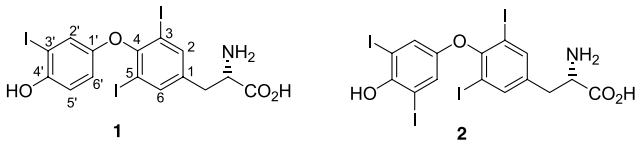

Figure 1. Chemical structures of L-T_3 (**1**) and L-T_4 (**2**), including ring-numbering of **1**.

Keywords: Thyroid hormone receptor; Thyroid hormone antagonist; Indirect antagonism; Benzyloxyphenyl derivatives; Thyroid hormone agonist; Thyrotoxicosis; Hyperthyroidism.
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0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2007.01.009

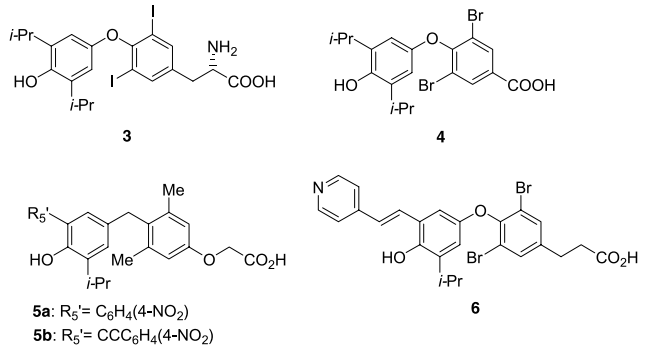


Figure 2. Chemical structures of direct antagonists **3**–**6**.

by the extensively studied compound **3**[6] and the more recent compounds **4**[7], **5a**,[8] **5b**[9] and **6**.[10]

Based on the concept of 'indirect antagonism' of estrogen receptors (ERs),[11,12] we previously showed that instead of direct antagonism, antagonism for TR also can be achieved through ligands that are smaller than direct antagonists and even agonists (indirect antagonism).[13] This work provided ligands where the ring containing the phenol moiety in thyromimetics (outer ring) was replaced with straight, branched or cyclic alkyl entities. The best binder among these new ligands, a cyclohexyl derivative (**7**), gave affinity comparable with already reported direct TR-antagonists **3**, **4** and **5a**, but less potent compared to the most recently reported direct TR antagonists **5b** and **6.**

Due to the greater ease of introducing a more variable set of functional groups at different positions, we decided to utilize benzyl instead of methylcyclohexyl as the outer ring. This strategy is outlined in Figure 3. Furthermore, in order to maximize affinity, and in accordance with what has been reported previously, we decided to keep the R^1 side-chain substituted with propionic acid and the R^3, R^5-positions with bromine.

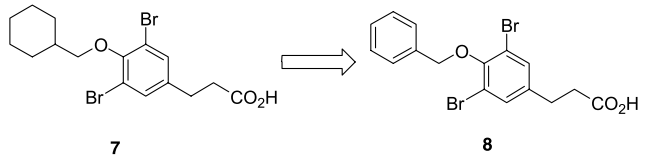
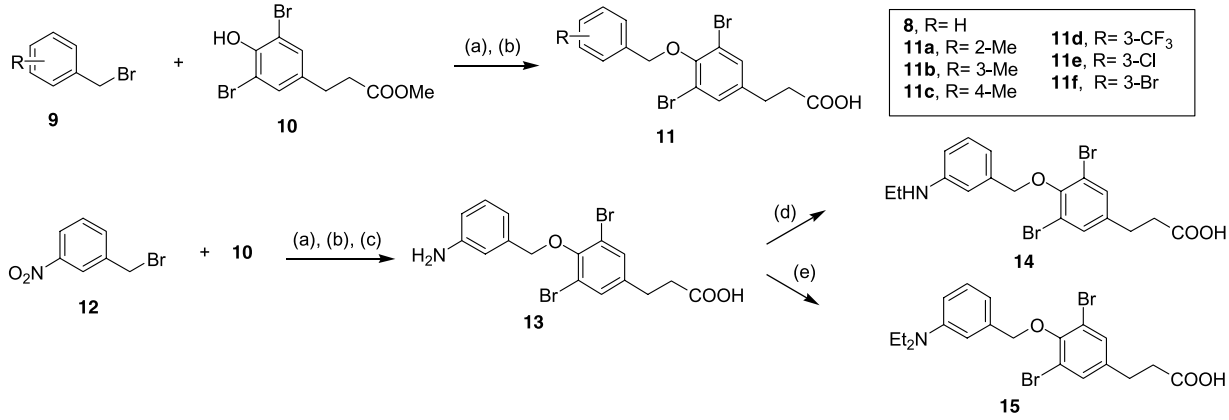


Figure 3. Chemical structures of **7** and **8**.

