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Discovery of a Novel Series of 6-Azauracil-Based Thyroid Hormone Receptor Ligands: Potent, TRβ Subtype-Selective Thyromimetics

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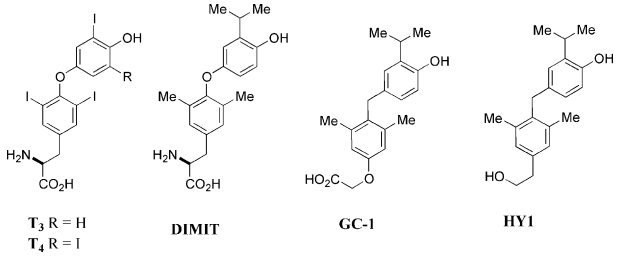
Abstract—In this communication, we wish to describe the discovery of a novel series of 6-azauracil-based thyromimetics that possess up to 100-fold selectivities for binding and functional activation of the β_1-isoform of the thyroid receptor family. Structure–activity relationship studies on the 3,5- and 3′-positions provided compounds with enhanced TRβ affinity and selectivity. Key binding interactions between the 6-azauracil moiety and the receptor have been determined through of X-ray crystallographic analysis.

It has been well established in humans that a hyperthyroid state leads to increased metabolic rate (thermogenesis).[1] However, attempts to enhance metabolic rate in obese patients via treatment with thyroxine (T_4), which is metabolized in various tissues to afford the active thyroid hormone T_3, leads to unacceptable cardiac side effects. Human cardiac tissue has been shown to contain predominately TRα_1, a major isoform of the thyroid hormone receptor (TR) family.[2] T_3 does not show preferential binding amongst the major TR isoforms (TRα_1, TRβ_1, and TRβ_2), suggesting that identification of a TRβ-specific agonist could lead to an agent which would retain the thermogenic potential, but would be devoid of the cardiac side effects.

To date there have been a limited number of reports on efforts to identify thyroid ligands that interact selectively with the various TR isoforms. During the course of our work, investigators at UCSF have reported that an oxyacetic acid analogue (GC-1) of DIMIT[3] is a potent thyromimetic which exhibits ~10-fold selectivity in binding to the TRβ subtype.[4] More recently, HY-1 a neutral analogue of GC-1 was discovered to be 5-fold more potent in activating TRβ mutant (R320C) relative to the wild-type.[5] In this communication, we wish to describe the discovery of appropriately functionalized 6-azauracils that are potent and TRβ-selective thyromimetics.



Screening of the Pfizer sample collection led to the discovery of several 6-azauracil-based diphenyl ethers (**1**)

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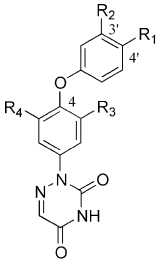
that bind to TRβ$_1$ with submicromolar potencies and modest (5- to 15-fold) selectivities versus the α$_1$-isoform of the thyroid receptor.[6] These compounds were originally prepared as part of an anticoccidial program in the Veterinary Medicine Division.[7,8] From a limited set of extant compounds, those derivatives containing a 4′-methylthioether substituent (R_1 = SMe) possessed the greatest TRβ$_1$ potencies and selectivities. Not surprisingly, the most potent analogue (**2**) identified in this initial screen contains a lipophilic substituent (R_2 = methyl) in the 3′-position, which overlaps with the iodine and isopropyl substituents in T_3 and DIMIT, respectively. Compound **2** possesses good binding affinity (K_i = 2 nM, T_3 K_i = 0.08 nM) for TRβ$_1$ and has a 6-fold lower affinity for the α$_1$-isoform of the thyroid receptor.



