Journal of Medicinal Chemistry

Article

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

Niyada Hin, Bridget Duvall, Dana Ferraris, Jesse Alt, Ajit G Thomas, Rana Rais, Camilo Rojas, Ying Wu, Krystyna M Wozniak, Barbara S. Slusher, and Takashi Tsukamoto

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b00482 • Publication Date (Web): 26 Aug 2015

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

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

Niyada Hin,[†] Bridget Duvall,[†] Dana Ferraris,[†] Jesse Alt,[†] Ajit G. Thomas,[†] Rana Rais,[†,‡] Camilo Rojas,[†,§] Ying Wu,[†] Krystyna M. Wozniak,[†] Barbara S. Slusher,[†,‡] Takashi Tsukamoto[†,‡]*

[†]Brain Science Institute, [‡]Department of Neurology, and [§]Department of Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD 21205, USA

CORRESPONDING AUTHOR *Phone: (410) 614-0982. Fax: (410) 614-0659. E-mail: ttsukamoto@jhmi.edu.

ABSTRACT: A series of 2-substituted 6-hydroxy-1,2,4-triazine-3,5(2*H*,4*H*)-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 IC_{50} values in the double-digit nanomolar range. The 6-hydroxy-1,2,4-triazine-3,5(2*H*,4*H*)-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 co-administration 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 efficacy,[2] 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. Since 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 since 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 **1**[10] and **2**[11] presumably originated from a prototype DAAO inhibitor, benzoic acid, have evolved into a new class of compounds containing carboxylate bioisosteres such as **3**[12] and **4**.[13] 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.[13] 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**[14] and **6**.[15] Structural insights gained from DAAO in complex with imino-DOPA[16] led to the discovery of the latest DAAO

inhibitors possessing an additional substituent that extends to the secondary binding site adjacent to the active site of DAAO. For example, our group exploited this secondary binding site using kojic acid derivatives such as compound **7** substituted at its 2-methyl group.[17] Similarly, Hondo et al. reported potent DAAO inhibitors **8a-b** based on a 4-hydroxypyridazin-3(2H)-one scaffold with a phenethyl group extending to the secondary binding site.[18] The secondary binding site was also exploited by carboxylate-based DAAO inhibitors such as **9**[19] and **10**.[20]

Recently, the pharmacokinetics profile of several representative DAAO inhibitors has been systematically compared in mice and rats.[21] 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.[22]

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 IC_{50} 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-dihydro-1,2,4-triazine-5,6-dione portion of ceftriaxone has caught our attention as its tautomer, 6-hydroxy-3-mercapto-2-methyl-1,2,4-triazin-5(2H)-one, has a structural feature common to other DAAO inhibitors. Interestingly, 6-hydroxy-2-methyl-1,2,4-triazine-3,5(2H,4H)-dione **11a**, one of the degradation products of ceftriaxone in aqueous solution,[23] inhibited DAAO with an IC_{50} value of 2.8 μM, prompting us to further examine the potential of 2-substituted 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-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(2H,4H)-dione scaffold. With

appropriate substitutions at the 2-position, some 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-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 co-administration 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 **12**[24] as a starting material (Scheme 1). Alkylation of the 5-position of **12** with alkyl halides mediated by bis(trimethylsilyl)acetamide (BSA)[25] gave 5-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 **11u-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 **16**[26] as a starting material (Scheme 2). Mitsunobu reaction of **16** and alcohols with the aid of DIAD and PPh_3 provided the 5-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 **23**. Synthesis of 2-phenyl derivative **26** involves Chan-Lam coupling of **16** to phenylboronic acid to give **24**. Subsequent benzyloxylation and deprotection by BBr_3 provided **26**.

As shown in Scheme 3, we synthesized two structurally close analogs of **11e**, where its carboxylate bioisostere moiety is slightly modified. 3-Thioxo derivative **30** was prepared using (2-

phenylethyl)hydrazine **27** as a starting material. Reaction of **27** with ammonium thiocyanate provided hydrazinecarbothioamide **28**,[27] which was subsequently treated with methyl chlorooxoacetate to give **29**.[28] Cyclization of **29** in the presence of DBU afforded the desired product **30**.[28] Uracil derivative **32** was prepared by BSA-mediated alkylation of 5-hydroxypyrimidine-2,4(1H,3H)-dione **31** with phenethyl iodide.

RESULTS AND DISCUSSION

The inhibitory potency of the synthesized compounds were determined using recombinant human DAAO as previously reported.[12] 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,4H)-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 nM 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. Analogs 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 towards 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,4H)-dione moieties. Other modifications at the linker (compounds **19f**, **22**, and **23**) were not as disruptive as the methyl group of **19e** though 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 nM 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).[18] In this model, the α-hydroxycarbonyl moiety forms key hydrogen bonds with Tyr228 and Arg283. It is conceivable that the 4-position nitrogen group of **11e** forms a hydrogen bond with the backbone carbonyl of Gly313 in a similar manner as the 2-nitrogen atom of 4-hydroxypyridazin-3(2H)-one moiety in **8a**. This explains the relatively high inhibitory potency of DAAO inhibitors with a 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione scaffold compared to the kojic acid based compounds[17] represented by **7** which lacks a nitrogen atom in the core ring. In contrast, the nitrogen atom at the 1-position of **11e** does not seem to be involved in the interaction with the active site of DAAO. This is consistent with the similar inhibitory potency of the uracil derivative **32**, in which the corresponding position is replaced by a carbon atom. 3-Thioxo derivative **30** was also as potent as **11e**, presumably due to the preservation of all of the key interactions involved in binding of **11e** to DAAO. The secondary binding pocket occupied by the 2-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 5, compound **11h** showed no ability to enhance plasma C_{max} of D-serine. However, its plasma clearance was substantially reduced for a sustained period of time, resulting in the significant increase in D-serine $AUC_{0\text{-}6h}$ (96.9 μg*h/mL) compared to that of D-serine alone treatment (43.3 μg*h/mL). The curve of D-serine concentration versus time in mice co-administered with **11h** is nearly identical to that of DAAO knockout mice treated with oral D-serine.[5] The lack of enhancement in D-serine plasma Cmax in mice by co-administration with **11h** is in a good agreement with the negligible levels of DAAO found in mouse liver as opposed to kidneys,[29] 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} 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 co-administered D-serine by reducing its clearance. The ability of compound **11h** to sustain constant levels of D-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. ^{1}H 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 using a Phenomenex Luna 5 μm C18 (2) column (21.2 mm × 250 mm, 5 μm). Analytical HPLC 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 × 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 **12** (0.30 g, 1.6 mmol) in acetonitrile (5 mL) was added *N,O*-bis(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 Na_2SO_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 further purification: ^{1}H NMR (DMSO-d_6) δ 3.44 (s, 3H), 12.50 (s, 1H).

6-(Benzyloxy)-2-methyl-1,2,4-triazine-3,5(2H,4H)-dione (14a). A mixture of **13a** (0.10 g, 0.49 mmol) and K_2CO_3 (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% $KHSO_4$ and EtOAc. The organic layer was dried over Na_2SO_4 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): ^{1}H NMR (DMSO-d_6) δ 3.36 (s, 3H), 5.12 (s, 2H), 7.37-7.46 (m, 5H), 12.16 (s, 1H).

6-Hydroxy-2-methyl-1,2,4-triazine-3,5(2H,4H)-dione (11a). To a solution of **14a** (0.060 g, 0.26 mmol) in methanol (5 mL) was added one spatula tip of 10% Pd/C. The mixture was shaken under hydrogen (30 psi) for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated to give 0.035 g of **11a** as a beige powder (94% yield): mp 259 °C; ^{1}H NMR (DMSO-d_6) δ 3.27 (s, 3H), 11.11 (s, 1H), 12.03 (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 3.5 min): retention time 5.05 min, *m/z* 144 $[M + H]^+$.

6-Bromo-2-isopentyl-1,2,4-triazine-3,5(2H,4H)-dione (13b). Compound **13b** was prepared as described for the preparation of **13a** except 1-iodo-3-methylbutane (1.6 equiv.) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether: yellow solid (27%

yield); ^{1}H NMR (DMSO-d_6) δ 0.89 ((d, J = 6.3 Hz, 6H), 1.50 (m, 2H), 1.60 (m, 1H), 3.82 (t, J = 7.3 Hz, 2H), 12.48 (s, 1H).

6-(Benzyloxy)-2-isopentyl-1,2,4-triazine-3,5(2H,4H)-dione (14b). Compound **14b** was prepared from **13b** as described for the preparation of **14a**: clear oil (36% yield); ^{1}H NMR (DMSO-d_6) δ 0.87 (d, J = 6.3 Hz, 6H), 1.49 (m, 2H), 1.54 (m, 1H), 3.73 (t, J = 7.1 Hz, 2H), 5.14 (s, 2H), 7.36-7.45 (m, 5H), 12.14 (s, 1H).

6-Hydroxy-2-isopentyl-1,2,4-triazine-3,5(2H,4H)-dione (11b). Compound **11b** was prepared from **14b** as described for the preparation of **11a**: grey solid (quantitative yield); mp 171 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (d, J = 6.3 Hz, 6H), 1.47 (m, 2H), 1.54 (m, 1H), 3.66 (t, J = 7.3 Hz, 2H), 11.65 (s, 1H), 11.94 (s, 1H). LCMS: retention time 1.04 min, *m/z* 200 $[M + H]^+$.

6-Bromo-2-(3,3-dimethylbutyl)-1,2,4-triazine-3,5(2H,4H)-dione (13c). Compound **13c** was prepared as described for the preparation of **13a** except 1-iodo-3,3-dimethylbutane (3.3 equiv.) was used in place of methyl iodide: clear oil (19% yield); ^{1}H NMR (DMSO-d_6) δ 0.92 (s, 9H), 1.53 (m, 2H), 3.82 (m, 2H), 12.50 (s, 1H).

6-(Benzyloxy)-2-(3,3-dimethylbutyl)-1,2,4-triazine-3,5(2H,4H)-dione (14c). Compound **14c** was prepared from **13c** as described for the preparation of **14a**: clear oil (80% yield); ^{1}H NMR (DMSO-d_6) δ 0.90 (s, 9H), 1.48 (m, 2H), 3.72 (m, 2H), 5.15 (s, 2H), 7.35-7.45 (m, 5H), 12.15 (s, 1H).

2-(3,3-Dimethylbutyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11c). Compound **11c** was prepared from **14c** as described for the preparation of **11a** except the material was purified by trituration with EtOAc/hexanes: white solid (77% yield); mp 219 °C; ^{1}H NMR (DMSO-d_6) δ 0.91 (m, 9H), 1.50 (m, 2H), 3.67 (m, 2H), 11.64 (s, 1H), 12.02 (s, 1H). LCMS: retention time 2.45 min, *m/z* 214 $[M + H]^+$.

2-Benzyl-6-bromo-1,2,4-triazine-3,5(2H,4H)-dione (13d). Compound **13d** was prepared as described for the preparation of **13a** except benzyl bromide (1.2 equiv.) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether: light tan solid (71% yield); ^{1}H NMR (DMSO-d_6) δ 5.02 (s, 2H), 7.29-7.38 (m, 5H), 12.59 (s, 1H).

2-Benzyl-6-(benzyloxy)-1,2,4-triazine-3,5(2H,4H)-dione (14d). Compound **14d** was prepared from **13d** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: white solid (70% yield); ^{1}H NMR (DMSO-d_6): δ 4.91 (s, 2H), 5.11 (s, 2H), 7.29-7.40 (m, 10H), 12.24 (s, 1H).

