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Substituted 3-(4-(1,3,5-triazin-2-yl)-phenyl)-2-aminopropanoic acids as novel tryptophan hydroxylase inhibitors

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ABSTRACT

Tryptophan hydroxylase (TPH) is a key enzyme in the synthesis of serotonin. As a neurotransmitter, serotonin plays important physiological roles both peripherally and centrally. Here we describe the discovery of substituted triazines as a novel class of tryptophan hydroxylase inhibitors. This class of TPH inhibitors can selectively reduce serotonin levels in murine intestine after oral administration without affecting levels in the brain. These TPH inhibitors may provide novel treatments for gastrointestinal disorders associated with dysregulation of the serotonergic system, such as chemotherapy-induced emesis and irritable bowel syndrome.

Serotonin (5-hydroxytryptamine, 5-HT) is an important substance involved in the modulation of behavior and control of gastrointestinal (GI) function. The rate-limiting enzyme of serotonin synthesis in the GI tract is tryptophan hydroxylase type I (TPH1). Serotonin is known to markedly influence bowel motility by activating at least five receptor types, namely 5-HT 1–4 and 7.[1] Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain, diarrhea, and constipation. The role of 5-HT in the pathophysiology of IBS has been investigated.[2] In IBS patients with diarrhea-predominant symptoms (IBS-D), the number of enterochromaffin cells is significantly enhanced[3] and the plasma 5-HT levels following a meal are significantly higher.[4] Furthermore, 5HT-3 antagonists are proven to be effective in the treatment of IBS-D.[5] These findings suggest that modulation of 5-HT in the gut via inhibition of TPH1 is a potential treatment for functional GI disorders.

We recently disclosed the discovery and structure–activity relationship (SAR) of small molecule TPH inhibitors, exemplified by **1** (Fig. 1).[6] These molecules can selectively reduce peripheral serotonin levels without affecting brain serotonin levels after oral administration. We also disclosed the key SAR for these TPH1 inhibitors. For example, we demonstrated that the L-phenylalanine moiety is essential for the TPH1 in vitro potency and cannot be replaced. On the other hand, the pyrazine core (e.g. compound **2**) can be replaced by pyrimidine (compound **1**) or triazine. In this Letter, we focus on the discovery of substituted 3-(4-(1,3,5-triazin-2-yl)-phenyl)-2-aminopropanoic acids as novel TPH1 inhibitors.

During the optimization of hit compound **2**, different heterocyclic replacements for the pyrazine moiety were investigated with regard to their inhibition of TPH1. Our results showed that the pyrazine core is sensitive to change but could be effectively replaced by 2-amino-triazine. Additional triazine derived analogs (**6a–l, 7a–m**) were prepared to investigate SAR. The general synthetic route is outlined in Scheme 1. Nucleophiles **3**, such as amines or alcohols, reacted with a variety of dichloro triazines **4** through nucleophilic aromatic substitution to form intermediate chlorides represented by **5**. Suzuki coupling of **5** with L-phenylalanine–boronic acid using bis(triphenylphosphine) palladium(II) dichloride as the catalyst and sodium carbonate as the base gave the target compounds **6** and **7** which were generally purified by preparative HPLC using a MeOH/water/TFA solvent system.

In vitro inhibitory potency (IC_{50}) was obtained using a continuous fluorescence assay which measured inhibition of purified, recombinant human TPH1.[7] Cellular potency (EC_{50}) was determined by measuring the inhibition of serotonin biosynthesis in the rat mastocytoma cell line RBL-2H3.[8] Compounds were examined in both the TPH1 fluorescence assay and RBL cellular as-

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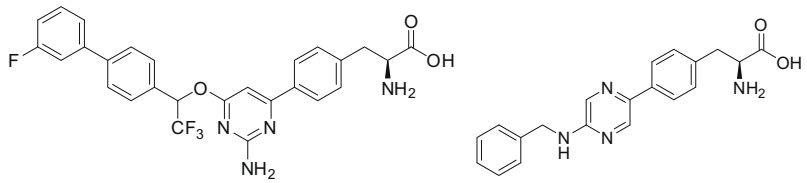


