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Design and Synthesis of a Novel Family of Triazine-Based Inhibitors of Sorbitol Dehydrogenase with Oral Activity: 1-{4-[3*R*,5*S*-Dimethyl-4-(4-methyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]triazin-2-yl}-(*R*) Ethanol

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Abstract—Two new templates, (*R*) 2-hydroxyethyl-pyridine and (*R*) 2-hydroxyethyl-triazine, were used to design novel sorbitol dehydrogenase inhibitors (SDIs). The design concept included spawning of these templates to function as effective ligands to the catalytic zinc within the enzyme through incorporation of optimally substituted piperazino-triazine side chains so as to accommodate the active site in the enzyme for efficient binding. This strategy resulted in orally active SDIs, which penetrate key tissues, for example, sciatic nerve of chronically diabetic rats. The latter template led to the design of the title inhibitor, **33**, which normalized the elevated sciatic nerve fructose by 96% at an oral dose of 10 mg/kg.

Introduction

Sorbitol dehydrogenase (SDH), the second enzyme in the polyol pathway (Fig. 1), in concert with NAD^+, oxidizes sorbitol to fructose, and reduces NAD^+ to NADH. Excess flux through SDH, as would be prevailing under diabetic conditions, creates an imbalance in the cytoplasmic NAD^+/NADH ratio. Williamson et al.[1] have coined the phrase 'pseudohypoxia' to describe the altered cytoplasmic redox state. They also have reported[2] that inhibition of SDH can restore the altered cytoplasmic red-ox state and can influence the early functional changes observed in experimental diabetes, including alterations in vascular albumin permeation, tissue blood flow, and nerve conduction velocity. Also, Geisen et al. have reported on the effects of inhibiting SDH on renal hyperfiltration[3] in diabetic rats. Because these early functional changes may contribute to eventual pathology in affected diabetic tissues, this report has spurred research targeted towards investigating the role of SDH in the field of diabetic complications.[4–7]

The first reported in vivo active sorbitol dehydrogenase inhibitor (SDI) is 4-(2-hydroxymethyl-pyrimidin-4-yl)-piperazin-1-sulfonic acid dimethylamide, **1**.[3] It is a relatively weak SDI, with a very short plasma half-life in diabetic rats.[8] The crucial role of 2-hydroxymethylpyrimidine of **1**, in liganding to the catalytic zinc atom in SDH and to manifest SDH inhibition activity is supported by the observation that isomers of **1**, **2** and **3**,[9] showed very little SDH inhibition activity and confirmed by the recently resolved single crystal X-ray structure of the ternary complex of recombinant human SDH with NADH and **1**.[10] The crystal structure shows that **1** is positioned in the active site such that both the oxygen of 2-hydroxymethyl and N-1 make contact with the zinc in SDH while the piperazine ring with its sulfamoyl side chain extends out towards the surface of the protein. The impact of replacing the hydroxymethyl group by (*R*) 2-hydroxyethyl group, as in **4**, in enhancing enzyme inhibition potency has also been reported.[9] Subsequent publications have disclosed the design and synthesis of even more potent and longer acting inhibitors, **5** and **6**. Critical contributors to higher potency and longer duration of action included the strategic placement of small lipophilic methyl groups on the piperazine linker and replacement of the metabolically vulnerable

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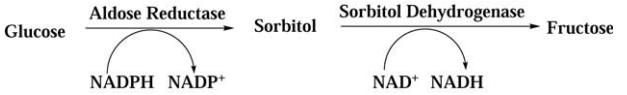


Figure 1. Polyol pathway.

dimethylsulfamoyl group by suitably substituted heterocycles.[11] The most potent inhibitors to date feature triazines substituted with methyl groups[12] (cf. **6**, $R_1 = R_2 = Me$). Structures **1**–**6** are depicted in Chart 1. Overall, our extensive SAR experience suggests that highly potent SDIs feature an effective arrangement of ligands to zinc (2-hydroxyethyl and pyrimidine N-1) and a moderately polar linker with lipophilic methyl groups (dimethylpiperazine) attached to planar nitrogen heterocycles (e.g., pyrimidine, triazine) substituted with lipophilic, small alkyl groups. The practice of seeking multiple chemo-types, in projects directed towards potential therapeutic target enzymes or receptors, is fairly well established in medicinal chemistry (e.g., aldose reductase inhibitors, angiotensin converting enzyme inhibitors and angiotensin II antagonists). We desired SDIs of new chemo-types. At present, there is no report of any in vivo active, non-pyrimidine SDI. Herein, we report our investigations towards the goal and disclose the first examples of new templates, (*R*)-2-hydroxyethyl-*s*-triazine (*R*)-2-hydroxyethyl-pyridine and (*R*)-2-hydroxyethyl-*s*-triazine (*R*)-2-hydroxyethyl-*s*-triazine, that we have used for the design of non-pyrimidine, orally active SDIs, effective in reducing accumulation of fructose in the sciatic nerve of streptozotocin diabetic rats.

Biological Testing

Potential SDIs were screened for in vitro activity against sheep liver and rat and human recombinant SDHs. Some compounds prepared very early in the program were tested only against sheep liver SDH. In general, we found that there were only minor differences in the responses of the inhibitors to different SDHs.[9,11,12] Inhibitors with IC_{50}s ≤ 250 nM were tested for oral in vivo activity in two streptozotocin diabetic rat models, acute and chronic, as measured by the ability of the inhibitors to lower fructose that becomes elevated in diabetic sciatic nerve (see Biological methods under Experimental for detailed procedures).

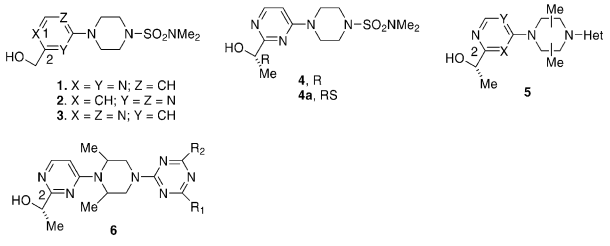


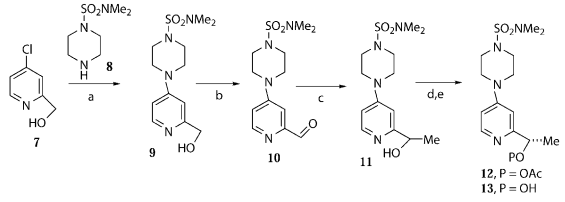
Chart 1.

Chemistry

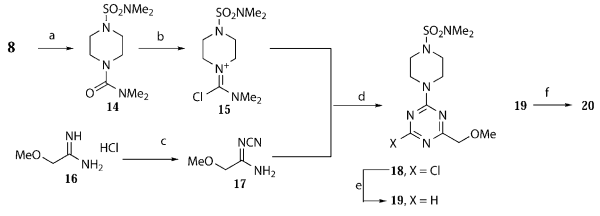
The pyridine targets, **9**, **11**, and **13**, were prepared according to Scheme 1. Reaction of 4-chloro-2-hydroxymethylpyridine,[13] **7**, with excess piperazine-1-sulfonic acid dimethylamide,[9] **8**, gave **9**. Oxidation of **9** to **10** followed by reaction with methylmagnesium bromide, gave the racemic alcohol, **11**. Exposure of **11** to lipase P30[9] in dioxane, separation of the resulting acetate, **12**, followed by base hydrolysis yielded **13**. The assignment of (*R*) configuration to the chiral center was not as rigorous as in the case of the corresponding pyrimidine alcohol, **4**.[9] It was based on the assumption that the lipase, as in the case of **4**, would specifically acylate the racemic alcohol substrate, **11**, to give the (*R*) acetate, **12**. It also turned out that **13**, like **4**, showed positive optical rotation (Scheme 1).

