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A library of novel allosteric inhibitors against Fructose 1,6-bisphosphatase

Sabrina Heng, Kimberly R. Gryncel, and Evan R. Kantrowitz*

Boston College, Department of Chemistry, Merkert Chemistry Center, Chestnut Hill MA 02467 USA

Abstract

The identification of a proper lead compound for fructose 1,6-bisphosphatase (FBPase) is a critical step in the process of developing novel therapeutics against type-2 diabetes. Herein, we have successfully generated a library of allosteric inhibitors against FBPase as potential anti-diabetic drugs, of which, the lead compound **1b** was identified through utilizing a virtual high-throughput screening (vHTS) system, which we have developed. The thiazole-based core structure was synthesized via the condensation of α -bromo-keotnes with thioureas and substituents on the two aryl rings were varied. **4c** was found to inhibit pig kidney FBPase approximately 5-fold better than **1b**. In addition, we have also identified **10b**, a tight binding fragment, which can be use for fragment-based drug design purposes.

Keywords

Fructose 1,6-bisphosphatase; Type-2 diabetes; ZINC database; Enzyme inhibition; Thiazole derivatives; Virtual high-throughput screening

1. Introduction

In 2007, it was estimated that 7.8 percent of the population in the United States (23.6 million people) have diabetes,¹ while the worldwide frequency of diabetes is expected to continue to grow by 6% annually, potentially reaching a total of 200 – 300 million cases by 2010.² In adults, type-2 diabetes, also known as non-insulin-dependent diabetes mellitus accounts for about 90 to 95 percent of all diagnosed cases of diabetes. The liver has a critical role in regulating endogenous glucose production from *de novo* synthesis (gluconeogenesis) or the catabolism of glycogen (glycogenolysis).³ Increased rates of hepatic glucose production are largely responsible for the development of overt hyperglycemia, in particular fasting hyperglycemia, in patients with diabetes.⁴ Therefore enzymes that regulate rate-controlling steps in the gluconeogenic or glycogenolytic pathways are obvious molecular targets for therapeutic interventions.⁵ As a rate-limiting and highly regulated enzyme in the gluconeogenesis pathway, fructose-1,6-bisphosphatase (FBPase) is an attractive target in the development of new anti-diabetic pharmaceuticals.

FBPase is a tetramer of four identical polypeptide chains (Mr 34,000/chain) and exists as a dimer of dimers.⁶ The enzyme exists in at least two distinct quaternary conformations called

* Corresponding author. Tel. +1 617 552 4558; e-mail: E-mail: evan.kantrowitz@bc.edu.

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R and T.⁷ The enzyme is subject to competitive substrate inhibition by fructose-2,6-bisphosphate⁸ and to allosteric inhibition by adenosine monophosphate (AMP). A “novel allosteric site” has also been identified at the center of the molecule where the four subunits converge.^{9,10} The enzyme does not exhibit substrate cooperativity but is cooperative with respect to the binding of AMP and metal cofactors.¹¹ Without effectors the enzyme exists in the R-quaternary structure. AMP induces the transition from the active R-state to the inactive T-state.¹²

Targeting the AMP binding site has historically been challenging due to the abundance of AMP-binding enzymes controlling other key biosynthetic pathways resulting in issues with specificity. Other difficulties that need to be overcome include the hydrophilic nature of AMP sites and their reliance on the negatively charged phosphate group of AMP for binding affinity.¹³ Target-based virtual database screening has become a useful tool for the identification of inhibitors for protein-ligand and protein-protein interactions.¹⁴ In light of the abovementioned challenges, virtual screening, in the use of high-performance computing to analyze chemical databases and prioritize compounds for synthesis and assay,¹⁵ then provides a more cost-effective approach to discovering allosteric inhibitors that bind to the desired allosteric site and yet are structurally distinct from the traditional AMP analogs.

In the present work, we have successfully generated a library of allosteric inhibitors against FBPsase of which, the lead compound was identified utilizing virtual high-throughput screening (vHTS) system, which we have developed. In this paper, the synthesis and the ability of the compounds in this library to inhibit FBPsase *in vitro* are also described, thus demonstrating how vHTS can be utilized to find and develop novel inhibitors against FBPsase.

2. Results

2.1 In Silico Screening

The identification of a proper lead compound for FBPsase is a critical step in the process of developing novel therapeutics against diabetes. To this end, target-based virtual database screening has become a useful tool for the identification of inhibitors for protein-ligand and protein-protein interactions.^{15,16} In our laboratory, we have in-place, a vHTS system that is set-up to screen millions of compounds against a desired target. The two essential components for a successful screen are the docking software and the database of small molecules. In an effort to make virtual screening more accessible to a broader community, Irwin et al.¹⁷ developed ZINC, a free database of structures of small molecules, many of them “drug-like” or “lead-like”. Virtual screening using the ZINC database have now been used for the development of inhibitors for a variety of targets including cyclooxygenase-2,¹⁸ anthrax edema factor¹⁹ and the H5N1 avian influenza virus.²⁰

The virtual high-throughput screening system we have developed consists of four parts: (1) a MySQL database containing entries of the molecules in the ZINC6 database in mol2, pdbq and mae format, (2) a set of unix tar files containing the executable program and associated auxiliary files for AUTODOCK,²¹ SUFLEX²² DOCK5,²³ and GLIDE^{24,25} (Schrödinger, Inc.), (3) a MySQL database for storage of the results of the docking calculations, and (4) a UNIX shell script that automates the process and provides the ability to distribute the computations over multiple computers.

Validation of AUTODOCK and DOCK5 were performed utilizing the structure of human FBPsase with AMP bound (PDB entry 1FTA). AMP was first removed from its site, and its coordinates changed so that AMP was in a different spatial position, distant from the actual binding site. AMP was then docked into the allosteric site of human FBPsase using the respective

docking programs. Both programs performed well in matching the docked conformation with the one observed in the crystal structure.

