Brief Articles

Novel Heteroaromatic Organofluorine Inhibitors of Fructose-1,6-bisphosphatase

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A broad group of compounds including substituted pyrazoles, pyrroles, indoles, and carbazoles were screened to identify potential inhibitor lead compounds of fructose-1,6-bisphosphatase (FBPase). Best inhibitors are (1*H*-indol-1-yl)(4-(trifluoromethyl)phenyl)methanone, ethyl 3-(3,5-dimethyl-1*H*-pyrrol-2-yl)-4,4,4-trifluoro-3-hydroxybutanoate, 3,5-diphenyl-1-(3-(trifluoromethyl) phenyl)-1*H*-pyrazole, and ethyl 3,3,3-trifluoro-2-hydroxy-2-(1-methyl-1*H*-indol-3-yl)propanoate. The IC_{50} values (3.1, 4.8, 6.1, and 11.9 μM) were comparable to that of AMP, the natural inhibitor of murine FBPase (IC_{50} of 4.0 μM). Docking programs were utilized to interpret the experiments.

Introduction

The inhibition of FBPase[a] has been an active area of research, as the number of individuals with obesity and impaired glucose tolerance in the prediabetic state continues to rise.[1] In recent years many new potential therapeutics have been screened and identified to inhibit gluconeogenesis. Some of the inhibitors showed excellent IC_{50} values, such as MB05032 (IC_{50}= 16 nM),[2] benzimidazole phosphonic acid ((IC_{50}= 90 nM),[3] or 10A (IC_{50}= 16 nM).[4] Most drug candidates that reached early clinical trials were later determined to have serious toxic effects or had delivery problems.[2−4] There are several drugs on the market (e.g., metformin, glyburide); however, because of toxic effects, their therapeutic use is limited.[5,6] Therefore, the search for the ideal drug to decrease high blood glucose levels continues. The control of gluconeogenesis, the process by which glucose is synthesized in the cell from carbon and non-sugar substrates, is a critical target for control of type II diabetes that results in hyperglycemia. Within the gluconeogenic pathway, the conversion of sugar fructose 1,6-bisphosphate (FBP) to fructose 6-phosphate and inorganic phosphate by fructose 1,6-bisphosphatase (FBPase), and the reverse reaction catalyzed by phosphofructokinase, must be delicately balanced to avoid hypoglycemia. The inhibition of fructose 1,6-bisphosphatase (FBPase) to control blood sugar levels has been found to be an effective method of treatment for laboratory rats in the prediabetic state.[4,7]

In this study we report new lead compounds for inhibition of FBPase. The major thrust of the experimental part of the work was to utilize fructose 1,6-bisphosphatase isolated from mouse liver homogenate to screen a series of small molecules: substituted pyrazoles,[8] *N*-heteroaryl(trifluoromethyl)hydroxyalkanoic acid esters,[9] *N*-sulfonylpyrroles, -indoles, -carbazoles,[10] *N*-acylindoles,[11] and other substituted pyrroles.[12] In addition, the human FBPase coordinates (PDB code 1FTA,

