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Design and synthesis of a novel series of [1-(4-hydroxy-benzyl)-1*H*-indol-5-yloxy]-acetic acid compounds as potent, selective, thyroid hormone receptor β agonists



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ABSTRACT

The design, synthesis, and structure activity relationships for a novel series of indoles as potent, selective, thyroid hormone receptor β (TR β) agonists is described. Compounds with >50× binding selectivity for TR β over TR α were generated and evaluation of compound **1c** from this series in a model of dyslipidemia demonstrated positive effects on plasma lipid endpoints in vivo.

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Thyroid hormone is an important mediator of a variety of biological processes including basal metabolic rate and lipid metabolism. Hyperthyroid individuals have an increased basal metabolic rate leading to weight loss and increased cholesterol metabolism and excretion leading to low serum cholesterol levels.¹ 3,5,3'-Triiodo-L-thyronine (T3), the major active thyroid hormone, is a potent agonist of TR α and TR β receptors. The utility of T3 as a treatment for obesity and hypercholesterolemia has been limited by unacceptable cardiac side effects. The major isoform of thyroid hormone receptor in human cardiac tissue is TR α .² This led to the speculation that identification of a TR β selective agonist would increase metabolic rate and beneficially affect cholesterol metabolism, be devoid of cardiac liabilities, and would therefore be a useful therapeutic agent.

Investigation of the structure activity relationships for thyroid hormone receptor modulators has been studied for several decades.³ Recently selective thyroid hormone receptor agonists that have higher affinity for TR β receptors compared to TR α receptors

http://dx.doi.org/10.1016/j.bmcl.2015.02.062 0960-894X/© 2015 Elsevier Ltd. All rights reserved. have been reported (SKF-94901, CGS-23425, CGS-26214, and GC-1,⁴ GC24,⁵ Pfizer azauracil,⁶ KB-141⁷).

Here we report the design and synthesis of a series of $[1-(4-hydroxy-benzyl)-1H-indol-5-yloxy]-acetic acid compounds as potent, selective, thyroid hormone receptor <math>\beta$ (TR β) agonists. Computational studies were performed by superimposing proposed compounds from this series with T3 using the published PDB X-ray crystal structure 1BSX of the TR β receptor.⁸

Modeling low energy conformations where the carboxylic acid was in a position to interact with key Arg residues and the phenol was in a position to interact with His-435 resulted in the indole core overlapping with the diiodo-phenyl ring of T3. In these conformations the 2- and 3-positions of the indole overlapped with one of the iodine atoms of T3 and the 7-position of the indole overlapped with the other iodine atom of T3, (see Fig. 1). A series of 2-, 3-, and 7-substituted indole analogs were designed and synthesized to investigate the effect of the indole ring substituents on affinity for TR β receptors and selectivity relative to TR α (see Table 1).

[1-(4-Hydroxy-benzyl)-1*H*-indol-5-yloxy]-acetic acid compounds (**1a-o**) were synthesized by alkylating the corresponding indoline

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Figure 1. Superimposition of T3 (green) and the indole system 1c (orange) in TR β (1BSX).

intermediate (**2**) through a reductive amination with the requisite benzaldehyde derivative (**3a**). This reaction worked well with the 7-H indoline intermediates. The reductive amination strategy with the 7-methyl and 7-chloro indoline intermediate did not give any of the desired product, therefore, these indoline intermediates were alkylated with the benzyl chloride (**3b**). The resulting alkylated indoline (**4**) was oxidized with DDQ to give the indole (**1**).⁹ The yield for this oxidation was higher for the 2-methyl or 3-methyl indoline oxidations compared to the oxidation of the unsubstituted indoline. The triisopropylsilyl ether was removed by treatment with TBAF and the ethyl ester was saponified with sodium hydroxide to give the targeted compounds (Scheme 1).

The intermediate indolines were prepared as described in Scheme 2. 5-Hydroxy-3-methyl-indole (**6**) was synthesized from 5-benzyloxy-gramine (**5**) by hydrogenation with Pd/C in ethanol

Table 1



Compound	\mathbb{R}^2	R ³	R ⁷	TR $\beta K_i (nM)$	TR αK_i (nM)	$K_{\rm i}$ TR β /TR α
1a	Н	Н	Н	1.04	13.45	13
1b	Me	Н	Н	1.12	16.33	15
1c	Н	Me	Н	0.38	7.74	20
1d	Н	Н	Me	0.26	1.09	4
1e	Н	Н	Cl	0.07	0.35	5
1f	Me	Н	Me	0.14	2.05	15
1g	Me	Н	Cl	0.04	0.38	10
1h	Н	Me	Me	0.18	2.77	15
1i	Н	Me	Cl	0.09	0.51	6

(Scheme 2a). Protection of the phenol (**6**) as the triisopropylsilyl ether was accomplished under standard conditions.¹⁰ Reduction of the indole ring with sodium cyanoborohydride in acetic acid gave the 3-methyl indoline intermediate (**8a**). The unsubstituted indoline intermediate (**8b**) was prepared in an analogous manner from 5-hydroxyindole (**6b**) (Scheme 2b). The 2-methyl indoline intermediate (**11**) was synthesized from 5-methoxy-2-methyl-indole (**9**) by reduction with sodium cyanoborohydride in acetic acid, removal of the methyl ether with boron tribromide, and protection of the phenol as the triisopropylsilyl ether (Scheme 2c).

The substituent at the 7 position was introduced by a directed metalation strategy on the *t*-butylcarbamate of the indoline (**12**).¹¹ The *t*-butyl carbamate was synthesized with ditert-butyldicarbonate and the carbamate was deprotonated with *sec*-butyl lithium in the presence of TMEDA. The resulting anion was allowed to react with either methyl iodide to give the 7-methyl derivative or dichloroethane to give the 7-chloro derivative (**2**) (Scheme 2d).