We then screened ~3 million compounds, which are characterized as purchasable in the ZINC6 database, against the AMP-binding site of human FBPase using a combination of the above mentioned programs. We then examined, in detail, a representative sample of the compounds that showed high-binding affinity. The eventual selection of the compounds for *in vitro* biochemical assays was based on the following rationales: (i) not all the *in silico* hits could be obtained commercially, thus limiting the number of compounds that could be directly tested *in vitro*, (ii) hits that were analogs of or were structurally similar to AMP were eliminated, (iii) only one member of a group of similar compounds was characterized thus maximizing chemical diversity, and (iv) the compounds had to be considered drug-like as defined by Lipinski's rule of 5.²⁶

2.2 Experimental Assays

The selected compounds were assayed for inhibitory activity using purified pig kidney FBPase, employing a coupled-enzyme assay with AMP as the control.²⁷ The degree of amino acid sequence conservation within the FBPase coding region among mammalian species is very high, with 90% of the residues identical and another 6% similar. Thus the pig kidney enzyme is an excellent model system to study enzyme inhibition with respect to human FBPase. In addition, the use of pig kidney enzyme was conducted to support our ongoing crystallographic work using this enzyme. Figure 1 illustrates some of the compounds from this screen that were found to inhibit pig kidney FBPase. As shown, the vHTS system successfully identified inhibitors of pig kidney FBPase with a broad spectrum of inhibitory activities, ranging from 10 mM to 20 μ M. Of these, we decided to pursue a more detailed structure activity relationship (SAR) study of **1**. Compound **1**, contains a thiazole ring, a functionality that has been found to be associated with a plethora of biological activities,²⁸ several of which have been developed into potent inhibitors of FBPase.^{9,29} More importantly, **1** was chosen because it has reasonable activity (IC_{50} of $31.6 \pm 1.6 \mu$ M) and possesses two aromatic rings that would allow for a variety of functional groups to be investigated.

2.3 Chemistry

The synthesis of the inhibitors shown in Table 1 was carried out by modifying reported procedures and is illustrated in Scheme 1. Where the primary thiourea could not be purchased commercially, it was easily synthesized by reacting the respective amine **20** – **24** with excess potassium thiocyanate in the presence of one equivalent concentrated HCl. In most cases, the pure primary thioureas could be isolated by precipitation and purified in good yield by crystallization.³⁰ The condensation of α -halogeno ketones **25** – **28** with the *N*-monosubstituted thioureas **11** – **15** via the Hantzsch reaction were originally accomplished by heating the reaction mixture under reflux, with varying reaction times from 7 h to 24 h.^{28,31} Although combinatorial chemistry has been widely adopted in drug discovery in recent years, it can be both labor and time intensive. Generating a small library of 29 compounds via a two-step synthetic pathway as described, would require setting up to 29 reaction vessels, each used twice. This was made more challenging when problems arose in the second synthetic step. Even after 24 hrs, overall yields were low (< 30%) and purifications were tedious.

In order to enhance the capabilities of combinatorial chemistry, we modified the second synthetic step according to the recent work by Kabalka and Mereddy,³² who demonstrated the use of microwave irradiation to promote the rapid one-pot synthesis of a series of 2-aminothiazoles. Using the same methodology, we were able to reduce the reaction times from 24 h to a mere 45 sec and increased the yields from less than 30% to greater than 90% after recrystallization.

Having designed an efficient route to the compounds in groups **1** - **8**, we were able to expand the library further by synthesizing the 2-aminothiazole fragments according to Scheme 2. Compounds **10a** – **10c** (Table 2), were synthesized from thiourea **19** according to the method described above.

3. Discussion

The ability of compounds **1** – **29** to inhibit the activity of pig kidney FBPase was determined using a coupled-enzyme assay²⁷ with AMP as the control, and the results obtained are showed in Tables 1 and 2. To validate our results, all 29 inhibitors were docked against the allosteric site of a pig kidney T-state crystal structure (PDB entry 1FRP) using the high precision mode (XP) mode in Glide (Schrödinger).²⁵ A graph of IC₅₀ values was plotted against the resultant docking score obtained from Glide. As showed in Figure 2, there is good agreement between the actual inhibitory activity against the pig kidney enzyme and the binding affinity as predicted by Glide.

The results displayed in Tables 1 and 2 revealed that substituting a hydroxyl at R₅ is essential for inhibition. This trend is consistent regardless of the substitution on ring 1, as inhibitors with hydroxyl functionality at R₅ resulted in improved inhibition over those without. Remarkably, while **7a**, even at 300 μM, showed no significant inhibitory activity against the enzyme, the R₅ hydroxyl substituted **7b** inhibited the enzyme at an IC₅₀ of 48 ± 3 μM. Likewise, a comparison of **5a** (IC₅₀ = 270 ± 14 μM) with **5b** (IC₅₀ = 15 ± 1 μM) showed that the R₅ hydroxyl substitution (**5b**) resulted in an ~18-fold improved inhibition. On the other hand, functionalizing R₅ with nitro instead of hydroxyl, eliminated inhibition. For example, a comparison of **3a** (IC₅₀ = 124 ± 6 μM) with **3b** (IC₅₀ = 48 ± 3 μM) and **3d** showed that the inhibitory activity of the inhibitor with an R₅ hydroxyl substitution (**3b**) improved 3-fold from **3a** while having its inhibitory activity eliminated when the hydroxyl is replaced by nitro at R₅ (**3d**). Similarly, a nitro group at R₅ in **8e** eliminates inhibition compared to **8a** (IC₅₀ = 2.5 ± 0.1 mM) and **8b** (IC₅₀ = 320 ± 4 μM).

Given the high concentration of positively charged residues present at the AMP binding site as shown in Figure 3, it is reasonable to assume that by adding a second hydroxyl at R₄, that the binding affinity of the inhibitors for the allosteric site might increase. This is true for groups **3** and **4** where the additional hydroxyl at R₄ improved enzyme inhibition. However, with the rest, the differences in inhibitory activity between the single and double hydroxyls were negligible.

Manipulation of the substituent positions on Ring 1 within the same functional group seemed to have little effect on activity. This is demonstrated by the relatively small changes in inhibitory activity between **1a** (IC₅₀ = 145 ± 11 μM) and **2a** (IC₅₀ = 104 ± 7 μM) where the sulfonamide was moved from R₃ to R₂. Similar observations can be made for the hydroxyl and nitro substitutions at R₁, R₂ and R₃. Given the relative size differences between these functional groups, this may suggest that the area where Ring 1 binds is not restrictive. However, between the different functional groups, there is a marked effect on the activity of the inhibitor. Overall, it would seem that hydroxyls are better for activity as opposed to sulfonamides and nitros. Inhibitors with no substitution on Ring 1 seemed to fare the worst as a group.

