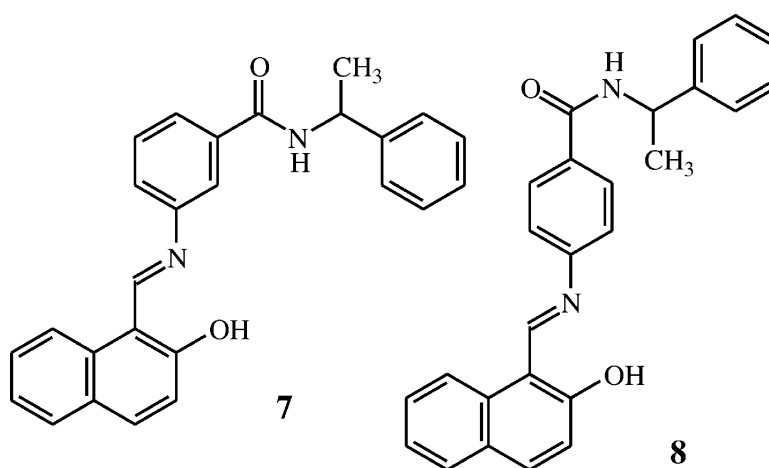Article

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Design, Synthesis, and Biological Evaluation of Sirtinol Analogues as Class III Histone/Protein Deacetylase (Sirtuin) Inhibitors

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In a search for potent inhibitors of class III histone/protein deacetylases (sirtuins), a series of sirtinol analogues have been synthesized and the degree of inhibition was assessed in vitro using recombinant yeast Sir2, human SIRT1, and human SIRT2 and in vivo with a yeast phenotypic assay. Two analogues, namely, 3- and 4-[(2-hydroxy-1-naphthalenylmethylene)-amino]-*N*-(1-phenylethyl)benzamide (i.e., *m*- and *p*-sirtinol), were 2- to 10-fold more potent than sirtinol against human SIRT1 and SIRT2 enzymes. In yeast in vivo assay, these two small molecules were as potent as sirtinol. Compounds lacking the 2-hydroxy group at the naphthalene moiety or bearing several modifications at the benzene 2′-position of the aniline portion (carbethoxy, carboxy, and cyano) were 1.3−13 times less potent than sirtinol, whereas the 2′-carboxamido analogue was totally inactive. Both (*R*)- and (*S*)-sirtinol had similar inhibitory effects on the yeast and human enzymes, demonstrating no enantioselective inhibitory effect.

Introduction

The reversible acetylation of histones is one of the better understood modes of gene regulation.[1−7] Specific patterns of gene expression are the result of the balance between the activities of histone acetyltransferases (HAT) and histone deacetylases (HDAC). The importance of this balance is underscored by the fact that perturbation of histone acetylation has been linked to several diseases including cancer.[3,8,9] Hypoacetylation of the ϵ-amino group of specific lysines in histone tails is responsible in large part for maintenance of eukaryotic genomes in the transcriptionally inactive or "silent" state, whereas transcriptionally active regions of the genome are typically hyperacetylated.

Three general classes of deacetylases have been identified in eukaryotes: class I, class II, and class III (sirtuin) HDACs.[10,11] Another deacetylase, maize HD2,[12] is structurally quite different from mammalian HDACs and has been attributed to a class of its own. Class I HDACs are predominantly nuclear and are expressed in most tissues and cell types. Class II HDACs are regulated by compartmentalization between the nucleus and cytoplasm through reversible phosphorylation, show a tissue-specific expression, and are subdivided into two subclasses, IIa and IIb, based on their sequence homology and domain organization. Class I/II HDACs are targeted to specific chromatin domains and have a Zn^{2+}-dependent mechanism of deacetylation to generate the free acetate and the deacetylated protein.

