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Discovery of oxazolo[4,5-*b*]pyridines and related heterocyclic analogs as novel SIRT1 activators

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ABSTRACT

SIRT1 is an NAD$^+$-dependent protein deacetylase that appears to produce beneficial effects on metabolic parameters such as glucose and insulin homeostasis. Activation of SIRT1 by resveratrol (**1**) has been shown to modulate insulin resistance, increase mitochondrial content and prolong survival in lower organisms and in mice on a high fat diet. Herein, we describe the identification and SAR of a series of oxazolo[4,5-*b*]pyridines as novel small molecule activators of SIRT1 which are structurally unrelated to and more potent than resveratrol.

SIRT1 is one of seven sirtuin members of the NAD$^+$-dependent deacetylases identified to date,[1] and has recently been linked to a number of metabolic functions, including activation of PGC-1α, a transcriptional coactivator that may regulate glucose metabolism.[2,3] In addition, modulation of SIRT1 could lead to an increase in mitochondrial biogenesis, a biological response that is similarly produced by calorie restriction.[4,5] With a net increase in mitochondria, skeletal muscle and adipose tissues display an improved glucose metabolism profile.[6] On this basis, activation of SIRT1 could serve as a novel approach to treat type II diabetes and other metabolic disorders. Additional validation for SIRT1 as a therapeutic target has come from transgenic mice that overexpress SIRT1, as these animals display phenotypes that are characteristic of an improved metabolic profile.[7] Resveratrol (**1**), a naturally occurring small molecule activator of SIRT1, has been reported to extend lifespan in yeast,[8] *Caenorhabditis elegans*, *Drosophila*[9] and rodents.[10] When administered to rodents, resveratrol has been demonstrated to improve metabolism and glucose tolerance, as well as overall physical performance in various stress tests.[11] Recent efforts in our laboratory have identified compounds capable of activating SIRT1 that are structurally distinct from and significantly more potent than **1**.[12]

A high-throughput in vitro fluorescence polarization assay was used to screen a commercial library of small molecules, which resulted in the identification of several novel scaffolds as SIRT1 modulators. This SIRT1 FP assay is a coupled assay where the first reaction is the deacetylation reaction of a p53-derived substrate catalyzed by SIRT1 and the second reaction is cleavage by trypsin at the newly exposed lysine residue.[14] Activation in this assay is measured both as the concentration of compound required to increase the enzyme activity by 50% ($EC_{1.5}$) as well as by the percentage maximum activation achieved at the highest doses of compound tested (max act). One notable series of SIRT1 activators identified through the HTS was the oxazolo[4,5-*b*]pyridines (OAP), as exemplified by compounds **2** and **3** (Fig. 1). These compounds showed moderate activation of SIRT1, with $EC_{1.5}$ = 6 μM, 240% max act, and 25 μM, 207% max activation, respectively. Importantly, these compounds were more potent than resveratrol, **1**, ($EC_{1.5}$ = 46 μM, 200% max act) and were amenable to analog synthesis at several different points on the scaffold. The focus of the initial analog libraries was twofold: to drive potency as well as to increase the solubility of the OAP compounds. Fortuitously, the attachment of solubilizing groups on specific points of the scaffold improved the inherent activity of the compounds. For example, substitution with the solubilizing groups amidoethyldimethylamine and dimethylamine on the central phenyl ring of the OAP provided compounds **4**, **5**, and **6**, with **5** activating SIRT1 at submicromolar levels ($EC_{1.5}$ = 0.7 μM, 240% max act), Table 1. Inclusion of the methylpiperazine moiety exhibited similar function. Compound **8** has a subtle methoxy positional rearrangement, which when combined with the methylpiperazine offers a boost in potency to an $EC_{1.5}$ value of 0.5 μM with 220% max activation. This trend was lost, however, if the solubilizing group was instead