In an effort to develop potent inhibitors against FBPase, part of our strategy is to use the fragment based approach to enhance inhibitor affinity. Thus, we investigated the effect that the phenylthiazole fragment **10a** – **10c** have on the enzyme activity. To our surprise, the fragment 4-(2-aminothiazol-5-yl)phenol **10b**, inhibited the enzyme with an IC₅₀ of 1.1 ± 0.1 μM, having better inhibitory activity than all the diphenylthiazole-based inhibitors found in

Table 1. In addition, the binding of **10b** at the allosteric site was observed in a low resolution X-ray crystallography data of the enzyme•**10b** complex that we had obtained.

In order to confirm that the FBPase inhibition by the achyrofurans was due to binding at the allosteric site and not the active site, a competition experiment was performed using the analog of AMP, 2',3'-O-(2,4,6-trinitrophenyl)adenosine 5-monophosphate (TNP-AMP). This analog has been shown to bind at the allosteric site of FBPase and exhibits fluorescence only when bound to the enzyme. TNP-AMP was added to the FBPases at a concentration equal to 0.5 times their respective K_d . Increasing concentrations of the respective inhibitors were added which resulted in a substantial diminution of the TNP-AMP fluorescence, indicating that these inhibitors may be competing with TNP-AMP at the allosteric site. Binding constants for the inhibitors could not be determined by this method due to the relatively low solubility of these compounds.

4. Conclusion

Compounds **1a** – **9b** represent a novel class of inhibitors against FBPase that are not analogs of AMP. The *in vitro* data in Table 1 and the observation of inhibitor electron density in a low-resolution structure of pig kidney FBPase•**1b** demonstrated how a vHTS system was used to identify and develop novel inhibitors against FBPase. By eliminating the sulfonamide on the original molecule (**1b**) as identified by the vHTS system, and adding a hydroxyl at R₂ to ring 1 and a second hydroxyl at R₄ to ring 3, we have designed and synthesized **4c** ($IC_{50} = 6 \pm 0.5 \mu M$) which has a 5-fold increased in potency over **1b** ($IC_{50} = 32 \pm 2 \mu M$) against pig kidney FBPase. Currently, one of the most potent allosteric inhibitor of FBPase is MB05032, which inhibits human liver FBPase at an EC_{50} of 16 nM, a value that is approximately 370-fold better than **4c**. However, in line with the fragment-based drug design strategy, a future goal is to obtain higher resolution X-ray data of the enzyme•**10b** complex so that we can use the resultant structure, with **10b** bound, in our vHTS system to screen for fragments that have a high (micromolar or better) affinity for the allosteric site. Thus, by synthetically linking the appropriate fragment with **10b**, we will be able to generate a more potent and specific inhibitor of FBPase. And although we only focused on developing a library based upon compound **1** in this work, our vHTS yielded many other potential hits, which we are currently in the process of further evaluating as inhibitors against FBPase.

5. Experimental

5.1. Isolation and purification of Pig kidney FBPase

Plasmid pEK284³³ was transformed into *Escherichia coli* strain EK1601.³⁴ Bacteria were cultured with vigorous agitation at 37° C in YT³⁵ media containing ampicillin at 100 µg per mL. Induction of T7 RNA polymerase was initiated by addition of 0.4 mM isopropyl-β-D-thiogalactopyranoside. After further growth for 16 - 22 h at 37 °C, the cells were harvested by centrifugation, broken open by sonication, and purified as described previously³⁴ with the modification of eluting from the Dyematrix Blue A column with a 50 mM KH₂PO₄ buffer at pH 7.5 containing 5 mM ATP instead of AMP. Pure fractions were pooled and dialyzed extensively against a buffer containing 50 mM KH₂PO₄ and 1 mM MgCl₂ at pH 7.5 before performing enzyme activity assays.

5.2. Measurement of FBPase Activity

FBPase activity was measured spectrophotometrically by employing the coupling enzymes phosphoglucose isomerase and glucose-6-phosphate dehydrogenase.²⁷ The reduction of NADP⁺ to NADPH was monitored directly at 340 nm. Specifically, buffer (0.2 M Tris, 4 mM MgCl₂, 4 mM (NH₄)₂SO₄, 0.1 EDTA, pH 7.5), 0.2 mM of NADP⁺, 1.4 units of phosphoglucose

isomerase and 0.5 units glucose-6-phosphate dehydrogenase, 0 – 300 μM of inhibitor and 9 ng of FBPase, were mixed in a cuvette and equilibrated at 30 °C. 70 μM of FBP was then added to initiate the reaction. A_{340} data were collected as a function of time using a JASCO V-630 spectrophotometer. After a lag phase, due to coupling, a straight line was fit to the progress curve and the slope was determined as ΔA_{340} per min. The amount of NADPH produced per min. was calculated using the 340 nm millimolar extinction coefficient of 6.22. At each inhibitor concentration, reactions were performed in duplicate. Inhibition curves were obtained for each compound by plotting the relative activity versus inhibitor concentration.

5.3. Fluorescence Measurements

Fluorescence data were collected using a JASCO FP-6300 spectrofluorometer. Excitation and emission wavelengths of 410 and 535 nm respectively were used for TNP-AMP (Molecular Probes). 0.15 mg of the respective FBPases, 0.08 mM MgCl_2 and 20 μM fructose 1,6-bisphosphate in 50 mM Tris-acetate buffer, pH 7.5, was added to a 2 mL cuvette and stirred. 3.5 μM of TNP-AMP was added to the cuvette and the emission data was obtained. Microliter volumes of each inhibitor were subsequently added to the cuvette and resultant emission data was obtained after each addition. The final concentration of each inhibitor in the enzyme solution was between 5 to 50 μM .