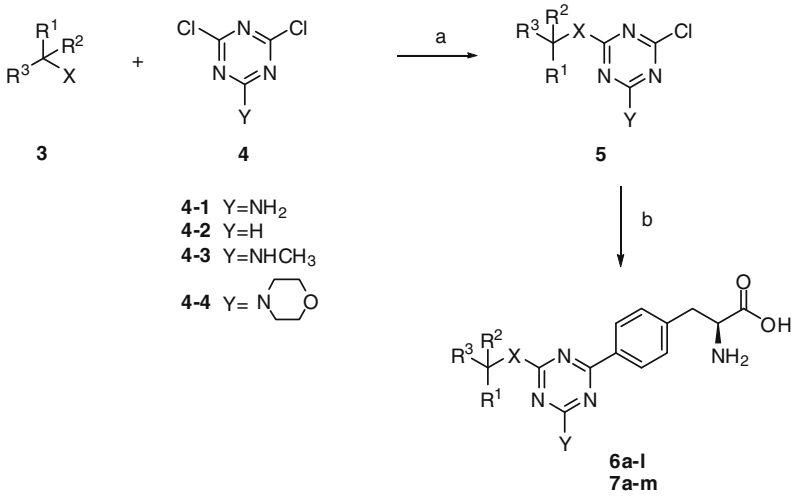
1 **2**

Figure 1. Chemical structures of compounds **1** and **2**.

say. As shown in Table 1, introduction of the 2-amino triazine resulted in compound **6a** having an IC_{50} of 0.5 μM against TPH1, which is slightly more potent than the HTS hit, pyrazine **2** (IC_{50} = 0.67 μM). The effect of varying substitutions on the distal phenyl ring was evaluated. A fourfold in vitro potency increase was observed when either electron withdrawing groups, such as a trifluoromethyl group (**6b**), or electron donating groups, such as methyl groups (**6c**) were substituted on the phenyl ring. However, these changes did not improve the cell potency. Replacement of the phenyl ring with cyclohexyl gave analog **6d** which was equipotent to the substituted phenyl derivatives. Using the published apo-structure of human TPH1, a homology model was constructed which suggested that the binding pocket could accommodate larger groups on the left side of the molecule. Biphenyl analogs were designed to test the hypothesis. Compared to **6a**, the *para*-biphenyl **6e** (IC_{50} = 0.06 μM, EC_{50} = 1.8 μM) increased the activity by eightfold in the biochemical assay. Moving the distal phenyl ring to the *ortho*-position resulted in **6f** (IC_{50} = 0.007 μM, EC_{50} = 2.2 μM), which further improved the primary potency. *meta*-Biphenyl **6g** (IC_{50} = 0.028 μM, EC_{50} = 1.4 μM) improved primary potency by twofold compared with **6e**. Interestingly, these biphenyl analogs improved the cellular potency more than sixfold compared with the corresponding phenyl analogs. Electronic effects on the biphenyl system were also investigated. Introduction of electron donating groups, such as a methyl group (**6i,** IC_{50} = 0.014 μM, EC_{50} = 2.3 μM) retained potency while electron withdrawing groups (e.g., **6j,** IC_{50} = 0.16 μM, EC_{50} = 4.1 μM) led to decreased in vitro potency. Phenoxyphenyl analog **6h** displayed fourfold reduced in vitro potency relative to **6g**. The addition of a benzylic α-methyl group as in **6k** (IC_{50} = 0.024 μM, EC_{50} = 2.5 μM) increased in vitro potency by more than twofold compared with the corresponding unsubstituted analog **6e**. We further tested our hypothesis by introducing a bulky fused ring system. Adamantane analog **6l** proved highly potent (IC_{50} = 0.019 μM) and demonstrated an additional threefold improvement in cell potency compared to **6k**.

