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# Discovery of spirocyclic proline tryptophan hydroxylase-1 inhibitors

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ABSTRACT

The central role of the biogenic monoamine serotonin (5-hydroxytryptamine, 5-HT) as a neurotransmitter with important cognitive and behavioral functions is well known. However, 5-HT produced in the brain only accounts for approximately 5% of the total amount of 5-HT generated in the body. At the onset of our work, it appeared that substituted phenylalanine derivatives or related aryl amino acids were required to produce potent inhibitors of TPH1, as significant losses of inhibitory activity were noted in the absence of this structural element. We disclose herein the discovery of a new class of TPH1 inhibitors that significantly lower peripherally 5-HT.



The central role of the biogenic monoamine serotonin (5-hydroxytryptamine, 5-HT) as a neurotransmitter with important cognitive and behavioral functions is well known. However, 5-HT produced in the brain only accounts for approximately 5% of the total amount of 5-HT generated in the body. It is estimated that 95% of peripheral 5-HT is produced by the gut enterochromaffin cells, with the vast majority (98%) being stored in platelets and the small remaining fraction circulating as free 5-HT. Outside the central nervous system, 5-HT acts as a hormone and vasoactive agent, regulating a large number of biological processes including gastric emptying, cardiovascular and genitourinary functions, and platelet aggregation.[1] Given 5-HT does not cross the blood–brain barrier, the central and peripheral serotonin systems are regulated independently.[2]

While drugs targeting the various 5-HT receptors or the 5-HT transporter have been developed to interfere with 5-HT signaling,[3] a further understanding of the pathologies associated with elevated serotonin levels is emerging, as genetic tools and selective pharmacological agents are becoming more readily available. For example, studies in mice lacking the gene for tryptophan hydroxylase-1 (TPH1), the rate-limiting enzyme in the biosynthesis of 5-HT from tryptophan, have implicated a role of 5-HT in GI motility, β-cells and hepatocytes proliferation, as well as effects on vessel contractility and cardiac myocyte proliferation.[2,4,5] Taken together, these results suggest that modulation of a dysfunctional peripheral 5-HT system may offer unexplored opportunities for the treatment of human diseases, such as carcinoid syndrome, fibrotic diseases, non-alcoholic fatty liver disease, obesity, atherosclerosis, allergic asthma, cancer, and pulmonary arterial hypertension.[6–12] In particular, this should be achievable by the selective inhibition of TPH1 in peripheral tissues.

One of the key challenges in studying the effects of inhibiting peripheral serotonin has been in either the identification of selective receptor antagonists, or more recently, potent inhibitors of TPH1.[13] Based on its ability to deplete 5-HT pools in vivo, *p*-chloro phenylalanine (*p*CPA, Fenclonine) was considered one of the first reported TPH1 inhibitors.[14] However, its direct binding to TPH1 in vitro is extremely weak ($IC_{50} > 100\ \mu M$), suggesting that its pharmacological activity is not directly related to enzyme inhibition.[15–17] *p*CPA was proven effective in treating chemotherapy-induced emesis,[18] as well as diarrhea in carcinoid tumor patients,[19–22] but was not developed further, in part due to its CNS distribution leading to the onset of depression and other behavioral impairments in patients and animals.[23] Other substituted phenylalanine derivatives, like *p*-ethynyl phenylalanine (*p*EPA), showed characteristics similar to *p*CPA, including long-term depletion of central 5-HT.[24,25]

More recently, bulkier phenylalanine derivatives have been reported to be potent and direct TPH1 inhibitors and to reduce intestinal 5-HT concentration without affecting brain 5-HT levels.[6,26–31] Most notably, Telotristat etiprate (a.k.a. LX1606) the orally bioavailable prodrug of the TPH1 inhibitor LX1033, is currently in Phase III clinical trials for the treatment of gastrointestinal symptoms associated with carcinoid syndrome in patients refractory to standard of care medication.[32]

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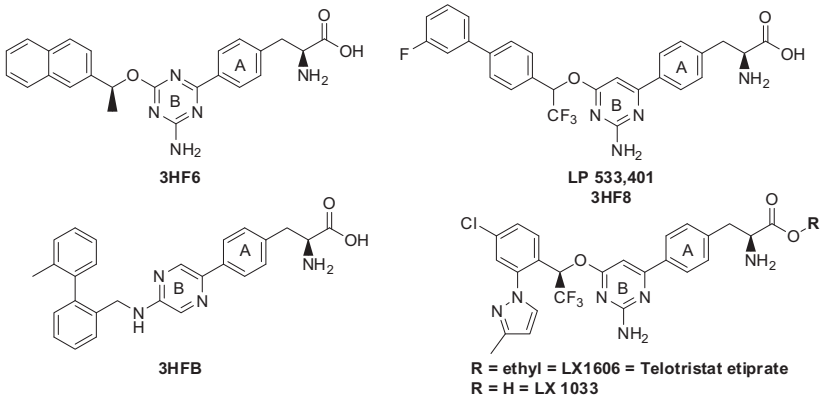


**Figure 1.** Structures of lexicon TPH1 inhibitors (pdb code for crystal structure provided).

At the onset of our work, it appeared that substituted phenylalanine derivatives or related aryl amino acids were required to produce potent inhibitors of TPH1, as significant losses of inhibitory activity were noted in the absence of this structural element.[30] In the first phase of our drug discovery project aimed at modulating peripheral 5-HT, we set out to discover new peripherally restricted chemotypes to inhibit TPH1 activity.

