6-Hydroxy-1,2,4-triazine-3,5(2H,4H)-dione Derivatives as Novel D-Amino Acid Oxidase Inhibitors

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Supporting Information

ABSTRACT: A series of 2-substituted 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione derivatives were synthesized as inhibitors of D-amino acid oxidase (DAAO). Many compounds in this series were found to be potent DAAO inhibitors, with IC50 values in the double-digit nanomolar range. The 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione pharmacophore appears metabolically resistant to O-glucuronidation unlike other structurally related DAAO inhibitors. Among them, 6-hydroxy-2-(naphthalen-1-ylmethyl)-1,2,4-triazine-3,5(2H,4H)-dione 11h was found to be selective over a number of targets and orally available in mice. Furthermore, oral coadministration of D-serine with 11h enhanced the plasma levels of D-serine in mice compared to the oral administration of D-serine alone, demonstrating its ability to serve as a pharmacoenhancer of D-serine.

INTRODUCTION

D-Serine, a full agonist at the glycine modulatory site of the N-methyl-D-aspartate (NMDA) receptor, has been actively explored as a potential therapeutic agent for the treatment of schizophrenia.1 Several clinical studies with oral D-serine have shown promising results for the treatment of not only positive symptoms but also negative symptoms which have not responded well to existing drugs.1 Further clinical development of D-serine, however, could be hampered by the high doses of D-serine (60−120 mg/kg) required for the optimal efficacy2 since D-serine was reported to be nephrotoxic in rats at high doses.3 D-Amino acid oxidase (DAAO, EC 1.4.3.3) is a flavoenzyme that catalyzes the oxidation of D-amino acids including D-serine to the corresponding α-keto acids. In mammals, DAAO is present in kidneys, liver, and brain. Because the highest DAAO activity is found in the kidneys,4 a substantial amount of orally administered D-serine is metabolized in the kidneys, contributing to its rapid clearance. Indeed, pharmacokinetics studies of D-serine in mice lacking DAAO unmasked the predominant role of DAAO in the plasma clearance of D-serine.5 Furthermore, D-serine-induced nephrotoxicity is most likely due to the production of hydrogen peroxide as a byproduct of DAAO-mediated oxidation because it does not occur in mutant rats lacking DAAO activity.6 These findings collectively suggest that inhibition of DAAO would exert dual beneficial effects on D-serine therapy: (i) enhancement of D-serine bioavailability and (ii) attenuation of D-serine induced nephrotoxicity.

In the past decade, concurrent with the functional studies of DAAO,7 substantial strides have been made in identifying potent and selective DAAO inhibitors of broad structural diversity (Figure 1).8,9 Carboxylate-based DAAO inhibitors 110 and 211 presumably originated from a prototype DAAO inhibitor, benzoic acid, have evolved into a new class of compounds containing carboxylate bioisosteres such as 312 and
Crystal structure of 4 bound to DAAO revealed that the α-hydroxycarbonyl moiety of 4 retains the key interactions seen between the DAAO active site and carboxylate-based inhibitors. Subsequently, a variety of other heterocyclic frameworks bearing an α-hydroxycarbonyl moiety were explored in the pursuit of new scaffolds for potent DAAO inhibitors including 5 and 6. Structural insights gained from DAAO in complex with imino-DOPA led to the discovery of a secondary binding site of DAAO in complex with imino-DOPA. For example, our group exploited this secondary binding site was also exploited by carboxylate-based DAAO inhibitors. Subsequent investigation revealed that most of these carboxylate bioisosteres are subject to glucuronidation in liver microsomes, presumably contributing to their poor oral bioavailability relative to those containing a carboxylate bioisostere. Subsequent investigation revealed that most of these carboxylate bioisosteres are subject to glucuronidation in liver microsomes, presumably contributing to their poor oral bioavailability.

Recently, the pharmacokinetics profile of several representative DAAO inhibitors has been systematically compared in mice and rats. Although most of the DAAO inhibitors were reported to show negligible distribution to the brain, the poor CNS permeability does not pose a major concern for our objective of increasing exposure to orally administered D-serine by inhibition of peripheral DAAO. To our surprise, however, carboxylate-based DAAO inhibitors exhibited superior oral bioavailability relative to those containing a carboxylate bioisostere. Subsequent investigation revealed that most of these carboxylate bioisosteres are subject to glucuronidation in liver microsomes, presumably contributing to their poor oral bioavailability.

As a part of our continuous effort to identify new orally available DAAO inhibitors, we have searched for a pharmacophore providing not only potent DAAO inhibition but also considerable resistance to glucuronidation. During the course of screening a library of FDA-approved small molecule drugs, ceftriaxone (Figure 2) was identified as a moderate DAAO inhibitor with an IC₅₀ value of 10 μM. Although ceftriaxone does not offer a particularly attractive molecular template for developing SAR studies, the 3-mercapto-2-methyl-1,2,4-triazine-3,5-dione portion of ceftriaxone has caught our attention as its tautomer, 6-hydroxy-1,2,4-triazine-3,5-dione, has a structural feature common to other DAAO inhibitors. Interestingly, 6-hydroxy-2-methyl-1,2,4-triazine-3,5-dione isomer 11a, one of the degradation products of ceftriaxone in aqueous solution, inhibited DAAO with an IC₅₀ value of 2.8 μM, prompting us to further examine the potential of 2-substituted 6-hydroxy-1,2,4-triazine-3,5-dione derivatives as new DAAO inhibitors. Here we present a new class of DAAO inhibitors based on the 6-hydroxy-1,2,4-triazine-3,5-dione scaffold. With appropriate substitutions at the 2-position, some 6-hydroxy-1,2,4-triazine-3,5-dione derivatives exhibited good potency as well as improved metabolic stability. One of these derivatives was also tested for its oral bioavailability as well as effects on D-serine plasma levels following oral coadministration with D-serine in mice.

CHEMISTRY

The majority of 2-substituted 6-hydroxy-1,2,4-triazine-3,5-(2H,4H)-dione derivatives were synthesized using 6-bromo-1,2,4-triazine-3,5-(2H,4H)-dione as a starting material (Scheme 1). Alkylation of the 2-position of 12 with alkyl halides mediated by bis(trimethylsilyl)acetamide (BSA) gave 2-substituted derivatives 13a–z. Subsequently, the 6-bromo group of 13a–z was replaced by the benzyloxy group by treating with benzyl alcohol in the presence of potassium carbonate to give 14a–z. The final products 11a–z were obtained by removing the benzyl group of 14a–z by either catalytic hydrogenation or boron tribromide. Anisole derivatives 15u–w are further converted into the corresponding phenolic derivatives 15u–w by boron tribromide.

Some derivatives were synthesized using 6-bromo-4-[(phenylmethoxy)methyl]-1,2,4-triazine-3,5-(2H,4H)-dione as a starting material (Scheme 2). Mitsunobu reaction of 16 and alcohols with the aid of DIAD and PPh₃ provided the 2-substituted derivatives 17a–f. Reaction with benzyl alcohol gave the benzyloxy protected intermediates 18a–f, which were subsequently converted into the desired products 19a–f by either catalytic hydrogenation or boron tribromide. Synthesis of ketone derivative 22 began with base-mediated coupling of 2-bromoacetophenone to 16. The product 20 was converted into 21 by treating with benzyl alcohol. The removal of benzyl and BOM groups from 21 by boron tribromide afforded 22. Synthetic intermediate 21 was also transformed to the hydroxyl derivative. Synthesis of 2-phenyl derivative 26 involves Chan–Lam coupling of 16 to phenylboronic acid to give 24. Subsequent benzyloxylolation and deprotection by BBr₃ provided 26.