As illustrated in Table 2, we continued to look at sizeable hydrophobic substitutions on the left part of the molecule. To this end, we decided to introduce a naphthalene moiety. Not surprisingly, naphthalene analog **7a** (Table 2) showed a good potency of 0.024 μM against TPH1. We next investigated the stereochemistry at the benzylic position. The (*R*)-2-methyl amino isomer **7b** (IC_{50} = 0.026 μM) demonstrated much better cellular potency than (*S*) isomer **7c** but maintained similar primary potency. Furthermore, we evaluated modulating the nature of the linker of **7b**. As shown in Table 2, **7d** (IC_{50} = 0.055 μM, EC_{50} = 1.7 μM) with a geminal dimethyl group exhibited a twofold decrease in enzyme potency and a fivefold decrease in cell potency compared with **7b.** Trifluoromethyl substituted alkoxy compound **7e** (IC_{50} = 0.32 μM, EC_{50} = 9.6 μM) and trifluoromethyl substituted amine **7f** (IC_{50} = 0.33 μM, EC_{50} = 6.3 μM) both proved detrimental to the enzyme and cellular potencies. N-methylation of **7b** resulted in **7g** (IC_{50} = 0.05 μM, EC_{50} = 0.48 μM), which showed a twofold decrease in primary potency and had little effect on cell potency. Changing the α-methyl group to ethyl, isobutyl, and isopropyl (**7h, 7i**, and **7j**, respectively) resulted in loss of cell potency. Efforts toward modifying the triazine substitution are also summarized in Table 2. Morpholino triazine **7k,** 2-*N*-methyl triazine **7l** and des-amino triazine **7m** all had reduced potencies.

An X-ray crystal structure of **7b** co-crystallized with TPH1 was obtained (Fig. 2) after our SAR exploration on the triazine series.[9] This crystal structure revealed that the carboxylate group of compound **7b** interacted with Arg257 through a charge–charge interaction and accepted two hydrogen bonds: one from the backbone nitrogen of Thr265 and another one from the hydroxyl group on



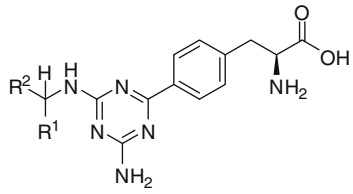
3 **4** **5**

4-1 Y=NH_2
4-2 Y=H
4-3 Y=$NHCH_3$
4-4 Y= N O

6a-l
7a-m

Scheme 1. General procedure for the synthesis of triazine analogs. Reagents and conditions: (a) 1,4-dioxane, K_2CO_3, reflux, 95%; (b) L-phenylalanine-boronic acid, $(PPh_3)_2PdCl_2$, 1 M Na_2CO_3, acetonitrile, microwave, 150 °C, 10 min, 92%; all compounds were characterized by ^1H NMR, LC/MS, and HPLC.

Table 1
In vitro inhibitory and cellular potencies of **6a–l**



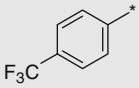
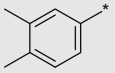
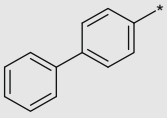
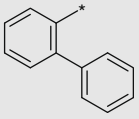
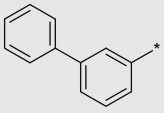
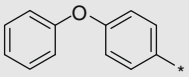
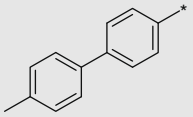
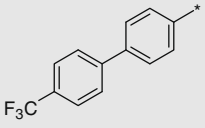
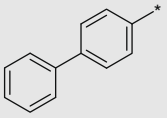
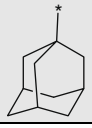
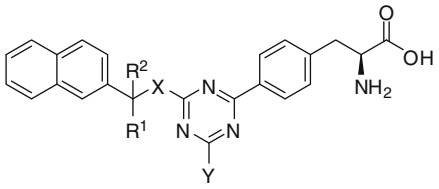
Example	R^2	R^1	TPH1 IC_{50} (μM)	RBL EC_{50} (μM)
6a		H	0.5	>11
6b		H	0.12	36
6c		H	0.10	>11
6d		H	0.12	>11
6e		H	0.06	1.8
6f		H	0.007	2.2
6g		H	0.038	1.4
6h		H	0.15	3.4
6i		H	0.014	2.3
6j		H	0.16	4.1
6k		CH_3	0.024	2.5
6l		CH_3	0.019	0.82