Class I/II HDACs are inhibited by trichostatin A (TSA) and other natural and synthetic compounds,[13] the majority of which are not able to distinguish between class I and class II HDACs. Exceptions include trapoxins,[13] cyclic hydroxamic acid containing peptide 1 (CHAP1),[14] and sodium butyrate (NaB),[15] which selectively inhibit classes I and IIa HDACs, and FK-228 which specifically inhibits class I HDACs.[16] In 2003, tubacin has been identified as a cell-selective HDAC6 inhibitor,[17−20] and a series of (aryloxopropenyl)pyrrolyl hydroxyamides has been recently described as class II selective HDAC inhibitors.[21,22]

Sirtuins are structurally and mechanistically distinct from other HDACs. They contain a conserved ∼300 amino acid catalytic domain that catalyzes a two-step reaction that requires NAD^+ as a cosubstrate. The first series of reactions is cleavage of the nicotinamide−ribose glycosidic bond of NAD^+, release of free nicotinamide, and formation of a relatively long-lived peptidyl imidate intermediate.[23] Second is the transfer of the acetyl group to ADP-ribose with production of *O*-acetyl-ADP-ribose (Figure 1).[24−27]

The sirtuin family of enzymes are named after their founding member, the Sir2 (silent information regulator 2) protein of *Saccharomyces cerevisiae*. In yeast, Sir2 is critical for transcriptional silencing at three specific loci: the telomeres, ribosomal DNA, and the silent mating type loci.[28,29] Sir2 and its homologues have gained considerable attention for their ability to mimic the diet known as caloric restriction, which extends lifespan in a variety of organisms, including yeast,[30] *Caenorhabditis elegans*,[31] rodents,[32] and probably pri-

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‡ Dipartimento di Genetica e Biologia Molecolare, Università degli Studi di Roma "La Sapienza".
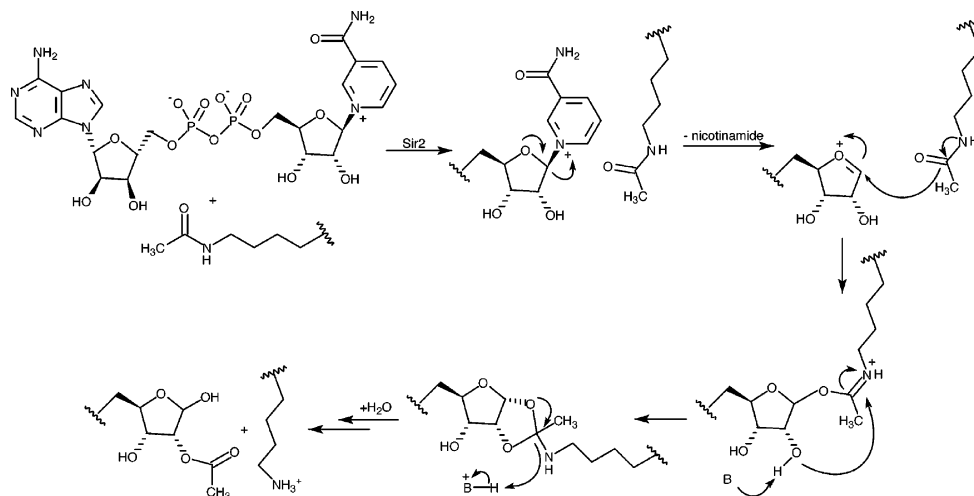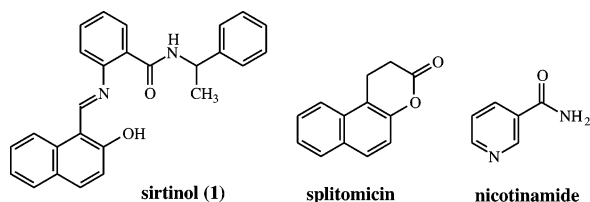‖ Istituto di Biologia e Patologia Molecolari.

Figure 1. Mechanism of deacetylation of sirtuins.

