Iodination of the isoquinoline moiety of T-0632 to generate photolabile analogs

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Abstract

Glucagon-like-peptide-1 receptor (GLP-1R) is a G protein-coupled receptor located on the β-cells of the pancreas. When the incretin hormone, glucagon-like-peptide-1 (GLP-1), binds to GLP-1R, it triggers intracellular signaling cascades that lead to insulin release from pancreatic β-cells. However, the half-life of GLP-1 in plasma is less than two minutes, which makes GLP-1 ineffective to administer as a drug for treatment of abnormal glucose levels. Previous studies have investigated GLP-1R agonists for use of glucose-lowering therapy. T-0632, a non-peptidic small molecule, is an inverse agonist of GLP-1R. Understanding the molecular interactions of binding between T-0632 and GLP-1R within the binding pocket can provide deeper insights to drug design for diabetes treatment. In order to achieve this, we want to create a library of T-0632 analogs possessing an azido group in a variety of locations around the molecule. Azido-analogs of T-0632, prepared through iodine displacement, will allow photolabeling studies of these interactions. We have focused on generating azide substitutions on the isoquinoline moiety of T-0632. Iodination of a tetrahydroisoquinoline has given three of four possible iodinated products, as confirmed by initial mass spectrometry results. Oxidation of this isoquinoline results in the isoquinoline precursor to the iodinated T-0632.
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I. INTRODUCTION

Certain peptides modulate the behavior of insulin production. These peptides, therefore, are of importance especially for individuals who have maturity-onset diabetes mellitus. Studies of glucagon behavior in human physiological conditions uncovered glucagon-like peptides that exhibit insulinotropic effects—glucagon-like peptide 1 (GLP-1) and its corresponding receptor, glucagon-like peptide 1 receptor (GLP-1R). Experimental results from Nauck et al. indicate that GLP-1 augments insulin production and secretion from pancreatic β-cells in hyperglycemic conditions and mediates insulin production through the release of somatostatin from δ-cells.

A. GLP-1

\[
\text{H\rightarrow A\rightarrow E\rightarrow G\rightarrow T\rightarrow F\rightarrow T\rightarrow S\rightarrow D\rightarrow V\rightarrow S\rightarrow S\rightarrow Y\rightarrow L\rightarrow E\rightarrow G\rightarrow Q\rightarrow A\rightarrow A\rightarrow K\rightarrow E\rightarrow F\rightarrow I\rightarrow A\rightarrow W\rightarrow L\rightarrow V\rightarrow K\rightarrow G\rightarrow R\rightarrow NH}_2
\]

Figure 1: Amino Acid sequence of the activated form of human GLP-1, which contains 30 amino acids.

While blood-glucose levels are the primary stimulus for insulin release, hormones released after oral ingestion of glucose also exhibit glucose mediation properties via insulin secretion. Intestinal L cells, in response to ingestion, release the naturally occurring incretin hormone, glucagon-like peptide 1 (GLP-1). GLP-1, upon binding with its receptor, GLP-1R, stimulates secondary signaling that triggers the production and release of insulin as well as the suppression of glucagon release. The insulinotropic and glucagonotropic actions of GLP-1 give rise to investigation of the peptide as a possible treatment for patients with Type 2 diabetes mellitus.

GLP-1 is a single, glycosylated polypeptide chain containing 30 amino acids, encoded by a separate exon in the proglucagon gene, which codes for 27 amino acids (Figure 1). The amino acid sequence of GLP-1 is highly conserved—the GLP-1 sequences of rat, hamster, human and bovine are identical, enabling study of the human GLP-1 peptide using animal subjects. Druker
et al. has shown that, upon binding, GLP-1 increases cAMP levels, which have been correlated with GLP-1-mediated insulin release. Structural studies of GLP-1 have also shown that the amino terminus is primarily involved in the signal transduction and biological potency of the peptide, while the carboxyl terminus is primarily responsible for recognizing and binding to receptors.

Although intravenous administration of the naturally produced hormone provides beneficial effects for the treatment of Type 2 diabetes mellitus, the native GLP-1 peptide has low stability in vivo with a half-life of only ~2-3 min. In vivo, GLP-1 is rapidly cleaved at its NH$_2$-end by the enzyme dipeptidyl peptidase 4 (DPP4). This cleavage results in an inactive, truncated form of GLP-1. The tendency for GLP-1 to rapidly inactivate poses a limitation for administration of the hormone and suggests a need for oral administration of small molecule, non-peptide ligands or stabilized proteins that can mimic the activity of GLP-1. Many strategies to develop GLP-1 analogs are under clinical development. One strategy is to inhibit DPP4, which would prevent the inactivation of GLP-1 and thus, prolong the action of GLP-1 and increase circulation of the activated hormone. Vildagliptin and sitagliptin are both orally active and rapidly absorbed drugs approved by the FDA aimed to inhibit DPP4 activity. The FDA has also approved GLP-1 peptide agonists, Liraglutide (Saxenda; Novo Nordisk), Exenatide (Byetta; AstraZeneca), and Dulaglutide (Trulicity; Eli Lilly), all of which are resistant to DPP4 degradation, and therefore exhibit a longer half-life in vivo than the native GLP-1 peptide. Finally, small molecular agonists and antagonists are under clinical development. These molecules ideally exhibit similar binding behavior to GLP-1 in vivo.
B. GLP-1R

The Glucagon-like peptide 1 receptor (GLP-1R) is a member of the class B subgroup within the larger family of G protein-coupled receptors (GPCRs) located on the β-cells of the pancreas. GPCRs are a family of proteins defined by seven transmembrane helices, an extracellular N terminus, ligand binding via the extracellular face, an intracellular C terminus, G protein-coupling, and signaling via the intracellular face. A significant structural feature consistent among the class B family of GPCRs is a long and complex extracellular amino-terminal domain containing six conserved cysteine residues that form disulfide bonds. Because these residues form disulfide bonds, they contribute strongly to the tightly folded structure of this family of receptors. Although this amino-terminal domain has also been suggested as the predominant domain for ligand binding, absolute binding structures have shown inconsistencies within the class B family GPCRs in the positioning of the ligand within these structures. These inconsistencies suggest variation in the binding mechanisms within this family of receptors.⁵

Accurate structural representations of GLP-1R have been particularly difficult to obtain because it is a transmembrane protein and has not been crystalized.

There are two major signal transduction pathways involving G protein-coupled receptors: the cAMP signal pathway and the phosphatidylinositol signal pathway. These signaling pathways enable the GLP-1 receptor to control the physiological response activated by GLP-1, and is one major factor in insulin production and secretion.⁶ Like other class B receptors, GLP-1R stimulates the production of the secondary messenger, cAMP, to trigger intracellular signaling cascades.⁶ In vivo mechanisms stimulated upon binding of GLP-1 to GLP-1R include receptor-mediated enhancement of glucose-induced insulin secretion from pancreatic β-cells, inhibition of gastric emptying with a delay in the gastrointestinal reabsorption of nutrients,
inhibition of glucagon secretion, and inhibition of food intake.\textsuperscript{6} Seshasai et al. investigated the use of the GLP-1R agonist, Taspoglutide, for treatment of Type 2 diabetes. Taspoglutide exhibited similar behavior to GLP-1, but exhibited a longer half-life. The data suggested that short-term, once-weekly administration of Taspoglutide was not associated with cardiovascular disease; however, long term data was not available because patients taking the drug developed gastrointestinal intolerance and hypersensitivity reactions and development of Taspoglutide was terminated.\textsuperscript{7} GLP-1R peptide agonists show successful binding potential; however, less is known about the specific binding mechanisms of these agonists with GLP-1R.

