Oxadiazole-carbonylaminothioureas as SIRT1 and SIRT2 Inhibitors

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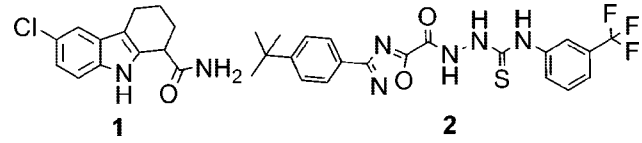
Abstract: A new inhibitor for human sirtuin type proteins 1 and 2 (SIRT1 and SIRT2) was discovered through virtual database screening in search of new scaffolds. A series of compounds was synthesized based on the hit compound (3-[[3-(4-*tert*-butylphenyl)1,2,4-oxadiazole-5-carbonyl]amino]-1-[3-(trifluoromethyl)phenyl]thiourea). The most potent compound in the series was nearly as potent as the reference compound (6-chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide).

Sirtuins have pathogenetic roles in cancer, diabetes, heart failure, neurodegeneration, and aging, and they are currently under investigation as new potential targets for drug discovery.[1,2] Several inhibitors of SIRT1[a] and SIRT2 have been reported,[3] including nicotinamide, EX-527 analogues (compound **1** in Figure 1),[4] splitomicin analogues,[5–7] sirtinol analogues,[8] cambinol,[9] 2-anilinobenzamides,[10] aristoforin,[11] and compounds reported by our group.[12–15]

We have reported a comparative model of SIRT1 and a binding mode for analogues of compound **1**.[16] This model of SIRT1 was applied for virtual database screening of novel inhibitors, which share a similar binding site with compound **1**. In this study, we report a novel hit compound (compound **2** (SPB 00466) in Figure 1 and Table 1) and a series of analogues. The hit compound possesses an oxadiazole-carbonylaminothiourea backbone, which is new for SIRT1 and SIRT2 inhibitors, and it inhibits SIRT1 at 192 μM and SIRT2 at 57 μM level (IC_{50}).

The proposed binding mode of **2** in the active site of SIRT1 is presented in Figure 2. The docking was performed with GOLD program, which is based on a genetic algorithm.[17] The results were visualized using the Sybyl 7.1 software package.[18] The *tert*-butylphenyl group of **2** binds at the deacetylation site of the acetylated lysine substrate in close contact with H363. The surrounding area consists of several hydrophobic residues, giving rise to hydrophobic interactions. The oxadiazole-carbonylaminothiourea backbone is bound in a pocket surrounded by a flexible loop (residues from 269 to 295). The oxadiazole ring acts as an H-bond acceptor forming an H-bond to the backbone NH of I347. The NH of the amino-thiourea group donates an H-bond to the carboxyl group of D348. The size of the binding pocket is restricted by the conformation of the flexible loop and the side chains of the residues in the flexible loop, especially by the residue F273, which is located closest to the deacetylation site.



1 **2**

Figure 1

The NAD^+−nicotinamide exchange reaction,[19,20] the reverse catalysis with binding of nicotinamide to ADP-ribose and NAD^+ resynthesis, underlies the inhibition of sirtuins by nicotinamide at the nicotinamide binding site.[1] We suggest that the binding of a high affinity inhibitor at this site, such as compound **1** or **2**,[4,16] may prevent the productive binding and subsequent cleavage of NAD^+. Napper et al. have also proposed that compound **1** binds after nicotinamide release and inhibits the release of one or both of the products, 2-*O*-acetyl-ADP-ribose and deacetylated peptide.[4] However, more kinetic analyses are needed to clarify the possible interaction of these inhibitors with the reaction intermediates or the products.

The synthetic route to the oxadiazole-carbonylaminothioureas and the oxadiazole-carbonylaminoureas is presented in Scheme 1. Arylnitriles **3**−**7** were reacted with hydroxylamine in aqueous ethanol to obtain hydroxyamidines **8**−**12**. Ethyl chloroacetate was added dropwise to the hydroxylamidines in dry pyridine/dichloromethane solution to yield compounds **13**−**17**. Stirring these compounds with hydrazine monohydrate in ethanol gave compounds **18**−**22**, respectively. The products **2**, **23**−**35** were obtained by adding the appropriate isothiocyanate or isocyanate in dry dimethylformamide (DMF) dropwise to a solution of compounds **18**−**22** in dry DMF. After stirring the mixtures overnight at room temperature (rt), the solvent was evaporated and the products were precipitated from toluene or ethanol/water.