As shown in Scheme 3, we synthesized two structurally close analogues of 11e, where its carboxylate bioisostere moiety is slightly modified. 3-Thioxo derivative 30 was prepared using (2-phenylethyl)hydrazine as a starting material. Reaction of 27 with ammonium thiocyanate provided hydrazinocarboxothioamide 28, which was subsequently treated with methyl chlorooxocacetate to give 29. Cyclization of 29 in the presence of DBU afforded the desired product. Derivative 32 was prepared by BSA-mediated alkylation of 5-hydroxypyrimidine-2,4(1H,3H)-dione 31 with phenethyl iodide.

![Figure 2. Ceftriaxone and compound 11a.](Image)
**RESULTS AND DISCUSSION**

The inhibitory potency of the synthesized compounds were determined using recombinant human DAAO as previously reported. In vitro DAAO inhibitory data are summarized in Tables 1 and 2. As shown in Table 1, 6-hydroxy-1,2,4-triazine-3,5(2H)-dione derivatives containing a variety of substituents at the 2-position displayed varying degrees of inhibitory potency against DAAO. All compounds but 11c and 26 were found to be potent DAAO inhibitors, with IC_{50} values in the low-micromolar to nanomolar range. Within the compounds containing an aromatic ring, phenethyl derivative 11e and naphthalen-1-ylmethyl derivative 11h exhibited the most potent inhibitory activity, with IC_{50} values of 70 and 50 nM, respectively. Incorporation of a heterocyclic ring (compounds 19a–d) did not result in improved potency. We conducted inhibitory kinetic studies of compound 11h at various concentrations of D-serine to determine its mode of inhibition. As shown in Figure 3, a double reciprocal plot of DAAO activity versus the D-serine concentrations produced a pattern indicative of competitive inhibition with a K_i value of 60 nM. Furthermore, escalation of FAD concentration from 10 to 100 μM did not affect IC_{50} value for 11h, providing additional supporting evidence that compound 11h competes with D-serine for the substrate-binding site distinct from the FAD-binding site of DAAO.

The potent inhibitory activity of 11e prompted us to conduct more focused SAR studies using 11e as a molecular template (Table 2). A variety of substitutions were examined at the terminal phenyl group of 11e (compounds 11j–z and 15u–w). Overall, there was no significant change in inhibitory potency, with IC_{50} values ranging from 40 to 270 nM. Analogues containing a large substituent such as compounds 11x–z retained IC_{50} values in the nanomolar range. Para substitutions were particularly well tolerated, as demonstrated by phenoxy substituted derivative 11y and phenyl substituted 11z with IC_{50} values of 80 and 50 nM, respectively. These substituents are presumably oriented toward the entrance of the DAAO active site, possessing a high degree of steric tolerance. In contrast, a notable decrease in potency was seen in compound 19e containing a branched methyl group at the linker connecting the phenyl and the 6-hydroxy-1,2,4-triazine-3,5(2H)-dione moieties. Other modifications at the linker (compounds 19f, 22, and 23) were not as disruptive as the methyl group of 19e although these compounds still showed somewhat lower potency compared to 11e. Compounds 30 and 32 possessing slightly modified carboxylate bioisosteres retained potent DAAO inhibitory activity with IC_{50} values 50 and 80 nM, respectively, nearly identical to that of 11e.

As illustrated in Figure 4, proposed binding mode of 11e (white) to the active site of DAAO is superposed onto the crystal structure of DAAO in complex with 8a (cyan). In this model, the α-hydroxycarbonyl moiety forms key hydrogen bonds with Tyr228 and Arg283. It is conceivable that the 4-position nitrogen atom of 4-hydroxypyridazin-3(2H)-one moiety in 8a is involved in the interaction with the active site of DAAO as superpositioned on the crystal structure of DAAO in complex with 8a.

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phenylethyl group of 11e appears quite spacious and capable of accommodating larger substituents owing to the movement of Tyr224 away from the active site. This is in a good agreement with the high inhibitory potency retained by compounds with bulky substituents such as compounds 11x–z.

Table 3 summarizes in vitro metabolic stability of selected compounds in mouse liver microsomes. As mentioned earlier, many of the DAAO inhibitors containing carboxylate bioisosteres are known to be subject to a varying degree of glucuronidation in liver microsomes.22 For example, a kojic acid based DAAO inhibitor 7 is extensively glucuronidated in mouse liver microsomes in the presence of UDPGA, while compound 8b with the 4-hydroxypyridazin-3(2H)-one scaffold shows some resistance to glucuronidation. 6-Hydroxy-1,2,4-triazine-3,5(2H,4H)-dione derivatives 11e and 11h were found to be completely resistant to glucuronidation in mouse liver microsomes. 3-Thioxo derivative 30 was also metabolically stable against glucuronidation. In sharp contrast, uracil-based DAAO inhibitor 32 was fully glucuronidated within 60 min of incubation. This trend appears consistent with our previous findings from other DAAO inhibitors, showing that increased topological polar surface area (tPSA) of the carboxylate bioisostere moiety contributes to higher resistance to glucuronidation.22 Compounds 11e and 11h were also stable in mouse liver microsomes in the presence of NADPH, a cofactor for CYP-dependent oxidation.

Further pharmacological characterization was conducted with compound 11h. No evidence of mutagenicity was observed in the Ames test using Salmonella typhimurium strain (TA100) in the presence or absence of metabolic activation by S9 mixture. Compound 11h was also tested in a panel of relevant in vitro assays (Table 4) including hERG channels, various sites of the NMDA receptors, and another class of flavoenzymes, monoamine oxidases A and B (MAO-A and MAO-B). Compound 11h showed no significant activity in any of these assays at the highest tested concentrations.

In vivo pharmacokinetic studies of 11h were carried out in CD1 mice by intravenous and oral administration (30 mg/kg). The plasma pharmacokinetic parameters are summarized in Table 5, along with those for 8b for direct comparison. The plasma clearance of 11h in mice was 10.2 mL/min/kg with a terminal half-life of 0.9 h following intravenous administration. Oral absorption of 11h was rapid, and the plasma peak concentration was generally observed within the first hour of oral administration. As predicted from the metabolic stability data (Table 3), the oral bioavailability of 11h (F = 79%) is significantly higher than that of 8b (F = 31%) despite the increased polarity. The improvement in oral bioavailability of 11h over 8b clearly illustrates the significant impact of glucuronidation on the pharmacokinetics of DAAO inhibitors. Following oral administration, compound 11h showed negligible brain penetration with a brain to plasma ratio of 0.01 in mice. Thus, compound 11h is unlikely to increase the brain levels of endogenous D-serine by inhibiting DAAO expressed in the brain. Given the favorable plasma exposure, however, compound 11h should be capable of inhibiting peripheral DAAO and minimize the metabolism of orally taken D-serine.

