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Discovery of 5-((5-chloro-2-methoxyphenyl)sulfonamido)nicotinamide (SBI-425), a potent and orally bioavailable tissue-nonspecific alkaline phosphatase (TNAP) inhibitor

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Abstract

Tissue-nonspecific alkaline phosphatase (TNAP) is an ectoenzyme crucial for bone matrix mineralization via its ability to hydrolyze extracellular inorganic pyrophosphate (ePP_i), a potent mineralization inhibitor, to phosphate (P_i). By the controlled hydrolysis of ePP_i, TNAP maintains the correct ratio of P_i to ePP_i and therefore enables normal skeletal and dental calcification. In other areas of the body low ePP_i levels lead to the development of pathological soft-tissue calcification, which can progress to a number of disorders. TNAP inhibitors have been shown to prevent these processes via an increase of ePP_i. Herein we describe the use of a whole blood assay to optimize a previously described series of TNAP inhibitors resulting in 5-((5-chloro-2-methoxyphenyl)sulfonamido)nicotinamide (SBI-425), a potent, selective and oral bioavailable compound that robustly inhibits TNAP *in vivo*.

Graphical abstract

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Tissue-nonspecific alkaline phosphatase (TNAP) is responsible for the regulation of extracellular matrix calcification in the skeleton and dental tissues. It is a member of the family of alkaline phosphatase (AP) enzymes that control dephosphorylation and transphosphorylation of a wide spectrum of substrates *in vitro*. In humans, four AP isozymes have been identified—one tissue-nonspecific (TNAP) and three tissue-specific—named according to the tissue of their predominant expression: intestinal (IAP), placental (PLAP) and germ cell (GCAP) APs. Modulation of activity of the different AP isozymes may have therapeutic implications in distinct diseases and cellular processes. The biological function of TNAP is to hydrolyze extracellular inorganic pyrophosphate (ePPi) to maintain the correct ratio of inorganic phosphate (Pi) to ePPi in skeletal tissues to enable normal skeletal and dental calcification. Elsewhere in the body, high ePPi levels prevent ectopic and other soft tissue calcification. Various disease states are characterized by the deficiency of ePPi which leads to abnormal calcification, including medical vascular calcification associated with chronic kidney disease (CKD)¹, as well as rare disorders such as pseudoxanthoma elasticum (PXE)², generalized arterial calcification of infancy (GACI)³, calcification of joints and arteries (CALJA)⁴, and Hutchinson-Gilford progeria syndrome (HGPS)⁵. Inhibition of TNAP as a means to raise ePPi and prevent soft-tissue calcification has emerged as a therapeutic strategy to treat a number of these disorders^{6–9}.

There have been numerous TNAP inhibitors described in the literature, with select compounds shown in Figure 1. Early inhibitors such as levamisole (**2**) and theophylline (**3**) show only very weak binding and are not selective against the other alkaline phosphatases¹⁰. A range of analogs replacing the phenyl group of levamisole have been generated, including thiophenes (**5**)¹¹, benzothiophenes (**6**)¹² and benzofurans (**7**)¹³, but these compounds in general show modest potency. There has also been a recent report of alkynyldihydrofuranones (**8**)¹⁴ as TNAP inhibitors, albeit with modest potency as well. Our earlier work on sulfonamides (**1**)¹⁵ and pyrazoles (**4**)^{16,17} generated potent and selective TNAP inhibitors, albeit with modest pharmacokinetics. Therefore we undertook an optimization campaign to develop a TNAP inhibitor that would be orally bioavailable and give robust TNAP inhibition *in vivo*. As part of this effort we developed an *in situ* TNAP biomarker (“whole blood”) assay measuring activity in minimally diluted blood plasma samples that simulates the compound environment *in vivo* to more accurately predict the level of TNAP inhibition *in vivo*¹⁸. When we screened the range of known TNAP inhibitors, we were surprised to find only modest activity at best. For example, our previous lead **1**, while <20 nM in our standard TNAP assay measuring the pyrophosphorolysis rate toward native substrate PPi *in vitro*, was >5 μ M in the whole blood assay. In addition compounds **2–4** all showed no activity in the whole blood assay. Therefore we used this assay as a critical secondary screen for our lead optimization efforts. Due to the observation that some activity was retained in the whole blood assay, we focused our efforts on the sulfonamide series with the areas of optimization highlighted in Figure 1. The results of our efforts are summarized in Tables 1–3 below. Prior work had shown the necessity of the sulfonamide linker, the quinolone or pyridine motif and the preference for a 2-methoxy group on the phenyl ring attached to the sulfonamide¹⁵; therefore we kept these moieties fixed. Our initial structure-activity relationship (SAR) studies focused on examining substituents in addition to the 2-methoxy group on the phenyl ring (Table 1). As shown in the table, we generated a range of