Schemes 2 and 3 present the synthetic route for the replacements of the carbonylaminothiourea moiety of the hit structure. In Scheme 2, Boc-glycine **36** was activated with ethyl chloroformate and coupled with the appropriate aniline to form compounds **37** and **38**. The Boc protecting group was removed, and the resulting intermediate products **39** and **40** were coupled with compounds **13** and **15** to yield compounds **41** and **42**, respectively. In Scheme 3, glutaric anhydride and the hydroxyamidines **8** and **10** were reacted to form the 4-aryl-[1,2,4]oxadiazol-5-yl]-butyric acids **43** and **44**. Activation with thionyl chloride to the corresponding acid halide and subsequent coupling with the appropriate aniline gave compounds **45** and **46**. The reference compound **1** was synthesized as a racemate as described in the literature.[4]

The in vitro assays for SIRT1 and SIRT2 activity were performed using the protocol of McDonagh et al.[21] based on the release of nicotinamide from NAD^+ during the reaction. The radioactively labeled nicotinamide was detected by thin layer chromatography technique[22] because the optic properties of some compounds disturb the use of fluorescent techniques. The detection of radioactively labeled nicotinamide indicates the magnitude of enzymatic activity indirectly, hence, the deacetylation of one acetylated substrate is in a stoichiometric relation with the cleavage of one NAD^+ to nicotinamide and *O*-acetyl-ADP-ribose.[22] The poor solubility of the compounds

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[a] Abbreviations: SIRT, human sirtuin type protein; sirtuin, Sir2 (silent information regulator 2).

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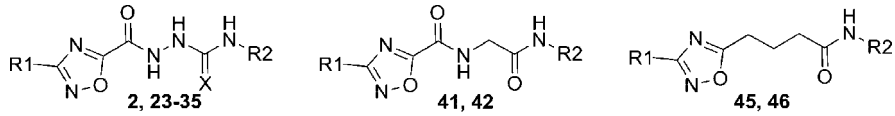
Table 1. SIRT1 and SIRT2 Activity Assay Results for Compounds **1**, **2**, **23**−**35**, **41**, **42**, **45**, and **46** (95% Confidence Intervals for IC_{50} Given in Parentheses).



compd	R1	X	R2	inhibition at 200 μM ± SD[a] (%) SIRT1	SIRT2	IC_{50} (μmol/L)[b] SIRT1	SIRT2
1 [c]				3 (1−5)	79 (45−140)		
2	4-*t*-Bu-Ph	S	3-CF3-Ph	59 ± 3	87 ± 2	192 (104−354)	57 (26−125)
23	4-*t*-Bu-Ph	S	4-CF3-Ph	70 ± 2	89 ± 15	325 (153−689)	109 (67−177)
24	4-*t*-Bu-Ph	O	3-NO2-Ph	18 ± 15	39 ± 2		
25	4-*t*-Bu-Ph	O	Ph	14 ± 7	38 ± 10		
26	4-*t*-Bu-Ph	S	Ph	−2 ± 5	29 ± 3		
27	4-*t*-Bu-Ph	O	*n*-Bu	22 ± 10	7 ± 2		
28	1-naphthyl	S	3-CF3-Ph	57 ± 16	56 ± 4	13 (5−37)	113 (64−200)
29	1-naphthyl	O	3-CF3-Ph	12 ± 10	51 ± 4		257 (179−395)
30	1-naphthyl	S	4-CF3-Ph	54 ± 6	71 ± 3	318 (140−723)	74 (47−115)
31	1-naphthyl	S	4-F-Ph	10 ± 3	35 ± 3		
32	Ph	S	4-CF3-Ph	29 ± 5	8 ± 11		
33	Ph	S	4-F-Ph	−22 ± 1	22 ± 2		
34	3-pyridyl	S	4-CF3-Ph	4 ± 3	−4 ± 26		
35	3-(Boc-NH)-Ph	S	4-CF3-Ph	69 ± 19	71 ± 4	168 (98−289)	129 (72−232)
41	4-*t*-Bu-Ph		3-CF3-Ph	20 ± 1	18 ± 10		
42	Ph		Ph	28 ± 8	17 ± 9		
45	4-*t*-Bu-Ph		3-CF3-Ph	17 ± 9	0 ± 4		
46	Ph		Ph	0 ± 12	5 ± 14		