The triazine target, **20**, was prepared by adapting an intriguing literature procedure for the preparation of certain substituted triazines.[14a,b] Compound **8** was reacted with *N*,*N*-dimethylaminocarbamoyl chloride to obtain **14**. A mixture of **14** and phosphorous oxychloride was heated for 30 m to obtain the iminochloride, **15**. Exposure of methoxy-acetamidine, **16** to cyanogen bromide resulted in *N*-cyano-acetamidine, **17**. Thermal cyclization of **15** with **17**, gave the chlorotriazine, **18**, which was sequentially dechlorinated and demethylated to yield the desired triazine, **20** (Scheme 2).

Unlike in the pyridine example, we started with chiral building blocks of established (*R*) configuration to synthesize the desired triazine targets. (*R*) 2-Benzyloxy-propionamide, 21,[15] was reacted with chlorosulfonyl isocyanate to obtain the urea, **22**, which was once more reacted with chlorosulfonyl isocyanate to obtain (*R*)-2-benzyloxy-*N*-ureidocarbonyl-propionamide, **23**. Base promoted cyclization of **23** gave the triazine-dione, **24**, which was heated with phosphorous oxychloride to obtain the dichloro-triazine, **25**. Coupling of **25** with **8** gave **26**, which upon catalytic reduction over Pd/C,



Scheme 1. Reagents and conditions: (a) Et_3N, reflux; (b) MnO_2, CH_2Cl_2, reflux; (c) MeMgBr, THF, reflux; (d) Lipase P30, dioxane, 45 °C, **4d**; (e) NaOH, MeOH, rt.

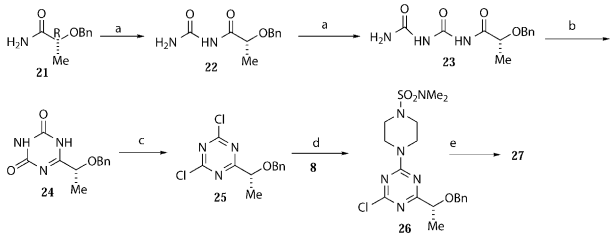


Scheme 2. Reagents and conditions: (a). $ClCONMe_2$–Et_3N–THF, rt; (b) $POCl_3$, 110 °C; (c) CNBr, EtOH–Et_3N; (d) MeCN, reflux; (e) Pd/C, H_2, EtOH; (f) BBr_3, CH_2Cl_2.

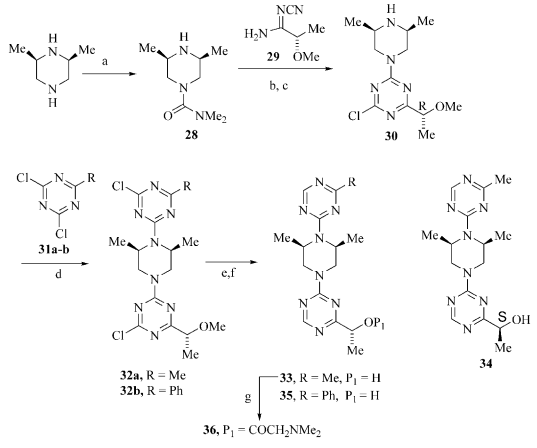
underwent both dechlorination and debenzylation to give the desired triazine, **27**. The enantiomeric purity of **27,** as determined by chiral HPLC, was 90% (Scheme 3).

The triazino-triazines, **33** and **35**, were prepared according to Scheme 4. *N*,*N*-Dimethylcarbomoyl dimethylpiperazine, **28**, prepared from 2,6-dimethylpiperazine, was heated with (*R*)-*N*-cyano-methoxy-propionamidine, **29** to obtain piperazino-triazine, **30**, (cf. preparation of **18**). Reaction of **30** with 1,3 di-chloro-5-methyl (**31a**, R = Me) and 1,3 di-chloro-5-phenyl (**31b**, R = Ph) triazines[16] gave, respectively, **32a** and **32b**. Dechlorination of these compounds followed by their subsequent demethylation by reaction with boron tribromide, gave the targets, with positive optical rotation. As in the case of **27**, **33**, and **35** had ee of 90%. Compound **33** was obtained in higher enantiomeric purity (98% ee) by preparative chiral HPLC. Sufficient sample of *S* enantiomer (94% ee), **34**, was obtained, by chiral separation. Esterification of the hydroxy group in **33** with dimethylamino acetyl chloride gave **36**.

Scheme 5 outlines the synthesis of the cyclopropyl analogue, **40**. Demethylation of **30** with boron tribromide gave **37**, which was first coupled with 1,3-dichloro-5-cyclopropyl-(1,3,5)-triazine, **38**, to obtain **39**, which was then dechlorinated to yield the target.



Scheme 3. Reagents and conditions: (a) $ClSO_2NCo$, MeCN, rt; (b) KOH, H_2O, rt; (c) $POCl_3$, $PhNEt_2$, 70 °C; (d) $NaHCO_3$, DMF; (e) Pd/C, $HCOONH_4$, IPO, 90 °C.

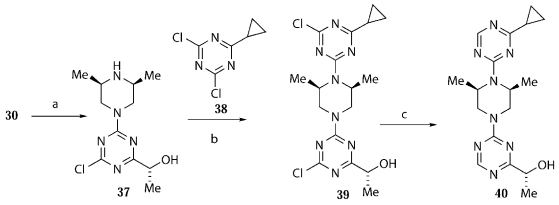


Scheme 4. Reagents and conditions: (a) $ClCONMe_2$, CH_2Cl_2; (b) $POCl_3$, DMF, 110 °C, (c) MeCN, reflux; (d) $NaHCO_3$, DMF, rt; (e) Pd/C, HCl, $HCOONH_4$, IPO; (f) BBr_3, CH_2Cl_2; (g) $ClCOCH_2NMe_2$, Et_3N.

Results and Discussion

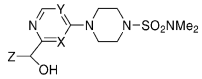
X-ray crystal structure of rat SDH shows that the triad, Cys44, His69 and Glu155, forms the core of ligands to the catalytic zinc in SDH.[17] Based on SAR relative to the different arrangements of pyrimidine nitrogen atoms, as in **2** and **3**, it was speculated that inhibitor **1** (pK_a, 6.1) would bind to the catalytic zinc in SDH through the N-1 of the pyrimidine and oxygen of the 2-hydroxymethyl group.[9] This speculation is now confirmed by Pauly et al.[10] through X-ray of the ternary complex of h-SDH with NADH and **1**. We first prepared hydroxymethylpyridine analogue, **9**, and found it to be a markedly weaker inhibitor (45% inhibition at 10 μM) than **1** (IC_{50} = 246 nM[9]). We expected the hydroxymethylpyridine moiety to serve as well as the hydroxymethylpyrimidine, to function as a zinc ligand. However, the higher pK_a of **9** (9.6) favors protonation, rather than coordination to zinc, at physiological pH. The racemate homologue, **11**, showed only incremental improvement in activity (IC_{50} = 5 μM), over **9**. As anticipated **13** showed a further increase in potency (IC_{50} = 2.5 μM), however small it was. In the absence of any change in pK_a, the trend in SDI potency is in agreement with SAR in the hydroxyethyl-pyrimidine series. Because **13** was still ~90× less potent than **4** in vitro, and because the higher pK_a of the pyridine template (99% protonated at blood pH) did not bode well for penetration into diabetic complications tissues, for example, peripheral nerve, we discontinued efforts around the hydroxyethyl-pyridine template. SDH inhibition data of these compounds is included in Table 1.