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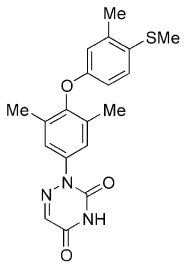

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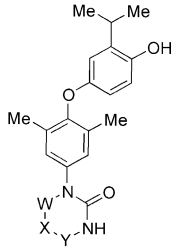


3 W-X = (N=CH), Y = (C=O)
4 W-X = (CH=CH), Y = (C=O)
5 W-X = (CH_2-CH_2), Y = (CH_2)

Neither **2** nor any of the other initial screening leads exhibit significant functional agonism of thyroid receptors. The lack of functional activity is due to the absence of the 4′-phenolic functionality present in classical thyromimetics which forms a hydrogen bond with a histidine residue in the TR binding pocket.[9] Incorporation of the key 4′-hydroxyl and 3′-isopropyl functionalities (**3**) produces ∼100× improvement in TRβ$_1$ binding affinity (K_i = 0.03 nM) and functional agonism in E25B2 cells.[10] Whereas DIMIT and T_3 possesses no TR isoform selectivity, compound **3** retains a modest 8-fold TRβ$_1$ selectivity, confirming that the 6-azauracil functionality is responsible for the TR isoform selectivity observed.

To gain insight into the structural features of the 6-azauracil moiety responsible for TR binding affinity, an X-ray crystal structure of **3** bound to TRβ$_1$ was generated (see Fig. 1).[11] In analogy to the binding modes for T_3 and GC-1,[12] the ionized imide functionality of the 6-azauracil forms a hydrogen-bonding array with Arg320 of TRβ$_1$. In contrast to the structures of T_3 and GC-1, an additional hydrogen bond interaction also occurs between the C-4 carbonyl of **3** and Arg316. A number of SAR findings relating to the 6-azauracil moiety are consistent with this binding motif defined by X-ray crystallography. Elimination of the acidic proton at N-3 of the azauracil ring (via *N*-methylation—data not shown) produces a > 50-fold reduction in receptor affinity. Removal of the nitrogen in the 6-position to afford the corresponding uracil **4** results in a 120-fold reduction in binding affinity for the TRβ1 receptor. While **4** loses significant TRβ$_1$ potency it still retains a TRβ$_1$/TRα selectivity (9-fold) that is comparable to that seen for the azauracil. Further simplification to the six-membered cyclic urea (**5**) completely eliminates TR binding (K_i > 10,000 nM). This alignment of N-3 acidity (**3** > **4** ≫ **5**) and binding potency further supports the importance of the Arg320 interaction with an ionized imide functionality.

Given the strict structural requirements of the 6-azauracil required for TR binding, further improvements in TRβ binding potency or selectivity would have to be driven through modification of the diphenyl ether core. However, since the only difference between the ligand binding domains of hTRβ and hTRα is a single residue (Ser277 in TRα for Asn331 in TRβ) there was some question as to whether modifications to the diphenyl ether would have any impact on TR isoform binding selectivity. While there have been a number of SAR reports on modifications to the diphenyl ether core (ether linker, 3,5- and 3′-positions) of T_3, few of these efforts have been directed towards enhancing TR isoform binding selectivity.

Initial efforts around the 3′-position focused on profiling a range of substituents, including aminomethyl, acylamino, sulfonyl, alkyl, aryl, alkylamino, arylamino, acyl, carboxamido and sulfonamido (data not shown). From this initial evaluation of 3′-functional groups, carboxamido- and sulfonamido-based substituents exhibited the greatest potential for identifying potent, TRβ-isoform selective thyromimetics.

To aid in rapid structure–activity relationship development in the 3′-position, synthetic routes were designed which allow for the incorporation of the amide or sulfonamide functionalities as the final step. The core 6-azauracil-based diphenyl ethers (**6**) are constructed utilizing methodologies previously reported.[7,8] The 3′-position of the diphenyl ether core can be readily and selectively functionalized by formylation, chlorosulfonylation or nitration, employing **6** or the corresponding phenol (Scheme 1). Oxidation of the 3′-formyl derivative to the carboxylic acid and methyl ether cleavage affords **8**. Activation of this o-hydroxyacid with *N*-hydroxysuccinimide provides a stable, activated ester

