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Letter

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(−) Arctigenin and (+) Pinoresinol Are Antagonists of the Human Thyroid Hormone Receptor β

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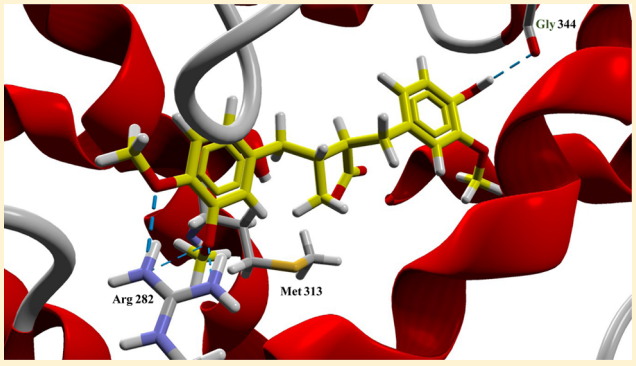
Supporting Information



ABSTRACT: Lignans are important biologically active dietary polyphenolic compounds. Consumption of foods that are rich in lignans is associated with positive health effects. Using modeling tools to probe the ligand-binding pockets of molecular receptors, we found that lignans have high docking affinity for the human thyroid hormone receptor β. Follow-up experimental results show that lignans (−) arctigenin and (+) pinoresinol are antagonists of the human thyroid hormone receptor β. The modeled complexes show key plausible interactions between the two ligands and important amino acid residues of the receptor.

1. INTRODUCTION

Compounds that make up the noncaloric components of the human diet have profound influence on the expression of genes and homeostatic regulations in biological systems although most molecular mechanisms involved in such regulations remain unknown. Phenolic and polyphenolic molecules constitute a major group of such compounds. There are over 500 structurally different dietary phenolic/polyphenol-like compounds. These include anthocyanins, chalcones, flavanols, flavones, isoflavones, phenolic acids, stilbenes, lignans, phenolic terpenes, hydroxycoumarins, etc. They are found in appreciable quantities in plant-derived edibles, such as fruits, vegetables, nuts, and seeds, as well as in many popular beverages.[1] Over the past two decades, epidemiological studies have shown that polyphenols promote vascular function, reduce hypertension, and lower the risk of cardiovascular diseases, neurodegenerative diseases, cancer, and stroke.[2,3] It is well-documented that the metabolic effects of these compounds are pleiotropic in nature.[4−6] The pleiotropy associated with these compounds seems to stem from their promiscuity toward numerous molecular targets, for example, multiple receptors or enzymes. It is becoming increasingly clear, however, that these compounds may not have therapeutic effects during pathological states but do have modulatory or hormetic effects that are largely beneficial in biological systems. These nontherapeutic effects are due, perhaps, to their relatively weak binding affinities to cognate receptors/molecular targets *in vivo* and to their susceptibility to phase II metabolic alterations.

The molecular targets of most polyphenols with reported biological activity remain unknown, but many are suspected to either activate or deactivate membrane-bound or cytosolic receptors. The isoflavones found in leguminous plants, for example, are known to have moderate binding affinities for the estrogen receptors. Isoflavones have been shown to have estrogenic effects which may or may not be advantageous, depending on the exposure levels and on the developmental or physiological state of the human subject.[7,8] Also, it was reported recently that some dietary phytochemicals perturb cell membranes and promiscuously alter protein function.[9] Human exposure to lignans occurs predominantly through consumption of flaxseeds and sesame seeds. Lignans are also present in lower amounts in broccoli, curvy kale, and apricots. It has been reported that enterolignans, such as enterodiol and enterolactone, have weak estrogenic activity.[1,10−12]

We report in this article that (−) arctigenin and (+) pinoresinol, lignans present in sesame seeds and olive oil, respectively, are antagonists of the human thyroid hormone receptor β (hTRβ), and we describe the molecular features that define the interactions between the receptor and the two lignans. Structurally, the hTRβ consists of an N-terminal domain (NTD), a DNA binding domain (DBD) which serves as the nuclear localization signal, and a C-terminal ligand binding domain (LBD). The LBD of hTRβ is made up of 12 alpha-helices. The binding cavity in the LBD is mainly hydrophobic but also contains a hydrophilic cavity. The hydrophobic portion is known to interact with the iodinated rings of thyroid hormone. Amino acid residues Arg 320, 316, and 282, as well as Asn 331, make up the hydrophilic pocket. This hydrophilic pocket mainly interacts with the polar substituent of thyroid hormone. In addition, amino acid residue His 435 in helix 11 of the ligand binding cavity serves as a hydrogen bond acceptor.[13,14]