In search of a template significantly less basic than pyrimidines (e.g., **4**) and pyridines for designing essentially non-basic SDIs (pK_a ~4), we decided to explore an *s*-triazine with hydroxyalkyl side chain as a potential zinc



Scheme 5. Reagents and conditions: (a) BBr_3, CH_2Cl_2; (b) $NaHCO_3$, DMF; (c) Pd/C, $HCOONH_4$, HCl, IPO.

Table 1. In vitro SDH inhibition data of sulfamoyl-pyridines and triazines



No.	X	Y	Z	IC_{50} (μM)		
				Sheep[a]	Rat[a]	Human[a]
9	CH	CH	H	>10		
11	CH	CH	Me (RS)	5		
13	CH	CH	Me (R)	2.5		
20	N	N	H	5		
27	N	N	Me (R)		0.20	0.22

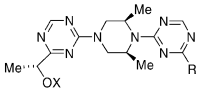
[a] n = 3, SE ≤20%.

ligand. Because *s*-triazine is non-basic and is strongly electron withdrawing, it was expected that SDIs according to the design strategy would have pK_as close to 4. This is supported by the stark contrast in pK_as between 2-aminopyrimidine (5.9), 2-aminotriazine (3.1). As there is very little change in pK_a from 2-aminopyrimidine to pyrimidine, **1** (6.1), it was expected that **20** would show a pK_a not much different than that of aminotriazine. The measured pK_a of **20** is 3.4. Because there would be very little protonation of **20** at pH 7.1, we hoped that **20** would compete more effectively for ligation to zinc, rather than to proton, and thus would be at least as potent an inhibitor as is **1**. However, **20** (IC_{50} = 5 μM), like **9**, was significantly less potent than **1**. Because there is very little information in the literature on zinc coordinating ability of 2-hydroxymethyltriazine, we are left with the obvious speculation that the potential liganding nitrogen atom of the *s*-triazine backbone may not be an effective coordinator to the catalytic zinc atom. However, as in the pyridine case, the hydroxyethyl compound, **27**, was more potent than **20**, thus reiterating the importance of enantiomeric ®-hydroxyethyl group. Because it was sufficiently potent in vitro (IC_{50} = 200 nM), it was tested in vivo, in the acute test, and was found to normalize the elevated fructose level in the diabetic rat sciatic nerve, by 50%, at an oral dose of 10 mg/kg. It was the first time that a non-pyrimidine template had yielded an inhibitor, which was active both in vitro and in vivo. However, **27** was markedly less potent than **4** (IC_{50} = 27 nM, ED_{50} = 1.6 mg/kg),[9] both in vitro and in vivo.

According to Johansson et al.,[17] the SDH substrate cleft is significantly more polar than the alcohol dehydrogenase substrate cleft. However, sorbitol can stack against the hydrophobic benzene ring of Phe118. Other hydrophobic amino acid residues within the cleft include Tyr200 and Tyr50. Our extensive SAR in the pyrimidine-based SDIs is in agreement with this subtle blend of polar and hydrophobic environment within the SDH active site cleft (vide supra), and it recently, led to the discovery of a highly potent pyrimidino-triazine family of SDIs[11] (cf. **6** with $R_1 = R_2 = Me$). Furthermore, in spite of the experience with **27**, the discovery of **6** was the impetus to investigate whether 2-hydroxymethyl- and 2-hydroxyethyl-triazines with potency enhancing piperazine-triazine side chains.

Substituents on the triazine not bearing the hydroxymethyl ligand included Me (**33**), c-Pr (**40**), and Ph (**35**). All compounds showed SDH inhibition activity both in vitro and in vivo (Table 2). SAR for in vitro activity in this series is in agreement with the pyrimidino-triazine series.[12] The Me and c-Pr analogues are nearly equipotent, while the Ph analogue is slightly less potent than both Me and c-Pr analogues. The Me analogue, **33**, showed the best combination of in vitro and in vivo activities. It was very potent orally giving near normalization of sciatic nerve fructose in both acutely (at 5 mg/kg) and chronically (at 10 mg/kg) diabetic rats. As expected the *S*-enantiomer, **34**, was less potent, both in vitro and in vivo. The distinctly different fructose inhibition responses with **33** and **35** are similar to those obtained with **4** and its *S*-enantiomer,[9] suggesting that the chiral hydroxyethyl group is fairly stable to in vivo oxido-reduction. The c-Pr analogue, **40**, was only slightly less potent than **33**, while the more lipophilic Ph analogue, **35**, was significantly less potent than **33**. Unlike, SDIs with a pyrimidine moiety[9,11] (e.g., **1**), the triazine-triazines were very poorly soluble in water, both at physiological pH and at acidic pH, as low as 2. We prepared **36**, a potential prodrug of **33,** with a basic dimethylamino side-chain, which was freely soluble in 10% HCl and in 1 M citric acid. As expected, it showed little SDI activity, in vitro. While it was hoped that the vastly improved opportunity for solubility of **36** in gastric fluids would manifest in greater vivo potency over **33**, the prodrug was only as potent as the parent. In the absence of pharmacokinetic data, it can be speculated that administration of **36** to diabetic rats did not result in more effective delivery of the parent drug to blood, nerve tissue, or both.

Table 2. In vitro and in vivo SDH inhibition data of triazino-triazines



No.	R	X	IC_{50} (nM)		% normalization of fructose		
			Rat[a]	Human[a]	mg/kg	Acute	Chronic
33	Me	H	42	59	10	114	96
					5	91	
34[b]	Me	H	400	390	5	35	
35	Ph	H	140	90	10	75	
36	Me	$COCH_2NMe_2$	—	—	5	89	
40	c-Pr	H	36	42	3	87	77[c]

[a] n = 3, SE for the assays, ≤15%.
[b] (*S*)-Enantiomer of **33**.
[c] Tested at 9 mg/kg.

Conclusion

We examined the potential of two new templates, (*R*)-hydroxyethyl-pyridine and (*R*)-hydroxyethyl-triazine for building novel SDIs. Because there was no precedent in the literature for either of these templates to serve as strong ligands to zinc, we investigated the possibility of spawning the new templates to construct novel SDIs, using extant SAR developed in the design of potent pyrimidine-based SDIs in our laboratory. Hydroxyethyl-pyridine SDI, **13**, was found to be a rather poor inhibitor. It is surmised that this compound is so basic that it is nearly fully protonated at physiological pH, thus depleting its potential ability to effectively ligand to the catalytic zinc in SDH. Progressing from **13**, we synthesized the first examples of non-basic non-pyrimidine SDIs, **20** and **27**. Inhibitor **27** showed good activity both in vitro and in vivo. This provided the impetus to append the earlier discovered potentiating substituted triazine side chains as in **6.** Several (*R*) hydroxyethyl-triazino-triazines from this approach were found to be

quite potent inhibitors, both in vitro and in vivo. Inhibitors, **33**, **35** and **40** showed IC_{50}s in the range of 40–90 nM and all were orally active in reducing fructose production in the sciatic nerve of streptozotocin diabetic rats. The best inhibitor in this series was **33**. It was quite potent and normalized sciatic nerve fructose by 114 and 96% at an oral dose of 10 mg/kg, in the acute and chronic tests, respectively. Inhibitor **40** was a close second to **33**, in vivo. Even the best triazine-triazine inhibitor, **33**, was still considerably less potent than the best members of the hydroxyethyl-pyrimidine series, for example, **6** ($R_1 = R_2 = Me$). It appears that ligand strengths of the templates increase in the order, hydroxyethyl-pyridine > hydroxyethyl-triazine >> hydroxyethyl-pyrimidine.