The peptide-binding model for many GPCRs is a two-step mechanism. In the first step, the C-terminal part of the ligand binds to the receptor N-terminal domain. This binding then enables the N-terminal ligand portion to bind to the receptor juxtamembrane domain, activating the receptor (Figure 2a).\textsuperscript{8} The binding mechanism of nonpeptide ligands to Class B GPCRs is less well-known, although nonpeptide antagonists exhibit binding activity to GLP-1R within the extracellular N-terminal region of the receptor. Figure 2b models the binding behavior of a nonpeptide antagonist with the CRF\textsubscript{1} receptor. The antagonist molecule binds to the juxtamembrane domain, producing a change in the receptor that blocks peptide binding on the J-domain. Because the peptide can no longer bind to the J-domain of the receptor, it can no longer activate the receptor.\textsuperscript{8}
Figure 2: Binding model of peptide (a) and nonpeptide (b) interaction with GPCRs. (a): (i) The C-terminus of the ligand (L) binds to the N-terminus of the receptor (R) (ii) The N-terminal region of the ligand weakly interacts with the juxtamembrane domain (J) of the receptor. (iii) The J-domain interaction with the ligand increases receptor interaction with the G-protein. (iv), and vice versa. (b): (i) Nonpeptide antagonist M (solid blue circle) binds within the J-domain. (ii) Antagonist interaction causes a possible conformational change within the J-domain that prevents peptide binding to the J-domain. This change has not been confirmed. (iii) By blocking peptide-J-domain interaction, the nonpeptide antagonist binding to the J-domain does not affect peptide binding to the N-domain, but prevents interaction with the G-protein, which stimulates cAMP signaling.8

The structure of the GLP1/GLP1R complex has also been studied using mutagenesis techniques. Wilmen et al. identified that substituting the amino acid, tryptophan, for alanine or phenylalanine, on the extracellular N-terminal region of the rat GLP-1 receptor results in complete loss of GLP-1 binding.9 The N-terminal domain of the GLP-1 receptor, when expressed in the absence of the transmembrane domains, also exhibits high affinity and specificity for GLP-1 binding. In addition, exchanging Trp39 on GLP-1R with alanine resulted
in a complete loss in GLP-1 binding, and replacing this amino acid with another aromatic amino acid, phenylalanine, did not lead to reappearance in binding. These observations suggest that the presence of the indole ring structure on tryptophan, rather than the mere presence of an aromatic residue, is crucial at this position. In 2008, Lin et al. estimated the 3-dimensional structure of GLP-1R/GLP-1 complex constructed using a protein folding model and molecular docking. The best scoring binding poses from the docking model were then subjected to long-time molecular dynamics simulations conducted on a hydrated lipid bilayer to mimic the real environment of GLP-1R (Figure 3a). The predicted extracellular domain of human GLP-1R matched closely with the corresponding crystal structure determined by Runge et al. (Figure 3b/c). Differences between the predicted structure and the crystal structure may be due to presence of the transmembrane domain or difference in solvent in the computational model.

![Figure 3: Predicted model of GLP-1R/GLP-1 complex.](a) GLP-1R/GLP-1 complex after 20ns MD simulation on a lipid bilayer. GLP-1R in green while GLP-1 as the purple ribbon. Solvent (water) not shown. (b) Predicted model of rat GLP-1R showing only the extracellular domain (residues 1-146). (c) Crystal structure of extracellular domain in human GLP-1R revealed by Runge et al. The difference between the predicted model and the crystal structure is colored in red.][10-11]
C. T-0632

In 2001, Tibadiuza et al. reported that the small molecule antagonist, T-0632, binds with 1.2 µM affinity to the human GLP-1R and exhibits almost 100-fold selectivity for the human receptor versus the highly homologous rat receptor. As an antagonist, T-0632 inhibits GLP-1 induced cAMP production, which prevents insulin production and secretion.

Because the rat GLP-1 receptor sequence has a similarity of 95.9% with the human GLP-1 receptor and because T-0632 selectively binds to the human receptor, using receptor chimeras would enable structural determination of the binding between the antagonist and the receptor. Replacing Trp33 on the N-terminal domain in the human receptor by the homolog in the rat receptor, serine, significantly decreased T-0632 affinity to close to the value observed in the rat wild type protein. Substitution of the remaining amino acids in the extracellular domain had no effect on T-0632 binding. This finding supports the conclusion that the single residue, Trp33, and the N-terminal domain are important affinity determinants of T-0632 binding.

Although the mechanisms of binding for some antagonists to GPCRs have been identified, the binding mechanism of T-0632 with GLP-1R is still unproven. Due to its antagonist behavior, Tibadiuza et al. speculated that T-0632 binds via the receptor’s extracellular N-terminal domain, but may hinder the second step that induces the G protein-couple and activates cAMP pathways. Because binding affinity is only moderately diminished if Trp33 is replaced by another amino acid with an aromatic side chain, such as phenylalanine, the role of tryptophan in the binding affinity of T-0632 may be due to aromatic stacking interactions between the indole of the amino acid and the aromatic groups of the T-0632 molecule.

T-0632 is also an antagonist for the type 1 cholecystokinin receptor (CCK1R), and its binding mechanism has been identified. In 1998, Taniguchi et al. showed that T-0632 had a
highly potent antagonistic action on CCK$_A$ receptors in several animal species. Oral administration of T-0632 in mice of up to a 1000-fold greater dose than exogenously administered CCK-8 for 30 days did not show any toxicity or changes in behavior.$^{12b}$ When docking T-0632 into a model of CCK receptors, Dong et al. found that the second extracellular loop moved out of the pocket between the fourth and fifth transmembrane helices, causing a change in the volume and shape of the allosteric pocket.$^{12a,13}$ Mutagenesis studies of residues facing the pocket within this loop indicated minimal to no loss of T-0632 binding with CCK1R for mutation of each residue normally present in the second extracellular loop region of CCK1R to the equivalent residues in CCK2R. Although the N-terminal domain was thought to be a primary contributor for T-0632 binding with CCK1R, mutating residues in the C-terminal domain of this extracellular loop affected T-0632 binding significantly. The length of the extracellular loop region is four residues shorter in the type-2 receptor and is known to be involved in the disulfide bond linking the extracellular loop with the top of a transmembrane helix. The results of the mutagenesis studies on these receptors suggest that, while the length of the extracellular loops region is four residues shorter in the Type II receptor and is otherwise highly conserved in sequence, the second extracellular loop of the Type I receptor is known to be involved in the disulfide bond linking the extracellular loop with the top of a transmembrane helix. This difference in tertiary structure of CCK1R relative to CCK2R may explain the difference in binding affinities of T-0632 to the Type 1 and Type 2 receptors. Because their chimeric approach depended strongly on conservation of structure, the observed data strongly support speculation of differences in conformation although the receptors are closely related in primary structure. By making modeling changes based on the experimental results, the findings of the experimental studies were justified using molecular modeling (Figure 4).
Figure 4: Molecular model of T-0632 docking to CCK1R. The new model described in Dong et al. is represented in cyan and the old model from Cawston et al. is displayed in gold. The movement of the second extracellular loop results in a change in volume and shape of CCK1R.\textsuperscript{12a}

Additionally, the hydrophobic residue, Trp209, significantly enhanced T-0632 binding affinity with CCK1R, and is predicted by the model to be adjacent to the hydrophobic isoquinoline moiety within T-0632 (Figure 5).\textsuperscript{12a} The binding distance between the hydrophobic isoquinoline and Trp209 is 6.86Å (Figure 5).
Figure 5: T-0632 binding pocket within CCK1R. Front and side views show T-0632 isoquinoline interacting with Trp29. T-0632 displayed as solid 3D molecule, with CCK1R shown as white ribbon. Trp29 emerges from CCK1R in the solid 3D point of view. All images were generated from Swiss pdb viewer via supplementary data from Dong et al. The Hydrogen Bond length is 6.86Å.
Figure 6: CCK1R residues near T-0632 (shown in blue). Arg197 was shown to strongly influence CCK binding to CCK1R, but is facing away from the pocket in which T-0632 binds with CCK1R. Ser208 and Leu200 are shown near T-0632 and mutations of these residues affect T-0632 binding. Trp209 was shown to strongly influence T-0632 binding affinity. Protein Data Bank file obtained from supplementary data from Dong et al.\textsuperscript{12a}

While the Arg197 residue has a significant effect on the binding of CCK to CCK1R, it had no effect on T-0632 binding. Figure 6 shows that the model of T-0632 binding to CCK1R predicts that Arg197 is directed away from the pocket, explaining the lack of effect on T-0632 binding. However, Ser208, when mutated to threonine, was found to bind T-0632 with approximately 3-fold lower affinity to CCK1R, while the natural ligand, CCK, bound normally with mutated CCK1R. In addition, replacing Leu200 with tryptophan significantly enhanced T-0632 binding affinity. The isoquinoline moiety of T-0632 is adjacent to the tryptophan residue substitution that was predicted to improve docking of T-0632 (Figure 6).\textsuperscript{12a}
Similarly, Tibadiuza et al., when replacing Trp33 in human GLP-1R with its homologous amino acid in the rat GLP-1R serine, found that T-0632 binding affinity decreased significantly and substitution of other amino acids in the human receptor to its rat equivalent, alone or in combination, had no effect on T-0632 binding. Replacing the tryptophan on human GLP-1R with another amino acid that possessed an aromatic side chain maintained T-0632 binding affinity, although at a lower level. Tibadiuza et al. speculated that the role of the tryptophan is based on aromatic stacking interactions with the aromatic groups of T-0632.

**D. Photoaffinity Labeling**

Since many GPCRs have multiple binding sites and have shown conformational changes even with very similar primary structures, interactions between different ligands on the same receptor can differ largely. Photoaffinity-labeling studies can provide structural insight into the binding pockets between certain ligands and their receptors. Photoaffinity labeling is a technique that allows a small molecule-labeling agent on a ligand that is chemically unreactive in the dark to be converted into its active form upon photolysis and covalently bond to groups in the receptor binding site. The receptor-binding site now labeled with this small molecule can then be analyzed to determine the single amino acid to which the molecule covalently bound. In order to obtain a thorough map of the amino acids of the receptor-binding site, multiple areas of the molecule must be capable of possessing the photoaffinity label.