2. EXPERIMENTAL DETAILS

2.1. Compound and Protein Structure Preparation. The ligands were drawn, and their geometries were optimized using the molecular mechanics force field (MMFF) algorithm in Spartan '10 for Windows.[15] Structural information about the

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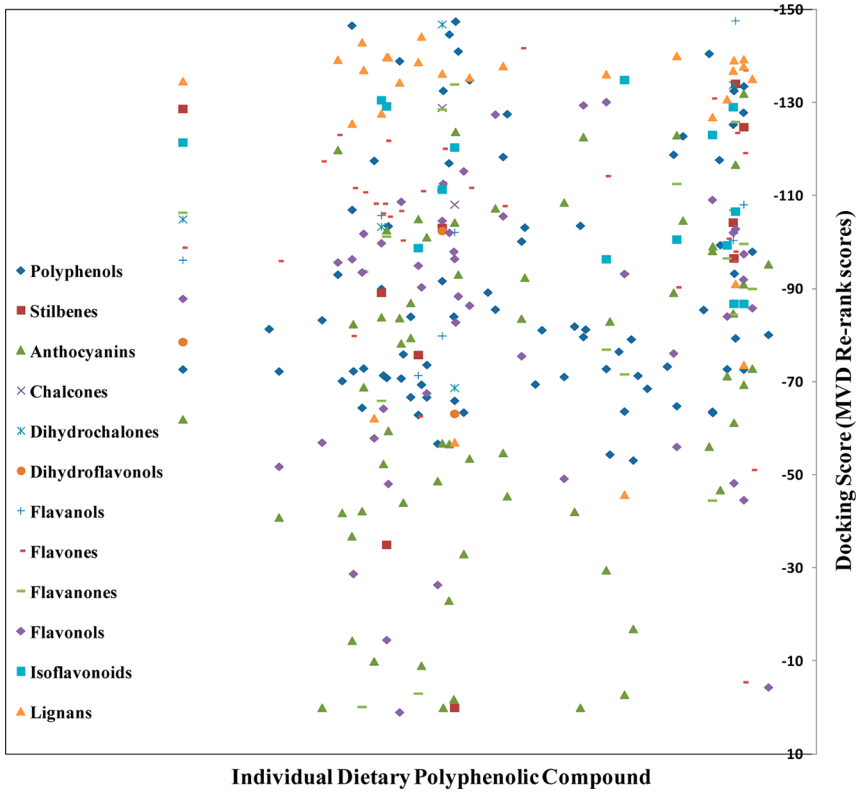

Figure 1. Docking scores of dietary polyphenolic compounds with the human thyroid hormone receptor β. The figure shows that lignans (▲, orange) have relatively high docking scores for the receptor.

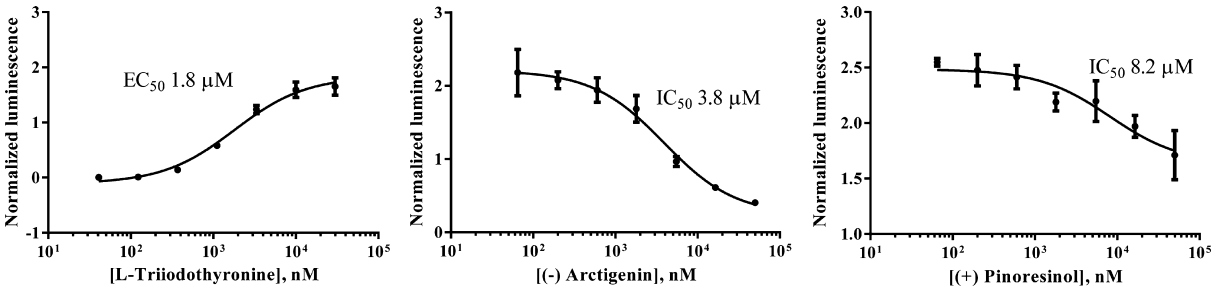

Figure 2. Antagonist activity of (−) arctigenin and (+) pinoresinol in a cell-based hTRβ reporter assay.

ligands was obtained from the Phenol-Explorer database.[1] The docking studies were carried out using the crystal structures of the ligand binding domain of hTRβ (PDB Id: 2pin, 3gws, 2j4a[13,16,17]) from the RCSB Protein Data Bank. The protein structures were used as rigid model structures. No relaxation was performed, and assignments of ionic charges on each protein structure were based on standard protonation states and the default templates of Molegro Virtual Docker (MVD).[18,19]