5.4. Chemistry

5.4.1. General—All materials were reagent grade and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel 60 aluminum backed plates (250 microns) from Sorbent Technologies and visualized by Abs_{254} irradiation. Flash chromatography was conducted with silica gel 60 (230 mesh) from AK Scientific Inc. All final products were characterized by ^1H NMR and HRMS. Purity of all final compounds were determined by HPLC. ^1H NMR spectra were recorded on a Varian 400 spectrometer. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.0) or with the solvent reference relative to TMS employed as the internal standard (CDCl_3 , δ 7.24 ppm; $\text{DMSO}-d_6$, δ 2.50). Standard abbreviations indicating multiplicity were used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad. Mass spectra were obtained at the Mass Spectrometry Facilities at Boston College. HPLC separations were performed on a Biologic Duo-Flow chromatography system (BioRad). Microwave reactions were performed using a General Electric Spacemaker II, operating at 400W.

5.4.2. Preparation of analogs

5.4.2.1. 4-Thioureidobenzenesulfonamide (11): A solution of sulfanilamide **20** (3g, 0.017 mol) in 15 mL of ethanol was stirred at room temperature while concentrated hydrochloric acid (37.4%, 2.14 mL) was added dropwise. The suspension that formed was heated to reflux and when all suspension had dissolved, a solution of potassium thiocyanate (2.6 g, 0.0255 mol) in 5 mL of ethanol was added to the suspension. The reaction mixture was stirred at reflux for 18 h. The precipitate formed upon cooling was dried under vacuum and recrystallized from ethanol to yield 3g (76.3%) of **11** as white solid; ^1H ($\text{DMSO}-d_6$): 7.28 (br, 2H), 7.70-7.75 (m, 4H), 10.21 (br, 1H).

5.4.2.2. 4-(4-(4-Hydroxyphenyl)thiazol-2-ylamino)benzenesulfonamide (1b): A solution of **11** (200 mg, 0.86 mmol) and 2-bromo-4'-hydroxyacetophenone **25** (188mg, 0.87 mmol) in 5 mL of ethanol were placed in the microwave cavity and subjected to MW irradiation at 71 °C (400 W). The reaction mixture was cooled to room temperature and solvent was removed under *vacuo*. The resultant solid was washed with ethanol and crystallized from ethanol / n-pentane to yield 280 mg (94%) of **1b** as a pale yellow solid; ^1H ($\text{DMSO}-d_6$): 6.73 (d, J = 8.8

Hz, 2H), 7.07 (s, 1H), 7.65-7.70 (m, 4H), 7.78 (d, $J = 8.8$ Hz, 2H), 10.57 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{14}N_3O_3S_2$, 348.0477; found 348.0486.

5.4.2.3. 4-(4-Phenylthiazol-2-ylamino)benzenesulfonamide (1a): (275 mg, 97%, white solid) was prepared in a similar manner as described for the synthesis of **1b** except using 2-bromoacetophenone **26**. (DMSO- d_6): 7.21 (br, 2H), 7.34 (m, 1H), 7.45 (d, $J = 9.6$ Hz, 2H), 7.46 (s, 1H), 7.80-7.90 (m, 4H), 7.96 (d, $J = 8.0$ Hz, 2H), 10.74 (bs, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{14}N_3O_2S_2$, 332.0527; found 332.0522.

5.4.2.4. 4-(4-(2,4-Dihydroxyphenyl)thiazol-2-ylamino)benzenesulfonamide (1c): (300 mg, 96%, brown solid) was prepared in a similar manner as described for the synthesis of **1b** except using 2-bromo-2'-4'-dihydroxyacetophenone **27**. 1H (DMSO- d_6): 6.36 (d, $J = 8.8$ Hz, 1H), 6.40 (br, 1H), 7.27 (s, 1H), 7.74-7.82 (m, 5H), 10.84 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{14}N_3O_2S_2$, 332.0527; found 332.0517. HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{14}N_3O_4S_2$, 364.0426; found 364.0443.

5.4.2.5 3-Thioureidobenzenesulfonamide (12): (800 mg, 60%, white solid) was prepared in a similar manner as described for the synthesis of **11** and by substituting sulfanilamide with 3-aminobenzenesulfonamide **21**. 1H (DMSO- d_6): 7.37 (s, 1H), 7.48-7.56 (overlapped, m, 2H), 7.70 (d, $J = 7.6$ Hz, 1H), 7.93 (bs, 2H), 9.95 (br, 2H)

5.4.2.6. 3-(4-Phenylthiazol-2-ylamino)benzenesulfonamide (2a): (560 mg, 90%, yellow solid) was prepared from **12** and according to the manner described for **1a**. 1H (DMSO- d_6): 6.82 (d, $J = 8.8$ Hz, 2H), 7.17 (s, 1H), 7.18 (d, $J = 9.6$ Hz, 1H), 7.76 (d, $J = 8.8$ Hz, 2H), 7.78 (d, $J = 7.6$ Hz, 2H), 7.85 (s, 1H), 7.86 (d, $J = 9.2$ Hz, 1H), 9.58 (s, 1H), 10.5 (s, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{14}N_3O_2S_2$, 332.0527; found 332.0517.

5.4.2.7. 3-(4-(4-Hydroxyphenyl)thiazol-2-ylamino)benzenesulfonamide (2b): (600 mg, 93%, brown solid) was prepared from **12** and according to the manner described for **1b**. 1H (DMSO- d_6): 6.52 (s, 1H), 6.78-6.86 (overlapped, m, 4H), 7.47 (d, $J = 7.2$ Hz, 2H), 7.80 (d, $J = 7.6$ Hz, 2H), 9.80 (br, 1H), 10.40 (br, 1H), 11.61 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{14}N_3O_3S_2$, 348.0477; found 348.0492.

5.4.2.8. 4-(4-Phenylthiazol-2-ylamino)phenol (3a): (158 mg, 93%, white solid) was prepared from commercially available 1-(4-hydroxyphenyl)thiourea **13** and according to the manner described for **1a**. 1H (DMSO- d_6): 6.76 (d, $J = 8.8$ Hz, 2H), 7.23 (s, 1H), 7.30 (t, $J = 7.4$ Hz, 1H), 7.42 (t, $J = 7.4$ Hz, 2H), 7.48 (d, $J = 6.8$ Hz, 2H), 7.89 (d, $J = 7.2$ Hz, 2H), 9.95 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{13}N_2OS$, 269.0749; found 269.0740.