2-Benzyl-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11d). Compound **11d** was prepared from **14d** as described for the preparation of **11a** except the hydrogenation was performed overnight under atmospheric pressure of hydrogen: light pink powder (81% yield); mp 242 °C; ^{1}H NMR (DMSO-d_6) δ 4.85 (s, 2H), 7.26-7.36 (m, 5H), 11.72 (bs, 1H), 11.94 (bs, 2H). 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 3.5 min): retention time 2.84 min, *m/z* 220 $[M + H]^+$.

6-Bromo-2-phenethyl-1,2,4-triazine-3,5(2H,4H)-dione (13e). Compound **13e** was prepared as described for the preparation of **13a** except phenethyl 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); ^{1}H NMR (DMSO-d_6): δ 2.94 (t, J = 7.5 Hz, 2H), 4.04 (t, J = 7.6 Hz, 2H), 7.22 (m, 3H), 7.28 (m, 2H), 12.53 (s, 1H).

6-(Benzyloxy)-2-phenethyl-1,2,4-triazine-3,5(2H,4H)-dione (14e). Compound **14e** was prepared from **13e** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: beige solid (74% yield); ^{1}H NMR (DMSO-d_6): δ 2.91 (t, J = 7.2 Hz, 2H), 5.05 (s, 2H), 3.96 (t, J = 7.2 Hz, 2H), 7.15 (m, 2H), 7.20 (m, 1H), 7.26-7.31 (m, 2H), 7.36-7.43 (m, 5H), 12.13 (s, 1H).

6-Hydroxy-2-phenethyl-1,2,4-triazine-3,5(2H,4H)-dione (11e). Compound **11e** was prepared from **14e** as described for the preparation of **11a** except a mixture of methanol and acetic acid (3:1) was used as a solvent and that the hydrogenation was performed at 50 psi for 1.5 h: light pink powder (60% yield); mp 200 °C; ^{1}H NMR (DMSO-d_6) δ 2.90 (t, J = 7.8 Hz, 2H), 3.87 (t, J = 7.6 Hz, 2H), 7.20 (m, 3H), 7.29 (m, 2H), 11.71 (s, 1H), 12.03 (s, 1H). LCMS: retention time 1.50 min, *m/z* 234 $[M + H]^+$.

6-Bromo-2-(3-phenylpropyl)-1,2,4-triazine-3,5(2H,4H)-dione (13f). Compound **13f** was prepared as described for the preparation of **13a** except (3-iodopropyl)benzene (1.6 equiv.) was used in place of methyl iodide: white solid (43% yield); ^{1}H NMR (DMSO-d_6): δ 1.94 (m, 2H), 2.63 (t, J = 7.7 Hz, 2H), 3.83 (t, J = 6.9 Hz, 2H), 7.15-7.22 (m, 3H), 7.25-7.29 (m, 2H), 12.45 (s, 1H).

6-(Benzyloxy)-2-(3-phenylpropyl)-1,2,4-triazine-3,5(2H,4H)-dione (14f). Compound **14f** was prepared from **13f** as described for the preparation of **14a**: clear oil (85% yield oil); ^{1}H NMR (DMSO-d_6): δ 1.93 (m, 2H), 2.60 (t, J = 7.7 Hz, 2H), 3.76 (t, J = 6.8 Hz, 2H), 5.13 (s, 2H), 7.18 (m, 3H), 7.24 (m, 2H), 7.35-7.46 (m, 5H), 12.09 (s, 1H).

6-Hydroxy-2-(3-phenylpropyl)-1,2,4-triazine-3,5(2H,4H)-dione (11f). Compound **11f** was prepared from **14f** as described for the preparation of **11a** except a mixture of methanol and acetic acid (3:1) was used as a solvent: light pink solid (85% yield); mp 153 °C; ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 2H), 2.60 (t, J = 7.6 Hz, 2H), 3.67 (t, J = 6.9 Hz, 2H), 7.20 (m, 3H), 7.25 (m, 2H), 11.65 (bs, 1H), 11.93 (bs, 1H). LCMS: retention time 1.87 min, *m/z* 248 $[M + H]^+$.

6-Bromo-2-(naphthalen-2-ylmethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13g). Compound **13g** was prepared as described for the preparation of **13a** except 2-(bromomethyl)naphthalene (1.2 equiv.) was used in place of methyl iodide. The reaction was heated overnight. The crude product was purified by trituration with cold diethyl ether: tan solid (56% yield); ^{1}H NMR (DMSO-d_6) δ 5.20 (s, 2H), 7.46 (dd, J = 1.5, 8.3 Hz, 1H), 7.51 (m, 2H), 7.22 (m, 1H), 7.86-7.92 (m, 4H), 12.62 (s, 1H).

6-(Benzyloxy)-2-(naphthalen-2-ylmethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14g). Compound **14g** was prepared from **13g** as described for the preparation of **14a**: white solid foam (76% yield); ^{1}H NMR (DMSO-d_6) δ 5.09 (s, 2H), 5.12 (s, 2H), 7.26-7.31 (m, 3H), 7.36 (m, 2H), 7.45 (dd, J = 1.8, 7.6 Hz, 1H), 7.50-7.53 (m, 2H), 7.85-7.92 (m, 4H), 12.27 (s, 1H).

6-Hydroxy-2-(naphthalen-2-ylmethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11g). To a solution of compound **14g** (0.26 g, 0.72 mmol) in dichloromethane (7.0 mL) was slowly added a 1.0 M solution of BBr_3 (1.4 mL, 1.4 mmol) at room temperature. The reaction was stirred for 1 h after which 2 additional

equiv. of BBr_3 were added. Stirring continued at rt for an additional hour and the reaction was quenched by the addition of water. The compound was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residual material was purified by preparative HPLC (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; ^{1}H NMR (DMSO-d_6) δ 5.03 (s, 2H), 7.44 (dd, J = 1.3, 8.3 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-Bromo-2-(naphthalen-1-ylmethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13h). Compound **13h** 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); ^{1}H NMR (DMSO-d_6) 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-(Benzyloxy)-2-(naphthalen-1-ylmethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14h). Compound **14h** 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); ^{1}H NMR (DMSO-d_6) δ 5.04 (s, 2H), 5.39 (s, 2H), 7.31 (s, 5H), 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 (11h). Compound **11h** 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 hydrogenation was performed at 20 psi for 3 h: white powder (90% yield); mp 260 °C (dec); ^{1}H NMR (DMSO-d_6) 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 **11h**. The salt was prepared by dissolving **11h** in 1.0 equiv of a 0.103 N volumetric solution of NaOH followed by the lyophilization.

6-Bromo-2-(2-(naphthalen-1-yl)ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13i). Compound **13i** 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: tan solid. (46% yield); ^{1}H NMR (DMSO-d_6) δ 3.41 (t, J = 7.5 Hz, 2H), 4.13 (t, J = 7.6 Hz, 2H), 7.43 (m, 2H), 7.55 (m, 2H), 7.81 (d, J = 8.3 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H), 12.56 (s, 1H).

6-(Benzyloxy)-2-(2-(naphthalen-1-yl)ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14i). Compound **14i** was prepared from **13i** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: yellow solid (56% yield); ^{1}H NMR (DMSO-d_6) δ 3.38 (t, J = 6.8 Hz, 2H), 4.06 (t, J = 7.1 Hz, 2H), 4.91 (s, 2H), 7.30 (m, 1H), 7.37-7.44 (m, 6H), 7.54 (m, 2H), 7.80 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 6.6 Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H) 12.15 (s, 1H).

6-Hydroxy-2-(2-(naphthalen-1-yl)ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11i). Compound **11i** was prepared from **14i** as described for the preparation of **11a** except a mixture of methanol and acetic acid (2:1) was used as a solvent: light pink powder (90% yield); mp 300 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.37 (t, J = 8.1 Hz, 2H), 3.93 (bs, 2H), 7.38 (bs, 1H), 7.44 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.8 Hz, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 8.20 (bs, 1H), 11.95 (s, 1H), 12.28 (d, 1H). LCMS: retention time 2.99 min, *m/z* 284 $[M + H]^+$.

6-Bromo-2-(2-chlorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13j). Compound **13j** was prepared as described for the preparation of **13a** except 3-chloro-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 (28% yield); ^{1}H NMR (DMSO-d_6) δ 3.07 (t, J = 7.1 Hz, 2H), 4.07 (t, J = 7.1 Hz, 2H), 7.26-7.29 (m, 2H), 7.34 (m, 1H), 7.41 (m, 1H), 12.54 (s, 1H).

6-(Benzyloxy)-2-(2-chlorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14j). Compound **14j** was prepared from **13j** as described for the preparation of **14a**: white solid (85% yield); ^{1}H NMR (DMSO-d_6) δ 3.05 (t, J = 6.7 Hz, 2H), 4.00 (t, J = 6.7 Hz, 2H), 4.92 (s, 2H), 7.22-7.27 (m, 4H), 7.34-7.43 (m, 5H), 12.15 (s, 1H).

2-(2-Chlorophenethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11j). Compound **11j** was prepared from **14j** as described for the preparation of **11g** with the exception that 2 equiv. of BBr_3 were used and that the crude product was purified by trituration with EtOAc/hexanes: white powder (39% yield); mp 211 °C; ^{1}H NMR (DMSO-d_6) δ 3.04 (t, J = 7.1 Hz, 2H), 3.91 (t, J = 7.1 Hz, 2H), 7.24-7.29 (m, 3H), 7.41 (m, 1H), 11.66 (s, 1H), 12.04 (s, 1H). LCMS: retention time 2.90 min, *m/z* 268 $[M + H]^+$.

6-Bromo-2-(3-chlorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13k). Compound **13k** was prepared as described for the preparation of **13a** except 3-chloro-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 (73% yield); ^{1}H NMR (DMSO-d_6) δ 2.95 (t, J = 7.2 Hz, 2H), 4.06 (t, J = 7.3 Hz, 2H), 7.18 (m, 1H), 7.27-7.34 (m, 3H), 12.53 (s, 1H).

6-(Benzyloxy)-2-(3-chlorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14k). Compound **14k** was prepared from **13k** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: beige powder (70% yield); ^{1}H NMR (DMSO-d_6) 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, 5H), 12.13 (s, 1H).

2-(3-Chlorophenethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11k). Compound **11k** was prepared from **14k** as described for the preparation of **11g** with the exception that 2 equiv. of BBr_3 were added at rt and that the reaction was stirred at rt for 1.5 h. The compound was purified by trituration with EtOAc/hexanes: beige powder (54% yield); mp 219 °C; ^{1}H NMR (DMSO-d_6) δ 2.92 (t, J = 7.1 Hz, 2H), 3.90 (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-chlorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13l). Compound **13l** 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); ^{1}H NMR (DMSO-d_6) 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-(Benzyloxy)-2-(4-chlorophenethyl)-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 reaction was heated over three days: white solid (72% yield); ^{1}H NMR (DMSO-d_6) 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 = 8.3 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 7.36-7.43 (m, 5H), 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 **11g** with the exception that only 2 equiv. of BBr_3 were used and that the compound was purified by trituration with EtOAc/hexanes: white solid (52% yield); mp 225 °C; ^{1}H NMR (DMSO-d_6) δ 2.90 (t, J = 7.2 Hz, 2H), 3.87 (t, J = 7.3 Hz, 2H), 7.20 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 11.69 (s, 1H), 12.03 (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 1.39 min, *m/z* 268 $[M + H]^+$.

6-Bromo-2-(2-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13m). Compound **13m** 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); ^{1}H NMR (DMSO-d_6) 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), 12.55 (s, 1H).

6-(Benzyloxy)-2-(2-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 three days and that the product was purified by trituration with EtOAc/hexanes: white powder

(65% yield); ^{1}H NMR (DMSO-d_6): 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, 5H), 12.17 (s, 1H).