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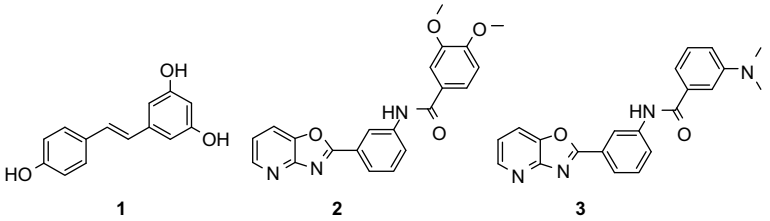
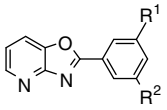
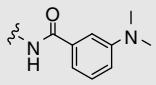
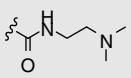
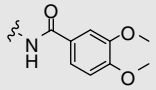
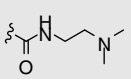
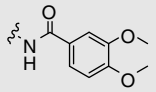
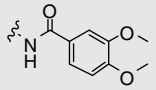
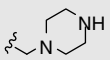
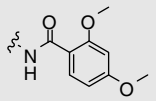
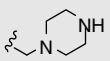


Figure 1. Resveratrol (**1**), and the oxazolo[4,5-*b*]pyridines (**2** and **3**).

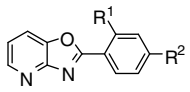
Table 1
Oxazolopyridines with solubilizing groups on central aryl ring

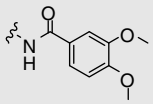
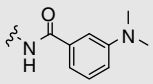
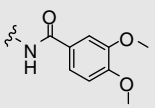
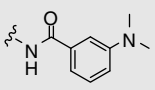


Compound	R^1	R^2	$EC_{1.5}$, μM (% max act)[a]
4			1.8 (330%)
5			0.7 (240%)
6			1.1 (200%)
7			1.1 (240%)
8			0.5 (220%)

[a] $EC_{1.5}$ values were determined from three separate titration curves. Each of the $EC_{1.5}$ values shown represents the mean of three determinations, with variations in individual values of <15%.

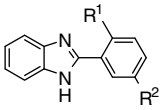
Table 2
Ortho- and *para*-substituted oxazolopyridines

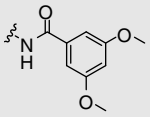
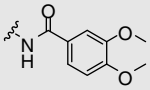
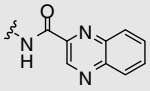
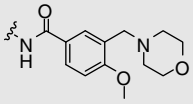
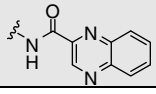
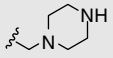


Compound	R^1	R^2	$EC_{1.5}$, μM (% max act)[a]
9		H	0.9 (170%)
10		H	4.4 (209%)
11	H		>100
12	H		>100

[a] $EC_{1.5}$ values were determined from three separate titration curves. Each of the $EC_{1.5}$ values shown represents the mean of three determinations, with variations in individual values of <15%.

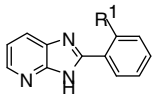
Table 3
Select benzimidazole SAR

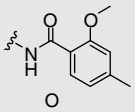
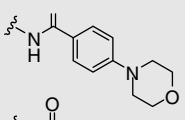
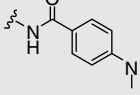
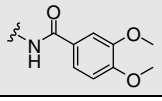


Compound	R^1	R^2	$EC_{1.5}$, μM (% max act)[a]
13		H	1.7 (180%)
14		H	0.4 (595%)
15		H	0.3 (253%)
16		H	0.5 (314%)
17			0.4 (820%)

[a] $EC_{1.5}$ values were determined from three separate titration curves. Each of the $EC_{1.5}$ values shown represents the mean of three determinations, with variations in individual values of <15%.

Table 4
Ortho-substituted azabenzimidazoles



Compound	R^1	$EC_{1.5}$, μM (% max act)[a]
18		4.1 (250%)
19		0.9 (273%)
20		0.7 (230%)
21		0.5 (270%)

[a] $EC_{1.5}$ values were determined from three separate titration curves. Each of the $EC_{1.5}$ values shown represents the mean of three determinations, with variations in individual values of <15%.

placed on the pyridyl ring of the OAP core; such compounds exhibited no activation of SIRT1 (data not shown).