5.4.2.9. 4-(4-(4-Hydroxyphenyl)thiazol-2-ylamino)phenol (3b): (154 mg, 91%, white solid) was prepared from **13** and according to the manner described for **1b**. 1H (DMSO- d_6): 6.76 (d, $J = 8.8$ Hz, 2H), 6.80 (d, $J = 8.8$ Hz, 2H), 6.95 (s, 1H), 7.44 (d, $J = 8.8$ Hz, 2H), 7.68 (d, $J = 8.8$ Hz, 2H), 9.97 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{13}N_2O_2S$, 285.0698; found 285.0698.

5.4.2.10. 4-(2-(4-Hydroxyphenylamino)thiazol-4-yl)benzene-1,3-diol (3c): (40 mg, 94 %, brown solid) was prepared from **13** and according to the manner described for **1c**. 1H (DMSO- d_6): 6.31 (d, $J = 8.4$ Hz, 1H), 6.32 (s, 1H), 6.81 (d, $J = 8.8$ Hz, 2H), 7.01 (s, 1H), 7.27 (d, $J = 8.8$ Hz, 2H), 7.53 (d, $J = 8.0$ Hz, 1H), 10.29 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{13}N_2O_3S$, 301.0647; found 301.0652.

5.4.2.11. 4-(4-(4-Nitrophenyl)thiazol-2-ylamino)phenol (3d): (176 mg, 95%, yellow solid) was prepared from **13** and according to the manner described for **1b** except using 2-Bromo-4'-

nitroacetophenone. ^1H (DMSO- d_6): 6.77 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 7.63 (s, 1H), 8.15 (d, J = 9.2 Hz, 2H), 8.29 (d, J = 9.2 Hz, 2H) 10.06 (br, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{12}\text{N}_3\text{O}_3\text{S}$, 314.0599; found 314.0612.

5.4.2.12. 1-(3-Hydroxyphenyl)thiourea (14): (480 mg, 44%, white solid) was prepared in a similar manner as described for the synthesis of **11** and by substituting sulfanilamide with 3-Aminophenol **22**. ^1H (DMSO- d_6): 6.52 (d, J = 8.2 Hz, 1H), 6.85 (d, J = 8.6 Hz, 1H), 7.00 (s, 1H), 7.10 (t, J = 8.0 Hz, 1H), 9.44 (s, 1H), 9.65 (s, 1H), 9.64 (s, 1H)

5.4.2.13. 3-(4-Phenylthiazol-2-ylamino)phenol (4a): (290 mg, 91%, white solid) was prepared from **14** and according to the manner described for **1a**. ^1H (DMSO- d_6): 6.50 (d, J = 8.4 Hz, 1H), 6.71 (s, 1H), 6.74-6.77 (m, 2H), 7.08 (t, J = 8.0 Hz, 1H), 7.24-7.28 (m, 1H), 7.34 (t, J = 7.6 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{OS}$, 269.0749; found 269.0748.

5.4.2.14. 3-(4-(4-Hydroxyphenyl)thiazol-2-ylamino)phenol (4b): (305 mg, 96%, pale green solid) was prepared from **14** and according to the manner described for **1b**. ^1H (DMSO- d_6): 6.37 (dd, J = 7.4, 1.8 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 7.01 (d, J = 8.4 Hz, 1H), 7.04 (s, 1H), 7.09 (t, J = 8.0 Hz, 1H), 7.31 (t, J = 2.2 Hz, 1H), 7.75 (d, J = 8.3 Hz, 2H), 10.09 (br, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_2\text{S}$, 285.0698; found 285.0700.

5.4.2.15. 4-(2-(3-Hydroxyphenylamino)thiazol-4-yl)benzene-1,3-diol (4c): (322 mg, 90%, brown solid) was prepared from **14** and according to the manner described for **1c**. ^1H (DMSO- d_6): 6.30-6.32 (overlapped, m, 2H), 6.42 (dd, J = 8.0, 2.4 Hz, 1H), 6.92-6.90 (m, 1H), 7.01 (t, J = 2.0 Hz, 1H), 7.126 (s, 1H), 7.126 (t, J = 8.0 Hz, 1H), 7.68 (d, J = 9.2 Hz, 1H), 10.25 (bs, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_3\text{S}$, 301.0647; found 301.0656.

5.4.2.16. 1-(2-Hydroxyphenyl)thiourea (15): (720 mg, 47%, white solid) was prepared in a similar manner as described for the synthesis of **11** and by substituting sulfanilamide with 2-aminophenol **23**. ^1H (DMSO- d_6): 6.76 (t, J = 7.2 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.93-6.99 (m, 2H), 7.76 (br, 1H), 8.92 (br, 1H), 9.75 (br, 1H).

5.4.2.17. 2-(4-Phenylthiazol-2-ylamino)phenol (5a): (293 mg, 92%, white solid) was prepared from **15** and according to the manner described for **1a**. ^1H (DMSO- d_6): 6.37-6.40 (m, 1H), 6.82 (d, J = 8.8 Hz, 2H), 7.00-7.02 (m, 1H), 7.04 (s, 1H), 7.10 (t, J = 8 Hz, 1H), 7.31 (t, J = 2.2 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 10.13 (br, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{OS}$, 269.0749; found 269.0751.

5.4.2.18. 2-(4-(4-Hydroxyphenyl)thiazol-2-ylamino)phenol (5b): (300 mg, 94%, white solid) was prepared from **15** and according to the manner described for **1b**. ^1H (DMSO- d_6): 6.37-6.39 (m, 1H), 6.82 (d, J = 8.8 Hz, 2H), 7.00-7.02 (m, 1H), 7.11 (t, J = 8 Hz, 1H), 7.30 (t, J = 2.2 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 10.13 (br, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_2\text{S}$, 285.0698; found 285.0704.

5.4.2.19. 4-(2-(2-Hydroxyphenylamino)thiazol-4-yl)benzene-1,3-diol (5c): (330 mg, 92%, dark brown solid) was prepared from **15** and according to the manner described for **1c**. ^1H (DMSO- d_6): 6.30 (d, J = 2.4 Hz, 1H), 6.32 (br, 1H), 6.86 (t, J = 7.3 Hz, 1H), 6.93-7.00 (m, 2H), 7.05 (s, 1H), 7.53 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 9.93 (br, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_3\text{S}$, 301.0647; found 301.0634.