[a] SD, standard deviation, (n = 2−3). [b] IC_{50} were determined with the NAD^+ based assay[21] for compounds that had over 50% inhibition at 200 μM for SIRT1 or SIRT2 (repeated at least three times). [c] **1** was tested as a racemate.

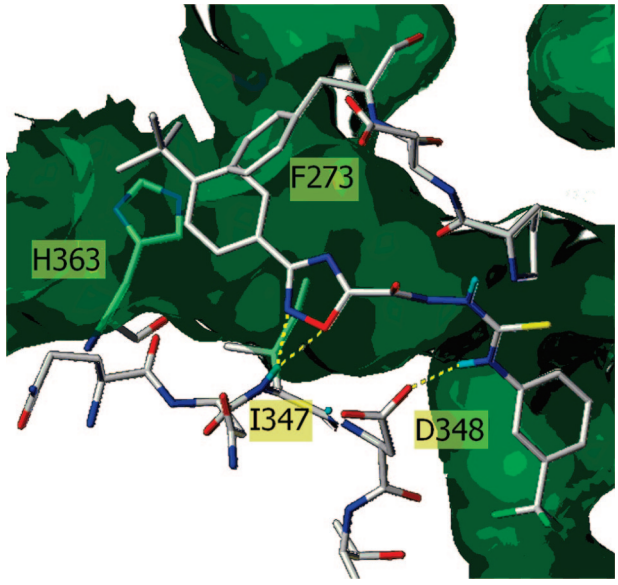
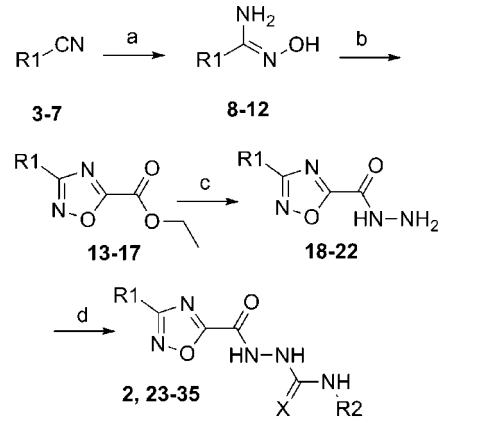


Figure 2. The binding mode of compound **2** in the SIRT1 model. The yellow dashed lines represent H-bond between the ligand and the residues at the active site. The green color visualizes the surface of the binding pocket.

Scheme 1[a]



R1 = 4-*t*-Bu-Ph in **2**, **3**, **8**, **13**, **18**, **23-27**; 1-naphthyl in **4**, **9**, **14**, **19**, **28-31**; Ph in **5**, **10**, **15**, **20**, **32**, **33**; 3-pyridyl in **6**, **11**, **16**, **21**, **34**; 3-(Boc-NH)-Ph in **7**, **12**, **17**, **22**, **35**

X = S in **2**, **23**, **26**, **28**, **30**, **31**, **32**, **33**, **34**, **35**; O in **24**, **25**, **27**, **29**

R2 = 3-CF_3-Ph in **2**, **28**, **29**; 4-CF_3-Ph in **23**, **30**, **32**, **34**, **35**; Ph in **25**, **26**; 4-F-Ph in **31**, **33**; *n*-Bu in **27**; 3-NO_2-Ph in **24**