Experimental

Melting points were determined on a Thomas-Hoover capillary melting point apparatus, and are uncorrected. ^{1}H NMR spectra were obtained on a Bruker AM-250 (Bruker Co., Billerica, MA, USA), a Bruker AM-300, a Varian XL-300 (Varian Co., Palo Alto, CA, USA), or a Varian Unity 400 at about 23 °C at 250, 300, or 400 MHz for proton. The solvent was $CDCl_3$, unless otherwise indicated. Chemical shifts are reported in parts per million (δ) relative to residual chloroform (7.26 ppm) or dimethylsulfoxide (2.49 ppm), as an internal reference. The peak shapes and descriptors for the peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; c, complex; br, broad; app, apparent. Low-resolution mass spectra were obtained under thermospray (TS) conditions on a Fisons (now Micromass) Trio 1000 Mass Spectrometer (Micromass Inc., Beverly, MA, USA), under chemical-ionization (CI) conditions on a Hewlett Packard 5989A Particle Beam Mass Spectrometer (Hewlett Packard Co., Palo Alto, CA, USA), or under atmospheric pressure chemical ionization (APCI) on a Fisons (now Micromass) Platform II Spectrometer. Optical rotations were obtained on a Perkin-Elmer 241 MC Polarimeter (Perkin-Elmer, Norwalk, CT, USA) using a standard path length of 1 dcm at about 23 °C at the indicated concentration in the indicated solvent.

Liquid column chromatography was performed using forced flow (flash chromatography) of the indicated solvent on either Baker Flash column (40 μm, J. T. Baker, Phillipsburg, NJ, USA) or Flash column60 (EM Sciences, Gibbstown, NJ, USA) in glass columns or using low nitrogen or air pressure in Flash 40™ or Flash 12™ (Biotage, Charlottesville, VA, USA) cartridges. Analytical and preparative chiral HPLC separations were done using chiralpak AD columns. Unless otherwise indicated the optical purity of chiral target analogues were at least 90% ee. Reactions requiring the use of hydrogen gas at pressures greater than 1 atmosphere were run using a Parr hydrogen apparatus (Parr Instrument Co., Moline, IL, USA). Unless otherwise specified, reagents were obtained from commercial sources.

4-[2-Hydroxymethyl)-pyridine-4-yl]-piperazine-1-sulfonic acid dimethylamide (9). A mixture of 4-chloro-2-hydroxymethyl-pyridine (21 mmol, 3.03 g), *N*,*N*-dimethylsulfamoyl-piperazine (37.1 mmol, 7.17 g) and Et_3N (42.2 mmol, 5.9 mL) was refluxed overnight. Excess Et_3N was removed and the residue was quenched with water (25 mL) and the resulting solid was filtered and the solid collected. This was crystallized from hot H_2O, (43%, 2.7 g): mp 150–151 °C. ^{1}H NMR (DMSO-d_6) δ 2.7 (s, 6H), 3.3 (m, 4H), 3.4 (m, 4H), 4.4 (s, 2H), 6.7 (dd, $J = 5.7$, 2.9 Hz, 1H), 6.9 (d, $J = 2.4$ Hz, 1H), 8.1 (d, $J = 5.7$ Hz, 1H).

4-(2-Formyl-pyridin-4-yl)-piperazin-1-sulfonic acid dimethylamide (10). To a solution of 4-[2-hydroxymethyl)-pyridine-4-yl]-piperazine-1-sulfonic acid dimethylamide, **8**, (0.65 mmol, 195 mg) in $CHCl_3$ (20 mL) was added manganese-di-oxide (0.6 g) and refluxed for 1.5 h. The reaction mixture was filtered and the filtrate was evaporated to obtain the crude product as an oil (68%, 133 mg). ^{1}H NMR (DMSO-d_6) δ 2.7 (s, 6H), 3.3 (m, 4H), 3.4 (m, 4H), 6.7 (dd, $J = 5.7$, 2.9 Hz, 1H), 6.9 (d, $J = 2.4$ Hz, 1H), 8.1 (d, $J = 5.7$ Hz, 1H), 9.4 (s, 1H).

4-[*R*,*S*-2-Hydroxyethyl)-pyridin-4-yl]-piperazine-1-sulfonic acid dimethylamide (11). To a solution of 4-(2-formyl-pyridin-4-yl)-piperazin-1-sulfonic acid dimethylamide, **10**, (4.4 mmol, 1.2 g), in dry THF, under a blanket of dry nitrogen, was added methylmagnesium bromide (3 M in Et_2O, 2.64 mL) and the reaction was refluxed for 3 h. The reaction was quenched with NH_4Cl and was extracted with CH_2Cl_2 (20 mL). The CH_2Cl_2 extract was washed with brine (10 mL) and the washed organic portion was collected, dried, and the residue was purified by flash column chromatography (5% MeOH/CH_2Cl_2 to yield the product (30%, 375 mg). ^{1}H NMR (DMSO-d_6) δ 1.5 (d, $J = 6.8$ Hz, 3H), 2.7 (s, 6H), 3.3 (m, 4H), 3.4 (m, 4H), 4.6 (q, $J = 6.8$ Hz, 1H), 6.7 (dd, $J = 5.7$, 2.9 Hz 1H), 6.9 (d, $J = 2.4$ Hz, 1H), 8.1 (d, $J = 5.7$ Hz, 1H).

Acetic acid 1-[4-(4-dimethylsulfamoyl-piperazin-1-yl)-pyridin-2-yl]-(*R*)-ethyl ester (12). A mixture of 4-[*R*,*S*-2-hydroxyethyl)-pyridin-4-yl]-piperazine-1-sulfonic acid dimethylamide, **11**, (1.11 mmol, 350 mg), vinyl acetate (111 mmol, 10.3 mL), dioxane (15 mL), and Lipase P30 (35 mg) was heated at 45 °C for 4 days. The reaction was filtered and the filtrate was evaporated to dryness. The residue was purified by flash column chromatography (5% MeOH/CH_2Cl_2 to obtain the product (84%, 167 mg): $[\alpha]_D$ +52.9° (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6) δ 1.5 (d, $J = 6.8$ Hz, 3H), 2.2 (s, 3H), 2.7 (s, 6H), 3.3 (m, 4H), 3.4 (m, 4H), 5.6 (q, $J = 6.8$ Hz, 1H), 6.7 (dd, $J = 5.7$, 2.9 Hz 1H), 6.9 (d, $J = 2.4$ Hz, 1H), 8.1 (d, $J = 5.7$, 1H).

4-[*R*-2-Hydroxyethyl)-pyridine-4-yl]-piperazine-1-sulfonic acid dimethylamide (13). A solution of the acetate, **12** (0.39 mmol, 139 mg), in dioxane (3 mL), MeOH (0.5 mL), and 1 N NaOH (0.25 mL) was stirred for 1 h at room temperature. The reaction was evaporated to dryness and the residue was slurried an a mixture of EtOAc/H_2O (1 mL/5 mL) and the resulting white solid

was collected (55%, 68 mg); mp 168–169 °C; $[\alpha]_D$ +24.0° (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6) 1.5 (d, *J* = 6.8 Hz, 3H), 2.7 (s, 6H), 3.3 (m, 4H), 3.4 (m, 4H), 4.6 (q, *J* = 6.8 Hz, 1H), 6.7 (dd, *J* = 5.7, 2.9 Hz 1H), 6.9 (d, *J* = 2.4 Hz, 1H), 8.1 (d, *J* = 5.7, 1H).