2-(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 1.5 h: white solid (83% yield); mp 194 °C; ^{1}H NMR (DMSO-d_6) δ 2.95 (t, J = 7.2 Hz, 2H), 3.88 (t, J = 7.2 Hz, 2H), 7.12 (m, 2H), 7.26 (m, 2H), 11.68 (bs, 1H), 11.99 (bs, 1H). LCMS: retention time 1.30 min, *m/z* 252 $[M + H]^+$.

6-Bromo-2-(3-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13n). Compound **13n** was prepared as described for the preparation of **13a** except 3-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 (66% yield); ^{1}H NMR (DMSO-d_6) δ 2.96 (t, J = 7.3 Hz, 2H), 4.06 (t, J = 7.3 Hz, 2H), 7.05 (m, 2H), 7.22 (m, 1H), 7.33 (m, 1H), 12.53 (s, 1H).

6-(Benzyloxy)-2-(3-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14n). Compound **14n** was prepared from **13n** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: tan solid (78% yield); ^{1}H NMR (DMSO-d_6) δ 2.94 (t, J = 6.8 Hz, 2H), 3.98 (t, J = 6.9 Hz, 2H), 5.06 (s, 2H), 6.96-7.05 (m, 3H), 7.29 (m, 1H), 7.36-7.43 (m, 5H), 12.13 (s, 1H).

2-(3-Fluorophenethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11n). Compound **11n** was prepared from **14n** 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 197 °C; ^{1}H NMR (DMSO-d_6) δ 2.93 (t, J = 7.3 Hz, 2H), 3.89 (t, J = 7.2 Hz, 2H), 7.03 (m, 3H), 7.31 (m, 1H), 11.89 (bs, 2H). LCMS: retention time 2.10 min, *m/z* 252 $[M + H]^+$.

6-Bromo-2-(4-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13o). Compound **13o** was prepared as described for the preparation of **13a** except 1-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 (42% yield); ^{1}H NMR (DMSO-d_6) δ 2.93 (t, J = 7.1 Hz, 2H), 4.03 (t, J = 7.3 Hz, 2H), 7.11 (m, 2H), 7.26 (m, 2H), 12.52 (s, 1H).

6-(Benzyloxy)-2-(4-fluorophenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14o). Compound **14o** was prepared from **13o** as described for the preparation of **14a**: white solid (81% yield); ^{1}H NMR (DMSO-d_6) δ 2.90 (t, J = 6.8 Hz, 2H), 3.95 (t, J = 6.9 Hz, 2H), 5.07 (s, 2H), 7.09 (m, 2H), 7.17 (m, 2H), 7.36-7.43 (m, 5H), 12.12 (s, 1H).

2-(4-Fluorophenethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (11o). Compound **11o** was prepared from **14o** 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 (73% yield); mp 193 °C; ^{1}H NMR (DMSO-d_6) 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 $[M + H]^+$.

6-Bromo-2-(2-methylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13p). Compound **13p** 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 (52% yield); ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 3H), 2.94 (m, 2H), 3.98 (m, 2H), 7.12 (m, 3H), 7.15 (m, 1H), 12.54 (s, 1H).

6-(Benzyloxy)-2-(2-methylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14p). Compound **14p** was prepared from **13p** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: beige powder (66% yield); ^{1}H NMR (DMSO-d_6) 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).

6-Hydroxy-2-(2-methylphenethyl)-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: grey solid (71% yield); mp 207 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (s, 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 $[M + H]^+$.

6-Bromo-2-(3-methylphenethyl)-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): ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 2.89 (t, *J* = 7.6 Hz, 2H), 4.01 (t, *J* = 7.6 Hz, 2H), 7.02 (m, 3H), 7.18 (t, *J* = 7.3 Hz, 1H), 12.50 (s, 1H).

6-(Benzyloxy)-2-(3-methylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14q). Compound **14q** 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); ^{1}H NMR (DMSO-d_6) 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, 3H), 7.16 (t, *J* = 7.6 Hz, 1H), 7.37-7.45 (m, 5H), 12.14 (s, 1H).

6-Hydroxy-2-(3-methylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11q). Compound **11q** 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 powder (82% yield); mp 210 °C; ^{1}H NMR (DMSO-d_6) 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 $[M + H]^+$.

6-Bromo-2-(4-methylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13r). Compound **13r** was prepared as described for the preparation of **13a** 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); ^{1}H NMR (DMSO-d_6) 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).

6-(Benzyloxy)-2-(4-methylphenethyl)-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); ^{1}H NMR (DMSO-

d_6) δ 2.24 (s, 3H), 2.86 (t, J = 7.2 Hz, 2H), 3.93 (t, J = 7.1 Hz, 2H), 5.06 (s, 2H), 7.03 (m, 2H), 7.06 (m, 2H), 7.36-7.43 (m, 5H), 12.12 (s, 1H).

6-Hydroxy-2-(4-methylphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11r). Compound **11r** was prepared from **14r** as described for the preparation of **11a** except the hydrogenation was performed at 30 psi for 2 h: white powder (54% yield); mp 219 °C; ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H), 2.85 (t, J = 7.5 Hz, 2H), 3.84 (t, J = 7.6 Hz, 2H), 7.08 (q, J = 8.1, 11.4 Hz, 4H), 11.68 (s, 1H), 12.02 (s, 1H). LCMS: retention time 2.49 min, *m/z* 248 $[M + H]^+$.

6-Bromo-2-(3-(trifluoromethyl)phenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13s). Compound **13s** was prepared as described for the preparation of **13a** except 1-(2-iodoethyl)-3-(trifluoromethyl)benzene (2.0 equiv.) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether: yellow solid (48%); ^{1}H NMR (DMSO-d_6) δ 3.06 (t, J = 7.2 Hz, 2H), 4.10 (t, J = 7.1 Hz, 2H), 7.55 (m, 4H), 12.53 (s, 1H).

6-(Benzyloxy)-2-(3-(trifluoromethyl)phenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14s). Compound **14s** was prepared from **13s** as described for the preparation of **14a**: white solid (85%); ^{1}H NMR (DMSO-d_6) δ 3.02 (t, J = 6.7 Hz, 2H), 4.01 (t, J = 6.8 Hz, 2H), 5.00 (s, 2H), 7.36 (m, 1H), 7.40 (m, 4H), 7.50 (m, 4H), 12.13 (s, 1H).

6-Hydroxy-2-(3-(trifluoromethyl)phenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11s). Compound **11s** was prepared from **14s** as described for the preparation of **11a**: white powder (85% yield); mp 201 °C; ^{1}H NMR (DMSO-d_6) δ 3.02 (t, J = 7.1 Hz, 2H), 3.93 (t, J = 7.2 Hz, 2H), 7.51 (m, 3H), 7.574 (m, 1H), 11.70 (s, 1H), 12.03 (s, 1H). LCMS: retention time 3.23 min, *m/z* 302 $[M + H]^+$.

6-Bromo-2-(4-(trifluoromethyl)phenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13t). Compound **13t** was prepared as described for the preparation of **13a** except 1-(2-iodoethyl)-4-(trifluoromethyl)benzene (2.0 equiv.) was used in place of methyl iodide. The crude product was purified using a Biotage Isolera One flash purification system with a silica gel flash cartridge (EtOAc/hexanes): yellow solid (69% yield); ^{1}H NMR (DMSO-d_6) δ 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-(trifluoromethyl)phenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14t). Compound **14t** was prepared from **13t** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: white solid (71% yield); ^{1}H NMR (DMSO-d_6) δ 3.01 (t, J = 6.7 Hz, 2H), 4.01 (t, J = 6.8 Hz, 2H), 5.02 (s, 2H), 7.38-7.42 (m, 7H), 7.62 (d, J = 8.1 Hz, 2H), 12.13 (s, 1H).

6-Hydroxy-2-(4-(trifluoromethyl)phenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11t). Compound **11t** was prepared from **14t** as described for the preparation of **11a** with the exception that a mixture of methanol and EtOAc (1:1) was used as a solvent and that the hydrogenation was performed overnight at 30 psi: gray solid (83% yield); mp 222 °C; ^{1}H NMR (DMSO-d_6) δ 3.01 (t, J = 7.1 Hz, 2H), 3.92 (t, J = 7.1 Hz, 2H), 7.42 (d, J = 7.8 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H), 11.71 (s, 1H), 12.02 (s, 1H). LCMS: retention time 2.99 min, *m/z* 302 $[M + H]^+$.

6-Bromo-2-(2-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13u). Compound **13u** was prepared as described for the preparation of **13a** with except of 1-(2-iodoethyl)-2-methoxybenzene (2.0 equiv.) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether: tan solid (57% yield); ^{1}H NMR (DMSO-d_6) δ 2.91 (t, J = 6.9 Hz, 2H), 3.73 (s, 3H), 4.01 (t, J = 7.0 Hz, 2H), 6.87 (dt, J = 1.0, 7.6 Hz, 1H), 6.93 (d, J = 7.6 Hz, 1H), 7.11 (m, 1H), 7.21 (m, 1H), 12.52 (s, 1H).

6-(Benzyloxy)-2-(2-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14u). Compound **14u** was prepared from **13u** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: white solid (63% yield); ^{1}H NMR (DMSO-d_6) δ 2.89 (t, J = 6.7 Hz, 2H), 3.74 (s, 3H), 3.94 (t, J = 6.7 Hz, 2H), 4.89 (s, 2H), 6.84 (t, J = 7.3 Hz, 1H), 6.93 (d, J = 8.1 Hz, 1H), 7.02 (dd, J = 1.8, 7.3 Hz, 1H), 7.20 (m, 1H), 7.35-7.41 (m, 5H), 12.12 (s, 1H).

6-Hydroxy-2-(2-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11u). Compound **11u** was prepared from **14u** as described for the preparation of **11a** with the exception that a mixture of methanol and EtOAc (1:1) was used as a solvent and that the hydrogenation was performed overnight under atmospheric pressure of hydrogen: yellow powder (83% yield); mp 166 °C; ^{1}H NMR (DMSO-d_6) δ 2.88 (t, J = 7.2 Hz, 2H), 3.75 (s, 3H), 3.84 (t, J = 7.2 Hz, 2H), 6.85 (dt, J = 1.0, 7.3 Hz, 1H), 6.93 (d, J = 7.8

Hz, 1H), 7.07 (dt, *J* = 1.5, 7.3 Hz, 1H), 7.20 (dt, *J* = 1.8, 8.1 Hz, 1H), 11.63 (s, 1H), 12.0 (s, 1H). LCMS: retention time 2.03 min, *m/z* 264 $[M + H]^+$.

6-Hydroxy-2-(2-hydroxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (15u). Compound **15u** was prepared from **11u** as described for the preparation of **11g** with the exception that 3 equiv. of BBr_3 were used and that the reaction was stirred for 1.5 h at rt: off-white solid (68% yield); mp 205 °C; ^{1}H NMR (DMSO-d_6) δ 2.83 (t, *J* = 7.3 Hz, 2H), 3.83 (t, *J* = 7.3 Hz, 2H), 6.68 (dt, *J* = 1.3, 7.6 Hz, ,1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.97-7.03 (m, 2H), 9.38 (s, 1H), 11.63 (bs, 1H), 11.97 (bs, 1H). LCMS: retention time 0.70 min, *m/z* 250 $[M + H]^+$.

6-Bromo-2-(3-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13v). Compound **13v** was prepared as described for the preparation of **13a** except 1-(2-iodoethyl)-3-methoxybenzene (2.0 equiv.) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether: tan solid (36% yield); ^{1}H NMR (DMSO-d_6) δ 2.91 (t, *J* = 7.5 Hz, 2H), 3.73 (s, 3H), 4.04 (m, 2H), 6.77 (m, 1H), 6.79 (m, 2H), 7.21 (t, *J* = 7.7 Hz, 1H), 12.53 (s, 1H).