5.4.2.20. N-(4-Nitrophenyl)-4-phenylthiazol-2-amine (6a): (700 mg, 93 %, orange solid) was prepared from commercially available 1-(4-nitrophenyl)-2-thiourea **16**, according to the manner described for **1a**. ^1H (DMSO- d_6): 7.35 (t, J = 6.6 Hz, 1H), 7.46 (t, J = 7.8 Hz, 2H),

7.57 (s, 1H), 7.85 (d, $J = 9.2$ Hz, 2H), 8.19 (d, $J = 11.6$ Hz, 2H), 8.28 (d, $J = 9.2$ Hz, 2H), 10.29 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{12}N_3O_2S$, 298.0650; found 298.0650.

5.4.2.21. 4-(2-(4-Nitrophenylamino)thiazol-4-yl)phenol (6b): (755 mg, 95%, orange solid) was prepared from **16** and according to the manner described for **1b**. 1H (DMSO- d_6): 6.83 (d, $J = 8.8$ Hz, 2H), 7.28 (s, 1H), 7.79 (d, $J = 8.4$ Hz, 2H), 7.94 (d, $J = 9.2$ Hz, 2H), 8.27 (d, $J = 9.6$ Hz, 2H), 11.05 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{12}N_3O_3S$, 314.0599; found 314.0600.

5.4.2.22. 1-(3-Nitrophenyl)thiourea (17): (742 mg, 52%, yellow solid) was prepared in a similar manner as described for the synthesis of **11** and by substituting sulfanilamide with 3-nitroaniline **24**. 1H (DMSO- d_6): 7.60 (t, $J = 8.0$ Hz, 1H), 7.83 (d, $J = 8.0$ Hz, 1H), 7.94 (d, $J = 8.4$ Hz, 1H), 8.62 (s, 1H), 10.11 (br, NH)

5.4.2.23. N-(3-Nitrophenyl)-4-phenylthiazol-2-amine (7a): (286 mg, 95%, orange solid) was prepared from **17** and according to the method described for **1a**. 1H (DMSO- d_6): 7.20-7.30 (m, 4H), 7.42 (dd, $J = 0.8, 8.0$ Hz, 1H), 7.60-7.67 (m, 2H), 7.76 (dd, $J = 7.8, 8.0$ Hz, 1H), 7.82 (t, $J = 2.0$ Hz, 1H), 7.89 (dd, $J = 8.2, 1.0$ Hz, 1H), 10.3 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{12}N_3O_2S$, 298.0650; found 298.0650.

5.4.2.24. 4-(2-(3-Nitrophenylamino)thiazol-4-yl)phenol (7b): (302 mg, 95%, yellow solid) was prepared from **17** and according to the method described for **1b**. 1H (DMSO- d_6): 6.84 (d, $J = 8.4$ Hz, 2H), 7.20 (s, 1H), 7.62 (t, $J = 8.2$ Hz, 1H), 7.79-7.82 (m, 3H), 7.95 (d, $J = 8.0$ Hz, 1H), 9.01 (t, $J = 2.4$ Hz, 1H), 9.62 (s, 1H), 10.78 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{12}N_3O_3S$, 314.0599; found 314.0606.

5.4.2.25. N,4-Diphenylthiazol-2-amine (8a): (763 mg, 92%, yellow solid) was prepared from commercially available phenylthiourea **18** and according to the method described for **1a**. 1H (DMSO- d_6): 6.99 (t, $J = 7.4$ Hz, 1H), 7.31-7.39 (m, 4H), 7.45 (t, $J = 7.6$ Hz, 2H), 7.76 (d, $J = 7.6$ Hz, 2H), 7.93 (d, $J = 7.2$ Hz, 2H), 10.40 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{13}N_2S$, 253.0799; found 253.0802.

5.4.2.26. N,4-Diphenylthiazol-2-amine (8b): (820 mg, 93%, white solid) was prepared from **18** and according to the method described for **1b**. 1H (DMSO- d_6): 6.81 (d, $J = 8.4$ Hz, 2H), 6.96 (t, $J = 6.6$ Hz, 1H), 7.06 (s, 1H), 7.33 (d, $J = 8.4$ Hz, 1H), 7.35 (d, $J = 7.6$ Hz, 1H), 7.71 (d, $J = 7.6$ Hz, 2H), 7.73 (d, $J = 8.4$ Hz, 2H), 10.22 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{13}N_2OS$, 269.0749; found 269.0748.

5.4.2.27. 4-(2-(Phenylamino)thiazol-4-yl)benzene-1,3-diol (8c): (887 mg, 95%, light brown solid) was prepared from **18**, and according to the method described for **1c**. 1H (DMSO- d_6): 6.31 (d, $J = 2.4$ Hz, 1H), 6.33 (s, 1H), 7.02 (t, $J = 7.2$ Hz, 1H), 7.14 (s, 1H), 7.37 (t, $J = 7.8$ Hz, 2H), 7.53 (d, $J = 7.2$ Hz, 2H), 7.66 (d, $J = 8.8$ Hz, 1H), 10.43 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{13}N_2O_2S$, 285.0698; found 285.0701.

5.4.2.28. 2-(2-(Phenylamino)thiazol-4-yl)phenol (8d): (837 mg, 95%, white solid) was prepared from **18**, and according to the method described for **1b** except using 2-Bromo-2'-hydroxyacetophenone **28**. 1H (DMSO- d_6): 6.87-6.92 (overlapped, m, 2H), 7.01 (t, $J = 7.4$ Hz, 1H), 7.16 (t, $J = 8.4$ Hz, 1H), 7.37 (t, $J = 7.4$ Hz, 2H), 7.43 (s, 1H), 7.57 (d, $J = 7.6$ Hz, 2H), 7.91 (d, $J = 7.6$ Hz, 1H), 10.40 (br, 1H). HRMS (ESI) $[M + H]^+$ calcd for $C_{15}H_{13}N_2OS$, 269.0749; found 269.0751.