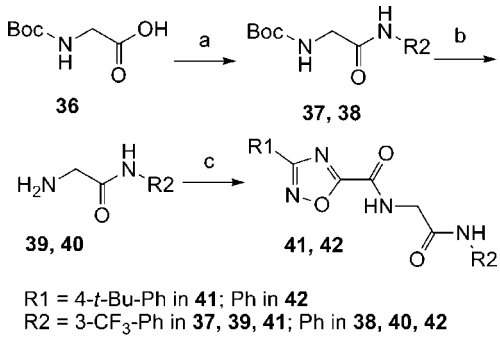
[a] Reagents and conditions: (a) hydroxylamine hydrochloride (1.5 equiv), sodium hydroxide (1.5 equiv), 80% ethanol/water, rt, 1−2 d; (b) ethyl chloroacetate (1.2 equiv), pyridine/dichloromethane, 0 °C−rt, 2−24 h; (c) hydrazine monohydrate (5 equiv), ethanol, rt, 1−24 h; (d) the appropriate isocyanate or isothiocyanate, dimethylformamide, 0 °C−rt, 1 d.

2, **23**−**35** presented difficulties to determine the IC_{50} values with high concentration samples. The IC_{50} values were determined for all compounds, which had over 50% inhibition at the concentration of 200 μM.

The inhibitory activity of the hit compound **2** was determined in vitro for SIRT1 (IC_{50} = 192 μM) and for SIRT2 (IC_{50} = 57 μM). These in vitro results were also confirmed by using a more physiological probe than the Biomol probe. The deacetylation of acetylated α-tubulin isolated from rat hippocampal stem cells were determined in vitro. The inhibitory activity of the hit compound **2** was demonstrated in this assay with SIRT1 at 200 μM and with SIRT2 at 50 μM concentration level (Supporting Information).

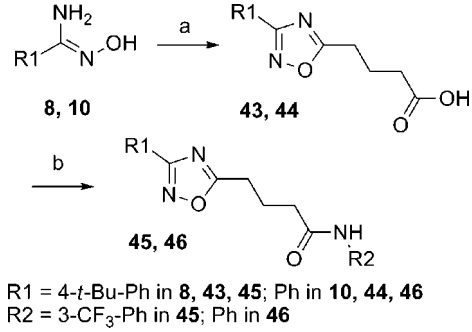
The synthesized compounds **1**, **2**, **23**−**35**, **41**, **42**, **45**, and **46** and their inhibitory activities for SIRT1 and SIRT2 are presented in Table 1. The most potent SIRT1 inhibitor in the series was compound **28** (IC_{50} = 13 μM). It was 15 times more potent than the hit compound **2** (IC_{50} = 192 μM). In addition, compound **28** was almost as potent as the reference compound **1** (IC_{50} = 3 μM), which is the most potent known SIRT1 inhibitor. Interestingly, compound **28** was the only compound in the series showing weak to moderate selectivity for SIRT1

Scheme 2[a]



[a] Reagents and conditions: (a) (i) triethylamine (1.1 equiv), ethyl chloroformate (1.1 equiv), dichloromethane, −20 °C, 30 min.; (ii) triethylamine (1.1 equiv), the appropriate aniline (1 equiv), rt, 1 d. (b) 25% Trifluoroacetic acid/dichloromethane, 0 °C−rt, 2 h. (c) Compd **13** or **15** (1.1 equiv), triethylamine (1.1 equiv), methanol, 40 °C, 2−24 h.

Scheme 3[a]



[a] Reagents and conditions: (a) glutaric anhydride, few drops of DMF, microwave 100 °C. (b) (i) Thionyl chloride (2.2 equiv), benzene, 60 °C, 1 h; (ii) the appropriate aniline (1.1 equiv), benzene, 0 °C−rt, 30 min.

similar to compound **1**, while the other compounds had a weak selectivity for SIRT2. Compound **2** (IC_{50} = 57 μM) was the most potent SIRT2 inhibitor in the series. Compounds **2**, **28** (IC_{50} = 113 μM), and **30** (IC_{50} = 74 μM) and the reference compound **1** (IC_{50} = 79 μM) are equipotent inhibitors for SIRT2.