To determine the effects of 11h on D-serine plasma levels, mice (n = 3 per time point) were dosed with 11h (30 mg/kg, po) along with D-serine (30 mg/kg, po) simultaneously. As shown in Figure S, compound 11h showed no ability to enhance plasma Cmax of D-serine. However, its plasma clearance was substantially reduced for a sustained period of time,

Table 1. Inhibition of Human DAAO by 6-Hydroxy-1,2,4-triazine-3,5(2H,4H)-dione Derivatives

| Compd | R   | IC50 (μM) | Compd | R   | IC50 (μM) |
|-------|-----|-----------|-------|-----|-----------|
| 11a   | CH3 | 2.8 ± 0.5 | 11g   |     | 0.22 ± 0.06 |
| 11b   |     | 0.26 ± 0.04 | 11h   |     | 0.05 ± 0.01 |
| 11c   |     | 40 ± 6    | 11i   |     | 0.10 ± 0.04 |
| 26    |     | >100      | 19a   |     | 0.76 ± 0.23 |
| 11d   |     | 5.1 ± 4.4 | 19b   |     | 0.33 ± 0.09 |
| 11e   |     | 0.07 ± 0.01 | 19c   |     | 0.44 ± 0.12 |
| 11f   |     | 1.7 ± 0.3 | 19d   |     | 0.31 ± 0.05 |

“Values are mean ± SD of at least four experiments.”

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resulting in the significant increase in D-serine AUC_{0−6h} (96.9 \mu g·h/mL) compared to that of D-serine alone treatment (43.3 \mu g·h/mL). The curve of D-serine concentration versus time in mice coadministered with 11h is nearly identical to that of DAAO knockout mice treated with oral D-serine. The lack of enhancement in D-serine plasma C_{max} in mice by coadministration with 11h is in a good agreement with the negligible levels of DAAO found in mouse liver as opposed to kidneys, where DAAO is abundant and most likely contributes to the plasma clearance of D-serine. Thus, the primary site of action for 11h in mice appears DAAO in kidneys, which explains its significant effects on plasma clearance of D-serine but not on plasma C_{max}.

Table 2. Inhibition of Human DAAO by Analogues of 11e

| compd | R2 | R3 | R4 | X1 | X2 | Y | Z | IC_{50} (\mu M) \textsuperscript{a} |
|-------|-----|-----|-----|-----|-----|---|---|-----------------------------|
| 11e   | H   | H   | H   | H   | H   | O | N | 0.07 ± 0.01 |
| 11j   | Cl  | H   | H   | H   | H   | O | N | 0.10 ± 0.05 |
| 11k   | H   | Cl  | H   | H   | H   | O | N | 0.06 ± 0.02 |
| 11l   | H   | H   | Cl  | H   | H   | O | N | 0.04 ± 0.01 |
| 11n   | F   | H   | H   | H   | H   | O | N | 0.08 ± 0.03 |
| 11a   | H   | F   | H   | H   | H   | O | N | 0.06 ± 0.02 |
| 11m   | H   | H   | F   | H   | H   | O | N | 0.05 ± 0.02 |
| 11p   | CH_{3} | H   | H   | H   | H   | O | N | 0.08 ± 0.03 |
| 11q   | H   | CH_{3} | H   | H   | H   | O | N | 0.07 ± 0.02 |
| 11r   | H   | H   | CH_{3} | H   | H   | O | N | 0.09 ± 0.02 |
| 11s   | H   | CF_{3} | H   | H   | H   | O | N | 0.10 ± 0.02 |
| 11t   | H   | H   | CF_{3} | H   | H   | O | N | 0.08 ± 0.04 |
| 11u   | OCH_{3} | H   | H   | H   | H   | O | N | 0.27 ± 0.07 |
| 11v   | H   | OCH_{3} | H   | H   | H   | O | N | 0.12 ± 0.03 |
| 11w   | H   | H   | OCH_{3} | H   | H   | O | N | 0.12 ± 0.03 |
| 15u   | OH  | H   | H   | H   | H   | O | N | 0.09 ± 0.04 |
| 15v   | H   | OH  | H   | H   | H   | O | N | 0.16 ± 0.05 |
| 15w   | H   | H   | OH  | H   | H   | O | N | 0.11 ± 0.03 |
| 11x   | H   | OPh | H   | H   | H   | O | N | 0.24 ± 0.05 |
| 11y   | H   | H   | OPh | H   | H   | O | N | 0.08 ± 0.02 |
| 11z   | H   | H   | Ph  | H   | H   | O | N | 0.05 ± 0.02 |
| 19e   | H   | H   | H   | CH_{3} | H   | O | N | 3.3 ± 0.6 |
| 19f   | H   | H   | H   | F   | F   | O | N | 0.40 |
| 22    | H   | H   | H   | O   | O   | O | N | 0.85 ± 0.13 |
| 23    | H   | H   | H   | OH  | H   | O | N | 0.47 ± 0.23 |
| 30    | H   | H   | H   | H   | H   | S | N | 0.05 ± 0.01 |
| 32    | H   | H   | H   | H   | H   | O | CH | 0.08 ± 0.01 |

\textsuperscript{a}Values are mean ± SD of at least four experiments except for compound 19f ($n = 2$).
value. Given the previously reported cross-species variation in \( IC_{50} \) values between human and rat forms of DAAO,\(^{13}\) caution needs to be taken in establishing the PK/PD relationship in mice as we have not measured inhibitory potency of 11h in mouse DAAO. It is worth noting, however, that 4-hydroxypyridazin-3(2H)-one derivatives represented by 8b showed little difference in inhibitory potency between human and mouse DAAO.\(^{18}\) Given their structural similarity to the 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione derivatives, we anticipate comparative inhibitory potencies of 11h against human and mouse forms of DAAO.

### CONCLUSIONS

In the past decade, substantial efforts have been made by multiple groups to develop DAAO inhibitors of potential therapeutic value. These efforts have not only provided structurally diverse DAAO inhibitors but also contributed to the cumulative knowledge in this area, including the use of carboxylate bioisosteres, exploitation of the secondary binding pocket, and the strategy to minimize glucuronidation. We took full advantage of the prior knowledge in guiding our efforts to develop a new class of DAAO inhibitors based on the 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione pharmacophore. The inhibitory potency of these compounds was dependent on the variation of the substituents at the 2-position. Many compounds containing an arylalkyl group at this position showed potent inhibitory activity against DAAO. Among these inhibitors, compound 11h exhibited good metabolic stability in liver microsomes and selectivity over other relevant targets. Compound 11h was also found to be orally available and enhance plasma levels of coadministered \( \Delta \)-serine by reducing its clearance. The ability of compound 11h to sustain high plasma levels of \( \Delta \)-serine should be particularly attractive for the treatment of chronic psychiatric disorders such as schizophrenia.

### EXPERIMENTAL SECTION

**General.** All solvents were reagent grade or HPLC grade. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All reactions were performed under nitrogen. Melting points were obtained on a Mel-temp apparatus and are uncorrected. \(^1H\) NMR spectra were recorded at 400 MHz. \(^{13}C\) NMR spectra were recorded at 100 MHz. The HPLC solvent system consisted of distilled water and acetonitrile, both containing 0.1% formic acid. Preparative HPLC purification was performed on an Agilent 1200 series HPLC system equipped with an Agilent G1315D DAD detector (detection at 220 nm) and an Agilent 6120 quadrupole MS detector. Unless otherwise specified, the analytical HPLC conditions involve a gradient of 20% acetonitrile/80% water for 0.5 min followed by an increase to 85% acetonitrile/15% water over 4 min and continuation of 85% acetonitrile/15% water for 3.5 min with a Luna C18 column (2.1 mm \( \times \) 50 mm, 3.5 μm) at a flow rate of 0.75 mL/min. All final compounds tested were confirmed to be of ≥95% purity by the HPLC methods described above. The model of compound 11h bound to human DAAO (Figure 4) was generated using AutoDock Vina.\(^{30}\)

