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Benzoxazole benzenesulfonamides as allosteric inhibitors of fructose-1,6-bisphosphatase

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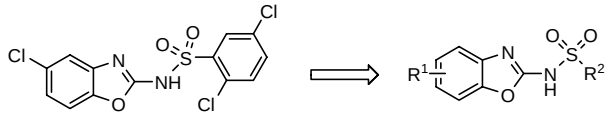
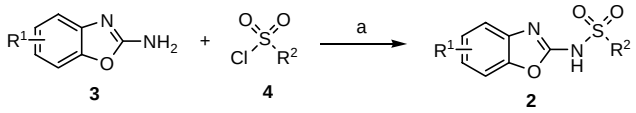
Abstract—A series of novel benzoxazole benzenesulfonamides was synthesized as inhibitors of fructose-1,6-bisphosphatase (FBPase-1). Extensive SAR studies led to a potent inhibitor, **53**, with an IC_{50} of 0.57 μM. Compound **17** exhibited excellent bioavailability and a good pharmacokinetic profile in rats.

In the previous paper,[1] we identified benzoxazole benzenesulfonamide **1** as a novel inhibitor of human fructose-1,6-bisphosphatase (hFBPase-1) with an IC_{50} of 3.4 μM in a colorimetric malachite green assay. X-ray crystallographic studies indicate that **1** has a unique binding mode, partially occupying the AMP allosteric regulatory site. In this communication, we will report the synthesis and structure–activity profile of benzoxazole benzenesulfonamide analogs **2**, leading to an improvement in potency.



1 **2**

Benzoxazole benzenesulfonamide analogs **2** were prepared by treating substituted benzoxazol-2-ylamines **3** with sulfonyl chlorides **4** in the presence of pyridine as shown in Scheme 1.[2] Both **3** and **4** were either commercially available or prepared according to the literature procedures.



3 **4** **2**

Scheme 1. Reagents and condition: (a) pyridine, CH_2Cl_2, microwave 130 °C.

A rapid systematic variation of substituents on the phenyl ring was accomplished in a parallel synthesis fashion through the key intermediate **5**. Compound **6** was prepared by Suzuki coupling reaction;[3] **7**, **8**, and **9** were prepared via corresponding copper assisted nucleophilic substitution reactions[4] (Scheme 2).

The introduction of substituents to the benzene ring was performed as Scheme 3. Treating **10** with either cyanogen bromide[5] or di(imidazole-1-yl)methanimine[6] afforded substituted benzoxazol-2-ylamine **11**, which, in turn, reacted with 2,5-dichlorobenzenesulfonyl chloride to form intermediate **12**. Coupling **12** with aryl boronic acid via Suzuki reaction gave rise to the desired compound **13**.

The inhibitory activity of synthesized molecules was evaluated by a colorimetric malachite green hFBPase-1 assay as described in our previous article.[1] The effects of replacement of the benzenesulfonamide on FBPase-1 inhibitory activity are summarized in Table 1. The aromatic character of this group appears to be crucial.

Keywords: Fructose-1,6-bisphosphatase; Enzyme inhibitor; Allosteric regulator; Benzoxazole benzenesulfonamide; Structure–activity studies; X-ray crystallography.
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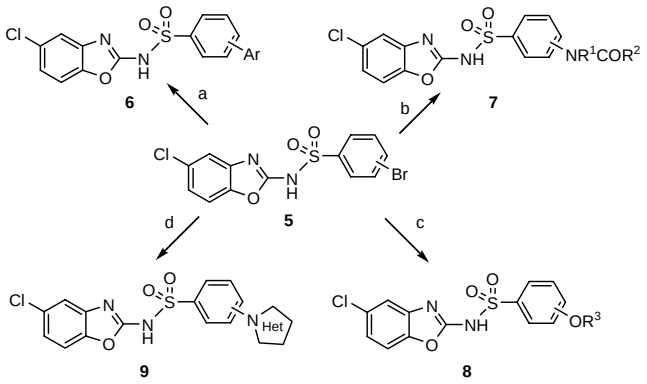
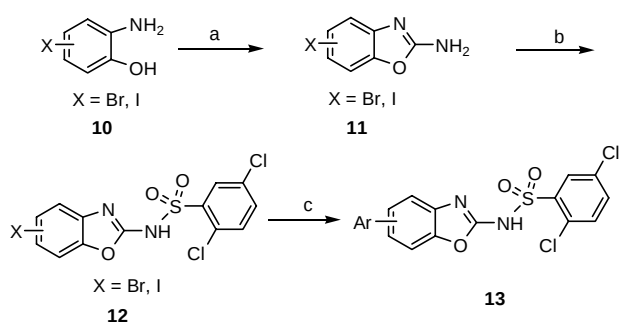


Scheme 2. Reagents and conditions: (a) $ArB(OH)_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3, DMF, microwave 150 °C; (b) R^2CONHR,[1] CuI, 1,10-phenanthroline, Cs_2CO_3, ODCB, microwave 200–220 °C; (c) R^3OH, CuI, 1,10-phenanthroline, Cs_2CO_3, tol, microwave 160–170 °C; (d) N-H heteroarene, CuI, 1,10-phenanthroline, K_2CO_3, NMP, 190 °C.