Compounds **2**, **28** (X = S, R2 = 3-CF3-Ph), and **23**, **30**, **32**, **34**, **35** (X = S, R2 = 4-CF3-Ph) and **31**, **33** (X = S, R2 = 4-F-Ph) were synthesized to study the modifications at the R1 position. 4-*tert*-butylphenyl, 1-naphthyl, and 3-(Boc-amino)-phenyl groups were preferred as R1 substituents and showed active compounds (**23**, **30**, and **35**). However, the compounds with the phenyl and 3-pyridyl groups as R1 substituents had a strongly decreased (**32**) or no detectable (**34**) inhibitory activity. Compounds **28**, **29** (R1 = 1-naphthyl, R2 = 3-CF3-Ph) and **25**, **26** (R1 = 4-*t*-Bu-Ph, R2 = Ph) were synthesized to study the modifications at the X position, but the results were not consistent. Compound **28** with the thiourea group showed a preference for SIRT1 over SIRT2 (IC_{50} = 13 μM and 113 μM, respectively). Compound **29** with the urea group was an equipotent SIRT2 inhibitor (IC_{50} = 257 μM, 51% at 200 μM) but clearly a weaker SIRT1 inhibitor (12% inhibition at 200 μM) as compared to compound **28**. Compounds **2**, **23**, **26** (R1 = 4-*t*-Bu-Ph, X = S), **28**, **30**, **31** (R1 = 1-naphthyl, X = S), **24**, **25**, **27** (R1 = 4-*t*-Bu-Ph, X = O), and **32**, **33** (R1 = Ph, X = S) were synthesized to study the modifications at the R2 position. The para and meta substituted (trifluoromethyl)-phenyl groups (**2** and **23**, **28** and **30**) were preferred over phenyl (**26**) or 4-fluorophenyl group (**31**) at the R2 position. However, all combinations with different R1 and X groups were not made. The intermediates **18**−**22**, which lack the R2 substituent, were also tested at the concentration of 200 μM, but they did not show inhibitory activity (not included in Table 1).

Compounds **41**, **42**, **45**, and **46** were synthesized to study the importance of the carbonylaminothiourea part of the backbone by replacing it with either a carbamoyl-methyl-amide or a butyramide linker. These compounds showed only weak inhibitory activities and confirmed the importance of carbonylaminothiourea part of the scaffold for the inhibitory activity.

In conclusion, a new oxadiazole-carbonylaminothiourea scaffold with inhibitory activity for SIRT1 and SIRT2 was found through virtual screening. The series of compounds based on this scaffold showed that each of the modified positions R1, R2, and X were important for the inhibitory activity. For example, replacement of aryl substituent with phenyl at either position (R1 or R2) was not tolerated. At the R1 position, bulky and lipophilic substituents were preferred over smaller or more polar substituents such as phenyl and pyridyl. Modifications at the R2 position indicated that the trifluoromethyl substituent of the phenyl group is important for the inhibitory activity. However, no specific binding has been observed for the trifluoromethyl group in the binding model. In addition to its high electronegativity, the trifluoromethyl group is known to increase the lipophilicity of the compounds and therefore the affinity of compounds in the hydrophobic binding sites, which may lead to increased inhibitory activity of these compounds. Modifications at the X positions indicated that the compounds based on the oxadiazole-carbonylaminothiourea scaffold were more potent than the compounds based on the oxadiazole-carbonylaminourea scaffold. The relatively high acidity of the NH thiourea protons compared to urea protons respectively is correlated with a strong hydrogen-bonding donor capability, thus providing efficient anchoring points of complementary functional groups such as carboxyl group of D348 in the proposed binding mode (Figure 2). The most potent compound **28** was nearly as potent as the previously reported reference compound **1**.

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Supporting Information Available: Database screening procedure, detailed experimental procedures for the syntheses, NMR spectra, ESI-MS results, and elemental analysis data for the new compounds and in vitro assay for SIRT1 and SIRT2 activities are described. This material is available free of charge via the Internet at http://pubs.acs.org.

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