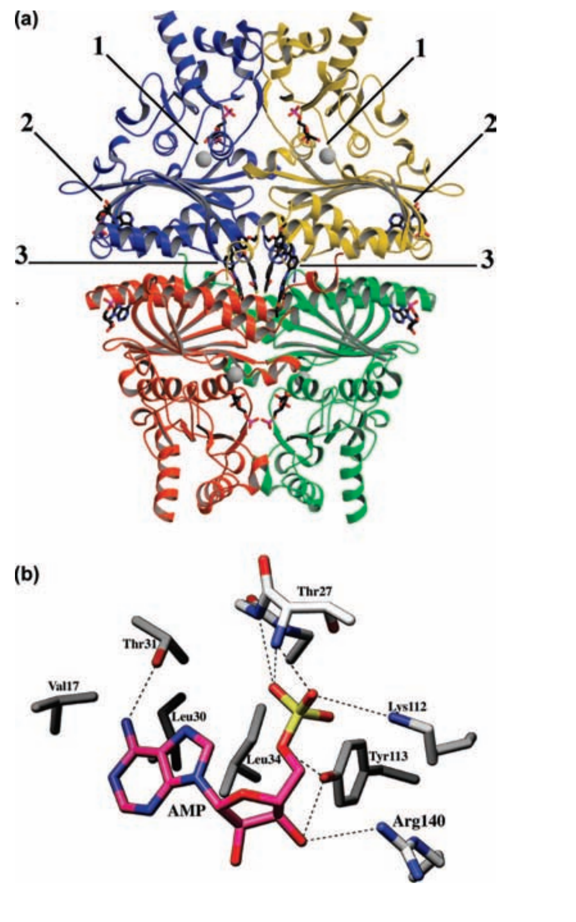

Figure 1. (a) The FBPase is a homotetramer. The three potential targets are shown: (1) the active site of FBPase, (2) the allosteric binding site of AMP, (3) the tetrameric allosteric inhibitor site. PDB coordinates 1KZ8[13,14] were used. The figure was drawn using POVScript+.[37] (b) A closeup of the AMP binding pocket is shown. The three programs used are Dock6, Autodock4, and Surflex and were tested using the crystallographic ligand AMP moved out of the binding pocket (as explained in results section). This figure was drawn using POV-Script+.[37] The three conformers of AMP were superimposed with a rmsd of <2.0 Å[5] from actual AMP atoms in the crystallographic structure.

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[a] Abbreviations: FBPase, fructose 1,6-bisphosphatase; FBP, fructose 1,6-bisphosphate; AMP, adenosine monophosphate; PDB, Protein Data Bank; RCSB, Rutgers Consortium of Structural Biology; 1FTA, PDB coordinates for the human fructose-1,6-bisphosphate in complex with adenosine monophosphate.

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Table 1. Results of Docking Studies and the Experimental IC_{50} Data of the Molecules Utilized in the Inhibition of FBPase

					X-score		
entry	inhibitor	Dock6,energy score	Autodock, estimated inhibition constant, K_i	Surflex, energy score	K_d	final binding energy (kcal/mol)	IC_{50} (μM)
1	**21**	−16.96	3.99×10^{-7}	2.84	5.70	−7.78	3.09
2	**7**	−16.05	1.14×10^{-4}	3.33	4.85	−6.62	4.81
3	**1**	−18.38	1.69×10^{-7}	3.61	6.26	−8.54	6.04
4	**9**	−17.33	4.11×10^{-7}	3.39	5.16	−7.03	11.86
5	**10**	−16.71	4.72×10^{-11}	2.97	5.05	−6.88	13.33
6	**5**	−14.01	7.05×10^{-5}	3.39	4.27	−5.83	25.74
7	**8**	−14.53	1.69×10^{-5}	3.30	4.91	−6.69	25.74
8	**11**	−15.37	5.85×10^{-7}	3.65	4.73	−6.45	35.97
9	**6**	−15.91	7.56×10^{-5}	2.96	4.60	−6.27	41.79
10	**2**	−18.62	2.23×10^{-7}	3.11	6.17	−8.42	75.45
11	**12**	−20.59	9.72×10^{-15}	2.08	4.47	4.47	76.15
12	**16**	−20.63	1.01×10^{-3}	1.67	4.55	−6.20	109.03
13	**17**	−21.73	8.15×10^{-14}	2.61	5.22	−7.12	119.26
14	**22**	−19.83	1.98×10^{-7}	2.81	5.94	−8.10	129.43
15	**3**	−19.00	9.43×10^{-8}	2.10	6.21	−8.47	150.60
16	**27**	−23.73	3.12×10^{-5}	2.81	5.19	−7.08	176.98
17	**19**	−25.66	5.68×10^{-4}	2.27	4.95	−6.75	184.95
18	**15**	−21.35	6.65×10^{-6}	2.73	4.39	−5.98	245.60
19	**26**	−18.53	7.93×10^{-7}	2.85	5.52	−7.53	>250
20	**14**	−20.36	4.93×10^{-6}	2.71	4.90	−6.68	>250
21	**18**	−22.22	1.36×10^{-7}	2.74	4.56	−6.22	>300
22	**23**	−17.79	9.60×10^{-5}	2.95	4.78	−6.52	>300
23	**4**	−12.94	2.20×10^{-7}	2.73	5.08	−6.92	>300
24	**20**	−25.66	5.68×10^{-4}	2.27	4.94	−6.75	>300
25	**28**	−22.92	6.29×10^{-8}	2.37	5.76	−7.82	>300
26	**24**	−19.02	3.01×10^{-8}	3.26	5.67	−7.73	>300
27	**25**	−16.40	2.76×10^{-7}	3.39	5.79	−7.90	>300
28	**13**	−22.76	3.31×10^{-6}	2.93	4.51	−6.16	>300

complexed with AMP) were utilized in docking studies to model these potential inhibitors. Previous studies of potent inhibitors of FBPase have been reported, but effective therapeutic drugs have not yet emerged, so the search for potent inhibitors of FBPase continues.