Karos's approach to discovering new TPH1 inhibitors stemmed from a structure based design methodology. Several X-ray co-crystal structures of TPH1 inhibitors bound in the absence of co-factor (pdb codes: 3HF6, 3HF8, and 3HFB) served as a starting point to model new ligands based on the key binding interactions (Fig. 1). A consistent pharmacophore feature of the clinically relevant TPH1 inhibitors to date has been the phenylalanine moiety believed to be a key recognition element for binding. This structural element may also be important to allow inhibitors access to the intracellular location of TPH1 via putative transporters, as required by the natural substrate tryptophan.

According to the published X-ray structures, the protein conformation is nearly identical in the regions binding to the amino acid, A-ring, and B-ring. However, to accommodate the different substitution patterns at the B-ring (*ortho* vs *para*) and diverse hydrophobic groups, TPH1 can adopt distinct conformations notably by repositioning the side-chain of Tyr235, thereby creating a new binding pocket. The structure of compound 3HF6 bound to TPH1 reveals that the large terminal naphthyl group occupies a site several angstroms away from the empty biopterin site, while for compounds LP 533,401 and 3HFB, Tyr235 adopts an alternate conformation and rotates towards the backbone of Glu267. Given this information, LP 533,401 represented a foundation and starting point for understanding some of the key binding requirements and to model new ligands (Fig. 2).

The binding conformation of LP 533,401 illustrates the three critical pharmacophore features of a phenylalanine-based TPH1 inhibitor (Fig. 3):

1. The amino acid head is involved in a H-bond network with Arg257 and Thr265.
2. The central core is typically a nitrogenous heterocyclic ring capable of forming a critical H-bond with a structurally conserved water molecule bound to the iron atom of TPH1.
3. The hydrophobic tail is an area on the inhibitor that is more tolerant of structural diversity as a result of the flexibility of Tyr235 side-chain.

Initially, potent TPH1 inhibition was reportedly only achieved with aromatic amino acid derivatives, in particular phenylalanine derivatives, and any significant deviation from this design resulted in a substantial loss in inhibitory potency.[30] However, visual examination of the known X-ray structures of inhibitors bound to TPH1 indicated that the phenylalanine binding pocket was large enough to tolerate alternative amino acids with three dimensional shapes. Thus, a variety of phenylalanine replacements were modeled to fit the binding site, synthesized, and the fully elaborated compounds

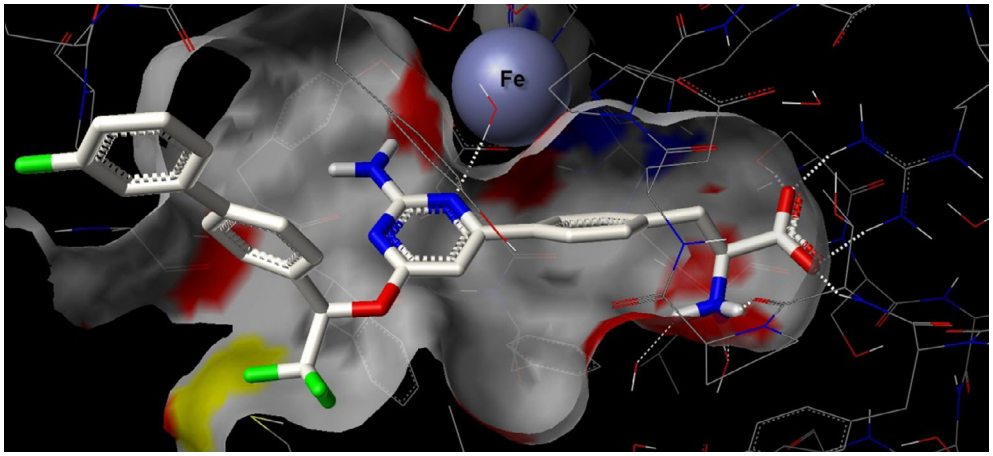


**Figure 2.** Binding conformation of LP 533,401 in TPH1 (pdb structure 3HF8). Dotted lines depict H-bond interactions between the inhibitor and TPH1 residues Arg257, Thr265, as well as conserved water molecules.