6-Bromo-2-methyl-1,2,4-triazine-3,5(2H,4H)-dione (13a). To a solution of 5-bromo-6-azauracil (0.30 g, 1.6 mmol) in acetonitrile (5 mL) was added N\(O\)-(trimethylsilyl) acetamide (4.0 mmol, 1.0 mL). The mixture was heated at 82 °C for 3 h, after which methyl iodide (0.12 mL, 1.9 mmol) was added. After 24 h, more methyl iodide (0.5 equiv) was added and the mixture was stirred for additional 24 h. The reaction mixture was concentrated, and the resulting residue was dissolved in dichloromethane, washed with water and brine, dried over \( \text{Na}_2\text{SO}_4 \), and concentrated to give 0.11 g of 13a as a black solid (33% crude yield). This material was used in the next step without

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**Table 3. Metabolic Stability of DAAO Inhibitors in Mouse Liver Microsomes**

| compd | tPSA (Å\(^3\)) | % remaining after 60 min incubation |
|-------|---------------|----------------------------------|
| 7     | 47            | 5 ± 1                            |
| 8b    | 62            | 59 ± 9                           |
| 11e   | 82            | ≥99 ± ≥99                        |
| 11h   | 82            | ≥99 ± ≥99                        |
| 30    | 97            | ≥99 ± ≥99                        |
| 32    | 70            | ≤1 ± ND                          |

\(^{a}\)Topological surface area of the carboxylate bioisostere moiety. \(^{b}\)Not determined.

**Table 4. In Vitro Pharmacological Characterization of 11h**

| assay                      | results                                      |
|----------------------------|----------------------------------------------|
| hERG channel               | no inhibition at concentrations up to 25 μM  |
| NMDA receptor (agonist)    | ≤5% inhibition at 10 μM                      |
| NMDA receptor (glucose)    | 22% inhibition at 10 μM                      |
| NMDA receptor (phencyclidine) | ≤5% inhibition at 10 μM                   |
| NMDA receptor (MK801)      | ≤5% inhibition at 10 μM                      |
| glycine receptor (strychnine sensitive) | ≤5% inhibition at 10 μM           |
| MAO-A                      | ≤5% inhibition at 10 μM                      |
| MAO-B                      | ≤5% inhibition at 10 μM                      |

**Table 5. Mouse Pharmacokinetics of 8b and 11h (30 mg/kg)**

| route | parameter | 8b\(^{a}\) | 11h |
|-------|-----------|------------|-----|
| iv    | CL (mL/min/kg) | 38.7 | 10.2 |
|       | Vd (L/kg) | 0.5 | 0.8 |
|       | T\(_{1/2}\) (h) | 0.2 | 0.9 |
|       | AUC\(_{int}\) (μg·h/mL) | 12.7 | 16.3 |
| po    | T\(_{max}\) (h) | 0.25 | 0.08 |
|       | C\(_{max}\) (μg/mL) | 6.6 | 13.8 |
|       | AUC\(_{int}\) (μg·h/mL) | 3.9 | 12.9 |
|       | brain to plasma ratio | ND\(^{b}\) | 0.01 |
| po/iv | F (%) | 31 | 79 |

\(^{a}\)Intravenous administration of 8b was conducted at 10 mg/kg. \(^{b}\)The value is dose-normalized to 30 mg/kg. \(^{c}\)Not determined.

Figure 5. Effects of compound 11h (30 mg/kg, po) on plasma pharmacokinetics of oral \( \Delta \)-serine (30 mg/kg) in mice.
further purification. ¹H NMR (DMSO-d₆) δ 3.44 (s, 3H), 12.50 (s, 1H).

6-(Benzoxyl)-2-methyl-1,2,4-triazine-3(2H,4H)-dione (14a). A mixture of 13a (0.10 g, 0.49 mmol) and K₂CO₃ (0.14 g, 1.0 mmol) in benzyl alcohol (1.0 mL) was heated overnight at 150 °C. The reaction mixture was partitioned between aqueous 10% KHSO₃ and EtOAc. The organic layer was dried over Na₂SO₄ and concentrated. The residual material was purified using a Biotage Isolera One flash purification system with a silica gel flash cartridge (EtOAc/hexanes) to give 0.070 g of 14a as a white solid (61% yield). ¹H NMR (DMSO-d₆) δ 4.91 (s, 3H), 5.42 (s, 2H), 7.29–7.40 (m, 10H), 12.24 (s, 1H).

6-(Benzoxyl)-2-methyl-1,2,4-triazine-3(2H,4H)-dione (11d). Compound 11d was prepared as described for the preparation of 11a, except benzyl bromide (1.2 equiv) was used in place of benzyl chloride. The crude product was purified by trituration with cold diethyl ether; yellow solid (80% yield). ¹H NMR (DMSO-d₆) δ 7.29 (m, 10H), 7.36 (m, 5H), 12.24 (s, 1H). LCMS: retention time 2.19 min, m/z 220 [M + H]⁺.

6-(Benzoxyl)-2-methyl-1,2,4-triazine-3(2H,4H)-dione (11e). Compound 11e was prepared as described for the preparation of 11d, except the product was purified by trituration with EtOAc/hexanes; beige solid (74% yield). ¹H NMR (DMSO-d₆) δ 7.36 (m, 5H), 12.24 (s, 1H). LCMS: retention time 2.19 min, m/z 220 [M + H]⁺.

6-(Benzoxyl)-2-(methyl-1,2,4-triazine-3(2H,4H)-dione (13e). Compound 13e was prepared as described for the preparation of 13a, except methyl iodide (2.5 equiv) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether; tan solid (64% yield). ¹H NMR (DMSO-d₆) δ 7.36 (m, 5H), 12.24 (s, 1H). LCMS: retention time 2.19 min, m/z 220 [M + H]⁺.

6-(Benzoxyl)-2-(2,4-dimethyl-1,2,4-triazine-3(2H,4H)-dione (14g). Compound 14g was prepared as described for the preparation of 14f, except the product was purified by trituration with EtOAc/hexanes; beige solid (71% yield). ¹H NMR (DMSO-d₆) δ 7.29 (m, 10H), 7.36 (m, 5H), 12.15 (s, 1H).
(20% acetonitrile/80% water for 5 min followed by an increase to 70% acetonitrile/30% water over 35 min and an increase to 100% acetonitrile over 10 min; flow rate 25 mL/min) to give 0.057 g of 11g as a white fluffy solid (29% yield); mp 264 °C. 1H NMR (DMSO-d6) δ 5.03 (s, 2H), 7.44 (dd, J = 1.3, 6.3 Hz, 1H), 7.49 (m, 2H), 7.80 (s, 1H), 7.88 (m, 3H), 12.07 (bs, 2H). LCMS (20% acetonitrile/80% water for 0.25 min followed by an increase to 85% acetonitrile/15% water over 1.5 min and continuation of 85% acetonitrile/15% water for 2.25 min; flow rate 1.25 mL/min): retention time 1.45 min, m/z 270 [M + H]+.

6-Benzyl-2-(naphthalen-1-ylmethyl)-1,2,4-triazine-3,5-(2H,4H)-dione (14h). Compound 14h was prepared as described for the preparation of 13a, except 1-(bromomethyl)naphthalene (1.2 equiv) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether; orange solid (65% yield). 1H NMR (DMSO-d6) δ 5.49 (s, 2H), 7.49 (m, 2H), 7.59 (m, 2H), 7.91 (m, 1H), 7.98 (m, 1H), 8.14 (d, J = 8.1 Hz, 1H), 12.64 (br s, 1H).