There are several potential targets, presented in Figure 1a, currently under intense scrutiny to inhibit the enzyme: (1) the active site which binds fructose 1,6-bisphosphate (FBP) and is inhibited in vivo by fructose 2,6-bisphosphate, (2) an allosteric regulatory site that binds AMP,[13] and (3) a tetrameric binding site at the dimer interface.[14] The AMP binding site (2) is presented in more detail in Figure 1b and targeted in this study.

Interestingly, an overwhelming majority of compounds identified in this study to be potential inhibitor lead compounds of FBPase belong to the large family of organofluorine compounds. Such compounds are of exceptional interest in biomedical applications, including drugs, and drug candidates, diagnostic agents, and research tools for metabolic studies.[15] Since the discovery of the beneficial effect of fluorine incorporation into organic molecules,[16] a steady stream of organofluorine compounds was introduced to the pharmaceutical sector. The most notable examples include steroidal, nonsteroidal anti-inflammatory, central nervous system drugs, anticancer, and antiviral agents.[17] In fact, these compounds represent approximately 20% of all drugs and three of the current top 10 best sellers are organofluorine products.

Methods

Synthesis of Inhibitor Candidates. All chemicals and solvents used for the synthesis of the inhibitor candidates were purchased from Aldrich. All the compounds used in this study were synthesized on the basis of methods described in the literature.[8−12] In each case the compounds were purified by flash chromatography. The identification and purity determination were carried out by gas chromatography−mass spectrometry and NMR spectroscopy (^{1}H, ^{13}C, and ^{19}F when applicable). All spectra were in agreement with the literature data. The full spectral characterization of the inhibitor candidates is available upon request.

Enzyme Assays. The acetone precipitated murine liver homogenate was resuspended in deoinized water and sterile-filtered and then dialyzed against 50 mM Tris buffer, pH 7.5, for 3 changes over 48 h at 4 °C. The crude protein extract was then run over gel filtration column (G25-150), and the fractions were analyzed by Bradford assay and SDS−PAGE. Fractions containing FBPase activity were pooled and redialyzed in 50 mM Tris, pH 7.5, loaded on a Matrex Gel Blue affinity column (Cibacron Blue 3GA dye coupled to cross-linked 5% agarose) and eluted in the presence of 1 mM FBP. Fractions were tested for activity, pooled, and redialyzed in 50 mM Tris, pH 7.5. The final concentration of wild-type enzyme was determined by absorption at 280 nm[18] and compared with the Bio-Rad version of the Bradford dye-binding assay.[19] The purity of the enzyme was verified by SDS−PAGE[20] and nondenaturing PAGE.[21,22]

The FBPase HTS assay measures phosphatase activity by colorimetric determination of released inorganic phosphate.[23,24] This colorimetric phosphate assay used an ammonium molybdate malachite green reagent. For all the assays presented in this study, this reagent was prepared with a 1:3 ratio of 4.2% ammonium molybdate and 0.05% malachite green oxalate. Specific activity was calculated from calibration curves, 2−20 μM, that were made with a standard KH_2PO_4 solution. The reaction was carried out at 37 °C for 3 min and then stopped by quenching the samples with dye reagent by adding 1 mL of the colorimetric phosphate assay reagent to each sample. The specific activity was estimated using the A_{660} to calculate the μmol of product formation using the standard KH_2PO_4 solution as a quantitative measure of inorganic phosphate present in the reaction mixture.