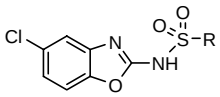
Scheme 3. Reagents and conditions: (a) BrCN (3 M in CH_2Cl_2) or di(imidazole-1-yl)methanimine, MeOH, rt; (b) 2,5-dichlorobenzenesulfonyl chloride, pyridine, CH_2Cl_2, microwave 130 °C; (c) $ArB(OH)_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3, DMF, microwave 150 °C.

Heteroaromatic (**15**, **16**) and fused aromatic rings (**17**, **18**) are well tolerated. Replacement of the phenyl ring with alkyl groups (**19**, **20**) dramatically diminishes the activity.

Further SAR studies focused on the effects of substituents of the phenyl ring (Table 2). Several substituents provide some improvement in potency, for instance, 3-Cl (**25**, IC_{50} = 1.3 μM) and 3-NO_2 (**26**, IC_{50} = 1.8 μM), represent a 2–3-fold boost in potency relative to initial lead **1**. In general, mono-substitution at the *ortho*-, *meta*- or *para*-position is desirable, but bulky groups such as *t*-Bu (**32**, IC_{50} = 24 μM) and 4-*t*-Bu-benzyloxy (**28**, IC_{50} > 50 μM) lead to reduced activity; *ortho*-, *ortho*-di-substitution (**36**, **37**) is strongly disfavored.

Manipulation of substituents on the benzoxazole ring also led to a series of new compounds (Table 3). The 4-position is the most restrictive site on the molecule; even small substituents such as iodo and methyl lead to a substantial loss of potency. This result is consistent with the previously reported crystallographic binding mode,[1] which indicates that 4-substituents are directed toward the interior of the FBPase tetramer. In contrast, the 7-position is directed toward the outside of the FPBase tetramer, allowing for a broader range of

Table 1. Inhibitory activity of selected FBPase-1 inhibitors



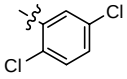
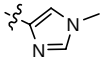
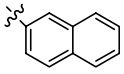
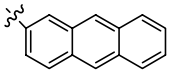
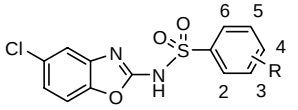
Compound	R	FBPase-1 IC_{50} (μM)
1		3.4
14		7.7
15		13
16		9.4
17		2.5
18		3.4
19	Me	>50
20	*n*-Bu	>50

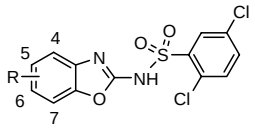
Table 2. Inhibitory activity of selected FBPase-1 inhibitors



Compound	R	FBPase-1 IC_{50} (μM)
14	H	7.7
21	2-Br	6.5
22	2-CN	6.0
23	2-Ph	3.8
24	2-(Imidazol-1-yl)	23
25	3-Cl	1.3
26	3-NO_2	1.8
27	3-Ph	9.8
28	3-(4-*t*-Bu-benzyloxy)	>50
29	4-F	2.8
30	4-OCF_3	7.8
31	4-Me	6.2
32	4-*t*-Bu	24
33	4-Ph	4.0
34	4-(3-Furanyl)	6.7
35	4-$NHCOCH_2CH_2CH_3$	12
36	2,6-Di-Cl	>50
37	2-Cl, 6-Me	>50

substituents, preferably hydrophilic. Small substituents are tolerated at the 5-position and are acceptable at the 6-position. A combination of 5-MeO and various 7-substituents significantly boosts the potency. Of note is **53**, in which a combination of 5-MeO with 7-(3-aminophenyl) gives a 6-fold boost in potency (IC_{50} = 0.57 μM) versus **1**. Compound **53** also shows im-

Table 3. Inhibitory activity of selected FBPase-1 inhibitors



Compound	R	FBPase-1 IC_{50} (μM)
38	4-I	>50
39	4-Me	55
40	4-(4-Pyridyl)	>50
41	5-Br	1.9
42	5-Me	6.4
43	5-*t*-Bu	32
44	5-(3-Furanyl)	4.0
45	6-Cl	8.1
46	6-Me	10
47	6-MeO	8.0
48	5-MeO, 7-(4-MeO-3-Pyridyl)	3.5
49	5-MeO, 7-(3-HO-Ph)	1.7
50	5-MeO, 7-(4-HO-Ph)	1.8
51	5-MeO, 7-(3-NH_2CH_2-Ph)	2.6
52	5-MeO, 7-(4-NH_2-Ph)	1.3
53	5-MeO, 7-(3-NH_2-Ph)	0.57