2.2. Docking Simulation and Scoring. Flexible ligand models were used for docking and postdocking geometry optimizations. Simulations were carried out using the ligand binding site of hTRβ. A docking sphere (15 Å radius) was placed on the binding sites of each crystal structure in order to allow different orientations of each ligand to be searched in the binding cavities and for multiple protein−ligand poses to be returned. The RMSD threshold for multiple cluster poses was set at <1.00 Å. The docking algorithm was set at maximum iterations of 1500 with a simplex evolution population size of 50 and a minimum of 30 runs for each ligand. Each binding site of oligomeric structures was searched, and docking scores of the lowest energy pose (based on the MVD rerank scores) for each ligand across all protein structures are presented in Tables S1−S10. The 2D representations of receptor−ligand interactions were prepared using Molecular Operating Environment (MOE).[20]

2.3. Human Thyroid Hormone Receptor β and Cell Viability Assays. The thyroid hormone receptor assay was carried out using the hTRβ (NR1A2) luciferase assay system from Indigo Biosciences (State College, PA) according to the manufacturer's instructions. (−) Arctigenin was obtained from Tocris Bioscience (Bristol, UK), and (+) pinoresinol was obtained from Sigma-Aldrich (St. Louis, MO). Human TRβ agonist L-triiodothyronine provided with the assay system kit was used as positive control for receptor activation. The activation/deactivation of receptor activity was monitored in 8 dose−response experiments with concentrations ranging from 65 nM to 50 μM for the lignans and 41 nM to 30 μM for the endogenous agonist. Reporter cell suspension (100 μL) was dispensed into 96-well assay plates, and 100 μL of test compounds in compound screening medium was added to the appropriate wells in triplicate.

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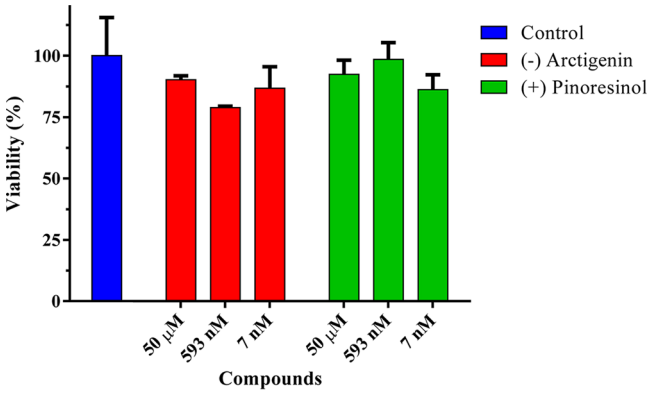

Figure 3. Effect of (−) arctigenin and (+) pinoresinol on the viability of reporter cells.

For antagonist mode assays, the reporter cell suspension was supplemented with 3.3 μM L-triiodothyronine shortly before test compounds in compound screening medium were added. The assay plates were placed at 37 °C in a humidified 5% CO_2 incubator for 24 h. After incubating for 24 h, the Luciferase Detection Reagent (100 μL) was added to each well and incubated for 15 min at room temperature, and luminescence was quantified using the Ascent Software on Labsystems Fluoroskan Ascent FL reader (Helsinki, Finland). EC_{50}/IC_{50} values were generated using GraphPad Prism 6.00 for Windows (La Jolla, CA). The effect of (−) arctigenin and (+) pinoresinol on the viability of the reporter cells was determined using the MTT assay. They were tested on the reporter cells using the MTT assay at the following concentrations: 50 μM, 593 nM, and 7 nM.[21] The viability of the treated cells was calculated based on the mean value of the no treatment control (100% viability).

3. RESULTS AND DISCUSSION

3.1. (−) Arctigenin and (+) Pinoresinol Are Antagonists of the hTRβ. Using molecular modeling tools to explore the structural compatibility between polyphenolic compounds and a wide range of molecular targets, we found that lignans have