4-Dimethylsulfamoyl-piperazine-1-carboxylic acid dimethylamide (14). To a solution of *N*,*N*-dimethylsulfamoyl-piperazine, 8, (5.2 mmol, 1.0 g) in THF (10 mL) and triethyl amine (0.75 mL) was added *N*,*N*-dimethylaminocarbamoyl chloride (5.2 mmol, 0.5 mL) and the reaction was stirred for 2 h at room temperature. The precipitated triethyl amine hydrochloride was filtered off and the filtrate was evaporated to obtain a white solid, which was crystallized from a 1:1 mixture of EtOAc and *n*-hexane to yield the title compound (88%). ^{1}H NMR δ 2.4 (s, 6H), 2.7 (s, 6H), 2.9 (m, 4H), 3.1 (m, 4H).

4-(Chloro-dimethylamino-methylene)-piperazine-1-sulfonic acid dimethylamide chloride (15). A mixture of 4-dimethylsulfamoyl-piperazine-1-carboxylic acid dimethylamide, **14**, (2 mmol, 530 mg) and phosphorus oxychloride (2 mmol, 0.2 mL) was heated to 110 °C for 0.5 h. After cooling, the reaction solidified to yield the title compound, which was immediately used in the next step below.

2-Methoxy-*N*-cyano-acetamidine. (17). To an ice-cold solution of 2-methoxy-acetamidine hydrochloride, **16**, (0.1 mol, 12.5 g) in ethanol (100 mL) and Et_3N (0.2 mol, 27.8 mL) was added dropwise a solution of cyanogen bromide in acetonitrile. After 1 h, the solvents were removed by evaporation and H_2O (100 mL) was added to the resulting residue. It was then extracted with EtOAc (2 × 100 mL). The EtOAc extract was collected, dried, filtered and the filtrate was evaporated to obtain a light yellow solid (72%, 8.2 g): mp 101–103 °C. NMR δ 3.4 (s, 3H), 3.7 (s, 2H), 5.8 (b, 1H), 6.5 (b, 1H).

4-(4-Chloro-6-methoxymethyl-[1,3,5]triazin-2-yl)-piperazine-1-sulfonic acid dimethylamide (18). 4-(chloro-dimethylamino-methylene)-piperazine-1-sulfonic acid dimethylamide chloride, **15**, was dissolved in acetonitrile (10 mL), to it was added 2-methoxy-*N*-cyano-acetamidine, **16** (2 mmol, 226 mg and refluxed for 2 h. Evaporation of the excess acetonitrile, gave a residue, which was purified by flash column chromatography to yield the title compound (58%, 410 mg): mp 143–144 °C. ^{1}H NMR δ 2.8 (s, 6H), 3.3 (m, 4H), 3.5 (s, 3H), 3.9 (b, 4H), 4.4 (s, 2H).

4-(4-Methoxymethyl-[1,3,5]triazin-2-yl)-piperazine-1-sulfonic acid dimethylamide (19). A mixture of 4-(4-chloro-6-methoxymethyl-[1,3,5]triazin-2-yl)-piperazine-1-sulfonic acid dimethylamide, **18** (1.0 mmol, 350 mg), palladium-carbon (100 mg), EtOH (10 mL), and NaOAc (2.4 mmol, 196 mg) was hydrogenated in a Parr shaker at 45 lb/sq inch for 1 h. The catalyst was filtered off and the filtrate was concentrated. The resulting white precipitate was filtered and the residue was purified by flash column chromatography (10% MeOH, CH_2Cl_2) to obtain the title compound (70%, 224 mg): mp 78–81 °C. ^{1}H NMR δ 2.8 (s, 6H), 3.3 (m, 4H), 3.5 (s, 3H), 3.9 (m, 4H), 4.4 (s, 2H), 8.6 (s, 1H).

4-(4-Hydroxymethyl-[1,3,5]triazin-2-yl)-piperazine-1-sulfonic acid dimethylamide (20). To an ice-cold solution of 4-(4-chloro-6-methoxymethyl-[1,3,5]triazin-2-yl)-piperazine-1-sulfonic acid dimethylamide, **19** (1.5 mmol, 474 mg) in CH_2Cl_2 (20 mL) was added dropwise a solution of boron tribromide (1 M in CH_2Cl_2, 3 mL). After 2 h, the reaction was quenched with H_2O (5 mL) and sufficient 10% KOH solution to raise the pH to 9. The CH_2Cl_2 layer was collected, dried, filtered and the filtrate was evaporated to a solid residue. This was crystallized from acetone to obtain the title compound (68%, 275 mg): mp 157–159 °C. ^{1}H NMR δ 2.8 (s, 6H), 3.3 (m, 4H), 3.9 (m, 4H), 4.4 (s, 2H), 5.2 (b. 1H), 8.6 (s, 1H).

2-Benzyloxy-*N*-ureidocarbonyl-(*R*)-propionamide (23). To a suspension of 2-benzyloxy-propionamide, **21**, (26.6 mmol, 4.77 g) in CH_3CN (100 mL) at room temperature was added dropwise chlorosulfonyl isocyanate (26.6 mmol, 4.1 mL) in CH_3CN (20 mL). After 1 h the reaction was concentrated, then carefully quenched with H_2O (20 mL) and allowed to stir at room temperature for 1 h. The precipitated solid (**22**) was filtered, collected and air-dried to obtain (2-benzyloxy-propionyl)-urea (62%, 3.66 g). ^{1}H NMR δ 1.2 (d, *J* = 6.5 Hz, 3H), 4.0 (q, *J* = 6.5 Hz, 1H), 4.4 (dd, *J* = 11.7 Hz, 2H), 7.3 (m, 5H), 7.7 (s, 1H), 10.0 (S, 1H). This compound was re-subjected to the above reaction conditions to convert (2-benzyloxy-propionyl)-urea to 2-benzyloxy-*N*-ureidocarbonyl-propionamide, using, (2-benzyloxy-propionyl)-urea (16.5 mmol, 3.66 g), chlorosulfonyl isocyanate (28.8 mmol, 2.5 mL), and acetonitrile (80 mL). The yield of 2-benzyloxy-*N*-ureidocarbonyl-propionamide was 59% (2.56 g). ^{1}H NMR δ 1.4 (d, *J* = 6.5 Hz, 3H), 4.2 (q, *J* = 6.5 Hz, 1H), 4.6 (dd, *J* = 12 Hz, 2H), 7.4 (m, 5H), 9.8 (s, 1H), 11.1 (s, 1H).

6-(1-Benzyloxy-(*R*)-ethyl)-1*H*-[1,3,5]triazine-2,4-dione (24). To an ice-cold suspension of 2-benzyloxy-*N*-ureidocarbonyl-propionamide, **23**, (9.4 mmol, 2.5 g) in H_2O (15 mL) was added KOH (28 mmol, 1.6 g) in water (10 mL). The reaction temperature was slowly raised to room temperature, and the reaction was allowed to stir for 1 h. Sufficient AcOH was added to adjust the pH of the reaction to 5, and the resulting cloudy solution was extracted with chloroform (3 × 20 mL). The $CHCl_3$ layer was collected, dried, filtered and the filtrate was concentrated to obtain a residue, which was triturated with IPE to obtain the title compound (74%, 1.72 g). ^{1}H NMR δ 1.4 (d, *J* = 6.5 Hz, 3H), 4.2 (q, *J* = 6.5 Hz, 1H), 4.6 (dd, *J* = 12 Hz, 2H), 7.4 (m, 5H), 11.2 (s, 1H), 12.1 (s, 1H).