5.4.2.29. 4-(4-Nitrophenyl)-N-phenylthiazol-2-amine (8e): (908 mg, 93%, orange solid) was prepared from **18**, and according to the method described for **1b** except using 2-bromo-4'-

nitroacetophenone. ^1H (DMSO- d_6): 7.00 (t, $J = 7.4$ Hz, 1H), 7.37 (t, $J = 8.0$ Hz, 2H), 7.741 (d, $J = 7.2$ Hz, 2H), 7.744 (s, 1H), 8.19 (d, $J = 9.2$ Hz, 2H), 8.31 (d, $J = 9.2$ Hz, 2H), 10.42 (bs, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{12}\text{N}_3\text{O}_2\text{S}$, 298.0650; found 298.0659.

5.4.2.30. Phenylthiazol-2-amine (10a): (444 mg, 96%, white solid) was prepared from thiourea **19** and according to the method described for **1a**. ^1H (DMSO- d_6): 6.88 (d, $J = 8.8$ Hz, 2H), 7.01 (s, 1H), 7.56 (d, $J = 8.8$ Hz, 2H), 8.90 (br, 2H), 9.95 (br, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_9\text{H}_9\text{N}_2\text{S}$, 177.0486; found 177.0481.

5.4.2.31. 4-(2-Aminothiazol-5-yl)phenol (10b): (480 mg, 95%, white solid) was prepared from **19** and according to the method described for **1b**. ^1H (DMSO- d_6): 7.25 (s, 1H), 7.44-7.52 (overlapped, m, 4H), 7.74 (d, $J = 8.0$ Hz, 2H), 8.80 (br, 2H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_9\text{H}_9\text{N}_2\text{OS}$, 193.0436; found 193.0429.

5.4.2.32. 4-(2-Aminothiazol-5-yl)benzene-1,3-diol (10c): (520 mg, 95%, yellow solid) was prepared from **19** and according to the method described for **1c**. ^1H (DMSO- d_6): 6.34 (dd, $J = 8.4, 2.0$ Hz, 1H), 6.44 (d, $J = 2.0$ Hz, 1H), 6.94 (s, 1H), 7.36 (d, $J = 8.8$ Hz, 1H), 8.79 (br, 1H), 9.80 (br, 1H). HRMS (ESI) $[\text{M} + \text{H}]^+$ calcd for $\text{C}_9\text{H}_9\text{N}_2\text{O}_2\text{S}$, 209.0385; found 209.0395.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and Notes

1. National Institute of Diabetes and Digestive and Kidney Diseases. National Diabetes Statistics, 2007 Fact Sheet. U.S. Department of Health and Human Services, National Institutes of Health; Bethesda, MD: 2008.
2. Amos AF, McCarty DJ, Zimmet P. *Diabet Med* 1997;14:S1. [PubMed: 9450510]
3. Benkovic SJ, deMaine MM. *Adv Enzymol* 1982;53:45. [PubMed: 6277165] Tejwani GA. *Advan Enzymol* 1983;54:121. [PubMed: 6303063]
4. Giaccari A, Morviducci L, Pastore L, Zorretta D, Sbraccia P, Maroccia E, Buongiorno A, Tamburrano G. *Diabetologia* 1998;41:307. [PubMed: 9541171]
5. Moller DE. *Nature* 2001;414:821. [PubMed: 11742415]
6. Mendicino J, Kratowich N, Oliver RM. *J Biol Chem* 1972;247:6643. [PubMed: 4342608]
7. Ke HM, Liang JY, Zhang Y, Lipscomb WN. *Biochemistry* 1991;30:4412. [PubMed: 1850623] Ke HM, Zhang Y, Lipscomb WN. *Proc Natl Acad Sci U S A* 1990;87:5243. [PubMed: 2164670]
8. Van Schaftingen E, Hers HG. *Proc Natl Acad Sci U S A* 1981;78:2861. [PubMed: 6265919]
9. Wright SW, Carlo AA, Carty MD, Danley DE, Hageman DL, Karam GA, Levy CB, Mansour MN, Mathiowetz AM, McClure LD, Nestor NB, McPherson RK, Pandit J, Pustilnik LR, Schulte GK, Soeller WC, Treadway JL, Wang IK, Bauer PH. *J Med Chem* 2002;45:3865. [PubMed: 12190310]
10. Wright SW, Hageman DL, McClure LD, Carlo AA, Treadway JL, Mathiowetz AM, Withka JM, Bauer PH. *Bioorg Med Chem Lett* 2001;11:17. [PubMed: 11140724]
11. Nimmo HG, Tipton KF. *Eur J Biochem* 1975;58:575. [PubMed: 171160]
12. Liang, JY.; Zhang, Y.; Huang, S.; Ke, H.; Lipscomb, WN. *Proc Robert A Welch Found Conf Chem Res*, 36th (Regulation of Proteins by Ligands). The Robert A. Welch Foundation; Houston: 1992. p. 57