6-(Benzyloxy)-2-(3-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14v). Compound **14v** was prepared from **13v** as described for the preparation of **14a** except the product was purified by trituration with EtOAc/hexanes: white solid (64% yield); ^{1}H NMR (DMSO-d_6) δ 2.89 (t, *J* = 7.1 Hz, 2H), 3.70 (s, 3H), 3.96 (t, *J* = 7.2 Hz, 2H), 5.06 (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-Hydroxy-2-(3-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11v). Compound **11v** was prepared from **14v** as described for the preparation of **11a** with the exception that a 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; ^{1}H NMR (DMSO-d_6) δ 2.88 (t, *J* = 7.6 Hz, 2H), 3.72 (s, 3H), 3.87 (t, *J* = 7.6 Hz, 2H), 6.75-6.78 (m, 3H), 7.20 (t, *J* = 8.0 Hz, 1H), 11.76 (bs, 1H), 11.99 (bs, 1H), 11.97 (bs, 1H). LCMS: retention time 1.80 min, *m/z* 264 $[M + H]^+$.

6-Hydroxy-2-(3-hydroxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (15v). Compound **15v** was prepared from **11v** as described for the preparation of **11g**: white solid (53% yield); mp 232 °C; ^{1}H

NMR (DMSO-d_6) δ 2.80 (t, J = 7.2 Hz, 2H), 3.83 (t, J = 7.2 Hz, 2H), 6.58 (m ,3H), 7.07 (t, J = 7.7 Hz, 1H), 9.32 (s, 1H), 11.69 (s, 1H), 12.04 (s, 1H). LCMS: retention time 0.37 min, *m/z* 250 $[M + H]^+$.

6-Bromo-2-(4-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13w). Compound **13w** was prepared as described for the preparation of **13a** except 1-(2-iodoethyl)-4-methoxybenzene (2.0 equiv.) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether: tan solid (58% yield); ^{1}H NMR (DMSO-d_6) δ 2.87 (t, J = 7.5 Hz, 2H), 3.71 (s, 3H), 3.99 (m, 2H), 6.84 (d, J = 8.6 Hz, 2H), 7.12 (m, 2H), 12.52 (s, 1H).

6-(Benzyloxy)-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 (70% yield); ^{1}H NMR (DMSO-d_6) δ 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), 7.36-7.44 (m, 5H), 12.12 (s, 1H).

6-Hydroxy-2-(4-methoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11w). Compound **11w** was prepared from **14w** as described for the preparation of **11a** with the exception that a mixture of methanol and EtOAc (1:1) was used as a solvent and that the hydrogenation was performed at 50 psi for 3 h: off-white solid (62% yield); mp 244 °C; ^{1}H NMR (DMSO-d_6) δ 2.83 (t, J = 7.5 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 $[M + H]^+$

6-Hydroxy-2-(4-hydroxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (15w). Compound **15w** was prepared from **11w** as described for the preparation of **11g**: light pink solid (54% yield); mp > 280 °C (decomp); ^{1}H NMR (DMSO-d_6) δ 2.78 (t, J = 7.6 Hz, 2H), 3.80 (t, J = 7.6 Hz, 2H), 6.65 (d, J = 8.3 Hz, 2H), 6.96 (d, J = 8.3 Hz, 2H), 9.22 (s, 1H), 11.66 (s, 1H), 12.01 (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 3.5 min): retention time 3.20 min, *m/z* 250 $[M + H]^+$.

6-Bromo-2-(3-phenoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13x). Compound **13x** was prepared as described for the preparation of **13a** except 1-(2-iodoethyl)-3-phenoxybenzene (2.0 equiv.)

was used in place of methyl iodide. Yield: 21% (white solid). ^{1}H NMR (DMSO-d_6): δ 2.93 (t, J = 7.1 Hz, 2H), 4.05 (t, J = 7.1 Hz, 2H), 6.85-6.67 (m, 2H), 6.95-7.01 (m, 3H), 7.10-7.15 (m, 1H), 7.29-7.39 (m, 3H), 12.53 (s, 1H).

6-(Benzyloxy)-2-(3-phenoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (14x). Compound **14x** was prepared from **13x** as described for the preparation of **14a**: white solid (52% yield); ^{1}H NMR (DMSO-d_6): δ 2.88 (t, J = 6.5 Hz, 2H), 3.96 (t, J = 6.5 Hz, 2H), 5.03 (s, 2H), 6.75 (m, 1H), 6.84-6.86 (m, 1H), 6.91-6.96 (m, 3H), 7.09-7.13 (m, 1H), 7.28-7.39 (m, 8H), 12.14, (bs, 1H).

6-Hydroxy-2-(3-phenoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11x). Compound **11x** was prepared from **14x** as described for the preparation of **11a**: tan solid (78% yield); mp 150-153 °C; ^{1}H NMR (DMSO-d_6): δ 2.89 (t, J = 7.2 Hz, 2H), 3.86 (t, J = 7.2 Hz, 2H), 6.79 (m, 1H), 6.83-6.86 (m, 1H), 6.95-6.98 (m, 3H), 7.10-7.14 (m, 1H), 7.28-7.40 (m, 3H), 11.86 (bs, 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 1.78 min, m/z 326 $[M + H]^+$.

6-Bromo-2-(4-phenoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (13y). Compound **13y** was prepared as described for the preparation of **13a** except 1-(2-iodoethyl)-4-phenoxybenzene (2.0 equiv.) was used in place of methyl iodide: tan solid (24% yield); ^{1}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-(Benzyloxy)-2-(4-phenoxyphenethyl)-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); ^{1}H 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, 7H), 12.14 (s, 1H).

6-Hydroxy-2-(4-phenoxyphenethyl)-1,2,4-triazine-3,5(2H,4H)-dione (11y). Compound **11y** was prepared from **14y** as described for the preparation of **11g**: white solid (69% yield); mp 207-209 °C; ^{1}H NMR (DMSO-d_6): δ 2.90 (t, J = 7.2 Hz, 2H), 3.88 (t, J = 7.3 Hz, 2H), 6.92-6.98 (m, 4H), 7.10-7.14 (m,

1H), 7.21 (m, 2H), 7.35-7.40 (m, 2H), 11.69 (bs, 1H), 12.04 (bs, 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 **13a** except 4-(2-iodoethyl)biphenyl (2.0 equiv.) was used in place of methyl iodide. The crude product was purified by trituration with cold diethyl ether: tan solid (59% yield); ^{1}H NMR (DMSO-d_6) δ 2.99 (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-(Benzyloxy)-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); ^{1}H 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), 7.58-7.64 (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 °C; ^{1}H NMR (DMSO-d_6) δ 2.95 (t, J = 7.6 Hz, 2H), 3.92 (t, J = 7.5 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.35 (t, J = 6.8 Hz, 1H), 7.45 (t, J = 7.6 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 7.1 Hz, 2H), 11.71 (s, 1H), 12.06 (s, 1H). LCMS: retention time 3.37 min, *m/z* 310 $[M + H]^+$.

4-(Benzyloxymethyl)-6-bromo-2-(2-(pyridin-2-yl)ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (17a). To a solution of **16** (0.30 g, 0.96 mmol), 2-(pyridin-2-yl)ethanol (0.12 g, 1.0 mmol), and triphenylphosphine (0.30 g, 1.1 mmol) in THF (5 mL) was added dropwise diisopropyl azodicarboxylate (0.23 mL, 1.2 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 min, then heated at 66 °C for 6.5 h. The reaction was concentrated and the residual oil was purified using a Biotage Isolera One flash purification system with a silica gel flash cartridge (EtOAc/hexanes) to give 0.34 g of

17a as a clear oil (85% yield): ^{1}H NMR ($CDCl_3$) δ 3.22 (t, *J* = 7.1 Hz, 2H), 4.37 (t, *J* = 7.6 Hz, 2H), 4.68 (s, 2H), 5.49 (s, 2H), 7.12-7.19 (m, 2H), 7.29-7.34 (mt, 5H), 7.61 (dt, *J* = 2.0, 7.8 Hz, 1H), 8.51 (d, *J* = 4.8 Hz, 1H).

6-(Benzyloxy)-4-(benzyloxymethyl)-2-(2-(pyridin-2-yl)ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (18a). To a solution of benzyl alcohol (0.13 mL, 1.2 mmol) in DMF (3 mL) was added 60% w/w sodium hydride (60% dispersion in mineral oil, 0.049 g, 1.2 mmol) at 0 °C. The mixture was stirred for 10 min and a solution of **17a** (0.34 g, 0.81 mmol) in DMF (5 mL) was added via syringe. The reaction was stirred at 0 °C for 30 min and quenched by the addition of water and EtOAc. The organic layer was dried over Na_2SO_4 and concentrated. The residual oil was purified using a Biotage Isolera One flash purification system with a silica gel flash cartridge (EtOAc/hexanes) to give 0.18 g of **18a** as an oil (50% yield): ^{1}H NMR ($CDCl_3$) δ 3.17 (t, *J* = 7.1 Hz, 2H) 4.27 (t, *J* = 5.6 Hz, 2H), 4.67 (s, 2H), 5.07 (s, 2H), 5.47 (s, 2H), 7.12 (m, 2H), 7.29-7.42 (m, 10H), 7.59 (dt, *J* = 2.0, 7.8 Hz, 1H), 8.54 (d, *J* = 5.1 Hz, 1H).

6-Hydroxy-2-(2-(pyridin-2-yl)ethyl)-1,2,4-triazine-3,5(2H,4H)-dione (19a). To a solution of **18a** (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 0.017 g of **19a** as a white solid (18% yield): mp 225 °C; ^{1}H 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 3.5 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* 235 $[M + H]^+$.

2-(2-(1H-Pyrazol-1-yl)ethyl)-4-(benzyloxymethyl)-6-bromo-1,2,4-triazine-3,5(2H,4H)-dione (17b). Compound **17b** was prepared as described for the preparation of **17a** except 2-(1H-pyrazol-1-

yl)ethanol was used in place of 2-(pyridin-2-yl)ethanol: clear oil (86% yield); ^{1}H NMR ($CDCl_3$) δ 4.36 (t, *J* = 5.9 Hz, 2H), 4.50 (t, *J* = 5.9 Hz, 2H), 4.67 (s, 2H), 5.47 (s, 2H), 6.24 (t, *J* = 2.1 Hz, 1H), 7.29-7.34 (m, 5H), 7.36 (m, 1H), 7.46 (m, 1H).

2-(2-(1H-Pyrazol-1-yl)ethyl)-6-(benzyloxy)-4-(benzyloxymethyl)-1,2,4-triazine-3,5(2H,4H)-dione (18b). Compound **18b** was prepared from **17b** as described for the preparation of **18a** except the reaction was stirred at 0 °C and warmed up to rt over a period of 4 h: clear oil (47% yield); ^{1}H NMR ($CDCl_3$): δ 4.27 (t, *J* = 5.9 Hz, 2H), 4.43 (t, *J* = 5.9 Hz, 2H), 4.67 (s, 2H), 5.05 (s, 2H), 5.46 (s, 2H), 6.21 (t, *J* = 2.1 Hz, 1H), 7.20 (m, 1H), 7.28-7.41 (m, 10H), 7.51 (m, 1H).