Chart 1. Known NAD+-Dependent (Class III) HDAC Inhibitors

mates.[33] Sir2-like proteins also deacetylate non-histone proteins, including the tumor suppressor p53,[34,35] the RNA polymerase I transcription factor TAFI68,[36] bovine serum albumin (BSA),[34] α-tubulin,[37] the archaeal chromatin protein Alba,[38] and acetyl-coenzyme A synthetase (ACS).[39,40] The human Sir2 ortholog, SIRT1, has been implicated in a variety of important disease-related processes including inflammatory responses, cell defense and survival, and fatty acid metabolism, and finding molecules that modulate these enzymes is considered a possible route for disease treatment.[41−44]

The novel product of the sirtuin reaction, *O*-acetyl-ADP-ribose (*O*AADPr) has attracted interest by virtue of its possible role in cell signaling. The initial enzymatic product is 2′-*O*AADPr, which then equilibrates with 3′-*O*AADPr in solution through a nonenzymatic, intramolecular transesterification reaction.[23−26] The physiological role of 2′- and 3′-*O*AADPr is still unclear. One clue, however, is the observation that microinjection of these compounds abrogates oocyte maturation and cell cycle progression during embryonic development.[45]

Molecules that inhibit the class I/II HDACs are ineffective in inhibiting sirtuins and vice versa. That said, class I/II HDAC inhibitors do induce differential changes in gene expression of sirtuin mRNAs in cultured cells, specifically up-regulation of SIRT2, -4, and -7 and down-regulation of SIRT1, -5, and -6 mRNAs.[46] A few specific sirtuin inhibitors have been reported to date, including sirtinol **1**,[47] splitomicin,[48] and nicotinamide[49,50] (Chart 1).

A number of splitomicin derivatives have been evaluated,[51,52] whereas little attention has been devoted to sirtinol **1** and its analogues, despite the fact that sirtinol, having a 2-hydroxy-1-naphthaldehyde moiety linked to a 2-amino-*N*-(1-phenylethyl)benzamide portion through a aldimine linkage, provides an interesting chemical template from which new **1** analogues are developed. Such compounds could provide insight into the inhibitory mechanism of **1** and may be useful tools for functional characterization and/or elucidation of the in vivo functions of these enzymes.
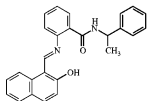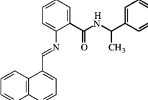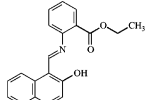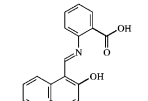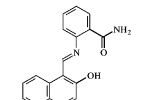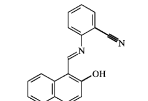
Accordingly, we prepared a number of **1** analogues by modification of the 2-hydroxynaphthyl group (compound **2**) or the benzamide function (compounds **3−6**) of the sirtinol structure. Two sirtinol isomers (*m*- and *p*-sirtinol **7** and **8**) and their enantiomerically pure forms ((*R*)- and (*S*)-sirtinol **9** and **10**) were also prepared (for structures, see Table 1). Compounds **2−10** were tested as inhibitors of yeast Sir2, human SIRT1, and human SIRT2 in vitro, with **1** as the reference compound. Moreover, phenotypic screening involving SIR2-mediated URA3 gene silencing (Figure 2) has been performed on *m*-, *p*-, (*R*)-, and (*S*)-sirtinol derivatives **7−10** to evaluate their ability to function in vivo.