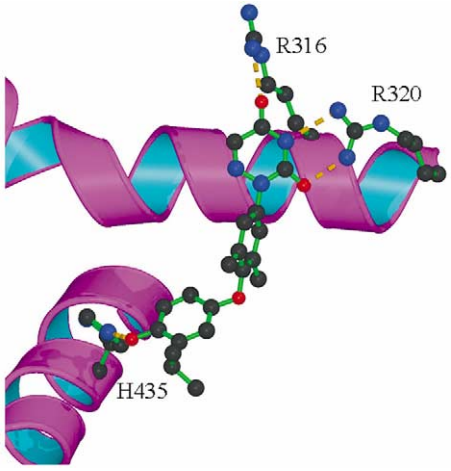
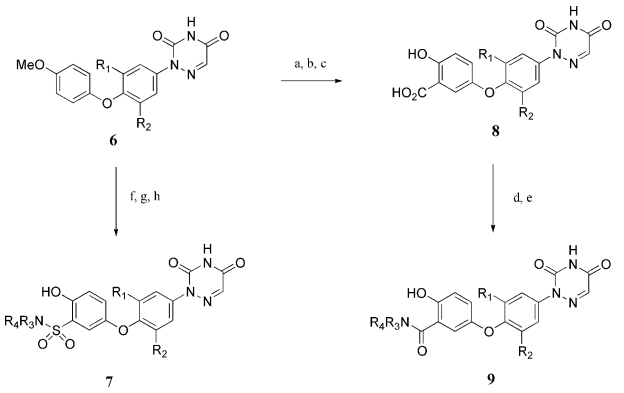


Figure 1. Binding of **3** to human TRβ$_1$.[11]

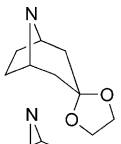
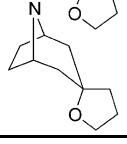


Scheme 1. Reagents and conditions: (a) hexamethylenetetramine, TFA, reflux (65%); (b) $NaClO_2$, 2-methylbutene, KH_2PO_4, *t*-BuOH, THF, H_2O (79%); (c) BCl_3, CH_2Cl_2, −78 °C to rt (78%); (d) *N*-hydroxysuccinimide, 1,3-dicyclohexylcarbodiimide, DME, 0 °C to rt (83%); (e) R_3R_4NH, TEA, DME (65–90%); (f) BBr_3, CH_2Cl_2, −78–0 °C (57%); (g) chlorosulfonic acid, 0 °C (50%); (h) R_3R_4NH (3 equiv), THF (45–80%).

that can be employed in the preparation of a range of 3′-amides (**9**). Synthesis of the corresponding 3′-sulfonamides (**7**) is accomplished through initial cleavage of the methyl ether of **6**. Condensation of this phenol with chlorosulfonic acid provides the 3′-chlorosulfonyl intermediate, which can be coupled with amines to form the target sulfonamides **7**.

Table 1 details a cross-section of a wide range of sulfonamide substituents that were profiled in the 3′-position of the diphenyl ether core. Incorporation of the piperidinylsulfonamide group into the core structure (**7a**) produces a 15-fold loss in TRβ binding affinity relative to isopropyl analogue **3**. As part of the initial profiling in this series, the piperidinyl sulfonamide substituent was held constant while the 3,5-substituents were varied (**7a–c**). As is observed for other 3′ substituents, potency and selectivity increases in the following order: Me, Me < Me,Cl < Cl,Cl. In the case of the **7c** binding affinity is restored to a level that is comparable to **3** and 4× more potent than T_3. In addition to enhanced potency, **7c** also possesses an ∼4-fold enhanced TRβ/TRα selectivity relative to **3**.

Table 1. Inhibition of [^{125}I]-T_3 binding to human $TR\beta_1$ and $hTR\alpha_1$ by 3′-sulfonamides[6]

Compd	R_1	R_2	N-R_3R_4	TR binding	
				$TR\beta_1$ K_i (nM)	α/β[a]
T_3				0.08	0.8
7a	Me	Me	Piperidinyl	0.46	14.8
7b	Me	Cl	Piperidinyl	0.13	21.2
7c	Cl	Cl	Piperidinyl	0.02	30.9
7d	Me	Cl	4-Methylpiperizinyl	13	8.2
7e	Me	Cl	Morpholinyl	27	13
7f	Cl	Cl	Cyclohexylamino	0.46	21.3
7g	Cl	Cl	Cyclobutylamino	0.40	55
7h	Cl	Cl	Anilinyl	0.10	14.3
7i	Cl	Cl	Indolinyl	0.23	13.5
7j	Cl	Cl		0.31	33.0
7k	Cl	Cl		0.28	86

[a]Ratio of TRα K_i/TRβ K_i.