6-Bromo-2-(naphthalen-1-ylmethyl)-1,2,4-triazine-3,5-(2H,4H)-dione (14i). Compound 14i was prepared from 13h as described for the preparation of 14a, except the product was purified by silica gel column chromatography (30% EtOAc in hexanes); white solid (40% yield). 1H NMR (DMSO-d6) δ 5.04 (s, 2H), 5.39 (s, 2H), 7.31 (s, 3H), 7.49 (d, J = 4.8 Hz, 2H), 7.57 (m, 2H), 7.91 (t, J = 4.8 Hz, 1H), 7.98 (m, 1H), 8.22 (d, J = 7.6 Hz, 1H), 12.29 (s, 1H).

6-Hydroxy-2-(naphthalen-1-ylmethyl)-1,2,4-triazine-3,5-(2H,4H)-dione (14j). Compound 14j was prepared from 14h as described for the preparation of 11a, with the exception that a mixture of methanol/ethyl acetate/acetic acid (1:1:0.1) was used as a solvent. The hydrogination was performed at 20 psi for 3 h; white powder (90% yield); mp 260 °C (dec). 1H NMR (DMSO-d6) δ 5.33 (s, 2H), 7.41 (d, J = 7.1 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 7.57 (m, 2H), 7.88 (d, J = 8.1 Hz, 1H), 7.96 (m, 1H), 8.16 (d, J = 7.5 Hz, 1H), 11.68 (s, 1H), 12.20 (s, 1H). LCMS (20% acetonitrile/80% water for 0.25 min followed by an increase to 85% acetonitrile/15% water over 1.5 min and continuation of 85% acetonitrile/15% water for 2.25 min; flow rate 1.25 mL/min): retention time 1.43 min, m/z 270 [M + H]+. The corresponding sodium salt was used for all in vivo studies involving compound 11b. The salt was prepared by dissolving 11b in 1.0 equiv of a 0.101 N volumetric solution of NaOH, followed by lyophilization.

6-Bromo-2-(2-(naphthalen-1-yl)ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13l). Compound 13l was prepared as described for the preparation of 13a, except 1-(2-iodoethyl)naphthalene (2.0 equiv) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether; yellow solid (46% yield). 1H NMR (DMSO-d6) δ 5.41 (q, J = 7.5 Hz, 2H), 6.13 (t, J = 7.6 Hz, 2H), 7.43 (m, 2H), 7.55 (m, 2H), 7.81 (t, J = 8.3 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H), 12.65 (s, 1H).

6-(Benzyl)-2-(2-(naphthalen-1-yl)ethyl)-1,2,4-triazine-3,5-(2H,4H)-dione (14l). Compound 14l was prepared from 13l as described for the preparation of 14a, except the product was purified by trituration with EtOAc/hexanes; white solid (72% yield). 1H NMR (DMSO-d6) δ 2.90 (t, J = 7.3 Hz, 2H), 3.98 (t, J = 6.9 Hz, 2H), 5.05 (s, 2H), 7.09 (m, 1H), 7.25−7.31 (m, 3H), 7.36−7.43 (m, 3H), 12.13 (s, 1H).

2-(3-Chlorophenyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11k). Compound 11k was prepared from 14k as described for the preparation of 11a, except the product was purified by trituration with EtOAc/hexanes; beige powder (70% yield). 1H NMR (DMSO-d6) δ 2.93 (t, J = 6.9 Hz, 2H), 3.98 (t, J = 6.9 Hz, 2H), 5.05 (s, 2H), 7.09 (m, 1H), 7.25−7.31 (m, 3H), 7.36−7.43 (m, 3H), 12.13 (s, 1H).

6-Bromo-2-(4-chlorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13m). Compound 13m was prepared as described for the preparation of 13a, except 1-chloro-4-(2-iodoethyl)benzene (1.6 equiv) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether; yellow solid (42% yield). 1H NMR (DMSO-d6) δ 2.94 (t, J = 7.3 Hz, 2H), 4.03 (t, J = 7.3 Hz, 2H), 7.25 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 12.52 (s, 1H).

6-(Benzyl)-2-(4-chlorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14f). Compound 14f was prepared from 13f as described for the preparation of 14a, except the reaction was heated over 3 days; white solid (72% yield). 1H NMR (DMSO-d6) δ 2.90 (t, J = 6.7 Hz, 2H), 3.96 (t, J = 6.9 Hz, 2H), 5.05 (s, 2H), 7.15 (d, J = 7.8 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 7.36−7.43 (m, 3H), 12.12 (s, 1H).

2-(4-Chlorophenethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11l). Compound 11l was prepared from 14l as described for the preparation of 11a, with the exception that only 2 equiv of BBr3 were used and that the crude product was purified by trituration with EtOAc/hexanes; white solid (54% yield); mp 219 °C. 1H NMR (DMSO-d6) δ 2.92 (t, J = 7.1 Hz, 2H), 3.09 (t, J = 7.2 Hz, 2H), 7.14 (m, 1H), 7.26−7.33 (m, 3H), 11.69 (s, 1H), 12.04 (s, 1H). LCMS: retention time 2.91 min, m/z 268 [M + H]+.

6-Bromo-2-(4-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13n). Compound 13n was prepared as described for the preparation of 13a, except 2-fluoro-4-(2-iodoethyl)benzene (2.0 equiv) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether; yellow solid (34% yield). 1H NMR (DMSO-d6) δ 2.98 (t, J = 7.2 Hz, 2H), 4.05 (t, J = 7.2 Hz, 2H), 7.15 (m, 2H), 7.29 (m, 2H), 8.01 (s, 1H).

6-(Benzyl)-2-(4-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14m). Compound 14m was prepared from 13m as described for the preparation of 14a, with the exception that the reaction was
heated for 3 days and that the product was purified by trituration with EtOAc/hexanes; white powder (65% yield).<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.96 (t, J = 6.8 Hz, 2H), 3.97 (t, J = 6.6 Hz, 2H), 4.95 (s, 2H), 7.11 (m, 2H), 7.17 (m, 1H), 7.24 (m, 1H), 7.36–7.41 (m, 4H), 12.17 (s, 1H).

2-(Fluorophenethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11m). Compound 11m was prepared from 14m as described for the preparation of 11a, with the exception that a mixture of methanol and ethyl acetate (1:1) was used as the solvent and that the hydrogenation was performed at 30 psi for 2 h; gray solid (71% yield); mp 207 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.31 (3H, 3H), 2.89 (t, J = 7.7 Hz, 2H), 3.81 (t, J = 7.7 Hz, 2H), 7.10 (m, 3H), 7.14 (m, 1H), 11.93 (bs, 2H). LCMS: retention time 2.42 min, m/z 248 [M + H]<sup>+</sup>.

Bromo-2-(2-(methylenephosphoryl))-1,2,4-triazine-3,5(2H,4H)-dione (13q). Compound 13q was prepared as described for the preparation of 13a, except 3-methyl-4-(2-iodoethyl)benzene (2.0 equiv) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether; tan solid (66% yield). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.39 (s, 3H), 2.89 (t, J = 7.7 Hz, 2H), 3.81 (t, J = 7.7 Hz, 2H), 7.02 (m, 3H), 7.18 (t, J = 7.3 Hz, 1H), 12.50 (s, 1H).