Many photoaffinity labeling studies have been done on the GLP-1/GLP-1R complex, revealing key residues in the extracellular matrix that are essential for ligand binding. Chen et al. identified regions of the GLP-1R juxtamembrane and amino-terminal domain using photolabile GLP-1 probes. Using a photolabile $p$-benzoyl-L-phenylalanine (Bpa probe) to replace a phenylalanine at the 12 and 6 positions of the ligand and a tyrosine at position 19 as a site for
radioiodination, Chen et al. characterized the ability of the probe to bind to GLP-1R. Each probe labeled GLP-1R specifically. Identification of the probe-labeled receptor sites verified that the site of labeling by the Bpa12 probe was between Leu144 and Lys197 within the juxtamembrane region of the amino-terminal domain of GLP-1R and the site of labeling by the Bpa6 probe was between Tyr205 and Trp214 of the extracellular loop. Furthermore, mutagenesis studies have shown that the extracellular loop of GLP-1R is important for ligand binding of GLP-1 amino terminal residues.\(^5\)

Miller et al. used photolabile probes to identify the locations of the receptor residue Leu141 above the first transmembrane segment and Trp297 within the second extracellular loop of the receptor for the GLP-1/GLP-1R complex. Trp297 was identified as unique among the family of Class B GPCR receptors. The observation that the amino terminus and mid-region probes identified amino acids belonging to separate extracellular loops, which is distinct from receptor labeling data of the closely related CFR\(_1\) receptor, suggest that ligand binding and activation of Class B GPCRs may vary by receptor.\(^{15}\) This observation motivates continued study of the binding site of GLP-1R with other ligands as well.

Finally, radioiodination is often paired with photoaffinity labeling studies. Dong et al. used radioiodination on T-0632 to perform a structural analysis on another GPCR, CCK1R. The radio-iodinated T-0632 was shown to bind tightly in a region of CCK1R that included exon 3, but exhibited no binding activity for the Type 2 CCK receptor. Exchanging these exon regions on the receptors verified this binding interaction. By targeting exon 3, Dong et al. determined tertiary structural differences between the Type 1 and Type 2 CCK receptors, although the two receptors are very similar in primary structure.
This project will employ an Aryl Azide as a photoaffinity-labeling reagent. Selectively synthesizing azido-T-0632 requires displacement of iodine via a nuclear substitution reaction. Radioactive I-125 can then be attached to azido-T-0632, serving as the radioactive marker of the ligand as the molecule is inserted into the receptor. When inserted into GLP-1R, Azido-T-0632, can be photolysed, resulting in a covalent bond with receptor residues. With the azide placed in different positions on the ligand, different versions of azido-T-0632 may bind to different receptor residues in GLP-1R. The receptor can be isolated and lysed to determine the position of the I-125 within the receptor. With a library of azido-T-0632 analogs, each binding to a different receptor residue in GLP-1R, we can gain better knowledge of the tertiary structure of T-0632 with GLP-1R.

E. \(\pi\)-stacking

Hunter et al. observed \(\pi\)-stacking interactions between porphyrins and other varieties of \(\pi\)-systems in organic solvents. Their proposed model of \(\pi\)-stacking interactions accounts for attractive interactions between aromatic \(\sigma\)- and \(\pi\)-frameworks as well as repulsions between \(\pi\)-electrons. In this model, the primary contributors to the stability of aromatic stacking interactions are van der Waals and electrostatic forces.\(^{16}\)

Van der Waals attractions are proportional to the area of \(\pi\)-overlap. Solvent also lowers the contribution of van der Waals interactions to the total \(\pi\)-stacking energy, and therefore, the hydrophobic effect can explain aggregation of nonpolar, aromatic regions of T-0632 with hydrophobic residues, such as tryptophan, on GLP-1R. The geometries attributed to this interaction have three types: face-to-face, edge-on/T-shaped, and staggered (Figure 7).
Figure 7: Types of π-stacking geometry. With van der Waals interactions being the only contribution to total π-stacking energy, the face-to-face geometry would be most dominant. However, because edge-on/T-shaped and staggered face geometries are more commonly observed experimentally, another interaction must be contributing to the total π-stacking energy. Images were generated in GaussView and calculations were done via Gaussian09. The electron density is shown for each ring, with red indicating areas of high electron density and blue as areas that are electron poor.

When optimizing geometry in Gaussian, a face-to-face starting position of two benzene molecules optimized to offset face geometry (Figure 8b). The offset face and edge-on/T-shaped are more often observed experimentally, which can be attributed to the attraction between the σ- and π-frameworks of the aromatic molecules. However, van der Waals interactions are not the force that controls experimentally observed geometries, or else π-overlap would be maximized.

Another large contribution to total π-stacking energy is the electrostatic repulsion between two approaching π-clouds. A simple model is shown in Figure 8a, where the dominant interaction is the repulsion between the two approaching π-clouds. The interaction is most repulsive when the π-systems are close together, due to Coulombic repulsion, and are considered separate from the σ-framework. The electrostatic repulsion between two π-systems explains the
more commonly observed edge-on/T-shaped and offset face \( \pi \)-stacking geometries. For these two systems, the aromatic \( \sigma \)-framework, which is electron poor, interacts favorably with the electron rich aromatic \( \pi \)-system.

![Figure 8: \( \pi \)-stacking geometry as a result of van Der Waals interactions and electrostatic repulsion. (b) and (c) were generated in Gaussian09 and GaussView. Electron density maps show electron rich areas indicated in red and electron poor areas in blue. (a) Simple model of electrostatic repulsion of two \( \pi \)-systems. (b) Geometry of a face-to-face benzene model becomes offset when optimized in Gaussian09. (c) Optimized geometry of two benzene rings when starting in an edge-on/T-shaped geometry generated by Gaussian09.](image)

Molecular modeling by Dong et al. observed that the tryptophan residue in CCK1R is adjacent to the isoquinoline moiety of T-0632 (Figure 6), and the fully \( \pi \)-stacked geometry does not seem to be dominant in the model. Though unproven, the binding pocket of T-0632 with GLP-1R may exhibit more stacked geometry between the isoquinoline moiety and tryptophan residue, but steric hindrance may prevent full face-to-face stacking, which is theoretically favored between \( \pi \)-deficient and \( \pi \)-rich hydrophobic molecules in polar solvent.

**F. Isoquinolines and Project Goals**

While the amino acid sequence for human GLP-1R is known, the binding pocket used by this small molecular agonist in the tertiary structure of GLP-1R remains unknown.
Computational modeling can speculate how the small molecule antagonist, T-0632, fits into the binding pocket and changes the shape of GLP-1R, but synthesis can provide more accurate results. In order to explore the binding structure of T-0632 with human GLP-1R, we need to create a library of T-0632 analogs possessing an azido group in a variety of locations around the molecule. Azido-analogs of T-0632, prepared through iodine displacement, will allow photoaffinity-labeling studies of these interactions. This project focuses on generating azide substitutions on the isoquinoline moiety of T-0632 from a tetrahydroisoquinoline. Oxidation of this tetrahydroisoquinoline will generate the isoquinoline precursor to the iodinated T-0632. Figure 9 illustrates the possible sites for iodination on the isoquinoline moiety of T-0632 using this chemistry.

![Chemical structure of T-0632. Starred are the possible sites for iodination on the isoquinoline moiety.](image)

Figure 9: Chemical structure of T-0632. Starred are the possible sites for iodination on the isoquinoline moiety.
II. Results and Discussion

A. 1,2,3,4-Tetrahydroisoquinoline-3-Carboxylic Acid (2)

Phenylalanine (1) and formaldehyde generate 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2) via a ring closure mechanism (Figure 10).

![Synthesis of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2).](image)

Figure 10: Synthesis of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2).\textsuperscript{17}

While GC/MS did not produce a peak for the molecular ion, a proton NMR in DMSO-\textit{d}_6 was used to identify the compound. The observed multiplets at δ4.3 ppm, δ3.5-3.8 ppm, and δ3.1 ppm show coupling patterns that are a result of the compound existing as a mixture of rotamers. Due to the chirality of the tertiary carbon, the alkyl protons can be \textit{cis} or \textit{trans} to the proton on the tertiary carbon. The multiplets observed exhibit evidence for \textit{cis} and \textit{trans} orientations in the \textsuperscript{1}HNMR. Presence of water prevented full resolution of the coupling pattern by protons at δ3.1-3.8 ppm. However, we observed a singlet at δ7.2 ppm, representing 4 aromatic protons, which provided strong evidence that the integration, chemical shift, and numbers of different protons confirmed ring closure (Figure 11).
Figure 11: $^1$HNMR spectrum of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2). DMSO-d$_6$ was used as solvent.