Chemistry

Condensation between 1-naphthaldehyde and 2-amino-*N*-(1-phenylethyl)benzamide[53] in acidic medium afforded the 2-[(1-naphthalenylmethylene)amino]-*N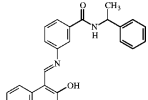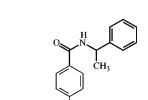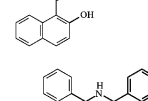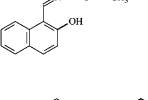*-(1-phenylethyl)benzamide **2**, which is the 2-dehydroxynaphthyl analogue of sirtinol **1** (Scheme 1). When 2-hydroxy-1-naphthaldehyde was heated with the appropriate aniline derivatives (ethyl 2-aminobenzoate, 2-aminobenzoic acid, 2-aminobenzamide, 2-aminobenzonitrile) in acidic medium, the desired sirtinol analogues **3−6** were obtained in high yields (Scheme 1). Catalytic reduction of known (*R/S*)-, (*R*)-, and (*S*)-2-nitro-*N*-(1-phenylethyl)benzamides[55,56] prepared by standard methods afforded the corresponding (*R/S*)-,[55] (*R*)-, and (*S*)-2-aminobenzamides **11−13**, which were in turn condensed with 2-hydroxy-1-naphthaldehyde in acidic medium to give sirtinol **1** and (*R*)- and (*S*)-sirtinol **9** and **10**, respectively (Scheme 2). Finally, the known 3- and 4-amino-*N*-(1-phenylethyl)benzamides **14**[53] and **15**[53] were condensed with 2-hydroxy-1-naphthaldehyde in acidic medium to give the two sirtinol isomers 3- and 4-[(2-hydroxy-1-naphthalenylmethylene)amino]-*N*-(1-phenylethyl)benzamide **7** and **8** (*m*-sirtinol and *p*-sirtinol) (Scheme 2).

Chemical and physical data of compounds **1−10**, **12**, and **13** are listed in Table 2.

Table 1. Yeast Sir2 (ySir2), Human SIRT1 (hSIRT1), and Human SIRT2 (hSIRT2) Inhibitory Activity of Sirtinol Analogues **1**−**10**[a]

compd	structure	ySir2		hSIRT1		hSIRT2	
		% inhbtn	$IC_{50} \pm SD$ (μM)	% inhbtn	$IC_{50} \pm SD$ (μM)	% inhbtn	$IC_{50} \pm SD$ (μM)
1 (sirtinol)		65.6	48 ± 4	44.5	131 ± 11	71.0	57.7 ± 9
2		10.0	ND[b]	33.0	ND	ND	ND
3		20.0	ND	9.0	ND	ND	ND
4		5.0	ND	26.0	ND	ND	ND
5		NI[c]		NI		ND	ND
6		15.0	ND	20.0	ND	ND	ND
7 (*meta*-sirtinol)		53.9	72 ± 3	63.0	59 ± 2	79.2	35.7 ± 2
8 (*para*-sirtinol)		59.8	33 ± 1	82.6	13 ± 2	80.2	25.9 ± 6
9 ((*R*)-sirtinol)		55.5	62 ± 5	61.8	55 ± 5	72.2	49.3 ± 6
10 ((*S*)-sirtinol)		57.0	66 ± 4	60.2	67 ± 4	73.5	39.4 ± 5

[a] Data represent the mean values of at least three separate experiments. [b] ND, not determined. [c] NI, no inhibition.

Results and Discussion

Enzyme Inhibition: Yeast Sir2, Human SIRT1, and Human SIRT2 Assays. The novel sirtinol analogues **2**−**10** together with sirtinol (**1**) as the reference drug have been evaluated for their ability to inhibit yeast Sir2 (ySir2), human SIRT1 (hSIRT1), and human SIRT2 (hSIRT2) enzymes. The results expressed as percent of inhibition at 100 μM and IC_{50} (50% inhibitory concentration) values are reported in Table 1.