Benzylxoy)-2-(3-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14n). Compound 14n was prepared from 13q as described for the preparation of 14a, except the product was purified by trituration with EtOAc/hexanes; yellow powder (62% yield). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.25 (s, 3H), 2.87 (t, J = 6.8 Hz, 2H), 3.94 (t, J = 7.0 Hz, 2H), 5.07 (s, 2H), 6.98 (m, 2H), 7.16 (t, J = 7.6 Hz, 1H), 7.37–7.45 (m, 5H), 12.14 (s, 1H).

Hydroxy-2-(3-methylenephosphoryl)-1,2,4-triazine-3,5(2H,4H)-dione (14l). Compound 14l was prepared from 14q as described for the preparation of 11a, with the exception that a mixture of methanol and ethyl acetate (1:1) was used as a solvent and that the hydrogenation was performed overnight at 30 psi; white solid (79% yield); mp 194 °C. LCMS: retention time 2.53 min, m/z 248 [M + H]<sup>+</sup>.

Bromo-2-(4-(methylenephosphoryl))-1,2,4-triazine-3,5(2H,4H)-dione (14r). Compound 14r was prepared from 13r as described for the preparation of 14a; white foam (72% yield). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.24 (s, 3H), 2.89 (t, J = 7.5 Hz, 2H), 4.00 (m, 2H), 7.10 (s, 4H), 12.52 (s, 1H).

Benzylxoy)-2-(4-(methylenephosphoryl))-1,2,4-triazine-3,5(2H,4H)-dione (14q). Compound 14q was prepared as described for the preparation of 11a, with the exception that a mixture of methanol and ethyl acetate (1:1) was used as a solvent and that the hydrogenation was performed overnight at 30 psi (82% yield); mp 210 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.27 (s, 3H), 2.86 (t, J = 7.3 Hz, 2H), 3.85 (t, J = 7.2 Hz, 2H), 6.99 (m, 3H), 7.18 (t, J = 7.8 Hz, 2H), 11.71 (s, 1H), 12.01 (s, 1H). LCMS: retention time 2.53 min, m/z 248 [M + H]<sup>+</sup>.

Bromo-2-(4-(methylenephosphoryl))-1,2,4-triazine-3,5(2H,4H)-dione (13r). Compound 13r was prepared as described for the preparation of 14a, except 4-methyl-4-(2-iodoethyl)benzene (2.0 equiv) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether; tan solid (63% yield). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.26 (s, 3H), 2.89 (t, J = 7.5 Hz, 2H), 4.00 (m, 2H), 7.10 (s, 4H), 12.52 (s, 1H).

Benzylxoy)-2-(4-(methylenephosphoryl))-1,2,4-triazine-3,5(2H,4H)-dione (14o). Compound 14o was prepared from 12o as described for the preparation of 11a, except the hydrogenation was performed under an atmospheric pressure of hydrogen for 3 h. The product was purified by trituration with EtOAc/hexanes; light-pink powder (76% yield); mp 213 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.89 (t, J = 7.3 Hz, 2H), 3.87 (t, J = 7.3 Hz, 2H), 7.10 (m, 2H), 7.22 (m, 2H), 11.72 (bs, 1H), 11.97 (bs, 1H). LCMS: retention time 1.95 min, m/z 252 [M + H]<sup>+</sup>.

Bromo-2-(2-(methylphosphoryl))-1,2,4-triazine-3,5(2H,4H)-dione (14p). Compound 14p was prepared as described for the preparation of 14a, except the product was purified by trituration with EtOAc/hexanes; beige powder (66% yield). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.30 (s, 3H), 2.90 (t, J = 7.5 Hz, 2H), 3.90 (t, J = 7.8 Hz, 2H), 5.07 (s, 2H), 7.03 (m, 1H), 7.09–7.12 (m, 2H), 7.14 (m, 1H), 7.36–7.45 (m, 5H), 12.16 (s, 1H).

Hydroxy-2-(2-(methylphosphoryl))-1,2,4-triazine-3,5(2H,4H)-dione (11p). Compound 11p was prepared from 14p as described for the preparation of 11a, with the exception that a mixture of methanol and ethyl acetate (1:1) was used as a solvent and the hydrogenation was performed at 30 psi for 2 h; gray solid (71% yield); mp 207 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.31 (3H, 3H), 2.89 (t, J = 7.7 Hz, 2H), 3.81 (t, J = 7.7 Hz, 2H), 7.10 (m, 3H), 7.14 (m, 1H), 11.93 (bs, 2H). LCMS: retention time 2.42 min, m/z 248 [M + H]<sup>+</sup>.
system with a silica gel flash cartridge (EtOAc/hexanes); yellow solid (69% yield). 1H NMR (DMSO-d$_6$) $\delta$ 3.04 (t, $J$ = 7.2 Hz, 2H), 4.08 (t, $J$ = 7.1 Hz, 2H), 7.46 (d, $J$ = 7.1 Hz, 2H), 7.64 (d, $J$ = 8.1 Hz, 2H), 12.53 (s, 1H).

6-(Benzyloxy)-2-(4-trifluoromethylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14a). Compound 14a was prepared from 13a as described for the preparation of 14a, except the product was purified by trituration with EtOAc/hexanes; white solid (64% yield). 1H NMR (DMSO-d$_6$) $\delta$ 1.95 (s, 3H), 2.89 (t, $J$ = 7.1 Hz, 2H), 3.70 (s, 3H), 3.96 (t, $J$ = 7.2 Hz, 2H), 5.02 (s, 2H), 6.72–6.78 (m, 3H), 7.19 (t, $J$ = 7.8 Hz, 1H), 7.36–7.44 (m, 5H), 12.14 (s, 1H).

6-Benzyloxy-2-(4-trifluoromethylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11v). Compound 11v was prepared for 14v as described for the preparation of 11v, except the mixture of methanol and EtOAc (1:1) was used as a solvent and that the hydrogenation was performed overnight at 30 psi; off-white solid (63% yield); mp 224 °C.

6-Hydroxy-2-(3-phenoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13u). Compound 13u was prepared as described for the preparation of 13a, except the product was purified by trituration with EtOAc/hexanes; white solid (70% yield). 1H NMR (DMSO-d$_6$) $\delta$ 2.84 (t, $J$ = 7.1 Hz, 2H), 3.69 (s, 3H), 3.91 (t, $J$ = 7.1 Hz, 2H), 5.06 (s, 2H), 6.82 (t, $J$ = 8.8 Hz, 2H), 7.05 (t, $J$ = 8.6 Hz, 2H), 7.17 (m, 2H), 7.35–7.44 (m, 5H), 12.12 (s, 1H).

6-Hydroxy-2-(4-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14w). Compound 14w was prepared from 13w as described for the preparation of 14a, except the product was purified by trituration with EtOAc/hexanes; white solid (63% yield). 1H NMR (DMSO-d$_6$) $\delta$ 2.83 (t, $J$ = 7.1 Hz, 2H), 3.71 (s, 3H), 3.82 (t, $J$ = 7.5 Hz, 2H), 6.83 (d, $J$ = 8.6 Hz, 2H), 7.09 (d, $J$ = 8.6 Hz, 2H), 11.74 (bs, 1H), 11.98 (bs, 1H). LCMS: retention time 1.75 min, m/z 264 [M + H]$^+$.
NMR (DMSO-d$_{6}$): δ 2.93 (m, 2H), 4.04 (m, 2H), 6.93–6.98 (m, 4H), 7.10–7.14 (m, 1H), 7.21–7.25 (m, 2H), 7.36–7.40 (m, 2H), 12.54 (s, 1H).