Thus, a series of ligands where the outer ring of a thyromimetic was replaced with a benzyl group containing various functional group substitutions was prepared as outlined in Scheme 1. Commercially available benzyl bromides **9** and **12** were coupled with phenol **10** in the presence of base, to give the appropriate benzyloxyphenyl derivatives; which were subsequently deprotected through saponification to give the end products **8**, **11a**–**f** and **13**. Further manipulation of **13** gave the monoethyl and diethyl-substituted anilines **14** and **15**, respectively.[14]

The results of a radioligand human binding assay for the human TRα1 and TRβ1, and its calculated TR-isoform selectivity is summarized in Table 1.

Affinity for TR-binding was marginally changed when the methylcyclohexyl entity (**7**) was replaced with a benzyl group (**8**), which encouraged us to start substituting the outer ring with additional hydrophobic bulk (i.e., methyl). From these results, it is obvious that optimal position of a methyl group is substitution at the R^3-position (**11b**) of the benzyl group, which was superior for TR-binding versus the other ligands with R^2-methyl (**11a**) or R^4-methyl (**11c**) substitution. Compared with the unsubstituted benzyl derivative (**8**), affinity for TR-binding approximately doubled for **11b**. Further substitution of the R^3-position with trifluoromethane (**11d**), chlorine (**11e**) and bromine (**11f**) revealed that **11f** was optimal in terms of affinity as well as for TR-receptor subtype selectivity. When the R^3-position was substituted with a secondary amine (**14**) and a tertiary amine (**15**) TR-affinity was even further improved, even though the subtype selectivity with these ligand was somewhat leaned towards $TR\beta_1$. Ligands **11f** and **14** had significantly improved affinities (lower IC_{50}'s) when compared with the previously reported 'indirect' ligand **7**, while affinity was at least within the same range as for the 'direct' antagonists **5a**, **5b** and **6**.



Scheme 1. Reagents and conditions: (a) K_2CO_3, acetone, Δ; (b) NaOH, MeOH, rt; (c) $Na_2S_2O_4$, EtOH, 70 °C; (d) $NaBH_4$, CH_3COOH, rt; (e) $NaBH_4$, CH_3COOH, 60 °C.

Table 1. Thyroid hormone receptor-binding affinities (IC_{50} or K_D's) of **1** and synthetic thyromimetics **4**, **5a**, **5b**, **6**–**8**, **11a**–**f** and **13**–**15**[a]

Compound	hTRα_1 (IC_{50})	hTRβ_1 (IC_{50})	hTRα_1/hTRβ_1[b]
1	0.24	0.26	0.54
4	1600	910	1.0
5a	200 ± 60	35 ± 12	5.7
5b	93 ± 29	20 ± 7	4.6
6	36 ± 3	22 ± 3	1.6
7	460	190	1.4
8	680	230	1.7
11a	1300	200	3.8
11b	360	120	1.8
11c	600	360	0.98
11d	370	160	1.4
11e	500	190	1.5
11f	99	60	0.97
13	2600	820	4.5
14	78	23	3.4
15	480	87	3.2

[a] IC_{50} values are expressed as nM and are calculated means of duplicate runs for **1**, **4**, **7**, **8**, **11a**–**f** and **13**–**15**. Data for **1**, **4**, **5a** **5b**, **6** and **7** are taken from Refs. 7–10, 13 and 15, 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, as well as experimental procedures, see Ref. 15 (and references cited therein).

Table 2. Thyroid hormone receptor-binding affinities (IC_{50}) of **1** and synthetic thyromimetics **11f** and **14**[a]

Compound	*h*TRAFα_1 (IC_{50})	hTRAFβ_1 (IC_{50})	*h*TRAFα_1 (EC_{50})	*h*TRAFα_1 (EC_{50})
11f	380 (80)[b]	230 (63)	NE[c]	NE
14	NE	NE	23 (88)	49 (76)

[a] IC_{50} and EC_{50} values are expressed as nM and are calculated means of duplicate runs for **11f** and **14**. The variability was approximately 25% between the runs. Experimental procedures for this assay can be found in Ref. 15 (and references cited therein).