2-(1-Benzyloxy-(*R*)-ethyl)-4,6-dichloro-[1,3,5]triazine (25). A mixture of 6-(1-benzyloxy-ethyl)-1H-[1,3,5]triazine-2,4-dione, **24**, (6.1 mmol, 1.5 g), phosphorus oxychloride (18.2 mmol, 1.7 mL), and diethyl aniline (1 mL) was heated at 70 °C for 1 h. Excess phosphorus oxychloride was removed and the residual oil was extracted with chloroform (2 × 20 mL); the extract was washed with

water (3 × 20 mL); the $CHCl_3$ layer was collected, dried, filtered and the filtrate was evaporated to obtain an oily product. This oily product was purified by flash column chromatography (90% hexane–EtOAc) to obtain the compound (35%, 611 mg). ^{1}H NMR δ 1.6 (d, J = 6.5 Hz, 3H), 4.0 (q, J = 6.5 Hz, 1H), 4.6 (dd, J = 12 Hz, 2H), 7.3 (m, 5H).

4-[4-(1-Benzyloxy-(*R*)-ethyl)-6-chloro-[1,3,5]triazin-2-yl]-piperazine-1-sulfonic acid dimethylamide (26). A mixture of 2-(1-benzyloxy-(*R*) ethyl)-4,6-dichloro-[1,3,5]triazine, **25**, (0.75 mmol, 212 mg), *N*,*N*-dimethylsulfamoyl piperazine, **8**, (0.75 mmol, 144 mg), $NaHCO_3$ (1.5 mmol, 125 mg), and DMF (3 mL) was stirred overnight at room temperature. EtOAc (15 mL) and H_2O (20 mL) were added and the EtOAc extract was collected and washed with H_2O (2 × 10 mL). The EtOAc layer was collected, dried and filtered, and the filtrate was evaporated to obtain the title compound (97%, 320 mg). ^{1}H NMR 1.5 (d, J = 6.6 Hz, 3H), 2.8 (s, 6H), 3.3 (m, 4H), 4.0 (m, 4H), 4.2 (q, J = 6.6, 1H), 4.6 (dd, J = 12 Hz, 2H), 7.3 (m, 5H); MS (AP+), 441.1

4-[4-(*R*)-1-Hydroxy-ethyl)-[1,3,5]triazin-2-yl]-piperazine-1-sulfonic acid dimethylamide (27). A mixture of 4-[4-(1-benzyloxy-*R* ethyl)-6-chloro-[1,3,5]triazin-2-yl]-piperazine-1-sulfonic acid dimethylamide, **26**, (0.73 mmol, 320 mg), (Pd/C 910%, 640 mg), HCl (2 M in ether, 4.4 mmol, 2.2 mL), ammonium formate (15 mmol, 915 mg) and IPO (10 mL) was heated at 90 °C for 2 h. The reaction was cooled and filtered, and to the filtrate was added chloroform (20 mL) and aq saturated $NaHCO_3$ (20 mL). The $CHCl_3$ layer was collected, dried, filtered and the filtrate was evaporated to dryness. The resulting residue was purified by flash column chromatography (96% $CHCl_3$/MeOH) to yield the title compound (59%, 136 mg): mp 124–125 °C. ^{1}H NMR δ 1.5 (d, J = 6.6 Hz, 3H), 2.8 (s, 6H), 3.3 (m, 4H), 4.0 (m, 4H), 4.4 (s, 2H), 4.6 (q, J = 6.6 Hz, 1H), 8.6 (s, 1H), 7.3 (m, 5H); MS (AP+), 317.1.

2,6-Dimethyl-piperazine-1-carboxylic acid dimethylamide (28). To an ice-cold solution of 2,6-dimethyl piperazine (179 mmol, 20.4 g), CH_2Cl_2 (200 mL), and (214 mmol, 29.9 mL) was added dropwise dimethylcarbamoyl chloride (179 mmol, 16.4 mL). After 4 h the reaction was quenched with a saturated $NaHCO_3$ solution and the CH_2Cl_2 layer was collected, dried, filtered and the filtrate was evaporated to dryness to obtain an orange oil (70%, 23.1 g). NMR δ 1.1 (d, J = 4 Hz, 6H), 2.4 (m, 4H), 2.7 (s, 6H), 2.9 (m, 2H).

(*R*)-2-Methoxy-*N*-cyanopropinamidine (29). To an ice-cold solution of *R* 2-methoxy-propinamidine[9] (3.8 mmol, 523 mg) in absolute EtOH (5 mL) and Et_3N (7.6 mmol, 1.1 mL) was added cyanogen bromide (2.9 M in CH_2Cl_2, 1.3 mL). After the addition, the reaction temperature was slowly raised to room temperature and stirred for 3 h. Evaporation of all volatile liquids gave a solid residue, which was extracted with EtOAc. The EtOAc layer was washed with water and the EtOAc layer was collected, dried, filtered and the filtrate was evaporated to dryness to obtain the title compound (85%, 410 mg). ^{1}H NMR δ 1.4 (d, J = 6.5, 3H), 3.1 (q, J = 6.5 Hz, 1H), 3.4 (s, 3H).

2-Chloro-4-(3R,5S-dimethyl-piperazin-1-yl)-6-(*R*-1-methoxy-ethyl)-[1,3,5]triazine (30). A mixture of 2,6-dimethyl-piperazine-1-carboxylic acid dimethylamide, **28** (51 mmol, 9.4 g) and phosphorus oxychloride (51 mmol, 4.8 mL) was heated at 110 °C for 30 min. After cooling the reaction to room temperature, 2-methoxy-*N*-cyanopropinamidine (51 mmol, 6.5 g) and acetonitrile was added and then refluxed for 2 h. The reaction mixture was evaporated to dryness and the residue was purified by flash column chromatography (90% CH_2Cl_2/MeOH) to obtain the compound (24%, 5.1 g). ^{1}H NMR δ 1.2 (dd, J = 5.6 Hz, 6H), 1.4 (d, J = 6.8 Hz, 3H), 2.8–3.1 (m, 4H), 3.4 (s, 3H), 4.1 (q, J = 6.8 Hz, 1H), 4.7 (m, 1H), 4.8 (m, 1H). MS (AP+), 286.0.

2-Chloro-4-[2-(4-chloro-6-methyl-[1,3,5]triazine-2-yl)-3*R*,5*S*-dimethyl-piperazin-1-yl]-6-(*R*-1-methoxy-ethyl)-[1,3,5]triazine (32a). A mixture of 2-chloro-4-(3,5-dimethyl-piperazin-1-yl)-6-(1-methoxy-ethyl)-[1,3,5]triazine, **30**, (1.12 mmol, 321 mg), 2,4-dichloro-6-methyl triazine, **31a** (1.12 mmol, 184 mg), $NaHCO_3$ (2.24 mmol, 189 mg) and DMF (3 mL) was stirred at room temperature overnight and was diluted with EtOAc (20 mL) and water (30 mL). The EtOAc extract was collected, dried, filtered and the filtrate was evaporated to a residue, which was purified by flash column chromatography (99% CH_2Cl_2/MeOH) to obtain the title compound (49%, 225 mg). ^{1}H NMR δ 1.2 (dd, J = 5.6 Hz, 6H), 1.4 (d, J = 6.8 Hz, 3H), 2.4 (s, 3H), 3.2 (m, 2H), 3.4 (s, 3H), 4.2 (q, J = 6.8 Hz, 1H), 4.7 (m, 1H), 4.8 (m, 1H), 5.0 (m, 2H). MS (AP+), 412.9.