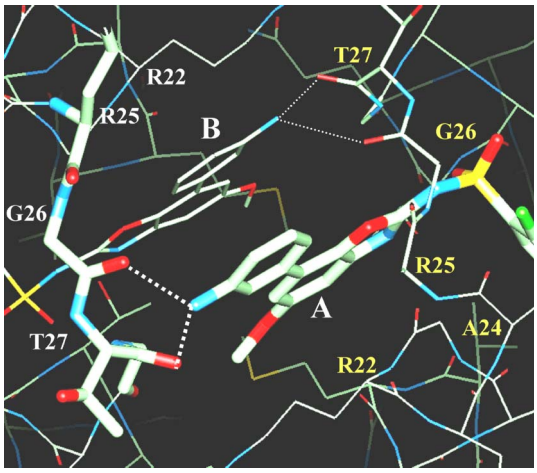


Figure 1. Binding mode of **53** in the FBPase tetramer. The view is approximately along one of the tetramer dyads and illustrates the binding mode of **53** in the periphery of the FBPase tetramer. Each molecule of inhibitor interacts with the main chain C=O of residues Gly26 and Thr27 across the dyad. Thick dotted lines (left) highlight the hydrogen-bonding interactions of the anilino group of molecule A of **53** with the main chain carbonyl oxygens of a second molecule of FBPase *trans*- across the twofold symmetry axis (white symbols and gray atoms). Thin dotted lines indicate the corresponding interaction for molecule B of **53** with the opposite monomer of FBPase (yellow symbols and stick bonds). There are four molecules of **53** per tetramer; H-bond distances range from ~2.7 to 3.6 Å. Aromatic stacking of the aniline groups across the symmetry axis is apparent.

proved potency in a 'coupled' assay format,[1] with an IC_{50} = 0.73 μM and K_i = 0.22 μM.

The X-ray crystal structure of **53**[7] confirms the previously observed binding mode for this series of inhibitors and is consistent with the SAR concepts outlined (Fig. 1). Similar to compound **1**,[1] the arylsulfonamide portion of **53** interacts with a part of the AMP allosteric

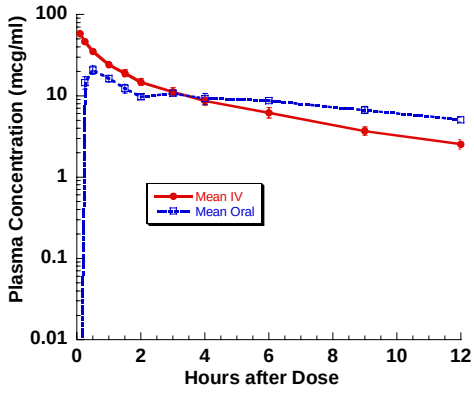


Figure 2. Rat pharmacokinetics of **17**.

regulatory site and the benzoxazole moiety protrudes into the space between subunits of the FBPase homotetramer. In addition to the AMP-site interactions, the amino group of the C7-substituent is exposed and makes two additional hydrogen-bonding interactions with the C=O of Gly26 and Thr27. Other analogs with 7-substituents capable of H-bonding (e.g., **48**, **49**) interact similarly with the protein backbone (data not shown) and demonstrate improved potency, suggesting that the latter may be the result of these H-bonding interactions.

Compound **17** was selected as a representative to evaluate the pharmacokinetic properties of these benzoxazole benzenesulfonamides in rats. At 5 mg/kg dose, compound **17** was shown to have an excellent oral bioavailability (>100%) with a long oral half-life (7.5 h) and a low plasma clearance (0.04 L/h kg) (Fig. 2).

In conclusion, we have synthesized a series of novel benzoxazole benzenesulfonamides as inhibitors of FBPase-1. Optimization of screening hit **1** led to compound **53** (IC_{50} = 0.57 μM) with a 6-fold boost in potency. X-ray crystal structures revealed that the appropriately selected 7-substituent of **53** forms two additional hydrogen bonding interactions with the enzyme, leading to the enhanced affinity. These results suggest opportunities to design even more potent FBPase-1 inhibitors. Compound **17** with an IC_{50} of 2.5 μM exhibited an excellent pharmacokinetic profile in rats. Further optimization studies as well as the pharmacological characterization of leading compounds will be reported in due course.

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7. Co-crystals of human FBPase-1 with **48** (and **53**) were grown by protocols similar to the ones used in Ref. 1 but displacing the seeding compound ZMP with the relevant ligand. The crystals with **48** were orthorhombic ($P2_12_12_1$) with cell constants $a = 67.4$, $b = 83.3$, $c = 277.5$ Å and diffracted to 2.8 Å resolution, using synchrotron radiation (Advanced Photon Source, APS, IMCA-CAT, 17-ID). The crystals contained one tetramer of FBPase and four molecules of **48** in the asymmetric unit. The crystals with **53** were orthorhombic ($P2_12_12_1$) with cell constants $a = 84.4$, $b = 108.7$, $c = 196.4$ Å and diffracted weakly only to approximately 3.5 Å, using synchrotron radiation (Advanced Photon Source, APS, IMCA-CAT, 17-ID). The crystals contained one tetramer of FBPase and four molecules of **53** in the asymmetric unit. The structures of FBPase with **48** and **53** were solved by molecular replacement using one FBPase tetramer and refined using standard methods. Crystallographic coordinates of FBPase complexed with **48** and **53** have been deposited at the Protein Data Bank with accession codes 2fie, 2fix.