A major breakthrough in the SAR of the OAP series was the determination that *ortho*-substitution of the amide linker on the central phenyl ring was optimal. A shift of the dimethoxyaryl amide and the dimethylaminoarylamide from the *meta*- to the *ortho*-positions resulted in significantly more active compounds. Compounds **9** and **10** have an $EC_{1.5}$ = 0.9 and 4.4 μM, respectively, with roughly twofold maximum activation (Table 2) as compared with the 6 and 25 μM values of their *meta*-counterparts, **2** and **3**. Interestingly, if the amide moiety was instead incorporated at the *para*-position of the central ring, all activity was lost, as exemplified by compounds **11** and **12**.

In addition to scanning for alternate functional groups to drive the potency of the OAP class, other bicyclic cores were explored as well. Appropriately substituted benzimidazoles were found to be potent SIRT1 activators. Selected SAR from this series is shown in Table 3. As with the oxazolopyridines, *ortho*-substitution of the amide linker was preferred. Compound **14** is an analog of the screening hit, **2**, and has much improved potency with an $EC_{1.5}$ = 0.4 μM and sixfold activation of the enzyme. The quinazoline amide functionality of compound **15** imparted improved potency as well. Solubilizing groups were again considered in an effort to improve solubility, and the substitution of an aminopiperazine moiety on the amido-aryl ring of **17** turned the benzimidazole into the first high-fold SIRT1 activator with a maximum activation of 820% ($EC_{1.5}$ = 0.4 μM).

A slight modification of the benzimidazole core gave rise to the analogous azabenzimidazoles, which were also found to similarly activate SIRT1. Compounds with tertiary amine solubilizing groups attached to the amido-aryl ring had good potency (**19** and **20**, Table 4).

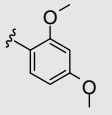
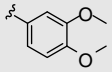
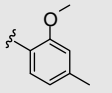
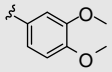
It is interesting to note that in this series, *meta*- and *para*-substitutions of the amide linker were well tolerated, unlike the SAR observed with the OAP and benzimidazole scaffolds (Table 5). This divergence in the activity of seemingly similar series will be better understood as the structural biology around SIRT1 progresses.

The synthetic routes to these bicyclic ring systems were straightforward, facilitating the synthesis of varied analogs.[13] The oxazolopyridine core was constructed through an initial condensation of 2-amino-3-hydroxypyridine and an aminobenzoic acid in the presence of PPA. Subsequent amide formation was carried out under microwave conditions in good yields. To incorporate solubilizing groups onto the central aryl ring, **26** was first combined with monomethyl-5-nitroisophthalate to form the amide, **30**. Cyclization to the bicyclic system was again carried out with PPA, and the resulting benzoic acid, **31**, was reduced and converted to the methylamine. Reduction of the nitro group and subsequent amide formation afforded the final substituted OAPs (Scheme 1).

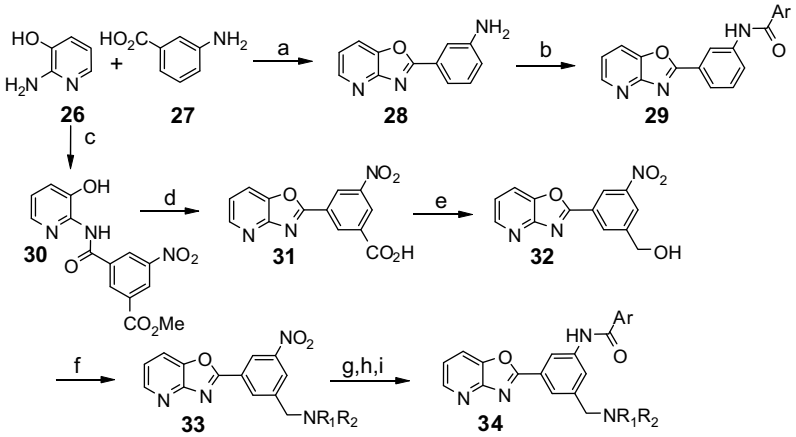
The benzimidazoles were synthesized by amide couplings with the commercially available benzimidazole-2-ylaniline, **35** (Scheme 2). The azabenzimidazole core was formed through a sulfur-mediated condensation of diaminopyridine and nitrobenzaldehyde (Scheme 3). The azabenzimidazole was then treated with SEMCl to provide a mixture of the protected nitrogen isomers, which were separated by column chromatography. The individual isomers were carried forward through subsequent nitro reduction and amide formation.