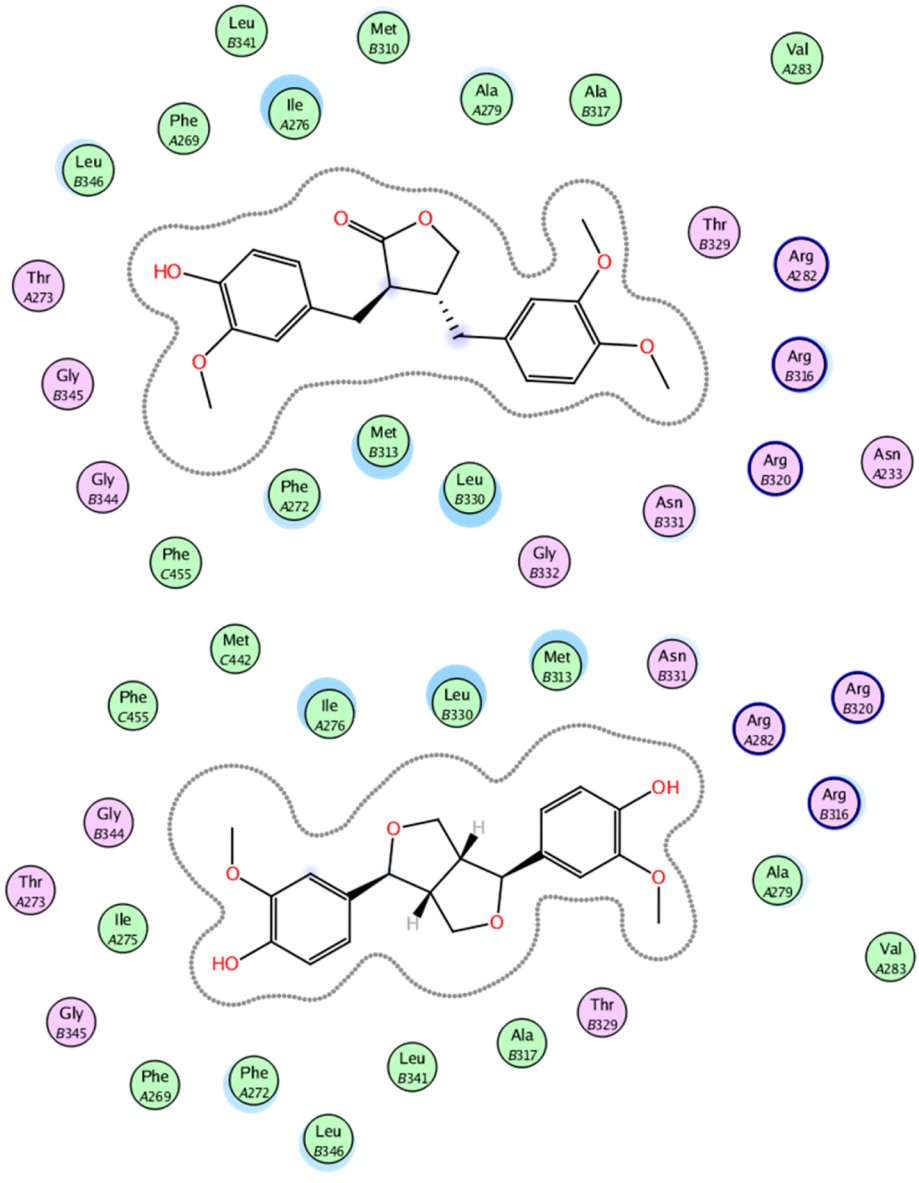

Figure 4. Amino acid residues predicted to interact with (−) arctigenin (top) and (+) pinoresinol (bottom).

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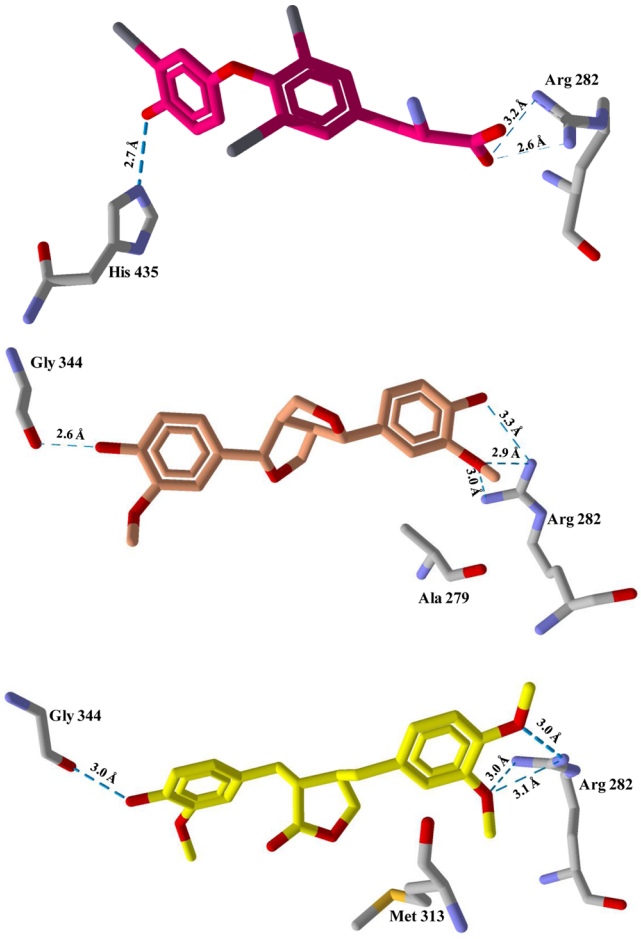