B. 2-Acetyl-1,2,3,4-Tetrahydroisoquinoline-3-Carboxylic Acid (2a)

Figure 12: Synthesis of 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a)

Acetylation of the amide on 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2) protects the amine from interacting with reagents in future reactions (Figure 12). The amine acts as a nucleophile on the acetic anhydride, resulting in the acetylated product. GC/MS showed peaks of
the desired product (2a) at 10.996 min as well as a peak corresponding to an acetylated and esterified product (4) 9.90 min in a 2:1 ratio, respectively. The acetylated and esterified product was confirmed by the fragmentation pattern in the mass spectrum (Figure 13). In order to purify the compounds for NMR characterization, the mixture was recrystallized in water before running NMR spectroscopy.
Figure 13: GC/MS for 2-acetyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) before recrystallization. (a) Mass Spectrum of 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) at 9.98 min. (b) Mass Spectrum of Esterified product of methyl-2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) at 11.03 min. Percent report of the sample was 66% (a) and 33% (b).
A proton NMR confirmed the downfield shifts of the alkyl protons as a result of the acetylation (Figure 16). Presence of N-CH₂ protons at δ4.6 ppm, increased coupling on the C-CH₂ protons δ3.1-3.5 ppm, and presence of the acetyl protons at δ2.15 ppm also provided evidence that the desired product was synthesized. While we expected the coupling pattern at δ4.6 ppm to have been a singlet, it is observed as a doublet. The coupling pattern at δ5.0 ppm, representing the proton on the tertiary carbon, should have been a triplet but is shown as a doublet of triplets, and the C-CH₂ protons at δ3.1-3.5 ppm should only couple to the single proton on the tertiary carbon but is shown as a multiplet. Furthermore, the acetyl protons, which should exist as a singlet, are present as a doublet. We hypothesize that the coupling pattern is a result that the acetyl protons exist as a mixture of rotamers (Figure 15). Due to the rotation of the bond about the tertiary carbon, protons on the alkyl groups can exist cis or trans to the carboxylic acid. The coupling pattern observed in the proton NMR is also a result of the rotation between the nitrogen atom and the acetyl carbon. This rotation is slow enough to be captured on the proton NMR due to the conjugation between the lone pair on the nitrogen atom and the carbonyl. Separation of the rotamers was unsuccessful, suggesting that 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) exists in equilibrium as a mixture of rotamers with the acetyl protons coupling to the proton on the tertiary carbon in two conformations.
Figure 14: $^1$H NMR spectra of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2) in blue and 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) in red. DMSO-$d_6$ was used as solvent for both products.

Figure 15: 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) existing as a mixture of rotamers, resulting in the coupling pattern observed by NMR spectroscopy.
A D$_2$O shake shows absence of the proton of the carboxylic acid and also the same coupling pattern among the alkyl protons, providing further evidence that the mixture of rotamers is a result of the acetyl protons coupling to the proton on the tertiary carbon in two orientations (Figure 16).

Figure 16: $^1$HNMR spectra with D$_2$O shake of 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) in DMSO-d$_6$.

C. Esterification and Acetylation of 1,2,3,4-Tetrahydroisoquinoline-3-Carboxylic Acid (2)

Iodination of 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) was attempted, but due to low solubility and interaction of the reagents with the carboxylic acid, resulting yield was so low that it prompted modifications of the procedure. In order to improve
the synthesis, the carboxylic acid was esterified and the amine was acetylated. The esterified and acetylated compound had improved solubility and overall yield of the subsequent iodination.

Methanol esterifies the carboxylic acid, while the acetic anhydride acetylates the amine. The overall yield improved with this synthesis, as GC/MS showed almost entirely pure product with a dominant peak at 10.203 min that had a molecular ion of 233, which matches that of the desired compound (4). NMR in chloroform-d (Figure 18) also showed that the coupling pattern of the alkyl protons support that the product exists in equilibrium as a mixture of rotamers due to the conjugation of the lone pair on the nitrogen atom and the adjacent carbonyl, which exists in a slow equilibrium in two orientations (Figure 17).

![Rotamer mixture of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4), resulting in the coupling pattern observed by NMR spectroscopy.](image)

Figure 17: Rotamer mixture of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4), resulting in the coupling pattern observed by NMR spectroscopy.
Figure 18: $^1$HNMR spectra of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2) in DMSO-d$_6$ (blue) and the esterified and acetylated product, methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) in chloroform-d (red).

D. Oxidation of Methyl 2-Acetyl-1,2,3,4-Tetrahydroisoquinoline-3-Carboxylate (4)

Ultimately, oxidation of methyl 2-acetyl-n-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (6) will generate the precursor to iodinated T-06. However, oxidation must occur after iodination in order to utilize the activating effects of the alkyl groups on methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4). Oxidation of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) yields methyl isoquinoline-3-carboxylate (5a) as one of
its products, which is a method that can be employed to oxidize of methyl 2-acetyl-n-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (6).

Oxidation of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) produced 3 purified products. GC/MS and NMR in chloroform-d characterized the products (Figure 19). GC/MS results showed the fully oxidized product (5a) with a molecular ion of 187, and two partially oxidized products with molecular ions of 266/268 (5b), which was a partially oxidized product that is the precursor to the fully oxidized product, and 247 (5c), which corresponds to the starting material oxidizing into a carbonyl (Figure 19).

Figure 19: GC/MS results from oxidation of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) yielding the compounds methyl isoquinoline-3-carboxylate (5a), methyl 4-bromo-3,4-dihydroisoquinoline-3-carboxylate (5b), and methyl 2-acetyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (5c).
(i) Methyl Isoquinoline-3-Carboxylate (5a)

The GC peak at 9.328 min showed the fully oxidized product with a molecular ion of 187. The singlets shifted downfield at δ8.7 ppm and δ9.4 ppm show presence of oxidation of the alkyl groups on methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4). The doublet of doublets at δ8.2 ppm and multiplet at δ7.8 ppm correspond to the 4 aromatic protons that had existed before oxidation, and the coupling pattern suggests that these protons also couple with the protons on the newly oxidized ring. The change in coupling pattern in the aromatic region justifies the absence of the alkyl protons that were present in the starting material. Finally, absence of the acetyl protons and downfield shifts of the protons corresponding to the ester at δ4.2 ppm further suggest successful oxidation (Figure 20).
Figure 20: $^1$HNMR spectra of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) in chloroform-d (blue) and methyl isoquinoline-3-carboxylate (5a) in chloroform-d (red).

(ii) Methyl 4-Bromo-3,4-Dihydroisoquinoline-3-Carboxylate (5b)

The GC/MS showing a molecular ion of 266/268 in a 1:1 ratio suggested that a brominated product, which is the precursor to fully oxidized methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4), was also produced. To identify the location of the bromine, an NMR in chloroform-d showed a doublet of doublets at δ5.7 ppm, which corresponded to the proton on the carbon that also contained the bromine (Br-CH). This doublet of doublets shows that the proton on the carbon containing the bromine couples with the proton on the tertiary carbon in two conformations, suggesting that the proton on the tertiary carbon can exist in both cis and trans orientations. Had bromination been on another carbon, the proton
would have been shifted more downfield because it would have been closer to the amine. The aromatic protons showed oxidation had occurred due to the doublet of doublets at δ7.3-7.6 ppm and doublet at δ8.2 ppm. Finally, due to the electron withdrawing behavior of the carbonyl and bromine, the proton of the chiral carbon is shifted downfield to δ5.3 ppm, further verifying the location of the partial oxidation and bromination (Figure 21).

Figure 21: 

![HNMR spectra](image)

Figure 21: $^1$HNMR spectra of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) in chloroform-d (blue) and methyl 4-bromo-3,4-dihydroisoquinoline-3-carboxylate (5b) in chloroform-d (red).

(iii) Methyl 2-Acetyl-1-Oxo-1,2,3,4-Tetrahydroisoquinoline-3-Carboxylate (5c).

GC/MS showed a third purified product with a molecular ion of 247. This compound results from substitution of the bromine on an alkyl group that oxidized into a carbonyl. Presence
of the singlets, corresponding to the acetyl and ester, show the alkyl groups did not oxidize into alkenes. In order to identify the location of the carbonyl on the alkyl carbons, NMR showed that the acetyl protons shifted downfield, suggesting that the carbonyl substituted close to the amine. Additionally, the absence of the multiplet that had corresponded to the alkyl protons adjacent to the amine provides further evidence that the carbonyl had substituted in that position. Had the carbonyl substituted closer to the methyl-ester, the quartets at δ5.3 and δ3.6 ppm would have been absent and the alkyl protons that would have been across the carbonyl would be more downfield than was observed due to the electron withdrawing characteristics of the amine (Figure 22). Because the alkyl protons at δ5.3 and δ3.6 ppm represent the alkyl protons closer to the methyl ester, we can be confident that the carbonyl substituted onto the alkyl carbon closer to the amine.
Figure 22: $^1$HNMR spectra of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate compound (4) in chloroform-d (blue) and methyl 2-acetyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (5c) in Chloroform-d (red).