The requisite benzaldehyde (3a) and benzyl chloride (3b) intermediates were synthesized as described in Scheme 3. 2-Bromo-anisole (13) was coupled with an alkyl Grignard reagent via a Kumada reaction to give alkylated anisole intermediate (14).¹² This anisole was subsequently deprotected with boron tribromide to afford the phenol (15). Several commercially available phenols (cyclopentyl, cyclohexyl, t-butyl, isopropyl, benzyl) were also used. Bromination with HBr in acetic acid and DMSO gave the desired *para* bromo intermediate (16). Silylation of the phenol with triisopropylsilyl chloride or t-butyldimethylsilyl chloride gave the desired silvl ether (17).¹⁰ Lithium–halogen exchange (*t*-butyl lithium or *n*-butyl lithium) followed by quenching with DMF provided the benzaldehyde intermediate (3a). The benzyaldehyde (3a) was used in the reductive aminations of the 7-H indolines. Alternatively, the benzyaldehyde was reduced with sodium borohydride to give the benzyl alcohols (19) which were converted to the appropriate benzyl chlorides (3b) by treatment with methanesulfonyl chloride. These benzyl chlorides (3b) were used for the alkylation of the 7-methyl and 7-chloro indolines.

The 2-methyl-7-chloro analog (**1g**) was the most potent TR β agonist in this series and it was a potent full agonist in the Gal 4 CTF cell-based assay (TR β EC₅₀ = 29 nM, TR α EC₅₀ = 181 nM). The 3-methyl analog (**1c**) was the most selective analog in this series (K_i TR α /TR β = 20) and it was selected for evaluation in vivo (see below). The 2,7-methyl (**1f**) and 3,7-dimethyl (**1h**) analogs were more selective than the 2-methyl-7-chloro (**1g**) or the 3-methyl-7-chloro (**1i**) analogs (K_i TR α /TR β = 15 and 10 for **6** and **7**, respectively, and K_i TR α /TR β = 15 and 6 for **8** and **9**, respectively).

We designed and synthesized analogs with different substituents *ortho* to the phenol in an attempt to improve the receptor selectivity for TR β receptors in the 3-methyl indole series. The results are summarized in Table 2.

The benzyl analog (**1k**) was found to be the most selective ($K_i \operatorname{TR}\alpha/\operatorname{TR}\beta$ = 68) and it was a full agonist in the Gal 4 CTF cell-based assay (TR β EC₅₀ = 406 nM, TR α EC₅₀ = 2466 nM). The improved receptor selectivity for the benzyl analog is similar to that recently reported for GC-24.⁵ The phenyl analog (**1n**) was found to be a weak partial agonist/partial antagonist. This result highlights the importance of the interactions between this region of the ligand for efficacy in the TR β receptor.

In an effort to assess in vivo properties compound **1c** was administered orally to fructose fed rats once a day for 7 days. A diet consisting of 60% fructose (Teklad #89247) was administered to six week old male Sprague-Dawley rats for three weeks. This diet and time duration significantly raised plasma cholesterol and triglycerides. Vehicle (10% Acacia) or Compound **1c** was then administered by oral gavage, q.d., in fructose-treated animals that were randomized into groups based on non-fasted serum cholesterol



Scheme 1. Synthesis of [1-(4-hydroxy-benzyl)-1H-indol-5-yloxy]-acetic acid compounds.



Scheme 2. Synthesis of indoline intermediates.

and triglyceride concentrations. Animals were maintained on fructose diet for the course of the study. After eight days, non-fasted animals were sacrificed and serum values of cholesterol and triglycerides were generated. A pooled sample of serum from each group was submitted to size exclusion chromatography to determine HDL content. Animals exhibited dose-dependent effects on lipoprotein-cholesterol and serum triglycerides (Table 3). HDL was elevated by up to 55% and triglycerides were reduced up to 64% while food intake was not affected.

In summary, we designed and synthesized a series of [1-(4-hydroxy-benzyl)-1H-indol-5-yloxy]-acetic acid compounds that were potent selective TR β agonists. The selectivity for TR β receptors was further improved by modification of the substituent



Scheme 3. Synthesis of benzaldehyde and benzylic chloride intermediates.

Table 2



Compound	R ^o	TR βK_i (nM)	TR αK_i (nM)	$K_i TR\beta/TR\alpha$
1c	i-Propyl	0.38	7.74	20
1j	t-Butyl	0.30	9.98	33
1k	Benzyl	0.54	36.53	68
11	Cyclopentyl	0.64	32.33	51
1m	CH ₂ -cyclohexyl	1.11	46.55	42
1n	Phenyl	3.14	89.89	29
10	Cyclohexyl	8.04	199.97	25

ortho to the phenol. Evaluation of compound **1c** from this series in a model of dyslipidemia demonstrated positive effects on plasma lipid endpoints.

Table 3

Dose (mg/kg)	HDL	Serum triglycerides
0.1	22	$204 \pm 10^{*}$
0.3	39	$214 \pm 21^{*}$
1.0	55	177 ± 20*
3.0	42	149 ± 22*
10.0	35	106 ± 19 [*]
Vehicle	-	296 ± 20

Compound **1c**, dosed once a day. HDL values are HDL-cholesterol (mg/dL) percent change from vehicle group from pooled samples for each group. Serum triglycerides are mg/dL. N = 6 per group.

 * Significantly different from vehicle group (ANOVA followed by Fisher HSD posthoc test, p <0.05).

Supplementary data

Supplementary data (Experimentals for the synthesis of compound **1c** and procedures for TR α and TR β binding and functional agonist assays.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.02.062.

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