When tested against ySir2, **1** showed 65.6% of inhibition at 100 μM, its IC_{50} being 48 μM (Table 1). Deletion of the hydroxyl group at the C2 position of the naphthalene moiety furnished a compound (**2**) that was 7-fold less potent than sirtinol, thus confirming the important role of the 2-hydroxy-1-naphthaldehyde moiety in inhibiting ySir2, as previously reported by Grozinger et al.[47] Replacement of the *N*-1-phenylethylamide moiety of sirtinol with a carbethoxy, carboxy, or cyano group lowered the Sir2 inhibitory activity of the derivatives (**3**, 3-fold; **4**, 13-fold; **6**, 4 times), and the insertion at the aniline C2 position of a carboxyamide portion resulted in a total loss of Sir2 inhibitory activity (compound **5**). When the *N*-1-phenylethylamide moiety was shifted from the ortho position to the meta or para position of the benzene ring (compounds **7** and **8**), a slightly less (1.5-fold, **7**) or more potent (1.5-fold, **8**) compound than **1** was obtained, respectively. Finally, (*R*)- and (*S*)-sirtinol (**9** and **10**) showed similar IC_{50}
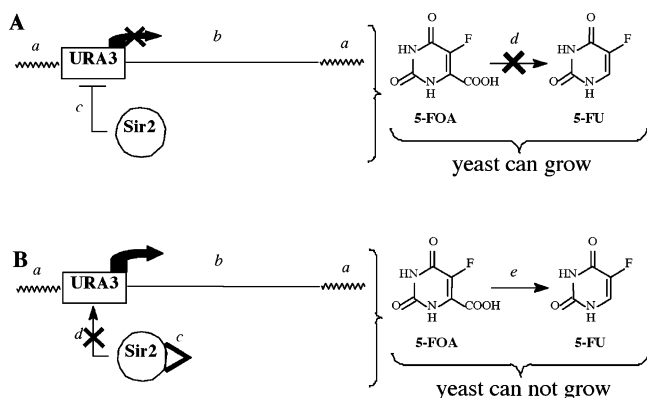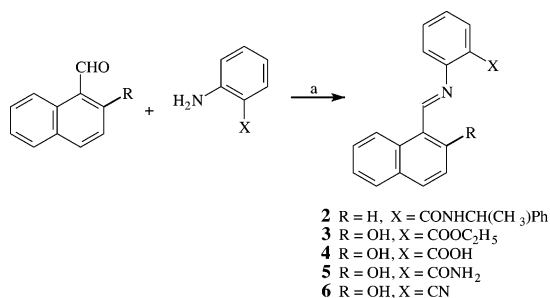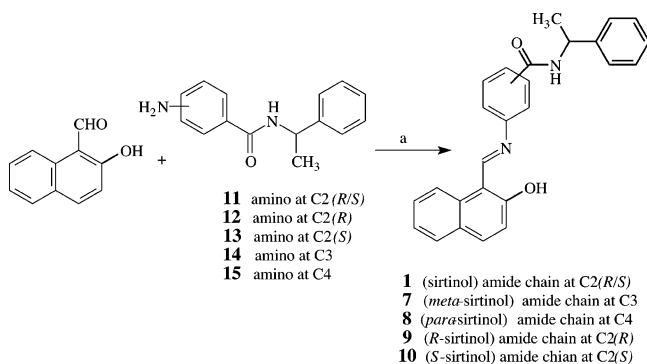
Figure 2. Sir2-regulated telomeric silencing assay. (A) (a) telomere; (b) chromosome VII; (c) Sir2 silences URA3 gene; (d) 5-FOA is not converted into 5-FU. (B) (a) telomere; (b) chromosome VII; (c) Sir2 inhibitor bound to Sir2; (d) Sir2 inhibition results in URA3 gene expression; (e) 5-FOA is converted into 5-FU.

Scheme 1[a]

2 R = H, X = CONHCH(CH$_3$)Ph
3 R = OH, X = COOC$_2$H$_5$
4 R = OH, X = COOH
5 R = OH, X = CONH$_2$
6 R = OH, X = CN

[a] (a) Acetic acid, benzene/ethanol, reflux.

Scheme 2[a]

11 amino at C2 *(R/S)*
12 amino at C2 *(R)*
13 amino at C2 *(S)*
14 amino at C3
15 amino at C4

1 (sirtinol) amide chain at C2*(R/S)*
7 (*meta*-sirtinol) amide chain at C3
8 (*para*sirtinol) amide chain at C4
9 (*R*-sirtinol) amide chain at C2*(R)*
10 (*S*-sirtinol) amide chian at C2*(S)*

[a] (a) Acetic acid, benzene/ethanol, 80 °C.

values, thus demonstrating the lack of enantioselectivity in sirtinol inhibitory activity (Table 1).