E. Iodination of Methyl 2-Acetyl-1,2,3,4-Tetrahydroisoquinoline-3-Carboxylate (4)

Because the two alkyl groups on the aromatic ring of methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) are activating and direct ortho/para, substitutions on the remaining 4 carbons are expected to be equally likely. The proposed synthesis iodinates the aromatic ring on methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4) in acidic conditions because in these conditions, the iodine on N-iodosuccinimide becomes positively charged, enabling it to substitute onto the activated aromatic ring (Figure 23).
The mixture of methyl 2-acetyl-\(n\)-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate isomers (6) prepared as described on page 67 was characterized by GC/MS (Figure 24). The three peaks at 12.06, 12.26, and 12.47 min, each showing the same fragmentation and molecular ion, verify that 3 of the 4 possible isomers had been synthesized in a 1:2:3 ratio (1.386%; 3.836%; 8.022% of the total sample, respectively). Another peak at 10.57 min comprised 64.694% of the total sample, which corresponded with the starting material (4), and a third peak at 9.57 min (19.65% of total) corresponded with an oxidized form of the starting material (5a).
Figure 24: GC/MS of methyl 2-acetyl-α-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate isomers (6). Peaks are identified on GC-Trace (top). All iodinated products observed a molecular ion of 359 and the same fragmentation (bottom).
Because the GC/MS showed a mixture of iodinated isomers, we needed to purify and separate each of the isomers at sufficient scale to characterize the substitution via NMR. The available chromatography methods to separate compounds on a scale large enough for NMR characterization separate based on polarity. Because the iodinated isomers only differ by the site of the iodination, separation of the isomers by polarity was expected to be very difficult. Fractions collected via flash chromatography still showed a mixture with substantial peaks at 9.36, 10.29, 11.97, 12.17, and 12.29 min, corresponding to the oxidized form of the starting material (5a), starting material (4), and three isomers of the product (6), respectively (Figure 25).
Figure 25: GC-Trace from separation of the crude product of methyl 2-acetyl-n-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate isomers (6) using Flash Chromatography on a silica stationary phase. Mobile phase was 80% dichloromethane:hexane, increasing to 100% dichloromethane. Three of the iodinated isomers were collected in the same fraction, as well as the starting material and the oxidized form of the starting material.

Reversed phase flash chromatography on a Biotage C18 reversed phase flash column also did not purify the iodinated isoquinolines enough to gather a resolved NMR, and the GC-Trace looked similar to that of effective phase flash chromatography (Figure 25). This led us to consider a more sensitive separation method, High Performance Liquid Chromatography (HPLC) with a reversed phase column. Upon HPLC, three fractions were collected that were
sufficiently volatile to generate a mass spectrum on GC/MS. Examination of the separated components by GC/MS showed that (a) represented starting material, and (b) and (c) were mixtures of the three iodinated isomers while (b) also contained a peak corresponding to an unknown compound with a molecular ion of 247 (Figure 26).

![HPLC gradient separation](image)

Figure 26: HPLC gradient separation of methyl 2-acetyl- n-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate isomers (6) via a 25 µL injection. All HPLC data were collected via an Agilent Eclipse C18 column 9.4mm X 25 cm with a 3 mL/min flow rate. Gradient (red) represents the percent of acetonitrile relative to deionized H₂O. Only peaks containing volatile compounds that were present on GC/MS are labeled and their corresponding identifications are shown below the chromatogram.

The reversed phase HPLC gradient successfully separated the starting material from the products, a result that previous methods had not been able to obtain. Since three iodinated
isomers eluted at the end of the HPLC and all had eluted in the same fraction, we attempted another reversed phase HPLC via an isocratic method at 30% acetonitrile in deionized H$_2$O. This particular concentration was chosen because the mixture was only soluble in at least 35% acetonitrile in deionized H$_2$O, and running the column with a lower amount of acetonitrile increases risk of having the isomers come out of solution while on the HPLC column.

The isocratic method was first run by injecting only 25 µL of the mixture of compounds that had been collected by flash chromatography. GC/MS were obtained for all fractions collected by HPLC, but only some of the fractions contained volatile compounds that resulted in a resolved GC-trace (Figure 27). The GC/MS results showed that fraction (a) corresponded to the starting material (4), and (b) corresponded to an unknown compound with a molecular ion of 247. Fractions (c), (d), and (e) all contained a combination of the iodinated isomers (6). GC/MS results of these fractions showed that (c) contained 3 of the iodinated isomers as well as an unknown compound with a molecular ion of 247. Both (d) and (e) showed evidence of 1 dominating isomer on the GC-trace. The dominant peak in the GC-Trace of (d) and (e) eluted at the same time using gas chromatography, which separates based on volatility, but eluted at different times using HPLC, which separates based on polarity. While volatility and polarity are similar across the iodinated isomers, the GC method suggests that the same isomer was collected in both fractions, while the HPLC chromatogram suggests that the isomers collected by each fraction were different. The iodinated isomers are similar in physical characteristics, which suggests the possibility that the GC eluted two different isomers at similar time periods. This hypothesis can be justified because the peak shapes on the chromatogram are symmetrical, suggesting that a different isomer eluted in each fraction and that separation of two iodinated isomers was successful. However, because this HPLC method was run with only a 25 µL
injection of sample (approximately 2 mg of crude product), the amount of material collected by each fraction was insufficient for a resolved NMR to characterize the separated iodinated isomers and prove that the two fractions eluted different isomers.

![HPLC Chromatogram](image)

**Figure 27:** Isocratic Reversed Phase HPLC chromatogram (30% acetonitrile in deionized H₂O) and identification of collected fractions using 25 µL injection. All HPLC data were collected via an Agilent Eclipse C18 column 9.4 mm X 25 cm with a 3 mL/min flow rate. Only peaks containing volatile compounds are labeled and characterization based on mass spectrum are identified.

The same isocratic HPLC method was run with a 200 µL injection (approximately 20mg of crude product). The method eluted compounds at the same times as the method using the smaller injection. Fractions containing iodinated isomers were compared to those collected with a 25 µL injection. All fractions collected in the method using a 200 µL injection showed a
mixture of iodinated isomers (6). GC/MS results showed that fraction (c) contained two dominant isomers as well as a peak representing the starting material. Fraction (d) showed 1 dominant iodinated isomer but also contained 2 peaks representing the oxidized form of the iodinated isomers, so a purified NMR could not be obtained. Fraction (e) also contained 1 dominant peak of an iodinated isomer, and 2 peaks representing 2 isomers of oxidized iodinated product (Figure 28). While (d) and (e) showed isolation of one iodinated isomer, the presence of the oxidized iodinated isomers makes the fraction impure and unable to be characterized via NMR. The mixture of isomers was stored at room temperature for 6 days in between the HPLC methods injecting 25 µL and 200 µL. Oxidation may have occurred during this time period, explaining the presence of oxidized isomers in fractions collected when the column was run with a 200 µL injection. A possible direction for successfully separating the isomers is to convert the ester to a carboxylic acid and oxidize the iodinated isomers. Because the carboxylic acid exhibits more polar characteristics than the ester, the same isocratic method may elute the iodinated and oxidized isomers containing a carboxylic acid at a much later time, which provides a possible alternative for separation of the iodinated isomers.
Figure 28: Comparison of fractions containing methyl 2-acetyl-α-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate isomers (6) via HPLC isocratic method at 25 µL and 200 µL injections (30% acetonitrile in dionized H$_2$O). All HPLC data were collected via an Agilent Eclipse C18 column 9.4 mm X 25 cm with a 3 mL/min flow rate. Peak labels correspond to those in Figure 27.

F. Computational Modeling

Chemical modeling in Gaussian09 was used to understand π-stacking behavior of the isoquinoline moiety of T-0632 with Trp33 in human GLP-1R, the amino acid that is understood to strongly affect binding affinity of T-0632 with the receptor. Wheeler et al. performed density functional theory (DFT) calculations to capture the interactions between the π-systems of aromatic molecules with and without substituents. Although methods have not been developed to only capture π-polarization effects on aromatic systems, DFT computational methods provide...
robust data and show the additional effect that substituents made to \( \pi \)-stacking interactions.