Once potent inhibitors were identified, to determine more detailed kinetic parameters (e.g., IC_{50}), the total volume of the assay mixture was increased to 200 μL to reduce experimental errors. Enzyme amount was adjusted to keep A_{660} between 0.1 and 0.5 to keep the percentage of product less than 20% of total substrate.[25]

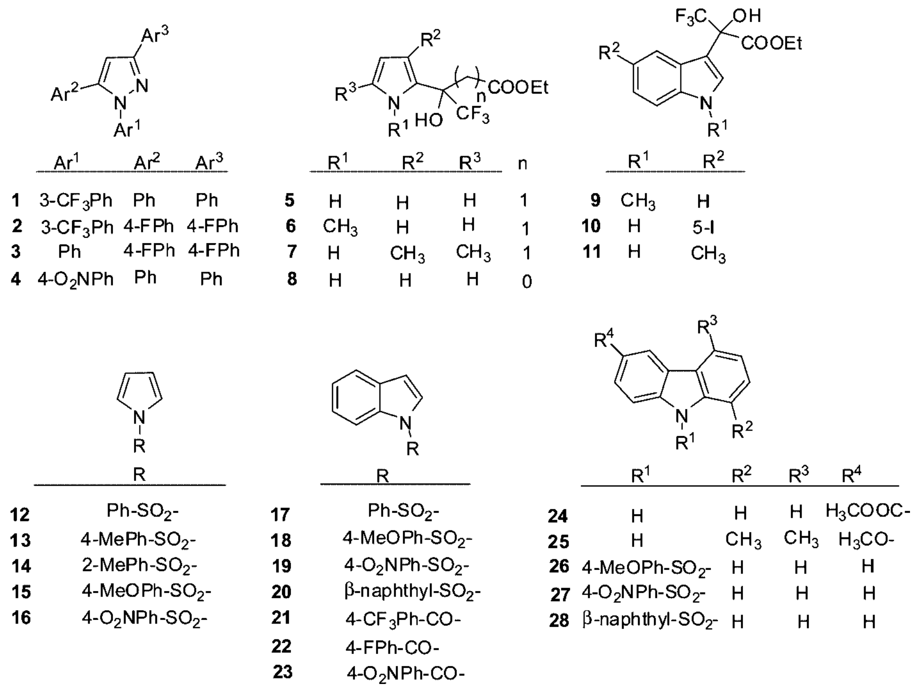

Figure 2. Schematic representation of the inhibitor candidates used in this study.

Docking Studies. The three-dimensional coordinates were downloaded from Rutgers RCSB.[26,27] The crystallographic ligand was removed from the binding pocket of interest. The energy-minimized ligand with full charges was loaded into the program PRODRG.[28] The format used was PDBQ for Autodock4[29] and mol2 format for Surflex[30] and DOCK6.1.[31]

The molecules were drawn in GaussView 2.1,[32] minimized by energy (HF/STO-3G), and converted to pdb then mol2 format (ChemDraw Ultra 9.0 and Dundee PRODRG). The pdb structure of protein 1FTA was obtained from Rutgers RCSB.[26,27]

By use of Surflex-Dock 2.0, a conformational search of rotatable bonds was performed and these conformations were aligned to the protein (protomol). The aligned fragments were scored and pruned, and the program presented the binding energy in log format (Table 1). The AutoDock4 performed the docking of the ligand to a set of grids describing the target protein. AutoDock4 studies resulted in estimated inhibition constants in log format shown in Table 1. Dock6 generated a set of overlapping spheres, which fill the active site. To orient a ligand within the active site, some of the sphere centers were "matched" with ligand atoms. Approximations were made to the usual molecular mechanics attractive and dispersive terms for use on a grid. The program presents energy scores in log format (Table 1). X-Score[33,34] was used to compare binding energies by analyzing binding strength between the protein and the ligand determined by electrostatic, hydrophilic, and hydrophobic interactions.

Results and Discussion

On the basis of earlier studies and detailed analysis of available databanks, we have selected several groups of compounds for this study. The general structure of the inhibitor candidates are illustrated in Figure 2. These compounds include several pyrazole, pyrrole, indole, and carbazole derivatives. The synthesis of these compounds have been carried out on the basis of our earlier work.[8−12] While these molecules are known compounds, they are not commercially available and thus have not been evaluated against FBPase inhibition. The biological potential of similar compounds has been already explored against Alzheimer's disease.[35]

As the first step, we have carried out enzyme inhibition assays using these compounds. A widely accepted protocol[23−25] has been used to evaluate the initial activity of our compounds. The inhibitors previously described above to inhibit amyloid fibrillogenesis[35] were first tested in in silico docking experiments. The compounds that yielded encouraging results in at least one of the three programs used were then tested in the laboratory for ability to inhibit FBPase reaction. The results of the assays and docking studies are summarized in Table 1.