2-(2-(1H-Pyrazol-1-yl)ethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (19b). To a solution of **19b** (0.14 g, 0.32 mmol) in methanol (5 mL) was added one spatula tip of 10% Pd/C. The mixture was hydrogenated overnight under an atmospheric pressure of hydrogen and filtered through a pad of celite. The filtrate was concentrated and the residual material was triturated with EtOAc/MeOH to give 0.060 g of compound **19b** as a white solid (83% yield): mp 275 °C (dec); ^{1}H NMR (DMSO-d_6) δ 4.01 (t, *J* = 6.2 Hz, 2H), 4.36 (t, *J* = 6.2 Hz, 2H), 6.20 (t, *J* = 2.1 Hz, 1H), 7.40 (d, *J* = 1.5 Hz, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 11.69 (s, 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 3.5 min; flow rate 0.25 mL/min; column Luna Plus C18, 2.1 mm × 100 mm, 1.8 μm): retention time 1.32 min, *m/z* 224 $[M + H]^+$.

2-(2-(1H-Benzo[d]imidazol-1-yl)ethyl)-4-(benzyloxymethyl)-6-bromo-1,2,4-triazine-3,5(2H,4H)-dione (17c). Compound **17c** was prepared as described for the preparation of **17a** except 2-(1H-benzo[d]imidazol-1-yl)ethanol was used in place of 2-(pyridin-2-yl)ethanol and that the compound was purified by trituration with EtOAc/hexanes: beige solid (72% yield); ^{1}H NMR ($CDCl_3$) δ 4.38 (t, *J* = 6.6 Hz, 2H), 4.56 (t, *J* = 6.4 Hz, 2H), 4.61 (s, 2H), 5.42 (s, 2H), 7.28-7.34 (m, 7H), 7.39 (m, 1H), 7.80 (m, 1H), 7.91 (s, 1H).

2-(2-(1H-Benzo[d]imidazol-1-yl)ethyl)-6-(benzyloxy)-4-(benzyloxymethyl)-1,2,4-triazine-3,5(2H,4H)-dione (18c). Compound **18c** was prepared from **17c** as described for the preparation of **18a**

with the exception that the reaction was stirred overnight at rt and that the compound was purified by a silica gel flash chromatography (1% NH_4OH/EtOAc,): white solid (34% yield); ^{1}H NMR ($CDCl_3$) δ 4.25 (t, *J* = 5.9 Hz, 2H), 4.47 (t, *J* = 5.9 Hz, 2H), 4.60 (s, 2H), 4.75 (s, 2H), 5.40 (s, 2H), 7.27-7.36 (m, 13H), 7.71 (s, 1H), 7.78 (m, 1H).

2-(2-(1H-Benzo[d]imidazol-1-yl)ethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (19c). Compound **19c** was prepared from **18c** as described for the preparation of **19b**: beige solid (18% yield); mp 260 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.99 (t, *J* = 5.8 Hz, 2H), 4.50 (t, *J* = 5.8 Hz, 2H), 7.18 (m, 1H), 7.23 (m, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 7.3 Hz, 1H), 8.14 (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 3.5 min; flow rate 0.25 mL/min; column Luna Plus C18, 2.1 mm × 100 mm, 1.8 μm): retention time 3.26 min, *m/z* 274 $[M + H]^+$.

2-(2-(1H-Pyrrolo[2,3-b]pyridin-1-yl)ethyl)-4-(benzyloxymethyl)-6-bromo-1,2,4-triazine-3,5(2H,4H)-dione (17d). Compound **17d** was 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); ^{1}H NMR ($CDCl_3$) 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), 7.31 (m, 1H), 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-(2-(1H-Pyrrolo[2,3-b]pyridin-1-yl)ethyl)-6-(benzyloxy)-4-(benzyloxymethyl)-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 (51% yield); ^{1}H NMR ($CDCl_3$) 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-(2-(1H-Pyrrolo[2,3-b]pyridin-1-yl)ethyl)-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 BBr_3 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 followed 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): beige powder (79% yield); mp 259 °C; ^{1}H NMR (DMSO-d_6) δ 4.03 (t, J = 4.8 Hz, 2H), 4.51 (t, J = 5.1 Hz, 2H), 6.43 (d, J = 3.3 Hz, 1H), 7.05 (dd, J = 2.8, 7.6 Hz, 1H), 7.46 (d, J = 3.3 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 8.16 (d, J = 4.3 Hz, 1H), 11.89 (bs, 2H). 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 3.5 min): retention time 0.45 min, *m/z* 274 $[M + H]^+$.

4-(Benzyloxymethyl)-6-bromo-2-(2-phenylpropyl)-1,2,4-triazine-3,5(2H,4H)-dione (17e). Compound **17e** was prepared as described for the preparation of **17a** except 2-phenylpropan-1-ol was used in place of 2-(pyridin-2-yl)ethanol: colorless oil (87% yield); ^{1}H NMR ($CDCl_3$) 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-(Benzyloxy)-4-(benzyloxymethyl)-2-(2-phenylpropyl)-1,2,4-triazine-3,5(2H,4H)-dione (18e). Compound **18e** was prepared from **17e** as described for the preparation of **18a**: colorless oil (52% yield); ^{1}H NMR ($CDCl_3$) δ 1.27 (d, J = 7.1 Hz, 3H); 3.24 (m, 1H), 3.99 (d, J = 7.1 Hz, 2H), 4.59 (s, 2H), 5.04 (qt, J = 12.4, 23.8 Hz, 2H), 5.43 (s, 2H), 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-phenylpropyl)-1,2,4-triazine-3,5(2H,4H)-dione (19e). Compound **19e** was prepared from **18e** as described for the preparation of **19b** with the exception that the product was purified by trituration with EtOAc/hexanes: beige solid (12% yield); mp 217 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (d, J = 7.1 Hz, 3H), 3.22 (m, 1H), 3.78 (d, J = 6.8 Hz, 2H), 7.22 (m, 3H), 7.28 (m, 2H), 11.65 (s, 1H), 11.99 (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 0.93 min, *m/z* 248 $[M + H]^+$.

4-(Benzyloxymethyl)-6-bromo-2-(2,2-difluoro-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (17f). Compound **17f** was prepared as described for the preparation of **17a** except 2,2-difluoro-2-phenylethanol was used in place of 2-(pyridin-2-yl)ethanol: colorless oil (95% yield); ^{1}H NMR (DMSO-d_6) δ 4.53 (s, 2H), 4.66 (t, J = 13.9 Hz, 2H), 5.30 (s, 2H), 7.27-7.36 (m, 5H), 7.50 (m, 3H), 7.56 (m, 2H).

6-(Benzyloxy)-4-(benzyloxymethyl)-2-(2,2-difluoro-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (18f). Compound **18f** was prepared from **17f** as described for the preparation of **18a** with the exception that 1.2 equiv. of benzyl alcohol and 1.2 equiv. of sodium hydride were used and that the reaction was stirred at rt for 2 h: white solid (59% yield); ^{1}H NMR (DMSO-d_6) δ 4.49 (s, 2H), 4.56 (t, J = 12.8 Hz, 2H), 4.83 (s, 2H), 5.27 (s, 2H), 7.26-7.33 (m, 5H), 7.40 (m, 5H), 7.50-7.58 (m, 5H).

2-(2,2-Difluoro-2-phenylethyl)-6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione (19f). Compound **19f** was prepared from **18f** as described for the preparation of **19b** with the exception that a mixture of methanol, EtOAc and acetic acid (1:1:0.1) was used as solvent. A mixture of compound **19f** and its *N*-hydroxylmethyl derivative was obtained as a beige solid. The residue was re-dissolved in methanol and treated with a catalytic amount of sodium carbonate and stirred over 2 days. The reaction mixture was acidified with a 10% $KHSO_4$ solution to pH4 and the precipitate was filtered, washed thoroughly with water and with 10% EtOAc/hexanes to give compound **19g** as a white powder (36% yield): mp 245 °C; ^{1}H NMR (DMSO-d_6) δ 4.38 (t, J = 14.0 Hz, 2H), 7.50 (m, 5H), 11.88 (m, 1H), 12.17 (m, 1H). LCMS: retention time 2.25 min, m/z 270 $[M + H]^+$.

4-(Benzyloxymethyl)-6-bromo-2-(2-oxo-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (20). To a suspension of NaH (0.14 g, 3.5 mmol) in DMF (2.5 mL) at rt was slowly added a solution of **16** (1.0 g, 3.2 mmol) in DMF (7 mL) via syringe. The mixture was stirred at rt for 1 h after which bromoacetophenone (0.70 g, 3.5 mmol) was added in one portion. The reaction was stirred for 3.5 h. The reaction mixture was partitioned between EtOAc and water. The organic layer was dried over Na_2SO_4 and concentrated. The residual material was purified by a Biotage Isolera One flash purification system with a silica gel flash cartridge (EtOAc/hexanes) to give 1.27 g of **20** as a white solid (92%

yield). ^{1}H NMR ($CDCl_3$) δ 4.72 (s, 2H), 5.40 (s, 2H), 5.54 (s, 2H), 7.31-7.39 (m, 5H), 7.54 (t, *J* = 7.6 Hz, 2H), 7.67 (m, 1H), 7.97 (m, 2H).

6-(Benzyloxy)-4-(benzyloxymethyl)-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: yellow oil (45% yield); ^{1}H NMR ($CDCl_3$) δ 4.72 (s, 2H), 5.16 (s, 2H), 5.29 (s, 2H), 5.53 (s, 2H), 7.29-7.41 (m, 10H), 7.54 (t, *J* = 7.3 Hz, 2H), 7.67 (m, 1H), 7.99 (m, 2H).

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 BBr_3 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); mp 220 °C; ^{1}H NMR (DMSO-d_6) δ 5.27 (s, 2H), 7.58 (t, *J* = 7.8 Hz, 2H), 7.71 (t, *J* = 7.6 Hz, 1H), 8.02 (d, *J* = 7.3 Hz, 2H), 11.78 (bs, 1H), 12.30 (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 0.26 min, *m/z* 248 $[M + H]^+$.

6-Hydroxy-2-(2-hydroxy-2-phenylethyl)-1,2,4-triazine-3,5(2H,4H)-dione (23). To a solution of **21** (0.17 g, 0.37 mmol) in a 2:1 mixture of methanol and EtOAc (15 mL) were added a small spatula tip of chloro(1,5-cyclooctadiene)rhodium(I) dimer and one drop of triethylamine. The mixture was stirred for 36 h under hydrogen (300 psi) using a mechanical stirrer. The reaction was filtered and concentrated to give a brown oil which was subsequently dissolved in dichloromethane (5 mL) and treated with a 1.0 M solution of BBr_3 in dichloromethane (1.5 mL, 1.5 mmol) for 1.5 h. The reaction was quenched by the addition of water. The mixture was concentrated and purified by preparative HPLC (10% acetonitrile/90% water for 5 min followed by an increase to 50% acetonitrile/50% water over 35 min and then an increase to 70% acetonitrile/30% water over 10 min; flow rate 15 mL/min) to give 0.024 g of **23** as a beige solid (26% yield): mp 202 °C; ^{1}H NMR (DMSO-d_6) δ 3.66 (dd, *J* = 4.0, 13.1 Hz, 1H), 3.85 (dd, *J* = 9.1, 13.4 Hz, 1H), 4.91 (dd, *J* = 4.3, 9.4 Hz, 1H), 5.52 (bs, 1H), 7.27-7.34 (m, 5H), 11.66

(s, 1H), 12.03 (s, 1H). LCMS (5% acetonitrile/95% water for 0.25 min followed by an increase to 40% acetonitrile/60% water over 1.5 min and continuation of 40% acetonitrile/60% water for 2.25 min; flow rate 1.25 mL/min): retention time 1.61 min, *m/z* 250 $[M + H]^+$.

4-(Benzyloxymethyl)-6-bromo-2-phenyl-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 (0.26 mL, 3.20 mmol), 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% $KHSO_4$ solution, dried over Na_2SO_4, 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); ^{1}H NMR ($CDCl_3$) 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).