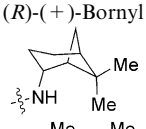
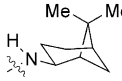
Attempts to introduce heteroatom functionality (**7d–e**) into the 3′ site result in significantly reduced binding affinity. These results are consistent with the highly lipophilic nature of the 3′-receptor binding pocket.[9] Sulfonamides derived from primary, aliphatic amines (e.g., **7f–g**) exhibit reduced binding affinities (10–20×) relative to tertiary sulfonamides of comparable size, though binding selectivity is not diminished. TRβ binding is not diminished to such a great extent when a primary, aromatic amine (**7h**) is incorporated. Unlike in the case for aliphatic amine-based sulfonamide substituents, aromatic amine-derived, tertiary sulfonamides such **7i** or the *N*-methylaniline analogue (data not shown) do not improve binding affinity.

Based on the above results a range of tertiary sulfonamide substituents derived from aliphatic amines were profiled. In general, 3′ substituents more sterically demanding than that present in **7c** lead to a reduction in $TR\beta_1$ affinity. One exception to this trend is **7j**, which incorporates a tropinone-based functionality. Because of the hydrolytic instability of the ketal moiety in **7j** the homologous spiro-tetrahydrofuranyl analogue **7k** was prepared.[13] Analogue **7k** binds potently and selectivity (86-fold) to $TR\beta_1$. In E25B2 cells **7k** is a full agonist, however it is ∼30-fold less active than T_3 (EC_{50} = 62 nM, T_3 = 2 nM) and is 28-fold selective versus TRα (EC_{50} ∼1.5 μM, T_3 = 2 nM).

Structure–activity trends for the 3′-carboxamido series were quite distinct from that seen for the sulfonamides (Table 2). Though piperidinyl derivative **9b** is more active than the 3-chloro-5-methyl analogue **9a**, it is ∼30-fold less potent in binding to $TR\beta_1$ than the corresponding sulfonamide (**7c**). Also in contrast to the 3′-sulfonamides, amides derived from primary amines produced a substantial improvement in potency (**9c** vs **9b**), though a decrease in the lipophilic character (**9d**) leads to a 10-fold reduction in potency. Another interesting point of divergence between the two series is that incorporation of a morpholinyl substituent did not significantly reduce potency (**9e** vs **9b**) in the carboxamide case.

Based on **9c**, a range of commercially available mono- and bicyclic-based secondary carboxamide substituents were evaluated. From this effort several bicyclic amine-based analogues (**9f–9h**) were discovered to possess potent $TR\beta_1$ binding affinity and selectivities of up to 100-fold versus TRα. As a follow-up to these findings, the enantiomeric, nopinone-derived amines were incorporated to provide **9i** and **9j**.[14] While both are potent (K_i ∼50 pM) binders, the R-enantiomer is substantially more selective. In functional assays **9i** is both a potent

Table 2. Inhibition of [^{125}I]-T_3 binding to human TRβ_1 and hTRα_1 by 3′-carboxamides[6]

Compd	R_1	R_2	N-R_3R_4	TR binding	
				TRβ_1 K_i (nM)	α/β[a]
T_3				0.08	0.8
9a	Me	Cl	Piperidinyl	1.9	75.3
9b	Cl	Cl	Piperidinyl	0.63	66.6
9c	Cl	Cl	Cyclohexylamino	0.07	33.8
9d	Cl	Cl	Cyclobutylamino	0.64	73.8
9e	Cl	Cl	Morpholinyl	0.72	30.1
9f	Cl	Cl	(±)-*exo*-2-Norbornyl	0.59	104.8
9g	Cl	Cl	(±)-*endo*-2-Norbornyl	0.16	32.2
9h	Cl	Cl	(*R*)-(+)-Bornyl	0.37	49.7
9i	Cl	Cl		0.06	58.2
9j	Cl	Cl		0.04	15

[a]Ratio of TRα K_i/TRβ K_i.

agonist (EC_{50} = 12 nM) and highly selective (77-fold) versus TRα.