Density functional theory has been particularly accurate in estimating van der Waals interactions with geometry results comparable to x-ray diffraction results.\(^{18}\)

We predict that the aromatic isoquinoline moiety on T-0632 exhibits \( \pi \)-stacking behavior with the amino acid, tryptophan on the human GLP-1R (Figure 29). Tryptophan is an electron rich aromatic amino acid; the nitrogen atom on the indole donates electron density to the aromatic \( \pi \)-system. The nitrogen atom of the isoquinoline moiety is electron withdrawing, making this aromatic \( \pi \)-system electron poor. The attraction between the two systems might favor more \( \pi \)-overlap in overall geometry.
Figure 29: Speculative prediction of π-stacking interactions between the isoquinoline moiety on T-0632 and tryptophan (blue)

Density functional theory calculations were initially obtained for the isoquinoline moiety of T-0632, which was isolated through the amine group, and tryptophan, which was modeled as if it were in sequence with a larger peptide (Figure 30).
The amine and carbonyl groups, which comprise the amino acid backbone of tryptophan, strongly influenced the results of the initial geometry optimizations (Figure 30). The observed repulsion shows the amino acid backbone on tryptophan strongly interacting with the isoquinoline moiety of T-0632, showing that this model was not able to capture π-stacking interactions due to the dominating electrostatic interactions that involved that amino acid backbone. Because these interactions are strong enough to negate any interactions between π-systems, our refined calculations only incorporated the aromatic π-systems of both molecules.

Figure 30: Geometry optimization of isoquinoline moiety of T-0632 (a) and tryptophan (b) in Gaussian09. (c) The optimized structure after geometry optimization in Gaussian09. Tryptophan contains a methyl-ketone group in place of a carboxylic acid to prevent extraneous electrostatic interaction in simulation. Electron density is shown from electron poor (blue) to electron rich (red).
In order to compare π-stacking interactions between the aromatic regions of isoquinoline and tryptophan, geometry optimizations were first performed on two naphthalene rings stacked in face-to-face, staggered, and edge-on, all in cis and trans, conformations. The results showed that staggered and edge-on starting positions optimized to lower binding energies than a face-to-face conformation for both cis and trans orientations, and are consistent with findings suggested by Hunter et al. (Figure 31). The computational results show that edge-on and staggered conformations are only more stable by 2.5 KJ/mol, which is equivalent to very weak hydrogen bonding interactions (1-2 KJ/mol), suggesting that π-stacking interactions are small relative to electrostatic effects, which are on the scale of tens to hundreds of KJ/mol.
Figure 31: Change in binding energy (KJ/mol) relative to the Face-to-Face (cis) orientation of naphthalene rings stacked in Face-to-Face, Staggered, and Edge-on (each cis/trans). Images show the optimized stacking geometry of each orientation.

However, binding energies for tryptophan and isoquinoline stacking suggest that these energies are not an accurate representation of optimal stacking geometry. When positioned in an unfavorable structure, the optimized geometry diverts from the original starting position as if the two molecules want to flip towards a more optimal geometry. However, the energy required to flip the molecule may be a barrier that the Gaussian program could not overcome, resulting in optimizations in local minima. The observed binding energy is therefore a local minimum of a stable structure rather than the energy that represents the interaction of the two moieties.
The IEFPCM solvent system was used as the solvent model for the system because other solvent models led to Gaussian09 errors. This model incorporates the electrostatic stability of the molecules, but does not account for the hydrophobic effect of these molecules in water. As a result of this solvent model, the optimized orientation may not be one in which the molecules are stacked on top of each other, which would minimize the hydrophobic interactions with water, but rather one that favors the electrostatic interactions between the molecules and water.

For starting positions stacked face-to-face in the cis orientation, in which both rings are stacked directly on top of each other, our hypothesis predicted that the optimal geometry would be conformation (2), but (1) exhibits the most overlap and (4) was calculated to have the most stable energy (Figure 32). Although it is neither the structure with the most overlap or lowest binding energy, (2) shows that the ring positions containing a nitrogen atom are positioned closer than the rings that do not contain a nitrogen atom, suggesting a possible attractive interaction between the rings that we predicted would have the greatest contribution to isoquinoline/indole stacking interactions.
Figure 32: Starting position and optimized geometries of isoquinoline and indole (tryptophan) in Face-to-Face (cis) orientation, such that both rings are stacked directly on top of each other. Nitrogen atom of isoquinoline is optimized in orientations such that it is placed Front left (1), Front Right (2), Back Right (3), or Back Left (4) relative to the viewer.

The nitrogen atom of the indole is always placed such that it faces the back right of the document.
The optimized structure for (1) in the face-to-face (cis) orientation is shown in Table 1. The two most positive (blue) and negative (red) Mulliken atomic charges are highlighted. The magnitudes of the observed Mulliken atomic charges are mostly neutral, suggesting that electrostatic interactions of the entire molecule may not play an important role in the geometry optimization. We observe that the most positive and negative sides of the molecules are positioned farthest from each other in the optimized orientation, suggesting that there may be another effect, perhaps electrostatic interactions with solvent, driving the optimization in this orientation.

The optimal structures for (3) and (4) show the compounds moving away from each other and reaching a local minimum, just before overcoming an energy barrier to settle into a more stable binding position. However, energy calculation for (4) was shown to be the most stable, which can be a result that the most positive and most negative atoms on the molecules are positioned closest to each other, even though the compounds exhibit no stacking interaction (Table 2). These geometry optimizations show that when two molecules are positioned in an orientation that is unfavorable, the geometry is optimized such that the compounds move in order to settle into a more stable orientation. However, flipping the molecule requires overcoming a large barrier, and the optimization settles into a local minimum. Geometry optimizations show that the energy landscape of the model is scattered with local minima, and global optimization may be better achieved via a more thorough Monte Carlo simulation or a similar model.
Table 1: Mulliken atomic charges, as calculated by Gaussian09, of the structure that exhibited the most hydrogen bonding in the Face-to-Face (cis) conformation (1). Optimized structure and atom labels are shown in the left most column. The two most negative Mulliken atomic charges are highlighted in red, while the two most positive Mulliken atomic charges are highlighted in blue. The Mulliken atomic charges calculated by Gaussian09 are primarily neutral.

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Table 2: Mulliken atomic charges, as calculated by Gaussian09, of the structure with the lowest binding energy in the Face-to-Face (cis) conformation (4). Optimized structure and atom labels are shown in the left most column. The two most negative Mulliken atomic charges are highlighted in red, while the two most positive Mulliken atomic charges are highlighted in blue. The Mulliken atomic charges calculated by Gaussian09 are primarily neutral.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Atom Number</th>
<th>Atomic Symbol</th>
<th>Mulliken Atomic Charge</th>
</tr>
</thead>
<tbody>
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<tr>
<td></td>
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<td></td>
<td>3</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>6</td>
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<tr>
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<td>C</td>
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<tr>
<td></td>
<td>25</td>
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<td>0.038075</td>
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<td></td>
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<tr>
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<td></td>
<td>36</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>41</td>
<td>O</td>
<td>-0.238076</td>
</tr>
</tbody>
</table>

The face-to-face (trans) optimizations, for which the isoquinoline is rotated 90° relative to the cis orientation, showed the most overlap when the rings were positioned such that the nitrogen atoms were stacked on top of each other (4). This structure most closely resembles our hypothesis, whereas the other structures showed potential movement away from the starting position (Figure 33).
Figure 33: Optimized geometries stacking isoquinoline and indole moieties face-to-face and in the *trans* orientation such that the isoquinoline is rotated 90° from the *cis* position. Gaussian 09 was unable to generate an optimized structure for (1). The nitrogen atom of isoquinoline is optimized in orientations such that it is placed Front left (1), Front Right (2), Back Right (3), or Back Left (4) relative to the viewer. The nitrogen atom of the indole is always placed such that it faces the back right of the document.
The Mulliken atomic charges of (4) in the face-to-face \textit{trans} orientation show the most positive side of the indole migrating towards the most negative side of the isoquinoline (Table 3). These interactions are most likely electrostatic. Electronegativities can explain which atoms in the molecules bear the most and least amount of electron density; therefore, the observed geometries calculated by Gaussian09 suggest that interactions between the molecules may be primarily driven by electrostatic interactions rather than by the stacking of their \(\pi\)-systems.

\textbf{Table 3:} Mulliken atomic charges of the structure that exhibited the most hydrogen bonding in the Face-to-Face (\textit{trans}) conformation (4). Optimized structure and atom labels are shown in the left most column. The two most negative Mulliken atomic charges are displayed in red, while the two most positive Mulliken atomic charges are displayed as blue.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Atom Number</th>
<th>Atomic Symbol</th>
<th>Mulliken Atomic Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 C</td>
<td>0.167788</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 C</td>
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<td></td>
</tr>
<tr>
<td>3 C</td>
<td>0.037533</td>
<td></td>
<td></td>
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<tr>
<td>5 N</td>
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<td>7 C</td>
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<td></td>
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<td>9 C</td>
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<td>21 C</td>
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</tr>
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<td></td>
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<td>25 C</td>
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<td>37 C</td>
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</tr>
<tr>
<td>41 O</td>
<td>-0.253783</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The staggered (cis) optimizations show migration of the structures toward a more T-shaped geometry, which suggests that the molecules were moving out of its starting position toward a more stable structure but getting stuck in a local minimum during the calculations. (1) exhibits the most overlap and also closely resembles (1) in the face-to-face (cis) orientation, suggesting that the starting positions optimized to a similar local minimum (Figure 34).