Table 2
In vitro inhibitory and cellular potencies of **7a–m**



	R^1	R^2	X	Y	TPH1 IC_{50} (μM)	RBL EC_{50} (μM)
7a	H	H	NH	NH_2	0.024	2.1
7b	CH_3(R)	H	NH	NH_2	0.026	0.3
7c	CH_3(S)	H	NH	NH_2	0.044	2.8
7d	CH_3	CH_3	NH	NH_2	0.055	1.7
7e	H	CF_3	O	NH_2	0.32	9.6
7f	H	CF_3	NH	NH_2	0.33	6.3
7g	H	CH_3 (R)	NCH_3	NH_2	0.05	0.48
7h	H	CH_2CH_3	NH	NH_2	0.15	2.3
7i	H		NH	NH_2	0.12	>11
7j	H		NH	NH_2	0.25	5.7
7k	H	H	NH		15.6	>40
7l	H	CH_3 (R)	NH	$NHCH_3$	0.61	10.2
7m	H	CH_3	NH	H	0.19	16.2

the side chain of Ser336. The amino group of the amino acid moiety in compound **7b** formed three hydrogen-bond interactions: one with the carbonyl oxygen of Thr265 and two with water molecules. The phenyl ring in **7b** made contacts with Pro268 and His272 through hydrophobic interactions. The triazine ring formed an edge-to-face π–π interaction with Phe313, being placed slightly more than 4 Å away from the ring plane.

Furthermore, one of the nitrogen atoms of the triazine ring was within hydrogen bond distance from one of the water molecules that coordinates the iron atom. Another nitrogen atom formed a hydrogen bond with a bridging water molecule, and the 2-amino group established an additional hydrogen-bond interaction with another water molecule.

The X-ray structure showed that the naphthalene moiety was sandwiched between the Tyr235 side chain (π–π-edge-to-face stacking) and Pro268 (hydrophobic interaction) and restricted access to the pocket that is normally occupied by the pterin cofactor.

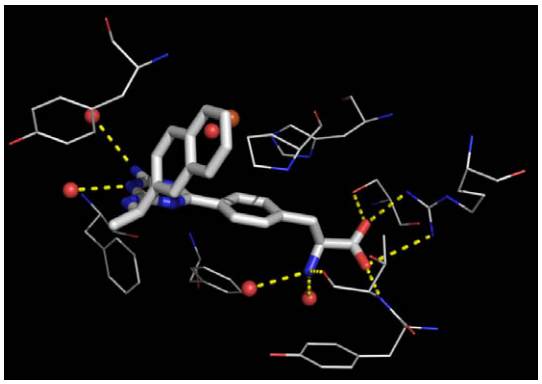


Figure 2. Crystal structure of TPH1 and **7b**.

The pharmacokinetic profile of compound **7b** was examined in two groups of C57-albino male mice. One group received an intravenous injection of 1 mg/kg, and another was orally dosed at 10 mg/kg. A low plasma clearance (CL = 0.5 L/h/kg) and a relatively small volume of distribution (V_{SS} = 0.5 L/kg) were observed following the intravenous injection. Following oral gavage dosing, the compound was poorly absorbed with a C_{max} = 0.5 μM, AUC = 1.6 μM h and an oral bioavailability of only 5%.

Compound **7b** was examined in vivo by administering the compound to mice by intraperitoneal injection (ip) and oral gavage (po) at a dose level of 100 mg/kg twice a day (200 mg/kg/day) for five days.[8] Levels of 5-HT in the blood and intestine were then measured. We observed a 17% decrease of 5-HT levels in the blood and a 38% decrease of 5-HT levels in duodenum following oral administration. There were a 26% decrease in blood 5-HT and a 37% decrease in duodenum 5-HT following IP administration. In a follow-up study, the compound was given at 100 mg/kg, twice a day by IP for eight days. A 50% decrease in blood 5-HT and a 57% decrease in duodenum 5-HT was observed in this case. Brain 5-HT levels, however, were not significantly altered.

In summary, we have described another novel class of potent TPH inhibitors. TPH inhibitors, such as **7b**, can selectively inhibit peripheral 5-HT synthesis without effects on the central nervous system because of very poor systemic exposure and the inability to cross the blood–brain barrier. This novel class of peripheral TPH inhibitors may provide potential treatments for a variety of gastrointestinal diseases caused by dysregulation of the serotonergic pathway in the periphery.

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9. Coordinates for the X-ray crystal structure of **7b** co-crystallized with TPH1 have been deposited at Protein Data Bank file name: 3HF6.