quinolines that were potent in the PPI assay but had a large shift in potency in the whole blood assay (Entries 1–5). Interestingly, the comparable pyridine analogs displayed a much smaller potency shift (see, for example **2** vs **6** and **3** vs **7**). It is unclear what the primary factors are that are driving the divergence in potency between the primary assay and the whole blood assay, but we hypothesize it is a combination of plasma protein binding/stability and physicochemical properties. Thus far we have not been able to ascertain a direct correlation to any one or several parameters however. In general, a range of substituents was tolerated, with a marked preference for substitution in the 5 position, with halogens being preferred. We therefore maintained the pyridyl motif for subsequent efforts. Early exploration of the SAR around the pyridyl ring indicated that substitution was not well tolerated in the 4- and 6- positions (data not shown); thus we focused on substitution at the 5-position. Groups such as methyl and chloro in that position gave weakly active analogs; in contrast substituted phenyl groups were reasonably potent, albeit not in the whole blood assay. Further work demonstrated that esters, acids and amides were also well tolerated, in particular carboxamide **27** (SBI-425) showing not only excellent potency in the PPI assay but also strong activity in the whole blood assay. Potency dropped off significantly with substituted amides (compounds **28–31**). With SBI-425 in hand, we then returned to investigating the substitution around the phenyl ring of the sulfonamide, with the results shown in Table 3. Consistent with previous results, halogens were preferred, with F, Cl and Br giving comparable potency and a similar shift between the PPI and whole blood assays.

A representative synthesis for our lead series of molecules is shown in Scheme 1¹⁹. Treatment of the chloroanisole with sulfonyl chloride gave sulfonyl chloride **36**, which was reacted with **38** (generated by esterification of aminonicotinic acid **37**). Amide formation gave SBI-425 in good yields. SBI-425 has been generated in >50g scale using this method.

We then examined the rodent pharmacokinetics of **27**, **32**, and **33** with the results shown in Table 4. In general, the compounds showed low to very low clearance, a good $t_{1/2}$ and good to excellent oral bioavailability. In particular, SBI-425 displayed very high oral exposure (AUC of >800 ug.hr/mL after a 10 mg/kg dose). Like earlier compounds from this series¹⁵, **SBI-425** was highly selective (>80 μ M) against the other alkaline phosphatases including IAP and PLAP. In addition, a Eurofins/Ricerca Hit Profiling screen against 35 targets at 10 μ M showed no cross reactivity with the exception of modest (>30 μ M) activity against CYP3A4.

To measure the level of TNAP inhibition *in vivo*, we dosed mice orally at 10 mg/kg and then measured the level of TNAP inhibition in plasma at various time points. As shown in Figure 2, a single oral dose of SBI-425 inhibits TNAP activity in plasma >75% out to 8 hours and ~50% at 24 hours.

In conclusion, we have developed a potent and selective TNAP inhibitor that gives robust inhibition *in vivo* after oral dosing. This compound has already demonstrated activity in blocking calcification in patient derived fibroblasts as well as in rodent models of GACI⁶ and PXE². Additional studies to examine the effect of TNAP inhibition in a broad range of disease states using SBI-425 are currently underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Select previously described TNAP inhibitors, including TNAP probe **1** (MLS-0038949) with areas for SAR exploration highlighted

Figure 2.
TNAP inhibition in mouse plasma after a 10 mg/kg oral dose of SBI-425

Scheme 1.