6-(Benzyloxy)-4-(benzyloxymethyl)-2-phenyl-1,2,4-triazine-3,5(2H,4H)-dione (25). Compound **25** was prepared from **24** as described for the preparation of **18a**: yellow oil (44% yield); ^{1}H NMR ($CDCl_3$) δ 4.76 (s, 2H), 5.25 (s, 2H), 5.58 (s, 2H), 7.29-7.33 (m, 5H), 7.38-7.40 (m, 5H, 7.44-7.47 (m, 5H).

6-Hydroxy-2-phenyl-1,2,4-triazine-3,5(2H,4H)-dione (26). Compound **26** was prepared from **25** as described for the preparation of **19a** with the exception that 4.0 equiv. of BBr_3 were used. After completion of the reaction, the reaction mixture was concentrated and the resulting residue was partitioned between EtOAc and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by trituration with EtOAc/hexanes: white powder (33% yield); mp 239 °C; ^{1}H NMR (DMSO-d_6) 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 (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) at rt. The white suspension was heated

at 78 °C for 50 h. The reaction was cooled to rt and the resultant solid was removed by filtration. The filtrate was concentrated by approximately one-half and the resultant solid was removed by filtration. This was repeated 4 times. The remaining filtrate was partitioned between EtOAc (50 mL) and satd. $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$ and concentrated. The crude material was purified by silica gel column chromatography (80% EtOAc/hexanes w/ 0.1% NH_4OH) to give 0.66 g of **28** as a white solid (16% yield): ^{1}H NMR (DMSO-d_6) δ 2.94 (m, 2H), 4.09 (m, 2H), 4.88 (s, 2H), 7.18-7.33 (m, 5H), 7.44 (br s, 2H).

Methyl 2-(2-carbamothioyl-2-phenethylhydrazinyl)-2-oxoacetate (29). To a solution of **28** (0.66 g, 3.4 mmol) in THF (15 mL) was added a solution of methyl chlorooxoacetate (0.41 g, 3.4 mmol) in THF (5 mL) over 2 min at rt. The mixture was stirred for 14 h and concentrated to dryness. The residue was dissolved in EtOAc (50 mL) and washed with satd. $NaHCO_3$ (25 mL). The organic layer was dried over $MgSO_4$ and concentrated. The crude material was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to give 0.30 g of **29** as a white solid (31% yield): 1H NMR ($CDCl_3$) 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).

6-Hydroxy-2-phenethyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one (30). To a solution of **29** (0.30 g, 1.1 mmol) in THF (5 mL) was added dropwise 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% $KHSO_4$. The organic layer was separated, dried over $MgSO_4$, and concentrated. The crude material was purified by silica gel chromatography (1% AcOH/EtOAc) to give 0.031 g of **30** as a yellow solid (11% yield): mp 190-230 °C;^{1}H NMR (CD_3OD) δ 3.11 (m, 2H), 4.41 (m, 2H), 7.19-7.31 (m, 5H). LCMS: retention time 2.90 min, m/z 250 $[M + H]^+$.

5-Hydroxy-1-phenethylpyrimidine-2,4(1H,3H)-dione (32). To a solution of 5-hydroxypyrimidine-2,4(1H,3H)-dione **31** (0.30 g, 2.3 mmol) in acetonitrile (15 mL) was added *N,O*-bis(trimethylsilyl)acetamide (2.0 mL, 8.2 mmol). The mixture was heated at 82 °C for 3 h after which phenethyl iodide (0.54 g, 2.3 mmol) was added via syringe. The reaction was heated for 48 h at same

temperature and the reaction mixture was concentrated in vacuo. The resulting residue was dissolved in dichloromethane and the organic solution was washed twice with water, dried over Na_2SO_4, and concentrated. The crude material was triturated with cold diethyl ether and filtered to give 0.080 g of **32** as a tan solid (15% yield): ^{1}H NMR (DMSO-d_6): δ 2.86 (t, J = 8.0 Hz, 2H), 3.79 (t, J = 8.0 Hz, 2H), 7.10 (s, 1H), 7.22-7.31 (m, 5H), 8.53 (s, 1H), 11.36 (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 0.45 min, m/z 233 $[M + H]^+$.

Human DAAO (hDAAO) purification. HEK cells expressing hDAAO were grown to confluence, harvested, washed, and re-suspended in ice-cold assay buffer (Tris buffer, 50 mM, pH 8.5) containing flavin adenine dinucleotide (FAD, 100μM) prior to sonication. The resulting cell lysate was centrifuged at 16,000 × g for 10 min at 4 °C and the hDAAO was purified using an UNO Q6 ion-exchange column pre-equilibrated in diluted assay buffer (10 mM, pH 8.5) on a FPLC system (Bio-Rad; Hercules, CA). The hDAAO was eluted by a linear gradient of 0 to 0.5 M NaCl and the fractions with the highest DAAO activity were combined and stored at -80 °C in 20% glycerol until usage. The specific activity with respect to D-serine was determined to be 35.0±0.2 μmole/min/mg, comparable to that of purified protein previously reported.[31] The final concentration of hDAAO in the assay described below was adjusted in such a manner that a rate of 2.0 mOD/min can be achieved for the oxidation of D-serine (5 mM) in the absence of a DAAO inhibitor.

In vitro DAAO assay. D-Serine was purchased from Bachem Biosciences Inc (King of Prussia, PA), horse radish peroxidase from Worthington Biochemical Corporation (Freehold, NJ) and *o*-phenylenediamine from Pierce Biotechnology, Inc (Rockford, IL). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO). A reliable 96-well plate D-amino acid oxidase (DAAO) assay was developed based on previously published methods.[32] Briefly, D-serine (5 mM) was oxidatively deaminated by hDAAO in the presence of molecular oxygen and flavin adenosine dinucleotide (FAD; 10 μM), to yield the corresponding α–keto acid, ammonia and hydrogen peroxide. The resulting

hydrogen peroxide was quantified using horseradish peroxidase (0.01 mg/mL) and *o*-phenylenediamine (180 μg/mL), which turns yellowish-brown upon oxidation. DAAO activity was correlated to the rate formation of the colored product, i.e., rate of change of absorbance at 411 nm. All reactions were carried out for 20 min at room temperature in a 100-μL volume in Tris buffer (50 mM, pH 8.5). Additionally, stock solutions and serial dilutions of potential DAAO inhibitors were made in 20:80 DMSO:buffer with a final assay DMSO concentration of 2%.

In vitro metabolic stability studies. The metabolic stability was evaluated using mouse liver microsomes. For the cytochrome P450 (CYP)-mediated metabolism, the reaction was carried out with 100 mM potassium phosphate buffer, pH 7.4, in the presence of NADPH regenerating system (1.3 mM NADPH, 3.3 mM glucose 6-phosphate, 3.3 mM $MgCl_2$, 0.4 U/mL glucose-6-phosphate dehydrogenase, 50 μM sodium citrate). Reactions, in duplicate, were initiated by addition of the liver microsomes to the incubation mixture (compound final concentration was 5 μM; 0.5 mg/mL microsomes). For the glucuronidation reaction, a test compound were added to TRIS-HCl buffer (50 mM, pH 7.5) with microsomes (0.5 mg/mL), along with $MgCl_2$ (8 mM), and alamethicin (25 μg/mL) and pre-incubated at 37ºC. The reaction was initiated (in duplicate) with UDPGA (2 mM; final concentration). Controls in the absence cofactors were carried out to determine the specific cofactor-free degradation. After a 60 min of incubation, aliquots of the mixture were removed and the reaction quenched by addition of two times the volume of ice cold acetonitrile spiked with the internal standard. Compound disappearance was monitored over time using a liquid chromatography and tandem mass spectrometry (LC/MS/MS) method.

In vivo pharmacokinetics. All procedures involving mice were approved by and conformed to the guidelines of the Institutional Animal Care Committee of the Johns Hopkins University. In vivo pharmacokinetics studies were performed using male CD1 mice (n = 3 for each time point for each group). Approximately 1 mL of whole 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 800 *g*, 4°C). Thereafter, the top layer of each tube (~400 μl plasma) was

aspirated via transfer pipette, dispensed into a clean non-heparinized microcentrifuge tube and stored at –80°C until subsequent analyses. Plasma levels of **8b** and **11h** 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[33] 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

Supporting Information

A csv file containing molecular formula strings. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.#####.

AUTHOR INFORMATION

Corresponding Author

*Phone, (410) 614-0982; fax, (410) 614-0659; E-mail, ttsukamoto@jhmi.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was in part supported by National Institutes of Health (R01MH091387 to T.T.) and the Johns Hopkins Brain Science Institute NeuroTranslational Drug Discovery program. We thank Mr. Reiji Tsukamoto for his technical assistance.

ABBREVIATIONS USED

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

REFERENCES

1. Nunes, E. A.; MacKenzie, E. M.; Rossolatos, D.; Perez-Parada, J.; Baker, G. B.; Dursun, S. M. D-serine and schizophrenia: an update. *Expert Rev. Neurother.* **2012,** *12*, 801-812.

2. Kantrowitz, J. T.; Malhotra, A. K.; Cornblatt, B.; Silipo, G.; Balla, A.; Suckow, R. F.; D'Souza, C.; Saksa, J.; Woods, S. W.; Javitt, D. C. High dose D-serine in the treatment of schizophrenia. *Schizophr. Res.* **2010,** *121*, 125-130.

3. Ganote, C. E.; Peterson, D. R.; Carone, F. A. The nature of D-serine--induced nephrotoxicity. *Am. J. Pathol.* **1974,** *77*, 269-282.

4. Curti, B.; Ronchi, S.; Simonetta, P. M., D- and L-Amino Acid Oxidases. In *Chemistry and Biochemistry of Flavoenzyme*, Muller, F., Ed. CRC Press: Boca Raton, FL, 1992; Vol. 3, pp 69-94.

5. Rais, R.; Thomas, A. G.; Wozniak, K.; Wu, Y.; Jaaro-Peled, H.; Sawa, A.; Strick, C. A.; Engle, S. J.; Brandon, N. J.; Rojas, C.; Slusher, B. S.; Tsukamoto, T. Pharmacokinetics of oral D-serine in D-amino acid oxidase knockout mice. *Drug Metab. Dispos.* **2012,** *40*, 2067-2073.

6. Maekawa, M.; Okamura, T.; Kasai, N.; Hori, Y.; Summer, K. H.; Konno, R. D-amino-acid oxidase is involved in D-serine-induced nephrotoxicity. *Chem. Res. Toxicol.* **2005,** *18*, 1678-1682.

7. Sacchi, S.; Caldinelli, L.; Cappelletti, P.; Pollegioni, L.; Molla, G. Structure-function relationships in human D-amino acid oxidase. *Amino Acids* **2012,** *43*, 1833-1850.

8. Ferraris, D. V.; Tsukamoto, T. Recent advances in the discovery of D-amino acid oxidase inhibitors and their therapeutic utility in schizophrenia. *Curr. Pharm. Des.* **2011,** *17*, 103-111.

9. Sacchi, S.; Rosini, E.; Pollegioni, L.; Molla, G. D-amino acid oxidase inhibitors as a novel class of drugs for schizophrenia therapy. *Curr. Pharm. Des.* **2013,** *19*, 2499-2511.

10. Adage, T.; Trillat, A. C.; Quattropani, A.; Perrin, D.; Cavarec, L.; Shaw, J.; Guerassimenko, O.; Giachetti, C.; Greco, B.; Chumakov, I.; Halazy, S.; Roach, A.; Zaratin, P. In vitro and in vivo pharmacological profile of AS057278, a selective D-amino acid oxidase inhibitor with potential anti-psychotic properties. *Eur. Neuropsychopharmacol.* **2008,** *18*, 200-214.