<table>
<thead>
<tr>
<th>Starting Position</th>
<th>Optimized Geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td>Staggered (cis)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 34: Starting position and optimized geometries of isoquinoline and indole (tryptophan) in Staggered (cis) orientation, such that both rings are positioned directly on top of one another and the isoquinoline is shifted to the right by 3-5 Å. Nitrogen atom of isoquinoline is optimized in orientations such that it is placed in the Front left (1), Front Right (2), Back Right (3), or Back Left (4) relative to the viewer. The nitrogen atom of the indole is always placed such that it faces the back right of the document. Gaussian 09 was unable to generate an optimized structure for (2).

The staggered (trans) optimizations all show the possibility that the structures were moving out of their original orientations to find a better position but settled into a local minimum (Figure 35). The rings on the isoquinoline and indole that contain a nitrogen atom show potential attractive interactions, which can explain why these parts of the molecules are positioned closest
to each other in the optimized geometries, even if the compounds shift their orientations relative to one another during calculations.

<table>
<thead>
<tr>
<th></th>
<th>Starting Position</th>
<th>Optimized Geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 35: Starting position and optimized geometries of isoquinoline and indole (tryptophan) in Staggered (trans) orientation, such that the isoquinoline is rotated 90° from the cis orientation. Nitrogen atom of isoquinoline is optimized in orientations such that it is placed in the Front left (1), Front Right (2), Back Right (3), or Back Left (4) relative to the viewer. The nitrogen atom of the indole is always placed such that it faces the back right of the document. A more thorough map of the energy landscape of these structures must be obtained in order to definitively determine the other potential local minima and the globally optimized structure. This very crude model suggests that the electrostatic interactions between the isoquinoline and indole moieties rather than π-stacking interactions dominate in the calculations toward optimized geometries. The more polar ends of the molecules are positioned closer as a result of attractive electrostatic forces although the molecules remain primarily neutral as indicated by the Mulliken atomic charges. Additional calculations can include constants that incorporate the hydrophobic effect, rather than just the electrostatic interactions of water.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Furthermore, the movement of the molecules through these iterations suggests that migration toward a globally optimized structure can be possible. Longer calculations and utilizing models such as Monte Carlo simulations may allow these structures to overcome local minima.

This model is also much smaller than the system in vivo, which includes the rest of T-0632 and GLP-1R as well as other molecular crowders, so steric hindrance can also play an important factor in the binding of T-0632 with GLP-1R. However, these preliminary calculations give an initial insight as to the orientation of the isoquinoline moiety of T-0632 with respect to tryptophan in GLP-1R, even if π-stacking interactions are not as strong as electrostatic forces. Experimental results via photoaffinity labeling may confirm the applicability of these calculations to the actual molecular interactions; confirmation of computational predictions emphasizes the importance of successfully selective iodination of the isoquinoline moiety of T-0632.
III. Experimental

A. General Information

All reagents were purchased from either Sigma Aldrich or Alfa Aesar and used without further purification unless otherwise noted. Air and water sensitive reactions were performed using oven-dried glassware fitted with a rubber septum under a positive pressure of nitrogen. Chemical shifts were reported in ppm units relative to TMS and obtained using a Bruker 300MHz NMR spectrometer. Coupling is denoted “s” for singlet, “d” for doublet, “t” for triplet, “q” for quartet, “m” for multiplet. GC/MS were obtained using Agilent 6890 Network GC system coupled with an electron impact Agilent 5937 Network Mass Selective Detector. The GC/MS used a method, “RAYANNE” (T_{init} = 120°C; T_{final} = 325°C; rate = 20°C/sec; mobile phase = He(g); constant flow mode; flow rate = 1.0 mL/min; injection volume = 1 µL; pressure = 11.6 psi), for analysis. Purification of compounds via flash chromatography was performed using the Biotage Flash chromatography system with pre-packed silica gel (size: 40-63 µm) varying dimensions and lengths. Eluents were detected using UV-absorbance at 254nm and identified using electron impact mass spectrometry. High Performance Liquid Chromatography (HPLC) was performed using a Waters 1525 Binary HPLC pump coupled to a Waters 2487 Dual Wavelength Absorbance Detector, detecting eluents at 254 nm. All HPLC data were collected via an Agilent Eclipse C18 column 9.4 mm X 25 cm with a 3 mL/min flow rate. All chemical modeling calculations were performed via the Gaussian09 software. All geometry and energy calculations were optimized to a minimum using a ground state DFT Default Spin method using following parameters: # opt b3lyp/cc-pvdz scrf=(iefpcm, solvent=water) geom=connectivity; Charge/mult: 0 1.
B. Overall Synthesis

Figure 36: Overall Synthesis. The experimental procedure for the synthesis of methyl 2-acetyl-n-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (6) isomers is detailed in the following pages. 1 = phenylalanine. 2a = 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. 2 = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. 3 = methyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate. 4 = methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate. 5a = methyl isoquinoline-3-carboxylate. 5b = methyl 4-bromoisoquinoline-3-carboxylate. 5c = methyl 2-acetyl-1-oxo-1,2-dihydroisoquinoline-3-carboxylate. 6 = methyl 2-acetyl-n-iodo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate isomers.

C. Synthesis of 1,2,3,4-Tetrahydroisoquinoline-3-Carboxylic Acid (2)
Phenylalanine (1), (12.5 g, 0.076 mols), concentrated hydrochloric acid, (125 mL, 65 eq.), 1,4-dioxane, (9 mL, 2 eq.), and 37% formaldehyde, (10 mL, 4.5 eq.), were heated to 75°C for at least 1 hour. Additional concentrated hydrochloric acid, (60 mL, 31.5 eq.), 1,4-dioxane, (2.5 mL, 0.5 eq.), and 37% formaldehyde, (5 mL, 2.25 eq.) were added and the reaction mixture was heated at 75°C for an additional 24 hours. After cooling the reaction vessel to room temperature, the product was collected by vacuum filtration and washed with cold 1,4-dioxane, giving 78% yield of product (10.0 g), in the form of a white powder. NMR (integration, coupling, corresponding protons on molecule) chemical shifts in DMSO-d$_6$ (Appendix 1): δ9.59 (2, s, -NH and -OH); δ7.3 ppm (4, s, Aromatic); δ4.3 ppm (1, m, O=C-CH); δ3.5-3.8 ppm (2, m, N-CH$_2$); δ3.1 ppm (2, d, C-CH$_2$).

**D. Synthesis of 2-Acetyl-1,2,3,4-Tetrahydroisoquinoline-3-Carboxylic Acid (2a)**

This reaction was not included in the final synthesis. The poor solubility of the compound made it difficult to use in the subsequent steps of our synthesis.

1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2), (1.0 g, 0.005 mols), was dissolved in pyridine and dichloromethane on ice (12 mL of each per every gram of reactant). Acetic anhydride, (2.3 mL, 5 eq.), was added and the reaction was left to stir for 24 hours. The dichloromethane was first evaporated, and then 6 mL of 2 M hydrochloric acid and 25 g of ice were added until the solution was acidic. The product was allowed to precipitate from the
solution. The product was vacuum filtered, washed with cold, dilute hydrochloric acid, and recrystallized from water with 1% yield (0.012 g) to form light pink crystals. GC/MS showed molecular ion of 219 at 10.996 min and molecular ion 233 at 9.90 min in 2:1 ratio, respectively.

NMR chemical shifts in DMSO-d$_6$ (Appendix 2): δ12.75 ppm (1, s, -OH); δ7.2 ppm (4, s, Aromatic); δ5.0 ppm (1, doublet of triplets, O=C-CH); δ4.6 ppm (2, m, N-CH$_2$); δ3.1-3.5 ppm (2, m, C-CH$_2$); δ2.15 ppm (3, d, O=C-CH$_3$). D$_2$O shake confirmed singlet at δ12.75 ppm belonged to the carboxylic acid.

