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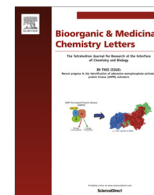

Cyclic peptide-based potent and selective SIRT1/2 dual inhibitors harboring N^{ε}-thioacetyl-lysine

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ABSTRACT

In the current study, we discovered that several N-terminus-to-side chain cyclic tripeptides harboring the catalytic mechanism-based SIRT1/2/3 inhibitory warhead N^{ε}-thioacetyl-lysine at their central positions exhibited a comparably strong inhibition (nM level) against the SIRT1/2-catalyzed N^{ε}-acetyl-lysine deacetylation reactions. Their dual SIRT1/2 inhibitory action was also found to be stronger than that against SIRT3/5/6. Considering the previous demonstration that a SIRT1/2 dual inhibition could be instrumental in achieving an anti-cancer effect on those cancers retaining the wild-type tumor suppresser p53 protein, these compounds could be employed as leads for developing novel anti-cancer agents.

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The sirtuin-catalyzed protein N^{ε}-acyl-lysine deacylation reaction has gained an increasing attention in recent years due to its intimate involvement in regulating critical cellular processes such as metabolism, gene transcription, and DNA damage repair.[1–3] Figure 1 depicts the β-nicotinamide adenine dinucleotide (β-NAD^+)-dependent sirtuin deacylation reaction.[4] The β-NAD^+-dependent nature for this enzymatic reaction catalyzed by the evolutionally conserved intracellular sirtuin family of enzymes[5] would imply that this enzymatic reaction would be involved in metabolic regulation. The identification of both histone and non-histone substrates for this enzymatic reaction[6] would also suggest that it would be involved in cellular processes beyond the histone-mediated epigenetic processes.

The physiological importance of the sirtuin-catalyzed deacylation reaction also suggests that this enzymatic reaction could also be the culprit for a variety of human diseases. It is now known that the sirtuin-catalyzed deacylation reaction also plays a pathological role in certain diseases such as cancer, metabolic and neurodegenerative diseases.[7–9] Besides the genetic targeting means (e.g., RNAi and the use of antisense oligonucleotides), pharmacological studies with the existing sirtuin inhibitors have also helped to explore the therapeutic potentials of the sirtuin-catalyzed deacylation reaction.[10–16] In the case of anti-cancer potential, previous cellular studies (with the cancer cell line retaining the wild-type tumor suppressor protein p53) strongly suggested that a SIRT1/2 dual inhibition would be instrumental in conferring an anti-cancer effect.[15] In the study, it was also found that the SIRT1/2 dual inhibitor salermide (chemical structure shown in Fig. 2) was able to inhibit cancer cell growth, however, the up-to-date most potent (nM level) and selective (versus SIRT2 and SIRT3) SIRT1 inhibitor EX-527 was unable to. This phenomenon could be attributed to the sharing of p53 protein as the native substrate by SIRT1 and SIRT2.[6] This cellular assay finding might also rationalize the observed tumor suppressive effect on animal models for cambinol and tenovin-6,[13,17] another two SIRT1/2 dual inhibitors also depicted in Figure 2. Therefore, a potent and selective dual SIRT1/2 inhibition seems to represent a viable approach to curbing the growth of those cancer cells that retain the wild-type p53 protein. It should be noted that a selective dual SIRT1/2 inhibition would presumably be also able to minimize the side effect from an off-target action of a SIRT1/2 dual inhibitor. In the current study, the dual SIRT1/2 inhibitory selectivity of the developed compounds was examined versus other human sirtuin family members whose N^{ε}-acyl-lysine deacylase activities were also well established, including SIRT3/5/6.

Abbreviations: β-NAD^+, β-nicotinamide adenine dinucleotide; MBHA, 4-methylbenzhydrylamine; SPPS, solid phase peptide synthesis; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic acid; DCM, dichloromethane; HBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; NMM, *N*-methylmorpholine; RP-HPLC, reversed-phase high performance liquid chromatography; HRMS, high-resolution mass spectrometry; IC_{50}, the inhibitor concentration at which an enzymatic reaction velocity is reduced by 50%.

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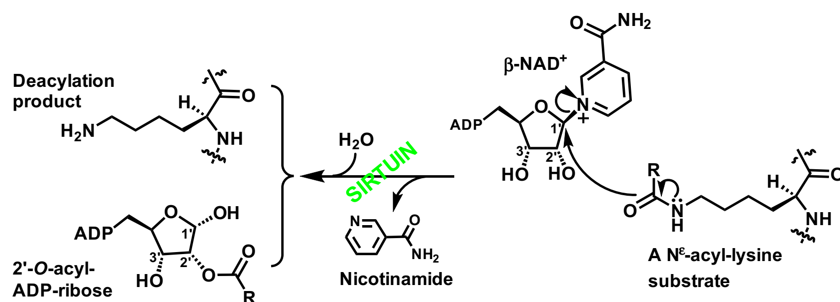

Figure 1. The β-NAD⁺-dependent sirtuin-catalyzed Nε-acyl-lysine deacylation reaction. Example Nε-acyl groups include acetyl (R is CH_3), crotonyl (R is $H_2C{=}CHCH_3$), succinyl (R is CH_2CH_2COOH), glutaryl (R is $CH_2(CH_2)_2COOH$), and myristoyl (R is $CH_2(CH_2)_{11}CH_3$).