6-(Benzyloxymethyl)-2-(4-pyridin-2-yl-ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14y). Compound 14y was prepared from 13y as described for the preparation of 14a; white solid (41% yield). 1H NMR (DMSO-d$_{6}$): δ 2.90 (t, J = 7.0 Hz, 2H), 3.96 (t, J = 7.0 Hz, 2H), 5.08 (s, 2H), 6.91–6.96 (m, 4H), 7.09–7.12 (m, 1H), 7.16–7.18 (m, 2H), 7.33–7.45 (m, 4H), 7.12 (s, 1H).

6-Hydroxy-2-(4-pyridin-2-yl-ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11y). Compound 11y was prepared from 10y as described for the preparation of 11g; white solid (69% yield); mp 207–209 °C. 1H NMR (DMSO-d$_{6}$): δ 2.90 (t, J = 7.2 Hz, 2H), 3.88 (t, J = 7.3 Hz, 2H), 6.90–6.98 (m, 4H), 7.10–7.14 (m, 1H), 7.21 (m, 2H), 7.35–7.40 (m, 2H), 11.69 (bs, 1H), 12.04 (s, 1H). LCMS (20% acetonitrile/80% water) for 0.25 min followed by an increase to 85% acetonitrile/15% water over 1.5 min and continuation of 85% acetonitrile/15% water for 2.25 min; flow rate 1.25 mL/min: retention time 1.80 min, m/z 326 [M + H]$^{+}$.

2-(2-(Biphenyl-4-yl)-ethyl)-6-bromo-1,2,4-triazine-3,5(2H,4H)-dione (13z). Compound 13z was prepared as described for the preparation of 11a, except 4-(2-isodicyclohexyl)benzyl (2.0 equiv) was used in place of benzyl isodicyclohexyl iodide. The product was purified by trituration with trichloroacetic acid; tan solid (55% yield). 1H NMR (DMSO-d$_{6}$): δ 2.90 (t, J = 7.5 Hz, 2H), 4.08 (t, J = 7.6 Hz, 2H), 7.31–7.37 (m, 3H), 7.45 (t, J = 7.6 Hz, 2H), 7.59–7.65 (m, 4H), 12.55 (s, 1H).

6-Benzoyl-2-(2-(biphenyl-4-yl)-ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14z). Compound 14z was prepared from 13z as described for the preparation of 14a; white solid (53% yield). 1H NMR (DMSO-d$_{6}$): δ 2.96 (t, J = 7.2 Hz, 2H), 4.00 (t, J = 7.1 Hz, 2H), 5.05 (s, 2H), 7.25 (d, J = 8.1 Hz, 2H), 7.34–7.46 (m, 8H), 5.87–5.84 (m, 4H), 12.16 (s, 1H).

2-(2-(Biphenyl-4-yl)-ethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11z). Compound 11z was prepared from 14z as described for the preparation of 11a, with the exception that a mixture of methanol and EtOAc (2:1) was used as a solvent and that the hydrogenation was performed overnight at 30 psi; white powder (89% yield); mp 254 and EtOAc (2:1) was used as a solvent and that the hydrogenation was performed overnight at 30 psi; white powder (89% yield); mp 254 °C.

To a solution of 17a (0.18 g, 0.40 mmol) in dichloromethane (8 mL) was added a 1.0 M solution of BBr$_{3}$ in dichloromethane (0.80 mL, 0.80 mmol) at rt. The reaction was stirred for 45 min, concentrated, and purified by preparative HPLC (0% acetonitrile/100% water for 5 min followed by an increase to 20% acetonitrile/80% water over 35 min and an increase to 40% acetonitrile/60% water for 10 min; flow rate 10 mL/min) to give 0.117 g of 19a as a white solid (18% yield); mp 225 °C. 1H NMR (DMSO-d$_{6}$): δ 3.17 (t, J = 5.8 Hz, 2H), 4.04 (t, J = 6.8 Hz, 2H), 7.59 (m, 2H), 8.07 (m, 1H), 8.63 (m, 1H), 11.64 (bs, 1H), 12.04 (s, 1H). LCMS (5% acetonitrile/95% water for 0.5 min followed by an increase to 40% acetonitrile/60% water over 4 min and continuation of 40% acetonitrile/60% water for 3 min; flow rate 0.25 mL/min; column Luna Plus C18, 2.1 mm × 100 mm, 1.8 μm); retention time 1.06 min, m/z 224 [M + H]$^{+}$.
prepared as described for the preparation of 17a, with the exception that 2-(1H-pyrrolo[2,3-b]pyridin-1-yl)ethanol was used in place of 2-(pyridin-2-yl)ethanol and that the reaction was stirred overnight: white solid (quantitative yield). 1H NMR (CDCl3) δ 1.27 (d, J = 6.3 Hz, 2H), 4.36 (t, J = 5.3 Hz, 2H), 4.67 (t, J = 5.6 Hz, 2H), 5.44 (s, 2H), 6.49 (d, J = 3.5 Hz, 1H), 7.00 (dd, J = 4.8, 7.8 Hz, 1H), 7.17 (d, J = 3.5 Hz, 2H), retention time: 7.34–7.36 (m, 4H), 7.86 (dd, J = 1.5, 7.8 Hz, 1H), 8.11 (dd, J = 1.5, 4.8 Hz, 1H).

2-(1H-Pyrrolo[2,3-b]pyridin-1-yl)-6-(benzoxyl)-1,2,4-triazine-3,5(2H,4H)-dione (18d). Compound 18d was prepared from 17d as described for the preparation of 18a, with the exception that the reaction was stirred for 4 h at rt and that the compound was precipitated from EtOAc; white solid (31% yield). 1H NMR (CDCl3) δ 4.28 (t, J = 5.3 Hz, 2H), 4.52 (s, 2H), 4.63 (t, J = 5.8 Hz, 2H), 4.66 (s, 2H), 5.43 (s, 2H), 6.42 (d, J = 3.5 Hz, 1H), 7.02 (m, 2H), 7.22 (dd, J = 1.8, 7.1 Hz, 2H), 7.29–7.36 (m, 8H), 7.84 (dd, J = 1.5, 7.8 Hz, 1H), 8.22 (dd, J = 1.5, 4.8 Hz, 1H).

2-(1H-Pyrrolo[2,3-b]pyridin-1-yl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (19d). Compound 19d was prepared from 18d as described for the preparation of 19a, with the exception that 4.0 equiv of BBr3 were used and that the reaction was stirred for 2 h at rt.

The crude material was purified by preparative HPLC (5% acetonitrile/95% water for 5 min by an increase to 35% acetonitrile/65% water over 35 min and an increase to 50% acetonitrile/50% water for 10 min; flow rate 15 mL/min;) to give a white solid (31% yield). 1H NMR (CDCl3) δ 1.31 (d, J = 6.8 Hz, 3H), 3.34 (m, 1H), 4.03 (dd, J = 7.8, 13.4 Hz, 1H), 4.13 (dd, J = 8.3, 13.4 Hz, 1H), 4.58 (s, 2H), 5.44 (s, 2H), 7.21 (m, 3H), 7.28–7.33 (m, 7H).