Figure 5. Key hydrogen bonding interactions (blue dash lines) between human TRβ and endogenous ligand L-triiodothyronine (top), (+) pinoresinol (middle), and (−) arctigenin (bottom).

relatively high docking scores for the ligand binding site of the human TRβ when compared to other dietary polyphenolic compounds. The docking scores from the simulations are presented in Figure 1, Tables S1−S10, and Figures S1 and S2. Lignans have not previously been reported as either agonists or antagonists of the human TRβ, so we tested the dietarily important lignans (−) arctigenin and (+) pinoresinol for their ability to activate or deactivate human TRβ.

The results show that (−) arctigenin and (+) pinoresinol are antagonists of the human TRβ with IC_{50} values of 3.8 μM and 8.2 μM, respectively (Figure 2). The lignans were also tested for possible cytotoxicity on the reporter cells, and the results show that the lignans were not toxic to the cells at the concentrations tested (Figure 3).

(−) Arctigenin has been reported as an inhibitor of cellular metabolism during glucose-deprived conditions. It has also been shown to inhibit the mitochondria complex 1, in addition to causing the activation of AMP-activated protein kinase in L6 myotubes and isolated skeletal muscles.[22,23] Perhaps this previously reported action of arctigenin may be related to its antagonism of the TRβ, although this remains to be tested.

3.2. Structural Motifs Involved in (−) Arctigenin and (+) Pinoresinol Interaction with hTRβ. To understand the molecular interactions that may be responsible for the activity of the two lignans at the receptor, the modeled complexes of the compounds and hTRβ were evaluated. Both lignans were predicted to interact with the following hTRβ amino acid residues: Phe 455, 269, and 272; Ala 234, 279, and 317; Arg 282 and 316; Asn 233 and 331; Ile 276 and 312; Thr 273 and 329; Val 283; Gly 332, 344, and 345; Leu 330, 341, and 346; Met 310, 313, and 442; Ser 314; and His 435 (Figure 4).

Hydrogen bonding interactions were predicted between the hydroxyl group of the lignans' 4-hydroxy-3-methoxyphenyl moiety and the backbone carbonyl group of hTRβ's Gly 344. The guanidinium side-chain of Arg 282 is also predicted to hydrogen bond with (−) arctigenin's 3,4-dimethoxyphenyl moiety and with (+) pinoresinol's 4-hydroxy-3-methoxyphenyl moiety (Figure 5). The strengths of the predicted hydrogen bonds are moderate and mostly electrostatic, based on the predicted lengths.[24] The endogenous agonist L-triiodothyronine is known to hydrogen bond with the guanidinium side-chain of Arg 282, as well as with His 435.[13,14,25] There are also significant steric interactions between (+) pinoresinol and Ala 279 of hTRβ and between (−) arctigenin and Met 313 of hTRβ (Figure 5).

■ CONCLUSION

From molecular docking simulations, we found that, relative to other dietary polyphenols, lignans have high structural compatibility with the ligand binding pocket of the thyroid hormone receptor β. Our experimental studies revealed that lignans (−) arctigenin and (+) pinoresinol are antagonists of the human thyroid hormone receptor β (hTRβ). (−) Arctigenin and (+) pinoresinol have low micromolar IC_{50} values and are predicted to interact with important amino acid residues such as

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Arg 282, His 435, Ala 279, and Met 313. Future work on the effects of these compounds on the multitude of TRβ target genes and on their ability to modulate the physiological roles of TRβ will be valuable. In addition, it would be of value to determine the importance, as well as the energetic contributions, of amino acid residues Gly 344 and Arg 282 to the interactions between the receptor and the lignans using experimental and computational mutational studies and molecular dynamics simulations.

■ ASSOCIATED CONTENT

Supporting Information

Tables S1−S10 and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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