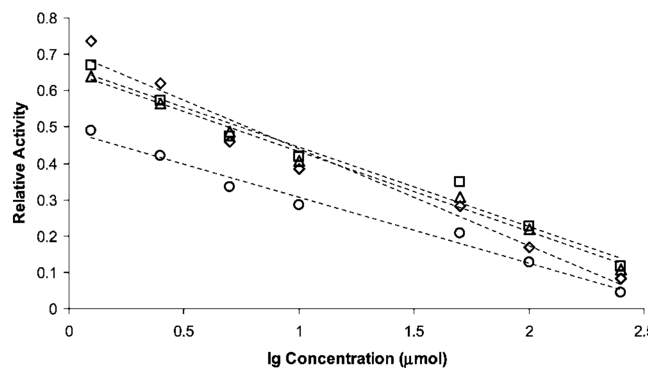

Figure 3. Effect of inhibitor concentration on the activity of FBPase for IC_{50} determination of the three inhibitor lead compounds and the natural inhibitor AMP: (◇) AMP; (△) **1**; (□) **7**; (○) **21**.

Interestingly, the best 10 compounds all had fluorine in their structure, to underline the beneficial effect of fluorine incorporation. Several of the molecules scored within ±2-fold of the natural inhibitor. On the basis of the docking results, 28 compounds were tested: 9 or 33% showed $IC_{50} > 250$ μM; 7 or 26% had 250 μM $> IC_{50} > 100$ μM; 2 or 7.4% had 100 μM $> IC_{50} > 50$ μM, and 9 or 33% had $IC_{50} < 50$ μM. Those that were within 2-fold of AMP, with either higher or lower IC_{50}, are considered lead compounds. Figure 3 shows the concentration vs activity functions for the three best inhibitors against AMP for comparison.

Although there is no clear agreement between the in silico docking results and the actual IC_{50} values, regarding the top 10 compounds, a correlation can be found between the theory and experimental data. Compound **21** with IC_{50} of 3.1 μM had a

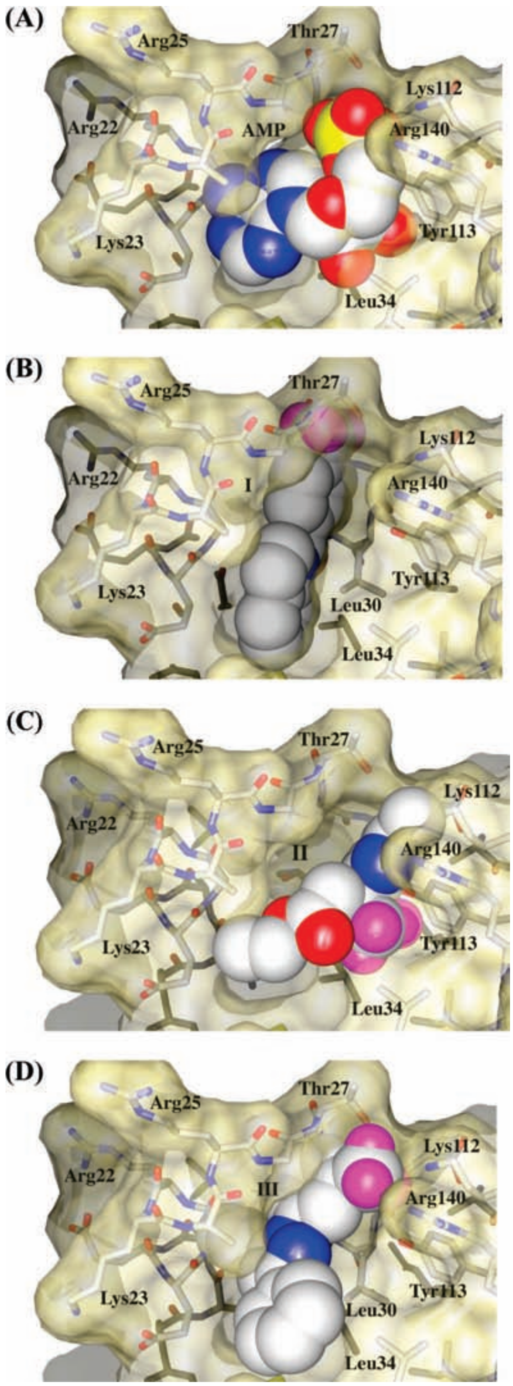

Figure 4. Comparison of AMP (A) in the AMP binding pocket of FBPase, PDB code 1FTA, with the docking results of the three best actual inhibitors (B) **21**; (C) **7**, and (D) **1** from Table 1: carbon, gray; fluorine, magenta; nitrogen, blue; oxygen, red; phosphorus, yellow.