1-{4-[3*R*,5*S*-Dimethyl-4-(4-methyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]triazin-2-yl}-*R*-ethanol (33). A mixture of 2-chloro-4-[2-(4-chloro-6-methyl-[1,3,5]triazine-2-yl)-3*R*,5*S*-dimethyl-piperazin-1-yl]-6-(1-methoxy-ethyl)-[1,3,5]triazine, **32a**, (051 mmol, 211 mg), Pd/C catalyst (10%, 84 mg), HCl (2 M in Et_2O, 0.76 mmol, 0.38 mL), ammonium formate (5.1 mmol, 322 mg) and IPO (8 mL) was stirred at 90 °C for 2 h. After cooling the reaction it was diluted with CH_2Cl_2 (20 mL) and was filtered. The filtrate was evaporated to dryness and the residue was partitioned between chloroform and aq saturated sodium bicarbonate. The $CHCl_3$ layer was collected, dried, filtered and the filtrate was evaporated to a residue, which was purified by flash column chromatography (99% CH_2Cl_2/MeOH) to obtain 2-[3*R*,5*S*-dimethyl-2-(4-methyl-[1,3,5]triazine-4-yl)-piperazin-1-yl]-4-(*R* 1-methoxy-ethyl)-[1,3,5]triazine (97%, 170 mg), [^{1}H NMR δ 1.2 (dd, J = 5.6 Hz, 6H), 1.4 (d, J = 6.8 Hz, 3H), 2.4 (s, 3H), 3.2 (m, 2H), 3.4 (s, 3H), 4.2 (q, J = 6.8 Hz, 1H), 4.7 (m, 1H), 4.8 (m, 1H), 5.0 (m, 2H), 8.5 (s, 1H), 8.6 (s, 1H). MS, AP+407.0] which was demethylated according to the procedure for preparation of **5**. This material (ee, 90%) was further purified using preparative chiral HPLC [column, Chiralpak AD (5 cm × 50 cm; eluent, hexane–isopropanol, 85:15; flow rate, 75 mL/min; detect., 250 nM; t_R, 60 min) to obtain the title compound (76%; ee, 98%)]: mp 136–138 °C; $[\delta]_D$ +14.4° (1.19 mg/mL, MeOH). ^{1}H NMR δ 1.2 (b, 6H),

1.5 (d, $J=6.8$ Hz, 3H), 2.4 (s, 3H), 3.2 (b, 2H), 4.6 (q, $J=6.8$ Hz, 1H), 4.8 (m, 2H), 5.0 (m, 2H), 8.4 (s, 1H), 8.5 (s, 1H). MS (AP+)S, 331.0.

1-{4-[3*R*,5*S*-Dimethyl-4-(4-methyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]triazin-2-yl}-(*S*) ethanol (34). Preparative chiral HPLC purification of 1-{4-[3*R*,5*S*-dimethyl-4-(4-methyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]triazin-2-yl}-(R) ethanol of 90% ee (**32**) according to the conditions described above gave the title compound (20%; ee, t_R, 62 min, 96%); mp 136–138 °C; $[\alpha]_D$ −13.8° (1.19 mg/mL, MeOH). ^{1}H NMR δ 1.2 (b, 6H), 1.5 (d, $J=6.8$ Hz, 3H), 2.4 (s, 3H), 3.2 (b, 2H), 4.6 (q, $J=6.8$ Hz, 1H), 4.8 (m, 2H), 5.0 (m, 2H), 8.4 (s, 1H), 8.5 (s, 1H); MS (AP+), 331.0.

Dimethylamino-acetic acid 1-{4-[3*R*,5*S*-dimethyl-4-(4-methyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]triazin-2-yl}-(*R*) ethyl ester (36). A mixture of 1-{4-[3*R*,5*S*-dimethyl-4-(4-methyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]-triazin-2-yl}-*R*-ethanol, **33**, (0.45 mmol, 150 mg), *N*,*N*-dimethylaminoacetyl chloride (2.7 mmol, 440 mg) and Et_3N (5.4 mmol, 0.75 mL) was refluxed overnight. The reaction mixture was evaporated to a residue, which was purified by flash column chromatography (94% CH_2Cl_2/MeOH) to obtain the title compound as a viscous oil (30%, 56 mg). ^{1}H NMR δ 1.2 (m, 6H), 1.6 (d, $J=6.5$ Hz, 3H), 2.4 (m, 9H), 3.2 (m, 2H), 3.3 (s, 2H), 4.7 (m, 2H), 5.0 (m, 2H), 5.6 (q, $J=6.5$ Hz, 1H), 8.5 (s, 1H), 8.5 (s, 1H).

2-Chloro-4-[2-(4-chloro-6-phenyl-[1,3,5]triazine-2-yl)-3*R*,S*5*-dimethylpiperazin-1-yl]-6-(1-methoxy-*R* ethyl)-[1,3,5]triazine (32b). A mixture of 2-chloro-4-(3*R*,5*S*-dimethyl-piperazin-1-yl)-6-(*R*-1-methoxy-ethyl)-[1,3,5]-triazine, **30**, (1.12 mmol, 321 mg), 6-phenyl-2,4-dichloro-triazine, **31b** (1.12 mmol, 184 mg), $NaHCO_3$ (2.24 mmol, 189 mg) and DMF (4 mL) was stirred at room temperature overnight and was diluted with EtOAc (20 mL) and water (20 mL). The EtOAc extract was collected, dried, filtered and the filtrate was evaporated to a residue, which was purified by flash chromatography (99% $CHCl_3$/MeOH), to give a white solid (48%, 225 mg). ^{1}H NMR δ 1.3 (m, 6H), 1.5 (d, $J=6.5$ Hz, 3H), 3.2 (m, 2H), 3.4 (s, 3H), 4.6 (q, $J=6.5$ Hz, 1H), 4.8 (m, 2H), 5.1 (m, 2H), 7.2–7.4 (m, 3H), 8.3 (b, 2H); MS (AP+), 475.3.

1-{4-[3,5-Dimethyl-4-(4-phenyl-[1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]triazin-2-yl}-ethanol (35). 2-Chloro-4-[2-(4-chloro-6-phenyl-[1,3,5]triazine-2-yl)-3*R*,*S*5-dimethyl-piperazin-1-yl]-6-(1-methoxy-*R* ethyl)-[1,3,5]triazine, **31b**, was reacted with boron tribromide according to the procedure for the preparation of **37** (54%). ^{1}H NMR δ 1.3 (m, 6H), 1.5 (d, $J=6.5$ Hz, 3H), 3.2 (m, 2H), 4.6 (q, $J=6.5$ Hz, 1H), 4.8 (m, 2H), 5.1 (m, 2H), 7.2–7.4 (m, 3H), 8.3 (b, 2H), 8.5 (s, 1H), 8.6 (s, 1H); MS (AP+), 393.

1-[4-Chloro-6-(3*R*,5*S*-dimethyl-piperazin-1-yl)-[1,3,5]triazin-2-yl]-(*R*) ethanol (37). To an ice-cold solution of 2-chloro-4-(3*R*,5*S*-dimethyl-piperazin-1-yl)-6-(*R*-1-methoxy-ethyl)-[1,3,5]triazine, **30**, (2.2 mmol, 631 mg) in methylene chloride (7 mL) was added boron tribromide (1 M CH_2Cl_2, 11.04 mmol, 11 mL) and the reaction was stirred for 2 h. After the reaction was allowed to come to room temperature, methylene chloride (10 mL) was added to it followed by a small quantity of water (1 mL) to quench the unreacted boron tribromide. Sufficient saturated aq sodium bicarbonate was added to raise the pH of the reaction solution to 8. The methylene chloride layer was collected, dried, filtered and the filtrate was evaporated to dryness to obtain a residue, which was purified by flash column chromatography (98% CH_2Cl_2/MeOH) to obtain the title compound (65%, 392 mg): mp 126–128 °C. $[\alpha]_D$ +13.0°. ^{1}H NMR δ 1.1 (m, 6H), 1.4 (d, $J=6.8$ Hz, 3H), 2.5 (m, 2H), 2.8 (m, 2H), 4.6 (m, 2H); MS (AP+), 272.0.