11. Sparey, T.; Abeywickrema, P.; Almond, S.; Brandon, N.; Byrne, N.; Campbell, A.; Hutson, P. H.; Jacobson, M.; Jones, B.; Munshi, S.; Pascarella, D.; Pike, A.; Prasad, G. S.; Sachs, N.; Sakatis, M.; Sardana, V.; Venkatraman, S.; Young, M. B. The discovery of fused pyrrole carboxylic acids as novel, potent D-amino acid oxidase (DAO) inhibitors. *Bioorg. Med. Chem. Lett.* **2008,** *18*, 3386-3391.

12. Ferraris, D.; Duvall, B.; Ko, Y. S.; Thomas, A. G.; Rojas, C.; Majer, P.; Hashimoto, K.; Tsukamoto, T. Synthesis and biological evaluation of D-amino acid oxidase inhibitors. *J. Med. Chem.* **2008,** *51*, 3357-3359.

13. Duplantier, A. J.; Becker, S. L.; Bohanon, M. J.; Borzilleri, K. A.; Chrunyk, B. A.; Downs, J. T.; Hu, L. Y.; El-Kattan, A.; James, L. C.; Liu, S.; Lu, J.; Maklad, N.; Mansour, M. N.; Mente, S.; Piotrowski, M. A.; Sakya, S. M.; Sheehan, S.; Steyn, S. J.; Strick, C. A.; Williams, V. A.; Zhang, L. Discovery, SAR, and pharmacokinetics of a novel 3-hydroxyquinolin-2(1H)-one series of potent D-amino acid oxidase (DAAO) inhibitors. *J. Med. Chem.* **2009,** *52*, 3576-3585.

14. Berry, J. F.; Ferraris, D. V.; Duvall, B.; Hin, N.; Rais, R.; Alt, J.; Thomas, A. G.; Rojas, C.; Hashimoto, K.; Slusher, B. S.; Tsukamoto, T. Synthesis and SAR of 1-hydroxy-1H-benzo[d]imidazol-2(3H)-ones as inhibitors of D-amino acid oxidase. *ACS Med. Chem. Lett.* **2012,** *3*, 839-843.

15. Katane, M.; Osaka, N.; Matsuda, S.; Maeda, K.; Kawata, T.; Saitoh, Y.; Sekine, M.; Furuchi, T.; Doi, I.; Hirono, S.; Homma, H. Identification of novel D-amino acid oxidase inhibitors by in silico screening and their functional characterization in vitro. *J. Med. Chem.* **2013,** *56*, 1894-1190.

16. Kawazoe, T.; Tsuge, H.; Imagawa, T.; Aki, K.; Kuramitsu, S.; Fukui, K. Structural basis of D-DOPA oxidation by D-amino acid oxidase: alternative pathway for dopamine biosynthesis. *Biochem. Biophys. Res. Commun.* **2007,** *355*, 385-391.

17. Raje, M.; Hin, N.; Duvall, B.; Ferraris, D. V.; Berry, J. F.; Thomas, A. G.; Alt, J.; Rojas, C.; Slusher, B. S.; Tsukamoto, T. Synthesis of kojic acid derivatives as secondary binding site probes of d-amino acid oxidase. *Bioorg Med Chem Lett* **2013,** *23*, 3910-3913.

18. Hondo, T.; Warizaya, M.; Niimi, T.; Namatame, I.; Yamaguchi, T.; Nakanishi, K.; Hamajima, T.; Harada, K.; Sakashita, H.; Matsumoto, Y.; Orita, M.; Takeuchi, M. 4-Hydroxypyridazin-3(2H)-one derivatives as novel D-amino acid oxidase inhibitors. *J. Med. Chem.* **2013,** *56*, 3582-3592.

19. Hopkins, S. C.; Heffernan, M. L.; Saraswat, L. D.; Bowen, C. A.; Melnick, L.; Hardy, L. W.; Orsini, M. A.; Allen, M. S.; Koch, P.; Spear, K. L.; Foglesong, R. J.; Soukri, M.; Chytil, M.; Fang, Q. K.; Jones, S. W.; Varney, M. A.; Panatier, A.; Oliet, S. H.; Pollegioni, L.; Piubelli, L.; Molla, G.; Nardini, M.; Large, T. H. Structural, kinetic, and pharmacodynamic mechanisms of D-amino acid oxidase inhibition by small molecules. *J. Med. Chem.* **2013,** *56*, 3710-3724.

20. Terry-Lorenzo, R. T.; Chun, L. E.; Brown, S. P.; Heffernan, M. L.; Fang, Q. K.; Orsini, M. A.; Pollegioni, L.; Hardy, L. W.; Spear, K. L.; Large, T. H. Novel human D-amino acid oxidase inhibitors stabilize an active site lid open conformation. *Biosci. Rep.* **2014,** *34*, 487-499.

21. Lange, J. H.; Venhorst, J.; van Dongen, M. J.; Frankena, J.; Bassissi, F.; de Bruin, N. M.; Besten, C.; de Beer, S. B.; Oostenbrink, C.; Markova, N.; Kruse, C. G. Biophysical and physicochemical methods differentiate highly ligand-efficient human D-amino acid oxidase inhibitors. *Eur. J. Med. Chem.* **2011,** *46*, 4808-4819.

22. Zimmermann, S. C.; Rais, R.; Alt, J.; Burzynski, C.; Slusher, B. S.; Tsukamoto, T. Structure-metabolism relationships in the glucuronidation of D-amino acid oxidase inhibitors. *ACS Med. Chem. Lett.* **2014,** *5*, 1251-1253.

23. Zajac, M.; Muszalska, I. Mechanism of ceftriaxone degradation in aqueous solution. *Acta. Pol. Pharm.* **1998,** *55*, 35-39.

24. J. Dudfield, P.; Le, V.-D.; D. Lindell, S.; W. Rees, C. Synthesis of C-ribosyl imidazo[2,1-f][1,2,4]triazines as inhibitors of adenosine and AMP deaminases. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2929-2936.

25. Pontillo, J.; Guo, Z.; Wu, D.; Struthers, R. S.; Chen, C. Synthesis of aryl-1,2,4-triazine-3,5-diones as antagonists of the gonadotropin-releasing hormone receptor. *Bioorg Med Chem Lett* **2005,** *15*, 4363-4366.

26. Leroy, I.; Dupont-Passelaigue, E.; Valeille, K.; Rival, Y.; Junquero, D. Derivatives of triazines and uracils, their preparation and their application in human therapeutics. U.S. Patent 8,618,287, Dec 31, 2013.

27. Jensen, K. A.; Anthoni, U.; Kägi, B.; Larsen, C.; Pedersen, C. T. Studies of thioacids and their derivatives. IX. Thiosemicarbazides. *Acta. Chem. Scand.* **1968,** *22*, 1-50.

28. Branch, C. L.; Eggleston, D. S.; Haltiwanger, R. C.; Kaura, A. C.; Tylerc, J. W. Synthesis of 6-Hydroxy-2-methyl-3-thioxo-2H-1,2,4-triazin-5-one. *Synth. Commun.* **2006,** *26*, 2075-2084.

29. Nagata, Y.; Shimojo, T.; Akino, T. D-amino acid oxidase in mouse liver—II. *Comp. Biochem. Physiol. B* **1988,** *91*, 503-504.

30. Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010,** *31*, 455-461.

31. Kawazoe, T.; Tsuge, H.; Pilone, M. S.; Fukui, K. Crystal structure of human D-amino acid oxidase: context-dependent variability of the backbone conformation of the VAAGL hydrophobic stretch located at the si-face of the flavin ring. *Protein Sci.* **2006,** *15*, 2708-2717.

32. Cook, S. P.; Galve-Roperh, I.; Martinez del Pozo, A.; Rodriguez-Crespo, I. Direct calcium binding results in activation of brain serine racemase. *J. Biol. Chem.* **2002,** *277*, 27782-27792.

33. Berna, M. J.; Ackermann, B. L. Quantification of serine enantiomers in rat brain microdialysate using Marfey's reagent and LC/MS/MS. *J. Chromatogr. B, Analyt. Technol. Biomed. Life Sci.* **2007,** *846*, 359-363.

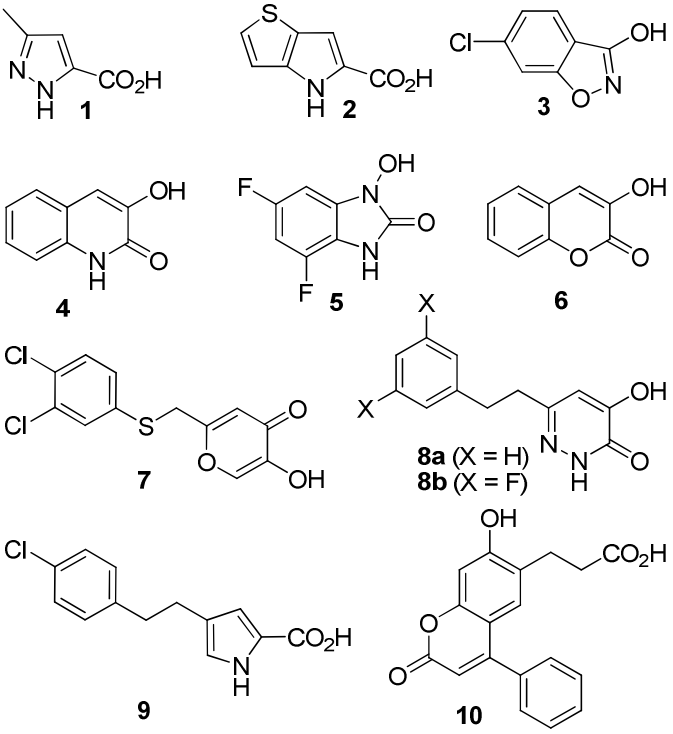


Figure 1. Representative inhibitors of DAAO

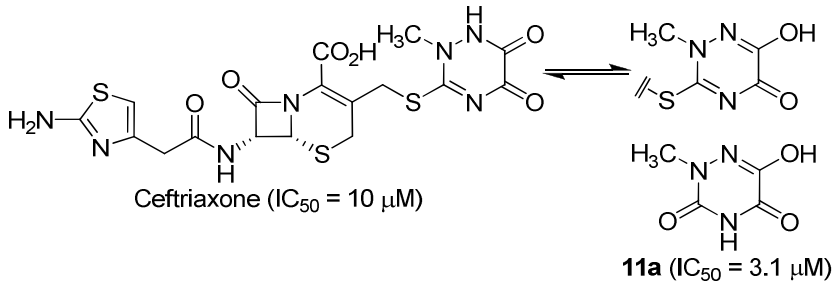


Figure 2. Ceftriaxone and compound **11a**.

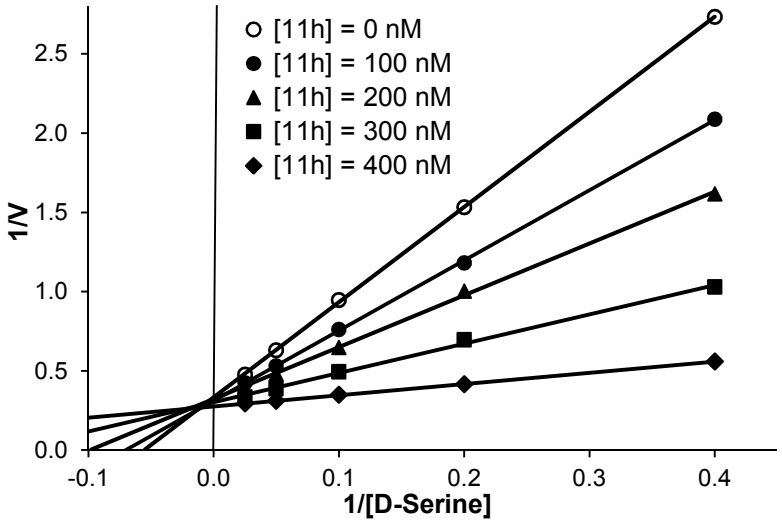


Figure 3. Double-reciprocal plot of the oxidation of D-serine by human DAAO in the presence of compound **11h**. The straight lines represent the least squares fit of the data obtained by plotting the reciprocal of the initial rate of change in the absorbance at 411 nm versus the reciprocal of the D-serine concentrations (mM).