In summary, oxazolo[4,5-*b*]pyridines were identified as novel activators of SIRT1 through high-throughput screening efforts. Diversification of the linker positions and aryl functionalities led to compounds with submicromolar activities, such as compound **8** ($EC_{1.5}$ = 0.5 μM, 220% max act). Further exploration of the bicyclic

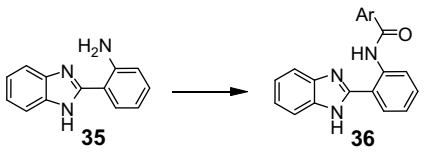
Table 5
Meta- and *para*-substituted azabenzimidazoles

Compound	Ar	$EC_{1.5}$, μM (% max act)[a]
22		0.5 (232%)
23		1.4 (324%)
24		2.3 (150%)
25		1.6 (180%)

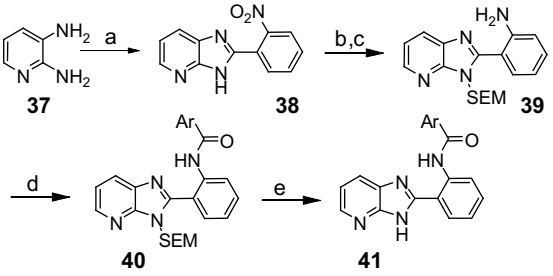
[a] $EC_{1.5}$ values were determined from three separate titration curves. Each of the $EC_{1.5}$ values shown represents the mean of three determinations, with variations in individual values of <15%.



Scheme 1. Reagents and conditions: (a) PPA, 180 °C, 80%; (b) ArCOCl, pyridine, microwave 160 °C, 10 min, 60–83%; (c) monomethyl 5-nitroisophthalate, HATU, DIEA, 90%; (d) PPA, 160 °C, 80%; (e) isobutylchloroformate, NMM, then $NaBH_4$, 68%; (f) MsCl, Et_3N, CH_2Cl_2 then NHR^1R^2, 75% over two steps; (g) sodium hydrosulfide hydrate, MeOH, H_2O, 88%; (h) ArCOCl, pyridine, microwave, 160 °C, 10 min, 50–75%; (i) TFA, CH_2Cl_2 as needed for BOC deprotection.



Scheme 2. Reagents and conditions: ArCOCl, pyridine, microwave, 160 °C, 10 min, 35–80%.



Scheme 3. Reagents and conditions: (a) 2-nitrobenzaldehyde, sulfur, neat, 120 °C, 52%; (b) NaH, DMF, SEMCl, 88% (mixture of regioisomers); (c) Pd/C (10%), H_2 (1 bar), EtOAc, MeOH, 72–89%; (d) ArCOCl, EtN_3, CH_2Cl_2, 30–80%; (e) 5 N HCl/EtOH, 1:1, 70 °C, 70–90%.

core provided two additional classes of SIRT1 activators, benzimidazoles and azabenzimidazoles. Within the benzimidazole series, high-fold activators of SIRT1 were discovered, such as compound **17**, showing eightfold activation of the enzyme ($EC_{1.5}$ = 0.4 μM). In total, these compounds represent the first reported SAR of small molecule activators of SIRT1. The understanding of the SAR within these series has led to more potent analogs which have shown efficacy in several rodent models of type 2 diabetes[14] and as such offer exciting opportunities for treatment of metabolic disease.

References and notes

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