Compared to its ability to inhibit ySir2, **1** was a weak inhibitor of hSIRT1, with 44.5% of inhibition at 100 μM and IC$_{50}$ = 131 μM (Table 1). The 2-dehydroxy analogue **2** and the 2′-carbethoxy (**3**), 2′-carboxy (**4**), and 2′-cyano (**6**) derivatives were less potent than **1** in inhibiting hSIRT1 (from 1.3- to 5-fold). As for ySir2, the 2′-carboxyamide **5** was totally inactive against hSIRT1. In contrast, 3-[(2-hydroxy-1-naphthalenylmethylene)amino]-*N*-(1-phenylethyl)benzamide (*m*-sirtinol) **7** was 2.2-fold more potent than sirtinol in inhibiting hSIRT1, and its para isomer **8** inhibited hSIRT1 10 times more efficiently than **1**, with IC$_{50}$ = 13 μM. Compounds **9** and **10** again showed similar inhibitory data, but they were 2-fold more potent than **1** in inhibiting human SIRT1 (Table 1).

Table 2. Chemical and Physical Data for Compounds **1**–**10**, **12**, and **13**

compd	mp, °C	recrystallization solvent	[α]$_D$, deg	yield, %	formula	anal.[a]
1	119–120	acetonitrile		94	C$_{26}$H$_{22}$N$_2$O$_2$	C, H, N
2	101–103	methanol		74	C$_{26}$H$_{22}$N$_2$O	C, H, N
3	oil			54	C$_{20}$H$_{17}$NO$_3$	C, H, N
4	180–182	acetonitrile		87	C$_{18}$H$_{13}$NO$_3$	C, H, N
5	238–240	acetonitrile		91	C$_{18}$H$_{14}$N$_2$O$_2$	C, H, N
6	139–141	acetonitrile		94	C$_{18}$H$_{12}$N$_2$O	C, H, N
7	125–126	acetonitrile		92	C$_{26}$H$_{22}$N$_2$O$_2$	C, H, N
8	128–130	acetonitrile		90	C$_{26}$H$_{22}$N$_2$O$_2$	C, H, N
9	123–124	acetonitrile	+3.3[b]	93	C$_{26}$H$_{22}$N$_2$O$_2$	C, H, N
10	117–119	acetonitrile	−3.9[b]	94	C$_{26}$H$_{22}$N$_2$O$_2$	C, H, N
12	157–159	CH$_2$Cl$_2$/*n*-hexane	+1.1[c]	91	C$_{15}$H$_{16}$N$_2$O	C, H, N
13	153–154	CH$_2$Cl$_2$/*n*-hexane	−1.0[c]	93	C$_{15}$H$_{16}$N$_2$O	C, H, N

[a] Analytical results were within ±0.4% of the theoretical values. [b] Concentration in MeOH: 0.001 g/mL. [c] Concentration in CHCl$_3$: 0.016 g/mL.

Since **1** is a better inhibitor of human SIRT2 than hSIRT1, compounds **7**–**10** were also tested against hSIRT2. In such assay, **1** showed 71% of inhibition at 100 μM with IC$_{50}$ = 57.7 μM. As for anti-hSIRT1 assay, both *m*-sirtinol (**7**) and *p*-sirtinol (**8**) were more potent than **1** in inhibiting hSIRT2 (from 1.6-fold (**7**) to 2.2-fold (**8**)), while **9** and **10** showed similar IC$_{50}$ values, **10** being slightly more active (1.5-fold) than **1** in this assay (Table 1).