predicted AutoDock4 inhibitory constant of $\sim 0.4 \times 10^{-6}$ and a Surflex score within 10% of natural inhibitor AMP at 2.84. Compound **7** with IC_{50} of 4.8 μM had a Surflex score 12% higher than AMP at 3.33. Compound **1** had IC_{50} of 6.1 μM and AutoDock4 score of 0.17×10^{-6}.

A comparison of the three highest scoring compounds was carried out with AMP by docking the lead compounds into the binding pocket of the AMP binding site of human FBPase (PDB code 1FTA) (Figure 4).

The actual crystallographic position of AMP is shown in Figure 1b and Figure 4A. There are several H-bonding interactions between the following residues and atoms of the AMP molecule: Thr27, Gly28, and Glu29 and oxygens of phosphate moiety; Arg140 and Tyr113 and the hydroxyl oxygens of the ribose ring of AMP; and the phosphate that is coordinated by Lys112 (Figure 1b and 4A). There are hydrophobic interactions of carbons of the heterocyclic ring and Val17, Leu30, and Leu34. Figure 4B−D show our best lead compounds from the inhibitor assays docked in the FBPase AMP binding domain (PDB code 1FTA). Note that the shape of the inhibitors fills nearly the entire AMP binding pocket with only a small space toward the bottom of the hydrophobic pocket. This shows that other hydrophobic functional groups may be added to enhance the binding. Figure 4B−D also show the trifluoromethyl moiety, which embeds the fluorine atoms into backbone interactions within phosphate binding pocket of AMP binding domain. Finally, AutoDock4 rotates the ligand until it fits into the AMP pocket in Figure 4D. The new version of AutoDock4 allows side chains to move slightly for an induced fit to the ligand. This might be misleading, as predicted binding here is far tighter than actual IC_{50} (10^{-6}). Even with inflated binding predicted in the 10^{-15} range, there is still empty space at the bottom of the hydrophobic pocket for tighter binding. In summary, docking results of all 28 compounds can be compared in the three programs.

In conclusion, we have identified three heterocyclic organofluorine lead compounds for FBPase inhibiton. A small scale automated docking utilizing the ZINC database[36] led us to identify classes of possible lead compounds. These compounds have been synthesized and have been targeted to high throughput colorimetric assay to screen compounds for FBPase inhibition. On the basis of the assays, a small group of compounds was identified as lead compounds. Then the interaction between these compound and FBPase was studied by utilizing three docking programs to understand how these inhibitors may bind the enzyme. The putative target for this study is the AMP binding pocket. However, as identified by others,[14] there exists an interfacial tetrameric binding pocket that requires a 2:1 ratio of tetramer to ligand binding. Since the affinity for this pocket is much smaller, some of these compounds may also be considered to be potential lead compounds for that site, as is shown in area 3 of Figure 1a.

References

(1) Convit, A.; Wolf, O. T.; Tarshish, C.; de Leon, M. J. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 2019–2022.

(2) Erion, M. D.; van Poelje, P. D.; Dang, Q.; Kasibhatla, S. R.; Potter, S. C.; Reddy, M. R.; Reddy, K. R.; Jiang, T.; Lipscomb, W. N. MB06322 (CS-917): a potent and selective inhibitor of fructose 1,6-bisphosphatase for controlling gluconeogenesis in type 2 diabetes. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 7970–7975.

(3) Erion, M. D.; Dang, Q.; Reddy, M. R.; Kasibhatla, S. R.; Huang, J.; Lipscomb, W. N.; van Poelje, P. D. Structure-guided design of AMP mimics that inhibit fructose-1,6-bisphosphatase with high affinity and specificity. *J. Am. Chem. Soc.* **2007**, *129*, 15480–15490.