1-{4-Chloro-6-[4-(4-chloro-6-cyclopropyl-[1,3,5]triazin-2-yl)-3*R*,5*S*-dimethyl-piperazin-1-yl]-[1,3,5]triazin-2-yl}-ethanol (39). A mixture of 1-[4-chloro-6-(3*R*,5*S*-dimethyl-piperazin-1-yl)-[1,3,5]triazin-2-yl]-(*R*) ethanol, **37**, (0.74 mmol, 200 mg), 2,4-dichloro-6-cyclopropyl-triazine, **38**, (0.74 mmol, 140 mg), $NaHCO_3$ (1.47 mmol, 124 mg) and DMF (4 mL) was stirred at room temperature overnight and was diluted with EtOAc (20 mL) and water (20 mL). The EtOAc extract was collected, dried, filtered and the filtrate was evaporated to a white solid (98%, 306 mg): ^{1}H NMR δ 1.0–1.2 (m, 4H), 1.3 (m, 6H), 1.5 (d, $J=6.8$ Hz, 3H), 2.0 (m, 1H), 3.2 (m, 2H), 4.6 (q, $J=6.8$ Hz, 1H), 4.8 (m, 2H), 5.0 (m, 2H); MS (AP+), 424.9.

1-{4-[4-(4-Cyclopropyl-[1,3,5]triazin-2-yl)-3*R*,5*S*-dimethyl-piperazin-1-yl]-[1,3,5]triazin-2-yl}-ethanol (40). A mixture of 1-{4-chloro-6-[4-(4-chloro-6-cyclopropyl-[1,3,5]triazin-2-yl)-3,5-dimethyl-piperazin-1-yl]-[1,3,5]triazin-2-yl}-ethanol, **39**, (0.72 mmol, 306 mg), Pd/C catalyst (10%, 122 mg), HCl (2 M in Et_2O, 1.08 mmol, 0.54 mL), ammonium formate (7.2 mmol, 454 mg) and isopropanol (7 mL) was stirred at 90 °C for 2 h. After cooling the reaction, it was diluted with methylene chloride (20 mL) and was filtered. The filtrate was evaporated to dryness and the residue was partitioned between chloroform and aq saturated sodium bicarbonate. The chloroform layer was collected, dried, filtered and the filtrate was evaporated to a residue, which was purified by flash column chromatography (99% CH_2Cl_2/MeOH) to obtain a solid, which was triturated with IPE to obtain the title compound (64%, 106 mg): mp 120–121 °C. ^{1}H NMR δ 1.0–1.2 (m, 4H), 1.3 (m, 6H), 1.5 (d, $J=6.8$ Hz, 3H), 2.0 (m, 1H), 3.2 (m, 2H), 4.6 (q, $J=6.8$ Hz, 1H), 4.8 (m, 2H), 5.0 (m, 2H), 8.4 (s, 1H), 8.5 (s, 1H); MS, (AP+), 357.2.

SDH inhibition activity

Recombinant r-, h- or s-SDH was purchased from Pan Vera Corp. (Madison, WI, USA) or Boehringer (Germany). Experimental compounds were dissolved at 5 mM in 20% (v/v) aqueous DMSO, and 25-μL aliquots were added to 0.2 mL of potassium phosphate buffer (pH 7.0) containing NAD^+, iodonitrotetrazolium violet (INT), and h- or s-SDH. Following a 15-min incubation at 25 °C, the reaction was initiated by the addition of 20 mM sorbitol (25 μL). Final concentrations in the

assay were 90 mM potassium phosphate, 1 mM NAD^+, 1 mM INT, 2 mM sorbitol, and 2 nM h-SDH or 8 nM s-SDH. Enzyme activity was assayed with a SLT340 ATTC plate reader (model 16-925, SLT Lab-Instruments, Austria), which measured the increase in the rate of INT reduction at 495 nm at 25 °C over 10 min. IC_{50}s were calculated using a log-linear regression analysis.

Nerve fructose assay in diabetic rats: acute model

Male CD Sprague–Dawley rats (175–225 g) were made diabetic by injection of STZ (17 mg/mL in 0.01 M citrate buffer, pH 4.5, 85 mg/kg body wt) into the tail vein of conscious rats. STZ was administered at approximately 9:00 a.m. on day 1. The animals were maintained with free access to food and water. Test compound was administered by oral gavage (volume of 5 mL/kg) at 4, 7, and 24 h after STZ administration. Four h after the final dose (28 h after STZ administration), animals were sacrificed by cervical dislocation. The left sciatic nerve was excised, weighed, and placed in 1 mL of ice-cold 6% perchloric acid and frozen for fructose analysis at a later date. Weighed sciatic nerves in 6% (w/v) perchloric acid (1 mL) were thawed and homogenized with a polytron (Kinematica, Switzerland). After the mixture was centrifuged, the decanted supernatant was neutralized by the addition of 3.0 M potassium carbonate (100 μL) and re-centrifuged. The fructose contents of the supernatants were determined enzymatically. Briefly, fructose was oxidized to 5-keto-fructose by fructose dehydrogenase (FDH) with concomitant reduction of resazurin to the highly fluorescent resorufin. Final assay concentrations were 0.2 M citric acid, pH 4.5, containing 13.2 M resazurin, 1.7 units/mL of FDH, and 0.068% (v/v) Triton X-100. Reaction mixtures were incubated for 60 min at room temperature in a closed drawer. The sample fluorescence was determined at excitation) (560 nm) and emission (580 nm), and slits of 5 mm each (Perkin-Elmer model LS50B fluorescence spectrophotometer). After the appropriate blanks from each sample were subtracted, nanomole of fructose in each sample was then determined by comparison with a linear regression of the fructose standards.

Nerve fructose assay in diabetic rats: chronic model

Male CD Sprague–Dawley rats (175–225 g) were made diabetic by the injection of STZ (17 mg/mL in 0.01 M citrate buffer, pH 4.5, 85 mg/kg body wt) into the tail vein of conscious rats. STZ was administered at approximately 9:00 a.m. on day 1. The animals were maintained with free access to food and water. On day 8, at 9:00 a.m., test compound was administered by oral gavage (volume of 5 mL/kg). Dosing continued once daily at 9:00 a.m. for 5 days. Four hours after the final dose (day 12), animals were sacrificed by cervical dislocation. The left sciatic nerve was excised, weighed, and placed in ice-cold 6% (w/v) perchloric acid (1 mL) and frozen for fructose analysis at a later date, using the methods described above.

For elemental analysis data, see the Appendix.

Appendix. Elemental analysis data

Compd	mol. formula	Calcd			Found		
		C	H	N	C	H	N
9	$C_{12}H_{20}N_4O_3S$	48.00	6.66	18.66	48.32	6.41	18.82
11	$C_{13}H_{22}N_4O_3S$	49.68	7.00	17.83	50.02	6.96	18.22
13	$C_{13}H_{22}N_4O_3S$	49.68	7.00	17.83	49.49	7.24	18.14
20	$C_{10}H_{18}N_6O_3S$	39.73	6.00	27.79	40.10	6.28	27.52
27	$C_{11}H_{20}N_6O_3S$	41.77	6.33	26.58	42.01	6.11	26.64
33	$C_{15}H_{22}N_8O$	54.50	6.70	33.40	54.43	7.00	33.56
34	$C_{15}H_{22}N_8O$	54.50	6.70	33.40	54.32	6.82	33.28
35	$C_{20}H_{24}N_8O$	61.22	6.12	28.57	61.46	6.39	28.77
36	$C_{24}H_{31}N_8O_2$	62.20	6.69	24.19	61.98	6.86	24.32
40	$C_{17}H_{24}N_8O$	57.30	6.74	31.46	57.58	6.55	31.72

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