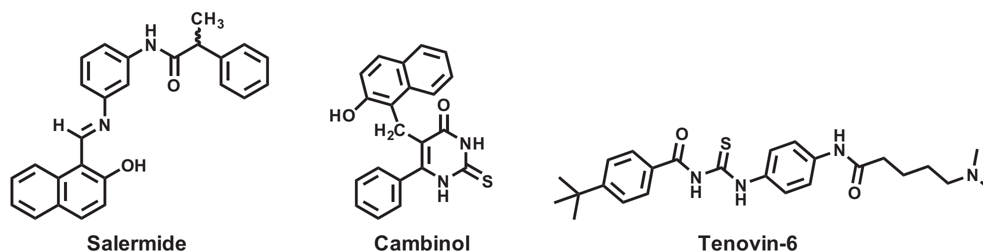

Figure 2. The three SIRT1/2 dual inhibitors that have been employed in previous cell-based and animal-based pharmacological studies.

Table 1
HRMS analysis of compounds **2–15**[a]

Compound	Ionic formula	Calculated *m/z*	Observed *m/z*
2	$[C_{30}H_{43}N_6O_5S_2]^+$	631.2731	631.2721
3	$[C_{31}H_{45}N_6O_5S_2]^+$	645.2887	645.2878
4	$[C_{32}H_{47}N_6O_5S_2]^+$	659.3044	659.3039
5	$[C_{33}H_{49}N_6O_5S_2]^+$	673.3200	673.3184
6	$[C_{32}H_{45}N_6O_5S]^+$	625.3167	625.3161
7	$[C_{33}H_{47}N_6O_5S]^+$	639.3323	639.3317
8	$[C_{34}H_{48}N_6O_5SNa]^+$	675.3299	675.3293
9	$[C_{35}H_{51}N_6O_5S]^+$	667.3636	667.3632
10	$[C_{25}H_{45}N_6O_5S]^+$	541.3167	541.3162
11	$[C_{26}H_{47}N_6O_5S]^+$	555.3323	555.3301
12	$[C_{27}H_{49}N_6O_5S]^+$	569.3480	569.3472
13	$[C_{28}H_{50}N_6O_5SNa]^+$	605.3456	605.3446
14	$[C_{24}H_{42}N_6O_5SNa]^+$	549.2830	549.2815
15	$[C_{20}H_{34}N_6O_5SNa]^+$	493.2204	493.2189

[a] All the compounds were measured with the positive ion mode of electrospray ionization, except for compound **6** which was measured with the positive ion mode of direct analysis in real time (DART) ionization.

Since the afore-mentioned SIRT1/2 dual inhibitors that have been employed in previous cell-based and animal-based studies all only have modest dual SIRT1/2 inhibitory potency and selectivity (versus SIRT3 as assessed) (see Refs. 13,15,17 and Table 2 in the current study), we were interested in the current study in employing the up-to-date most powerful catalytic mechanism-based SIRT1/2/3 inhibitory warhead *N*ε-thioacetyl-lysine (discovered previously by our laboratory[18] and that of Denu[19]) to see if stronger SIRT1/2 dual inhibition could be realized. As depicted in Figure 3A, the catalytic mechanism-based SIRT1/2/3 inhibition by a *N*ε-thioacetyl-lysine-containing inhibitor has been demonstrated to result from its processing by a sirtuin as an alternate substrate with the formation of the stalled *S*-alkylamidate intermediate along the catalytic coordinate.[18,19]

We previously discovered that the simple *N*ε-thioacetyl-lysine-containing tripeptide **1** shown in Figure 3B behaved as a potent SIRT1/2/3 inhibitor with a slightly selective (~2-fold) dual SIRT1/2 inhibition among these three sirtuins (IC_{50} (SIRT1/2/3, μM): 2.1/2.4/4.5).[20] In the current study, we have designed fourteen macrocyclic analogs of compound **1**, i.e., compounds **2–15** depicted in Figure 3C. Our design of these analogs was inspired by (i) the demonstrated potential of peptide chain macrocyclization in enhancing the target binding affinity of a ligand[21] and (ii) the demonstrated importance of the amino acid side chains immediately surrounding the *N*ε-acetyl-lysine residue in a peptide substrate in mediating its binding interactions at a sirtuin active site.[4] Therefore, we anticipated that the SIRT1/2 dual inhibition potency and selectivity (versus SIRT3) of compound **1** could be both enhanced by such design.

The first issue that we need to address in this design was to determine the macrocycle attaching points; for this, the X-ray co-crystal structure of the archaeal sirtuin Sir2–Af2 complexed with a *N*ε-acetyl-lysine peptide substrate[22] was used as a guide. As suggested from the 3-dimensional rendering of this co-crystal structure depicted in Figure 3D, a macrocycle linking the α-NH at the −1 position of compound **1** (corresponding to the α-NH of K381 of the bound substrate) and the side chain at the +1 position of compound **1** (corresponding to the side chain of L383 of the bound substrate) would be accommodatable at a sirtuin active site. Based on visual inspection, suberic acid connecting the α-NH and the ε-NH, respectively at the −1 and +1 positions of compound **1**, via two amide bonds could be a reasonable linking unit for constructing the macrocyclic analogs of compound **1**. It should be noted that compound **15** (with a succinic acid linker) was found in the current study to be a much weaker SIRT1/2/3 inhibitor than compound **14** (with the suberic acid linker) (vide infra).

Since the green regions in the above 3-dimensional rendering have the lowest degree of amino acid residue conservation among the ten sirtuins used in the study of Ref. 22, we decided to vary the side chain identity at the −1 position of compound **1** (corresponding to the side chain of K381 of the bound substrate) and the side chain length at the +1 position of compound **1**, with the hope that this low amino acid sequence conservation among different sirtuins could be exploited to enhance the inhibitory selectivity among different sirtuins for the designed compounds.

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