(4) Dang, Q.; Kasibhatla, S. R.; Reddy, K. R.; Jiang, T.; Reddy, M. R.; Potter, S. C.; Fujitaki, J. M.; van Poelje, P. D.; Huang, J.; Lipscomb, W. N.; Erion, M. D. Discovery of potent and specific fructose-1,6-bisphosphatase inhibitors and a series of orally-bioavailable phosphoramidase-sensitive prodrugs for the treatment of type 2 diabetes. *J. Am. Chem. Soc.* **2007**, *129*, 15491–15502.

(5) Salpeter, S.; Greyber, E.; Pasternak, G.; Salpeter, E. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus: systematic review and meta-analysis. *Arch. Intern. Med.* **2003**, *163*, 2594–2602.

(6) Turner, R. C.; Holman, R. R.; Stratton, I. M.; Cull, C. A.; Matthews, D. R.; Manley, S. E.; Frighi, V.; Wright, D.; Neil, A.; Kohner, E.; McElroy, H.; Fox, C.; Hadden, D. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* **1998**, *352*, 854–865.

(7) van Poelje, P. D.; Potter, S. C.; Chandramouli, V. C.; Landau, B. R.; Dang, Q.; Erion, M. D. Inhibition of fructose 1,6-bisphosphatase reduces excessive endogenous glucose production and attenuates hyperglycemia in Zucker diabetic fatty rats. *Diabetes* **2006**, *55*, 1747–1754.
(8) Lange, S. M.; Schmidt, A.; Outerbridge, V.; Török, B. Synthesis of pyrazoles by a one-pot tandem cyclization−dehydration approach on Pd/C/K-10 catalyst. *Synlett* **2007**, 1600–1604.
(9) Abid, M.; Török, B. Synthesis of heteroaryl(trifluoromethyl)hydroxyalkanoic acid esters by highly efficient solid acid-catalyzed hydroxyalkylation of indoles and pyrroles with activated trifluoromethyl ketones. *Adv. Synth. Catal.* **2005**, *347*, 1797–1803.
(10) Abid, M.; Teixeira, L.; Török, B. Triflic acid controlled successive annelation of aromatic sulfonamides: an efficient one-pot synthesis of *N*-sulfonyl pyrroles, indoles, and carbazoles. *Tetrahedron Lett.* **2007**, *48*, 4047–4050.
(11) Abid, M.; De Paolis, O.; Török, B. A novel pot synthesis of *N*-acylindoles from primary aromatic amides. *Synlett* **2008**, 410–412.
(12) Abid, M.; Spaeth, A.; Török, B. Solvent-free solid acid-catalyzed electrophilic annelations: a new green approach for the synthesis of substituted five-membered N-heterocycles. *Adv. Synth. Catal.* **2006**, *348*, 2191–2196.
(13) Wright, S. W.; Carlo, A. A.; Danley, D. E.; Hageman, D. L.; Karam, G. A.; Mansour, M. N.; McClure, L. D.; Pandit, J.; Schulte, G. K.; Treadway, J. L.; Wang, I. K.; Bauer, P. H. 3-(2-Carboxyethyl)-4,6-dichloro-1*H*-indole-2-carboxylic acid: an allosteric inhibitor of fructose-1,6-bisphosphatase at the AMP site. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2055–2058.
(14) Wright, S. W.; Carlo, A. A.; Carty, M. D.; Danley, D. E.; Hageman, D. L.; Karam, G. A.; Levy, C. B.; Mansour, M. N.; Mathiowetz, A. M.; McClure, L. D.; Nestor, N. B.; McPherson, R. K.; Pandit, J.; Pustilnik, L. R.; Schulte, G. K.; Soeller, W. C.; Treadway, J. L.; Wang, I. K.; Bauer, P. H. Anilinoquinazoline inhibitors of fructose 1,6-bisphosphatase bind at a novel allosteric site: synthesis, in vitro characterization, and X-ray crystallography. *J. Med. Chem.* **2002**, *45*, 3865–3877.
(15) Hiyama, T., Ed. *Organofluorine Compounds*; Springer: Berlin, 2001. Kirsch, P. *Modern Fluoroorganic Chemistry: Synthesis, Reactivity, Applications*; Wiley-VCH: New York, 2004. Soloshonok, V. A. Ed. *Fluorine-Containing Synthons*; ACS Symposium Series; American Chemical Society: Washington, DC, 2005.
(16) Fried, J.; Sabo, E. F. *J. Am. Chem. Soc.* **1954**, *76*, 1455–1456.
(17) Soloshonok, V. A., Ed. *Enantiocontrolled Synthesis of Fluoro-organic Compunds: Stereochemical Challenges and Biomedicinal Targets*; Wiley: New York, 1999. Ramachandran, P. V., Ed. *Asymmetric Fluoroorganic Chemistry*; ACS Symposium Series; American Chemical Society: Washington, DC, 2000. Prakash, G. K. S.; Beier, P. *Angew. Chem., Int. Ed.* **2006**, *45*, 2172.