[b] Values within parentheses refer to % antagonism or agonism, respectively.

[c] NE, no effect or very weak effect.

The two most promising ligands in terms of affinity and selectivity in the TR-binding assay, **11f** and **14**, were tested in a reporter cell assay employing Chinese hamster ovary cells stably transfected with *h*TRα_1 or hTRβ1 and an alkaline phosphate reporter gene downstream from a thyroid hormone receptor response element (TRAFα1 and TRAFβ1) as shown in Table 2.

Interestingly, **14** behaves like an agonist in the transactivation assay, while **11f** displays an expected TR-antagonism. This might be due to the H-binding capacity of **14** to His-435 (TRβ_1-numbering),[16] which eventually by virtue of its own can re-arrange the ligand binding pocket into an agonist conformation. As mentioned before in the literature this property is likely to be important for functional activity.[3]

A representative graph for TRAFα_1 with **11f** is shown in Figure 4. The graphs clearly show that **11f** is a competitive antagonist for TRα_1 in this cell system.

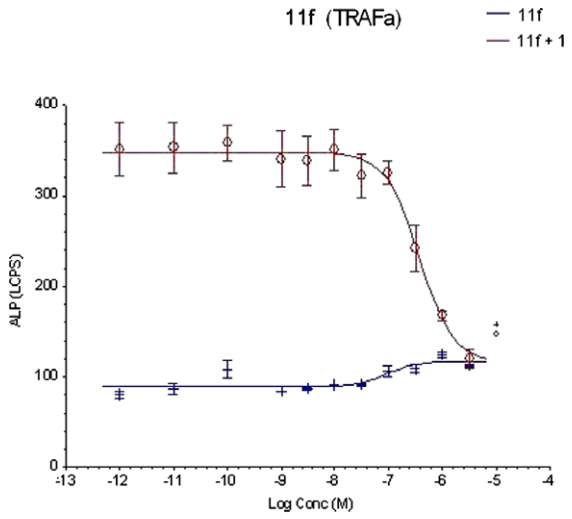


Figure 4. Transcriptional effects of **11f** + **1** (L-T_3) (red curve) and **11f** only (blue curve) on CHOK1-cells (Chinese hamster ovary cells), stably transfected with hTRα_1 and with an alkaline phosphate (ALP) reporter gene downstream to a thyroid response element (TRAFα_1).[17] The *y*-axis is expressed as light units emitted from ALP and the *x*-axis as log concentration of added ligand. The concentration of **11f** required for 50% inhibition of L-T_3 (**1**) is approximately 90 nM. The response value for each concentration of ligand is the mean of triplicate determinations ±SD for each value indicated.

Table 3. Various nuclear receptor-binding affinities (IC_{50}) for **11f**[a]

	AR	ERα	GR	PR	MR
IC_{50}	7700	29,000	14,000	2500	8700

[a] IC_{50} values are expressed as nM and are calculated means of duplicate runs. AR, androgen receptor; ERα, extrogen alpha receptor; GR, glucocorticoid receptor; PR, progesterone receptor; MR, mineral corticoid receptor. The data are the result of a single measurement and constructed and measured analogously to the TR-binding assay described in Ref. 15.

As **11f** appeared to be the overall best ligand in terms of affinity and antagonism action, it was further tested for other nuclear receptors (NRs). This is shown in Table 3. The result of this NR-receptor screen revealed that selectivity for most receptors was almost >100 times, except for the progesterone receptor (PR) where selectivity for TRα_1 over PR binding is only 25 times. This finding clearly represents a potential problem and further structure–activity relation (SAR) work has to be conducted in order to avoid this unwanted activity.

In summary, we have shown that the concept of 'indirect antagonism' among NRs might be further rationalized also for TRs. Compared with our previous work, incremental improvement for affinity was achieved for this class of ligands. Compared with already reported 'direct' TR-antagonists, affinity for this 'indirect' TR-antagonist **11f** was clearly within the same range. Optimal affinity, selectivity, and antagonist activity were found with **11f**, but most probably there is a limitation to size of groups that can be accommodated within this region by the ligand-binding pocket of TR, as well as a dependence on the exact orientation. We also revealed that the

presence of an H donator in the outer ring will turn this class of ligands into agonists in a transactivation assay.

Acknowledgment

Beatriz Campos is acknowledged for her support in compound handling and recording of MS-spectra.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.009.

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