Phenotypic Screening: Telomeric Sir2-Mediated URA3 Gene Silencing. Compounds **1** and **7**–**10** were evaluated for Sir2 in vivo inhibitory activity using a functional test for yeast telomeric silencing. The yeast strain contained a URA3 reporter gene integrated into the subtelomeric region of chromosome VII-L where it is silenced by Sir2.[56] In the presence of 5-fluoroorotic acid (5-FOA), loss of transcriptional silencing in this strain and the corresponding increase in URA3 expression are lethal (Figure 2).[57]

As shown in Figure 3, the growth of the URA3-tagged strain was dramatically reduced relative to the untreated cells (in the upper panel, the sir2Δ strain was used as a positive control for complete loss or silencing of Sir2 activity) in a dose-dependent manner by the addition of 5.6, 16.7, and 25.0 μM Sir2 inhibitors **1** and **7**–**10**. No cytotoxic effect was observed for these inhibitors at 25.0 μM (lower panel).

Conclusion

A series of sirtinol analogues **2**–**10** have been synthesized, and their ability to inhibit yeast Sir2, human SIRT1, and human SIRT2 has been compared to that of sirtinol (**1**). Phenotypic assay, based on the inhibition of Sir2-mediated telomeric URA3 gene silencing, was performed on **1** and **7**–**10**. In vitro assays showed that compounds **2**–**4** and **6**, lacking the 2-hydroxy group at the naphthalene moiety (**2**) or bearing several modifications at the benzene 2′-position of the aniline portion (carbethoxy (**3**), carboxy (**4**), and cyano (**6**)) of sirtinol **1**, were 3- to 13-fold less potent than **1** against yeast Sir2 or were 1.3- to 5-fold less potent against human SIRT1. The 2′-carboxamido analogue **5** was totally inactive in both enzyme assays. 3-[(2-Hydroxy-1-naphthalenyl-methylene)amino]-*N*-(1-phenylethyl)benzamide **7** and 4-[(2-hydroxy-1-naphthalenylmethylene)amino]-*N*-(1-phenylethyl)benzamide **8** (*m*- and *p*-sirtinol, respec-
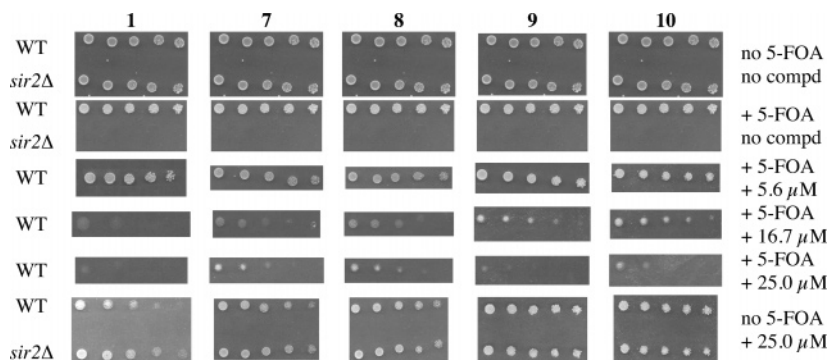
Figure 3. Yeast phenotypic screening performed on **1** and **7−10**. The cell grown is evaluated (after 3−4 days of incubation at 30 °C), comparing the spot areas consisting of 3-fold dilution of growing cells on selective medium.

tively) have IC_{50} values similar to those of **1** for yeast Sir2, while against hSIRT1 and hSIRT2 they were from 1.6 to 10 times more potent than **1**. Furthermore, in phenotypic screening **7** and **8** were endowed with the same potent Sir2 inhibitory activity as **1**. Interestingly, in the **7** and **8** structures the hydrogen bond observed in sirtinol **1** between the naphthyl 2-hydroxy group, the aldimine nitrogen, and the carbonyl of the amide function[58] is considerably less favorable.

Sir2 and Sir2-like inhibitory assays as well as phenotypic screening showed that there is no enantioselective effect of (*R*)-sirtinol (**9**) and (*S*)-sirtinol (**10**), **9** and **10** inhibitory data being similar to each other as well as to **1**. In the hSIRT1 assay, **9** and **10** were 2-fold more potent than **1** in inhibiting the enzyme.