To date, a detailed receptor structure-based understanding of the impact on TR binding of these 3′ modifications has been thwarted by an inability to co-crystallize ligands containing a 3′-substituent larger than isopropyl. It is presumed that modifications of the 3′-position produce conformational/positional shifts that negatively affect binding around the polar pocket (vicinity of Ser277) of TRα.

A series of 3′-sulfonamide and 3′-carboxamide analogues of the screening lead **2** have been prepared. Several of these compounds have been shown to be potent and TRβ-selective thyromimetics. The potential for these selective agents to increase metabolic rate, while limiting cardiovascular side effects, will be the subject of future reports.

References and Notes

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6. Compounds (20 mM in 100% DMSO) were serially diluted in 100% DMSO, then diluted in assay buffer (5 mM Tris–HCl, pH 8.0; 50 mM NaCl; 2 mM EDTA; 10% (v/v) glycerol; 1 mM DTT) containing 0.4 nM [^{125}I]-T_3 (specific activity = 2200 Ci/mmol). High Five insect cell nuclear extract, prepared from cells engineered to express either human TRα_1 or TRβ_1, was diluted in assay buffer, then combined with an equal volume of each compound dilution (final ^{125}I-T_3 = 0.2 nM). After incubating at room temperature for 90 min, bound ^{125}I-T_3 was separated from free using a filtration manifold (Millipore #MHAB N45). Radioactivity was quantitated in a Wallac Microbeta plate scintillation counter, and IC_{50} or K_i values were calculated using non-linear regression analysis (GraphPad/Prism).
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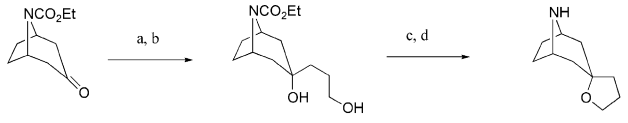
10. Cells (E25B2) were transiently transfected (using lipofectin) with cDNAs encoding either human TRα_1 or TRβ_1 together with a plasmid containing firefly luciferase under the control of a thyroid hormone response element (DR + 4). Following transfection, the cells were distributed into 96-well plates and compounds and/or T_3 were added to the culture medium and luciferase activity was measured in cell lysates approximately 18 h later.
11. Recombinant human TRβ ligand-binding domain (residues 204-461) was expressed in *Escherichia coli*. Isolation and purification of the protein was performed using anion exchange, size exclusion and cation exchange chromatography, maintaining the presence of **3** throughout the purification. Crystals of the complex were grown by hanging-drop vapor diffusion. The reservoir solution contained 1.8 M $(NH_4)_2SO_4$ in 0.1 M Na Hepes, pH 6.0. The crystals were monoclinic, space group P2(1), with unit cell dimensions a = 42.92, b = 105.31, c = 55.99 Å, b = 98.19°, and contained two molecules of the protein–ligand complex in the asymmetric unit. Diffraction data to 2.2 Å resolution were collected on an RAXIS-IIc detector mounted on a Rigaku Ru200 X-ray generator. The structure was solved by molecular replacement, using the coordinates of a the structure of human TRβ complexed with T3, solved earlier (data not shown). The atomic coordinates have been deposited in the Protein Data Bank, http://www.rcsb.org (PDB ID code 1N46).
12. Wagner, R. L.; Huber, B. R.; Shiau, A. K.; Kelly, A.; Cunha Lima, S. T.; Scanlan, T. S.; Apriletti, J. W.; Baxter, J. D.; West, B. L.; Fletterick, R. J. *Mol. Endocrin.* **2001**, *15*, 398.
13. Tropane-based amine **11** was synthesized from commercially available *N*-carboethoxy-4-tropanone via the sequence outlined below:



Reagents: (a) allylmagnesium chloride, THF, −78 °C (95%); (b) BH_3·THF, THF, then NaOH (aq), 3% aq H_2O_2, MeOH (63%); (c) *p*-toluenesulfonyl chloride, pyridine, 5 °C (55%); (d) hydrazine hydrate, ethylene glycol, reflux (80%).
14. The amines utilized in the preparation of **9i** and **9j** were synthesized from the enantiomers of nopinone utilizing the following procedure. Ipaktschi, J. *Chem. Ber.* **1984**, *117*, 856.