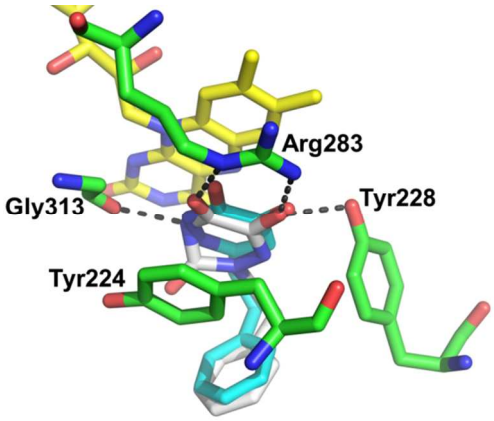


Figure 4. Proposed binding mode of **11e** (white) to the active site of DAAO (3W4K). Key residues and FAD are shown in green and yellow, respectively. Hydrogen-bonding interactions between **11h** and the key residues are shown as gray dashed lines. Compound **8a** (cyan) of 3W4K is also shown for comparison.

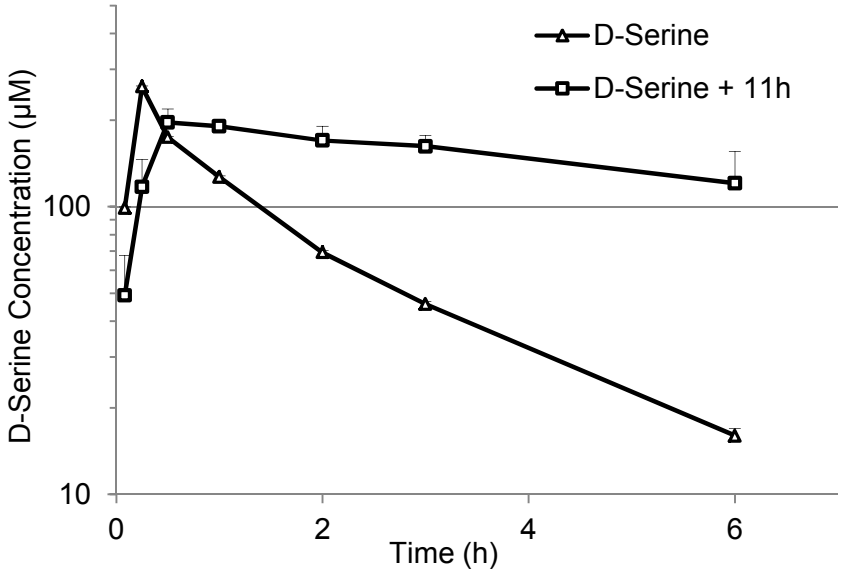
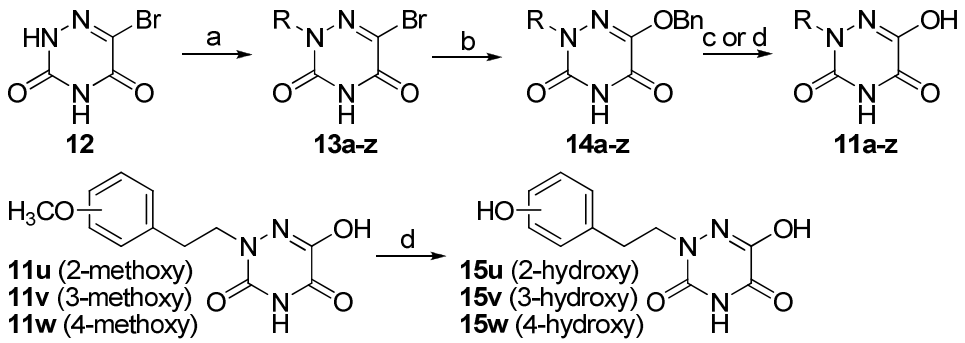
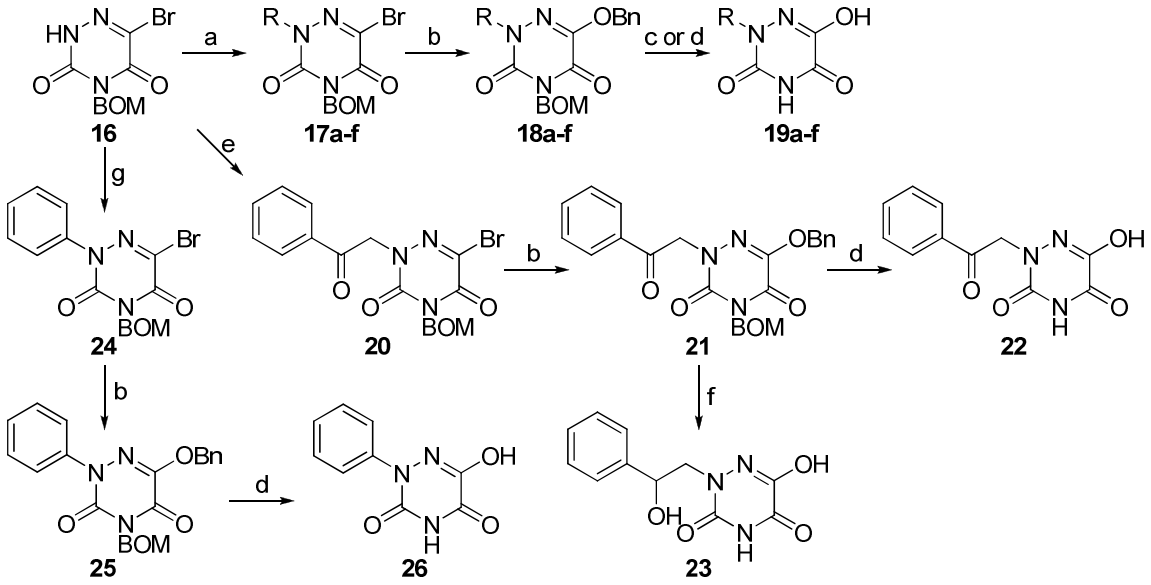


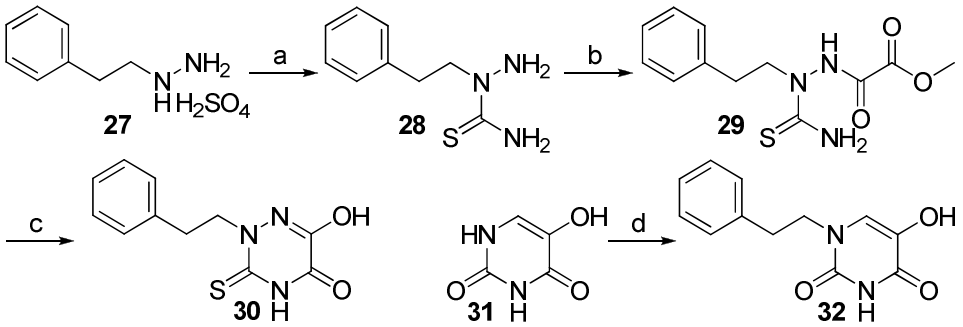
Figure 5. Effects of compound **11h** (30 mg/kg, p.o.) on plasma pharmacokinetics of oral D-serine (30 mg/kg) in mice.



Scheme 1. Synthesis of **11a-z** and **15u-w**. Reagents and conditions: (a) RI or RBr, BSA, acetonitrile, 82 °C; (b) K_2CO_3, BnOH, 150 °C; (c) H_2, Pd/C, MeOH, rt; (d) BBr_3, dichloromethane, rt.

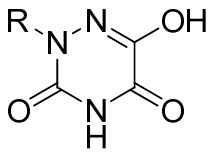


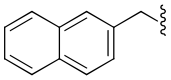
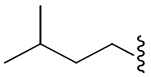
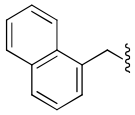
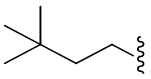
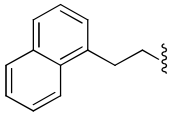
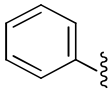
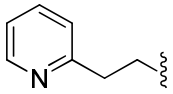
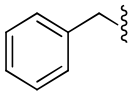
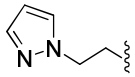
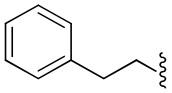
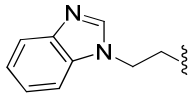
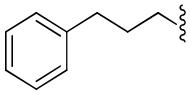
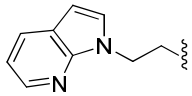
Scheme 2. Synthesis of **19a-f**, **22**, **23**, and **26**. Reagents and conditions: (a) ROH, Ph_3P, DIAD, THF, 66 °C; (b) BnOH, NaH, DMF, 0 °C; (c) H_2, Pd/C, MeOH, rt; (d) BBr_3, dichloromethane, rt; (e) $BrCH_2COPh$, NaH, DMF, rt; (f) (i) H_2, $[Rh(COD)Cl]_2$, Et_3N, MeOH-EtOAc, rt; (ii) BBr_3, dichloromethane, rt; (g) phenylboronic acid, pyridine, copper(II) acetate, dichloromethane, rt.



Scheme 3. Synthesis of **30** and **32**. Reagents and conditions: (a) NH_4SCN, EtOH, 78 °C; (b) methyl chlorooxoacetate, THF, rt; (c) DBU, THF, 50 °C; (d) $PhCH_2CH_2I$, BSA, acetonitrile, 82 °C.

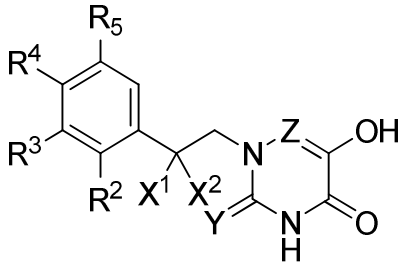
Table 1. Inhibition of human DAAO by 6-hydroxy-1,2,4-triazine-3,5(2H,4H)-dione derivatives.



Compd	R	IC_{50} (μM) [a]	Compd	R	IC_{50} (μM) [a]
11a	CH_3	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

[a] Values are mean ± SD of at least four experiments.

Table 2. Inhibition of human DAAO by analogs of **11e**.



Compd	R^2	R^3	R^4	X^1	X^2	Y	Z	IC_{50} (μM) [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
11m	F	H	H	H	H	O	N	0.08 ± 0.03
11n	H	F	H	H	H	O	N	0.06 ± 0.02
11o	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	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

[a] Values are mean ± SD of at least four experiments except for compound **19f** (n = 2).

Table 3. Metabolic stability of DAAO inhibitors in mouse liver microsomes

cmpd	tPSA (Å^2)	% remaining after 60 min incubation	
		w/ UDPGA	w/ NADPH
7	47	5	ND[b]
8b	62	59	92
11e	82	≥99	≥99
11h	82	≥99	≥99
30	97	≥99	87
32	70	≤1	ND[b]

[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 (Glycine)	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
	V_d (L/kg)	0.5	0.8
	$T_{1/2}$ (h)	0.2	0.9
	AUC_{last} (μg*h/mL)	12.7[b]	16.3
po	T_{max} (h)	0.25	0.08
	C_{max} (μg/mL)	6.6	13.8
	AUC_{last} (μg*h/mL)	3.9	12.9
	Brain to plasma ratio	ND[c]	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.

Table of Contents Graphic