13. Erion MD, Kasibhatla SR, Bookser BC, van Poelje PD, Reddy R, Gruber HE, Appleman JR. *J Am Chem Soc* 1999;121:308.
14. Schneidman-Duhovny D, Nussinov R, Wolfson HJ. *Curr Med Chem* 2004;11:91. [PubMed: 14754428]
15. Ghosh S, Nie A, An J, Huang Z. *Curr Opin Chem Biol* 2006;10:194. [PubMed: 16675286]
16. Zhong S, Macias AT, MacKerell AD Jr. *Curr Top Med Chem* 2007;7:63. [PubMed: 17266596]
17. Irwin JJ, Shoichet BK. *J Chem Inf Model* 2005;45:177. [PubMed: 15667143]
18. Sudha KN, Shakira M, Prasanthi P, Sarika N, Kumar ChN, Babu PA. *Bioinformation* 2008;2:325. [PubMed: 18685718]
19. Chen D, Misra M, Sower L, Peterson JW, Kellogg GE, Schein CH. *Bioorg Med Chem* 2008;16:7225. [PubMed: 18620864]
20. Nandi T. *Bioinformation* 2008;2:240. [PubMed: 18317572]
21. Goodsell DS, Morris GM, Olson AJ. *J Mol Recognit* 1996;9:1. [PubMed: 8723313]
22. Jain AN. *J Med Chem* 2003;46:499. [PubMed: 12570372]
23. Shoichet BK, Bodian DL, Kuntz ID. *J Comp Chem* 1992;13:380.
24. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS. *J Med Chem* 2004;47:1739. [PubMed: 15027865]
25. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL. *J Med Chem* 2004;47:1750. [PubMed: 15027866]
26. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. *Adv Drug Deliv Rev* 2001;46:3. [PubMed: 11259830]
27. Riou JP, Claus TH, Flockhart DA, Corbin JD, Pilgis SJ. *Proc Natl Acad Sci U S A* 1977;74:4615. [PubMed: 200922]
28. Holla BS, Malini KV, Rao BS, Sarojini BK, Kumari NS. *Eur J Med Chem* 2003;38:313. [PubMed: 12667698]
29. Erion MD, van Poelje PD, Dang Q, Kasibhatla SR, Potter SC, Reddy MR, Reddy KR, Jiang T, Lipscomb WN. *Proc Natl Acad Sci U S A* 2005;102:7970. [PubMed: 15911772] Tomoharu T, Toshiyaus T, Takahide N, Takahiro Y, Osamu K. *PCT Int Appl* 2006;181CODEN:PIXXD2 WO 2006104030 A1 20061005
30. Herr RJ, Kuhler JL, Meckler H, Opalka CJ. *Synthesis* 2000;11:1569.
31. Bailey N, Dean AW, Judd DB, Middlemiss D, Storer R, Watson SP. *Bioorg Med Chem Lett* 1996;6:1409. Bramley SE, Dupplin V, Goberdhan DGC, Meakins GD. *J Chem Soc, Perkin Trans 1* 1987;3:639.
32. Kabalka GW, Mereddy AR. *Tetrahedron Let* 2006;47:5171.
33. Lu G, Giroux EL, Stec B, Kantrowitz ER. *Pro Sci* 1996;5:2333.
34. Giroux E, Williams MK, Kantrowitz ER. *J Biol Chem* 1994;269:31404. [PubMed: 7989306]
35. Miller, JH. *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory; Cold Spring Harbor: 1972.
36. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. *Proc Natl Acad Sci U S A* 2001;98:10037. [PubMed: 11517324]
37. DeLano, WL. The PyMol molecular graphics system, DeLano Scientific LLC, San Carlos, CA, USA. 2002. <http://www.pymol.org>

AMP IC_{50} 1.3 μ M

1 IC_{50} 32 μ M

2 IC_{50} 750 μ M

3 IC_{50} 20 μ M

4 IC_{50} 3 mM

Figure 1.
Structures and IC_{50} of AMP and various inhibitors identified by the vHTS system.

Figure 2.

Graph of the Glide²⁵ docking Score (high-precision XP mode) of compounds **1a** – **10c** versus their experimentally determined IC₅₀ values. A more negative Glide docking score corresponds to tighter binding and therefore predicts better inhibition. This graph was prepared using Kaleidagraph with two outlying points removed (**3d** and **10b**).

Figure 3. X-ray crystal structure of AMP bound to pig kidney FBPase at the allosteric site. (PDB entry 1RDZ). The electrostatic potentials calculated using the Adaptive Poisson-Boltzmann Solver³⁶ were map on a surface representation of the protein created using PyMol.³⁷

Scheme 1.

Reagents and conditions: (i) conc. HCl, potassium thiocyanate, EtOH, reflux, 18 h (ii) EtOH, MW (71 °C, 400 W), 45 sec.

Scheme 2.

Reagents and conditions: (i) EtOH, MW (71 °C, 400 W), 45 sec.

Table 1

Inactivation of pig kidney FBPase by inhibitors **1a** – **9b**. Assays were performed in 0.2 M Tris, 4 mM MgCl₂, 4 mM (NH₄)₂SO₄, 0.1 EDTA, pH 7.5.

Ring 1 Ring 3

1a - 9b

Compound	R ₁	R ₂	R ₃	R ₄	R ₅	FBPase, IC ₅₀ (μM)
1a	H	H	SO ₂ NH ₂	H	H	145 ± 11
1b	H	H	SO ₂ NH ₂	H	OH	32 ± 2
1c	H	H	SO ₂ NH ₂	OH	OH	55 ± 0.5
2a	H	SO ₂ NH ₂	H	H	H	104 ± 7
2b	H	SO ₂ NH ₂	H	H	OH	50 ± 6
3a	H	H	OH	H	H	124 ± 6.0
3b	H	H	OH	H	OH	48 ± 3.0
3c	H	H	OH	OH	OH	11 ± 0.5
3d	H	H	OH	H	NO ₂	ND ^a
4a	H	OH	H	H	H	35 ± 4
4b	H	OH	H	H	OH	13 ± 0.5

Ring 1

Ring 3

1a - 9b

Compound	R ₁	R ₂	R ₃	R ₄	R ₅	FBPase, IC ₅₀ (μM)
4c	H	OH	H	OH	OH	6 ± 0.5
5a	OH	H	H	H	H	270 ± 14
5b	OH	H	H	H	OH	15 ± 1
5c	OH	H	H	OH	OH	20 ± 2
6a	H	H	NO ₂	H	H	ND ^b
6b	H	H	NO ₂	H	OH	246 ± 12
7a	H	NO ₂	H	H	H	ND ^b
7b	H	NO ₂	H	H	OH	48 ± 3
8a	H	H	H	H	H	2500 ± 110
8b	H	H	H	H	OH	318 ± 4
8c	H	H	H	OH	OH	343 ± 4
8d	H	H	H	OH	H	119 ± 4
8e	H	H	H	H	NO ₂	ND ^b

Ring 1

Ring 3

1a - 9b

Compound	R ₁	R ₂	R ₃	R ₄	R ₅	FBPase, IC ₅₀ (μM)
9a ^c	H	COOH	H	H	H	571 ± 5.0
9b ^c	H	COOH	H	H	OH	48 ± 4

^a Approximately 87 % inhibition at 100 μM.

^b No detectable inhibition observed.

^c Commercially available compounds.

Table 2

Inactivation of pig kidney FBPase by inhibitors **10a** – **10c**. Assays were performed in 0.2 M Tris, 4 mM MgCl₂, 4 mM (NH₄)₂SO₄, 0.1 EDTA, pH 7.5.

10a -10c

Compounds	R ₄	R ₅	FBPase, IC ₅₀ (μM)
10a	H	H	1392 ± 68
10b	H	OH	1.1 ± 0.1
10c	OH	OH	93 ± 5