6-Hydroxy-2-(2-phenoxypyphenyl)-1,2,4-triazine-3,5(2H,4H)-dione (20). Compound 20 was described as described for the preparation of 18a, with the exception that 2.2 equiv of benzyl alcohol and 2.2 equiv of sodium hydride were used and that the reaction was stirred for 4 h at rt and then an increase to 70% acetonitrile/30% water over 10 min; flow rate 15 mL/min;)

3.5 Hz, 1H), 7.02 (m, 2H), 7.22 (dd, J = 1.8, 7.1 Hz, 2H), 7.29–7.36 (m, 8H), 7.84 (dd, J = 1.5, 7.8 Hz, 1H), 8.22 (dd, J = 1.5, 4.8 Hz, 1H).

6-(Benzyloxymethyl)-6-bromo-2-(2-oxo-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (21). Compound 21 was prepared from 20 as described for the preparation of 18a, with the exception that 2.2 equiv of benzyl alcohol and 2.2 equiv of sodium hydride were used and that the reaction was stirred for 10 min. The crude material was purified by trituration with EtOAc/hexanes; white solid (63% yield). 1H NMR (CDCl3) δ 4.72 (s, 2H), 5.40 (d, 2H), 5.54 (s, 2H), 7.02–7.07 (m, 3H), 7.14–7.20 (m, 3H), 7.25 (m, 2H), 7.29–7.33 (m, 5H), 7.38–7.41 (m, 5H).

6-Hydroxy-2-(2-oxo-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (22). Compound 22 was prepared from 21 as described for the preparation of 19a, with the exception that 4.0 equiv of BBr3 were used and that the reaction was stirred for 10 min.

2-Hydroxy-2-(2-oxo-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (23). To a solution of 21 (0.17 g, 0.37 mmol) in 4-(benzoxyl)methyl)-2-(2-oxo-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (24). To a solution of 16 (0.10 g, 0.16 mmol) in dichloromethane (25 mL) were added pyridine (0.26 mL, 3.20 mmol) and then an increase to 50% acetonitrile/50% water over 35 min; flow rate 1.25 mL/min;)

6-Hydroxy-2-(2-diﬂuoro-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (24). To a solution of 16 (0.50 g, 1.6 mmol) in dichloromethane (25 mL) were added pyridine (0.56 mL, 3.20 mmol),

6-Hydroxy-2-(2-diﬂuoro-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (24). To a solution of 16 (0.50 g, 1.6 mmol) in dichloromethane (25 mL) were added pyridine (0.56 mL, 3.20 mmol),

6-Hydroxy-2-(2-diﬂuoro-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (24). To a solution of 16 (0.50 g, 1.6 mmol) in dichloromethane (25 mL) were added pyridine (0.56 mL, 3.20 mmol),

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phenylboronic acid (0.39 g, 3.20 mmol), and copper(II) acetate (0.44 g, 2.4 mmol). The mixture was stirred at rt overnight and filtered through a pad of Celite. The filtrate was concentrated, and the resulting residue was partitioned between EtOAc and water. The organic layer was washed with a 10% KHSO₄ solution, dried over Na₂SO₄, and concentrated. The residue was purified by flash column chromatography using EtOAc/hexanes as eluent to give 0.62 g of 24 as a white solid (quantitative yield).

1H NMR (CDCl₃) δ 4.77 (s, 2H), 5.61 (s, 2H), 7.30–7.37 (m, 5H), 7.42 (m, 1H), 7.46–7.49 (m, 4H).

- Phenylhydrazinecarbothioamide (28). To a suspension of 27 (5.00 g, 21.3 mmol) in ethanol (50 mL) was added ammonium thiocyanate (1.60 g, 21.0 mmol). The mixture was stirred for 50 h. The reaction was cooled to rt, and the resulting solid was removed by filtration. The filtrate was concentrated by approximately one-half, and the resultant solid was removed by filtration. This was repeated four times. The remaining filtrate was partitioned between EtOAc (50 mL) and satd NaHCO₃ (20 mL). The organic layer was dried over MgSO₄, and concentrated. The crude material was purified by silica gel column chromatography (80% EtOAc/hexanes w/0.1% NH₄OH) to give 0.66 g of 28 as a white solid (16% yield).

1H NMR (CDCl₃) δ 7.32 (t, J = 7.3 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.49 (m, 2H), 11.85 (bs, 1H), 12.23 (s, 1H). LCMS (20% acetonitrile/80% water for 0.25 min followed by an increase to 85% acetonitrile/15% water over 1.5 min and continuation of 85% acetonitrile/15% water for 2.25 min; flow rate 1.25 mL/min): retention time 1.08 min, m/z 206 [M + H]⁺.

- 1-Phenethylhydrazinecarbothioamide (29). To a solution of 28 (0.30 g, 1.1 mmol) in THF (15 mL) was added a solution of DBU (0.32 g, 2.1 mmol) in THF (3 mL) at rt. The mixture was stirred at rt for 14 h and then heated to 50 °C for 30 min. The reaction was concentrated to dryness. The residue was dissolved in EtOAc (50 mL) and washed with satd NaHCO₃ (25 mL). The organic layer was dried over MgSO₄, and concentrated. The crude material was purified by silica gel column chromatography (5% MeOH/CH₂Cl₂) to give 0.30 g of 29 as a white solid (31% yield).

1H NMR (CDCl₃) δ 3.09 (t, J = 7.03 Hz, 2H), 3.88 (s, 3H), 4.32 (m, 2H), 6.03 (s, 2H), 7.25–7.37 (m, 5H), 8.22 (brs, 1H).

- 1-Phenethylhydrazinecarbothioamide (29). To a solution of 28 (0.30 g, 1.1 mmol) in THF (15 mL) was added a solution of DBU (0.32 g, 2.1 mmol) in THF (3 mL) at rt. The mixture was stirred at rt for 14 h and then heated to 50 °C for 30 min. The reaction was concentrated to dryness. The residue was partitioned between EtOAc and 5% KHSO₄.

1H NMR (CDCl₃) δ 3.11 (m, 2H), 4.41 (m, 2H), 7.19–7.31 (m, 1H). LCMS: retention time 2.90 min, m/z 250 [M + H]⁺.

- 1-Phenethylhydrazinecarbothioamide (29). To a solution of 28 (0.30 g, 1.1 mmol) in THF (15 mL) was added a solution of DBU (0.32 g, 2.1 mmol) in THF (3 mL) at rt. The mixture was stirred at rt for 14 h and then heated to 50 °C for 30 min. The reaction was concentrated to dryness. The residue was partitioned between EtOAc and 5% KHSO₄.

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1H NMR (CDCl₃) δ 3.11 (m, 2H), 4.41 (m, 2H), 7.19–7.31 (m, 1H). LCMS: retention time 2.90 min, m/z 250 [M + H]⁺.
blood was collected from each animal by cardiac puncture into heparinized microcentrifuge tubes, capped, gently inverted a few times, and stored on wet ice until centrifugation (10 min at 800g, 4 °C). Moreover, the top layer of each tube (~400 μL plasma) was aspirated via transfer pipet, dispensed into a clean nonheparinized micro-centrifuge tube, and stored at ~80 °C until subsequent analyses. Plasma levels of 8b and 11b were analyzed using an Agilent 1290 HPLC system coupled to an Agilent 6520 QTOF-MS system. Plasma D-serine level analyses were carried out using the previously reported method involving derivatization with Marfey's reagent,30 followed by the quantification of the derivatized D-serine using an Agilent 1290 HPLC system coupled to an Agilent 6520 QTOF-MS system.

■ ASSOCIATED CONTENT

2 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00482.

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DAAO, D-amino acid oxidase; AUC, area under the curve; BOM, benzyloxymethyl; tPSA, topological polar surface area

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