Synthesis of **SBI-425**. i. ClSO_3H , 85% ii. SOCl_2 , MeOH iii. Pyridine, DMAP, 90% iv. NH_4OH , H_2O , 75%

Table 1

SAR elucidation of the phenyl ring

Cmpd	R ¹	Core	TNAP (PPI) IC ₅₀ (μM) ^a	TNAP (pNPP, whole blood) IC ₅₀ (μM) ^a
1 (MLS-0038949)	5-OMe	A	0.015	6.14
2	5-Br	A	0.006	>20
3	5-Cl	A	0.006	4.51
4	5-CF ₃	A	0.026	>20
5	5-CN	A	0.038	NT
6	5-Br	B	0.043	0.543
7	5-Cl	B	0.084	0.795
8	5-CF ₃	B	0.183	3.01
9	5-CN	B	0.322	NT
10	4-OMe	B	10.3	NT
11	4-Cl	B	4.17	NT
12	4-CH ₃	B	0.896	3.55
13	5-CO ₂ Me	B	1.51	NT
14	5-CONH ₂	B	1.49	NT
15	5-Ph	B	0.876	>20

^aValues are means of at least three experiments, standard deviations were within 20% of reported values.

NT= Not tested

Table 2

SAR elucidation around the pyridyl core

Cmpd	R ¹	R ²	TNAP (PPi) IC ₅₀ (μM) ^a	TNAP (pNPP, whole blood) IC ₅₀ (μM) ^a
16	Cl	2-Me-Ph	0.178	NT
17	Cl	3-Me-Ph	0.160	NT
18	Cl	4-Me-Ph	0.147	NT
19	Cl	4-OMe-Ph	0.023	16.0
20	Cl	4-Cl-Ph	0.198	NT
21	OMe	3-Pyridyl	0.120	NT
22	OMe	4-Pyridyl	0.073	>20
23	Cl	3-Pyridyl	0.073	NT
24	Cl	4-Pyridyl	0.073	>20
25	Cl	-CO ₂ Me	0.065	>20
26	Cl	-CO ₂ H	0.134	>20
27 SBI-425	Cl	-CONH ₂	0.016	0.105
28	Cl		0.109	>20
29	Cl		0.193	>20
30	Cl		0.310	NT

Cmpd	R ¹	R ²	TNAP (PPi) IC ₅₀ (μM) ^a	TNAP (pNPP, whole blood) IC ₅₀ (μM) ^a
31	Cl		0.671	NT

^aValues are means of at least three experiments, standard deviations were within 20% of reported values.

NT= Not tested

Table 3

Further SAR elucidation around the phenyl ring

Cmpd	R ¹	R ³	TNAP IC ₅₀ (μM) ^a	TNAP (pNPP, whole blood) IC ₅₀ (μM) ^a
27 SBI-425	Cl	OMe	0.016	0.105
32	F	OMe	0.019	0.109
33	Br	OMe	0.014	0.121
34	OCF ₃	OMe	0.131	1.56
35	Cl	OCF ₃	0.432	18.7

^aValues are means of at least three experiments, standard deviations were within 20% of reported values.

Table 4Selected mouse PK parameters for compounds **27**, **32** and **33**^a

Compds	Cl _p ^b (mL/min/kg)	Vd ^b (L/kg)	C _{max} ^c (ug/mL)	AUC ^c (ug.hr/mL)	t _{1/2} ^c (hr)	%F
27, SB1-425	5.14	1.03	178	848	2.3	58
32	16.8	0.88	63	141	3.7	>100
33	1.83	0.31	133	603	2.2	70

^a Compounds dosed 2 mpk iv and 10 mpk po.^b iv,^c po