(18) http://www.expasy.org/6/16/08.
(19) Bradford, M. M. A rapid and sensitive method of quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(20) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.
(21) Davis, B. J. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **1964**, *12*, 404–427.
(22) Ornstein, L. Disc electrophoresis. I. Background and theory. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 321–349.
(23) Itaya, K.; Ui, M. A new micromethod for the colorimetric determination of inorganic phosphate. *Clin. Chim. Acta* **1966**, *14*, 361–366.
(24) Chen, L.; Roberts, M. F. Overexpression, Purification, and analysis of complementation behavior of *E. coli* SuhB protein: comparison with bacterial and archaeal inositol monophosphatases. *Biochemistry* **2000**, *39*, 4145–4153.
(25) Chen, L.; Roberts, M. F. Cloning and expression of the inositol monophosphatase gene from *Methanococcus jannaschii* and characterization of the enzyme. *Appl. Environ. Microbiol.* **1998**, *64*, 2609–2615.
(26) Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W.; Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410.
(27) Feng, Z.; Chen, L.; Maddula, H.; Akcan, O.; Oughtred, R.; Berman, H. M.; Westbrook, J. Ligand depot: a data warehouse for ligands bound to macromolecules. *Bioinformatics* **2004**, *20*, 2153–2155.
(28) Schuettelkopf, A. W.; van Aalten, D. M. F. PRODRG, a tool for high-throughput crystallography of protein−ligand complexes. *Acta Crystallogr.* **2004**, *D60*, 1355–1363.
(29) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olsen, A. J. J. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *Comput. Chem.* **1998**, *19*, 1639–1662.
(30) *Surflex-Dock*, version 2.0; Tripos International (1699 South Hanley Rd, St. Louis, MO, 63144).
(31) Shoichet, B. K.; Kuntz, I. D. Protein docking and complementarity. *J. Mol. Biol.* **1991**, *221*, 327–346.
(32) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J., Barone, V., Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G., Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A., Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W., Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.9; Gaussian, Inc.: Pittsburgh, PA, 1998.
(33) Wang, R.; Lu, Y.; Wang, S. Comparative evaluation of 11 scoring functions for molecular docking. *J. Med. Chem.* **2003**, *46*, 2287–2303.
(34) Wang, R.; Lai, L.; Wang, S. Further development and validation of empirical scoring functions for structure-based binding affinity prediction. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 11–26.
(35) Török, B.; Abid, M.; London, G.; Esquibel, J.; Török, M.; Mhadgut, S. C.; Yan, P.; Prakash, G. K. S. Highly enantioselective organocatalytic hydroxyalkylation of indoles with ethyl trifluoropyruvate. *Angew. Chem., Int. Ed.* **2005**, *44*, 3086–3089. Török, M.; Abid, M.; Mhadgut, S. C.; Török, B. Organofluorine inhibitors of amyloid fibrillogenesis. *Biochemistry* **2006**, *45*, 5377–5383. Abid, M.; Teixeira, L.; Török, B. Triflic acid-catalyzed highly stereoselective Friedel−Crafts aminoalkylation of indoles and pyrroles. *Org. Lett.* **2008**, *10*, 933–935.
(36) Irwin, J. J.; Shoichet, B. K. ZINC, a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.* **2005**, *45*, 177–182.
(37) Fenn, T. D.; Ringe, D.; Petsko, G. A. POVSript+: a program for model and data visualization using persistence of vision ray tracing. *J. Appl. Crystallogr.* **2003**, *36*, 944–947.

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