**E. Synthesis of Methyl 1,2,3,4-Tetrahydroisoquinoline-3-Carboxylate (3)**

![Molecular diagram](image)

1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2), (10 g, 0.05 mols), was heated at reflux with 4 M hydrochloric acid in 1,4-dioxane, (20 mL, 1.5 eq.), and methanol, (100 mL, 50 eq.), for 72 hours. The methanol was then evaporated before the acetylation. Yield had not been obtained because the next step of the synthesis was done in the same pot and assumed 100% yield. GC/MS yields a single peak at 9.38 min with molecular ion of 191 to confirm the product before proceeding to (F).
F. Synthesis of Methyl 2-Acetyl-1,2,3,4-Tetrahydroisoquinoline-3-Carboxylate

In the same pot as the synthesis described in (E), sodium carbonate, (5 g, 3 eq.), and tetrahydrofuran (THF), (25 mL), were added for 30 minutes on ice. Acetic anhydride, (5.5 mL, 5 eq.), was then added and stirred for 48 hours at room temperature. The amount of reagents added was calculated as if the synthesis described in (E) generated 100% yield. After 48 hours, the THF was evaporated and the organic layer was extracted from water into dichloromethane. The solution was then dried with magnesium sulfate and the organic solvent was evaporated. The product was purified using flash chromatography in ethyl acetate:hexane (1:1) with increasing strength of ethyl acetate to 100% ethyl acetate and analyzed using GC/MS and NMR in chloroform-d. Chromatography generated 43% yield (5.6 g) of the pure product. GC/MS produced a single peak at 10.203 min with a molecular ion of 233. HNMR chemical shifts (Appendix 3): δ7.2 ppm (4, s, Aromatic); δ5.4 ppm (1, d, O=C-CH); δ4.3 ppm (2, doublet of doublets, N-CH2); δ3.6 ppm (3, d, O-CH3); δ3.3 ppm (2, m, C-CH2); δ2.3 ppm (3, d, O=C-CH3).

CNMR chemical shifts (Appendix 4): δ171.5 (C=O); δ170.6 (C=O); 6 at δ126.2-δ129.8 (Aromatic); δ55.3 (CH); δ52.2 (O-CH3); δ46.3 (N-CH2); δ30.5 (C-CH2); δ21.9 (O=C-CH3).
G. Synthesis of Methyl Isoquinoline-3-Carboxylate (5a)

Purified methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4), (1.0 g, 0.004 mols), and N-bromosuccinimide, (1.53 g, 2 eq.), in dichloromethane, (100 mL per every 5 g of reactant), was stirred at room temperature for at least 3.5 hours. The solution turned a bright red color. The mixture was washed thrice with aqueous potassium carbonate solution, (100 mL per every 5 g of reactant), then washed twice with water, (100 mL per every 5 g of reactant), and then dried using magnesium sulfate. The solvent was evaporated, giving a yellow oil and purified by flash column chromatography in dichloromethane:methanol (starting at 100% dichloromethane up to 5% methanol). The synthesis generated 25% yield of crude product. GC/MS showed 3 products with molecular ion 187 (9.328 min), 266/268 (10.753 min) and 247 (10.071 min). NMR in chloroform-d confirmed the products:

(i) Molecular ion = 187 (5a) (Appendix 5)

δ9.4 ppm (1, s, N-CH); δ8.7 ppm (1, s, O=C-C-CH); δ8.2 ppm (doublet of doublets) and δ7.8 ppm (multiplet) integration of 4, corresponding to remaining 4 aromatic protons; δ4.2 ppm (3, s, O-CH₃)
(ii) Molecular ion = 266/268 (5b) (Appendix 6)

$\delta 8.2$ ppm (1, d, N=CH); $\delta 7.3$-7.6 ppm (4, doublet of doublets, Aromatic); $\delta 5.7$ ppm (1, doublet of doublets, Br-CH); $\delta 5.3$ ppm (1, d, O=C-CH); $\delta 2.8$ ppm (3, s, O-CH$_3$).

(iii) Molecular ion = 247 (5c) (Appendix 7)

$\delta 8.1$ ppm and $\delta 7.1$ ppm (1 each, doublet, para aromatic protons); $\delta 7.3$ ppm and $\delta 7.5$ ppm (1 each, d, ortho aromatic protons); $\delta 5.3$ ppm (1, q, O=C-CH); $\delta 3.6$ ppm (3, d, O-CH$_3$); $\delta 3.6$ ppm (1, q, C-CH$_2$); $\delta 2.8$ ppm (3, d, O=C-CH$_3$).

H. Synthesis of Methyl 2-Acetyl-$n$-Iodo-1,2,3,4-Tetrahydroisoquinoline-3-Carboxylate (6)

Oven-dried glassware wrapped in aluminum foil to shield the reaction from light was used throughout this reaction. Purified methyl 2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4), (3.5 g, 0.025 mols), was dissolved in glacial acetic acid, (5 mL, 6 eq.), and sulfuric acid, (0.133 mL ,0.1 eq), on ice. After 10 minutes, $N$-iodosuccinimide, (5.07 g, 1.1 eq.), was added while being shielded from light, and the reaction vessel was flushed with nitrogen gas for 30 minutes. The flush was completed in the dark. After the flush was complete, the reaction was left to stir at room temperature for 6 hours. The solution, a red color, was diluted with dichloromethane, and extracted using water and dichloromethane. The organic layer was then
washed in sodium thiosulfate, which turned the solution from a red color to a light yellow color. The organic layer was dried with anhydrous magnesium sulfate and the solvent was evaporated. Crude yield of the mixture was 2.5 g (from 3.5 g starting material). A GC/MS of the mixture confirmed 3 peaks at 12.06 min, 12.26 min, and 12.47 min with a molecular ion of 359, as well as a fourth peak at 10.29 min with a molecular ion of 233, and a fifth peak at 9.36 min with a molecular ion of 187. Purification of each iodinated isomer for NMR was attempted on a Biotage flash chromatography system (Observation wavelength = 254 nm, Monitor wavelength = 220 nm) using a silica stationary phase on a gradient of 1:1 ethyl acetate:hexane, increasing the concentration of ethyl acetate to 100%. Purification was also attempted with reversed phase C18 flash chromatography via the Biotage system (Observation wavelength = 254 nm, Monitor wavelength = 220 nm) using 1% methanol in deionized water, increasing the concentration of methanol to 10%. However, flash chromatography failed to purify the iodinated isomers, so the fractions containing the isomers were then collected for separation via HPLC. HPLC was run with a 25 µL injection volume and used a gradient method of 20% acetonitrile in deionized water, increasing the concentration of acetonitrile to 95% over 36 min. The results of this method led to development of an isocratic method of 30% acetonitrile in deionized water over 28 min. The isocratic method was run with both 25 µL and 200 µL injection volumes. All HPLC methods used an Agilent Eclipse C18 9.4 mm X 25 cm reversed phase column with maximum 200 µL injection volume at a flow rate of 3 mL/min.

I. Computational Modeling

Geometries were optimized for two napthalenes as comparison with the isoquinoline and indole, which comprises the R-group of tryptophan (Figure 37). Geometry optimizations for each pair were first performed on each individual molecule using B3LYP/ccVDZ in Gaussian09.
Next, the individual compounds were brought together in the same document for binding calculations. The compounds were initially positioned 5Å apart and solvent effects using the IEFPCM solvation model with B3LYP/ccVDZ calculations were incorporated for computing stacking interactions.

Figure 37: Structures of compounds for geometry optimization in Gaussian09. (1) Two naphthalene rings. (2) Isoquinoline moiety of T-0632 and indole of tryptophan.

Geometries were optimized for compounds in different starting positions. The naphthalenes were optimized stacked face-to-face with both rings on top of each other (cis) and with one ring rotated 90° (trans), staggered cis and trans, and edge-on with both edges of one ring facing the other naphthalene (cis) and with one edge of one ring facing the other naphthalene (trans) (Figure 38). The isoquinoline and tryptophan were optimized in face-to-face and staggered in both cis and trans conformations. The number of optimizations quadrupled due to the asymmetry of the compounds. The placement of the nitrogen atom in the isoquinoline was optimized in four positions and was placed cis (both rings were placed on top of each other) and
trans (the isoquinoline was rotated 90°) in each position (Figure 39). In each simulation, the compounds were initially positioned 5Å apart.

<table>
<thead>
<tr>
<th>Napthalene Stacking</th>
<th>Face-to-Face (cis)</th>
<th>Face-to-Face (trans)</th>
<th>Staggered (cis)</th>
<th>Staggered (trans)</th>
<th>Edge-on (cis)</th>
<th>Edge-on (trans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting position</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure 38: Starting positions for two napthalene molecules for stacking interaction computations. Each starting position (Face-to-Face, Staggered, and Edge-on) was positioned in a cis and trans conformation. Both rings positioned in the same long or short conformation defined the cis orientation. The conformation was trans if one compound was positioned in the shorter orientation while the other compound was positioned in the long orientation. Computational methods were performed using B3LYP/cc-VDZ in IEFPCM solvation model with the compounds initially positioned 5Å apart using B3LYP/cc-VDZ computational method without a solvation model.
Figure 39: Isoquinoline/Indole Stacking Starting Positions. The vertical column headings refer to the nitrogen positioned on the isoquinoline. Compounds positioned in the same long orientation were considered *cis*, while one positioned in its shorter orientation relative to the other’s long orientation was considered *trans*. Each *cis* and *trans* conformation was positioned in a Face-to-Face, Staggered, and Edge-on starting position 5Å apart using B3LYP/cc-VDZ computational method in IEFPCM solvation model.
Works Cited:


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![HNMR spectrum of 2-acetyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (2a) in DMSO-d$_6$.](image)
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