Docking studies were attempted on **7** and **8** to ascertain their possible binding mode by using the published structure of the archaeal Af2 enzyme[35] to dock the inhibitors into NAD^+ and the peptide binding site. Nevertheless, such studies failed to generate any testable hypotheses to reveal the mode of binding of **7** and **8** (see Figure A in Supporting Information).

Further computational and synthesis efforts are in progress to design new sirtinol-related Sir2 and Sir2-like inhibitors.

Experimental Section

Chemistry. Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Perkin-Elmer Spectrum One instrument. ¹H NMR spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer. Chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethyl-silane (Me_4Si). All compounds were routinely checked by TLC and ¹H NMR. TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with spots visualized by UV light. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ∼20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results are within ±0.40% of the theoretical values. All chemicals were purchased from Aldrich Chimica (Milan, Italy) or Lancaster Synthesis GmbH, (Milan, Italy) and were of the highest purity.

Syntheses. The specific examples presented below illustrate general synthesis methods. As a rule, samples prepared for physical and biological studies were dried in high vacuum over P_2O_5 for 20 h at temperatures ranging from 25 to 110 °C, depending on the sample melting point.

General Procedure for the Synthesis of Sirtinol Analogues 2−8. Example: 4-[(2-Hydroxy-1-naphthalenyl-methylene)amino]-*N*-(1-phenylethyl)benzamide (8). A

mixture of 2-hydroxy-1-naphthaldehyde (0.5 g, 2.1 mmol) and 4-amino-*N*-(1-phenylethyl)benzamide **15**[53] (0.4 g, 2.1 mmol) in 30 mL of absolute ethanol/benzene (2:1) in the presence of a catalytic amount of glacial acetic acid was heated at reflux for 4 h. After the mixture was cooled at room temperature, a yellow solid was formed, which was collected by filtration, washed with $CHCl_3$, and purified by crystallization. ¹H NMR (DMSO-d_6) δ 1.47−1.48 (d, 3 H, CHC*H*₃), 5.16−5.19 (q, 1 H, C*H*CH₃), 6.94−6.96 (d, 1 H, NH), 7.20−7.21 (m, 1 H, naphthyl H-3), 7.29−7.54 (m, 7 H, Ph and benzene H-3′,5′), 7.69−7.76 (m, 2 H, naphthyl H-6,7), 7.89−8.01 (m, 3 H, naphthyl H-8 and benzene H-2′,6′), 8.46−8.48 (m, 1 H, naphthyl H-5), 8.83−8.85 (m, 1 H, naphthyl H-4), 9.64 (s, 1 H, CH=N), 14.25 (s, 1 H, OH). Anal. ($C_{26}H_{22}N_2O_2$) C, H, N.

Assays of Recombinant Yeast Sir2 and Human SIRT1. Recombinant His-tagged yeast Sir2p, His-tagged human SIRT1, and His-tagged human SIRT2 were purified and assayed for deacetylase activity using the HDAC fluorescent activity assay (AK-500, BIOMOL Research Laboratories).[59] This assay system allows detection of a fluorescent signal upon deacetylation of a histone substrate when treated with developer. Fluorescence was measured on a fluorometric reader (Wallac Victor III fluorescence plate reader, Perkin-Elmer) with excitation set at 360 nm and emission detection set at 450 nm. Reactions consisted of either 3 μg of ySir2 or 1 μg of SIRT1 or SIRT2, incubated with 250 μM acetylated histone substrate, 1 mM dithiothreitol, and a range of inhibitor concentrations as described. Reactions with the yeast and human proteins were carried out at 30 and 37 °C, respectively, for 60 min. Assays were performed in the presence of 200 μM NAD^+ and each of the inhibitors at 0, 20, 75, 100, 150, or 300 μM.

Telomeric Silencing Assay. Wild type (13978, mat α, adh4::URA3-Tel(VII-L); ppr1::HIS3; leu2; lys2; trp1; ura3; his3; ade2; met15)[56] and sir2Δ (14053, mat α, adh4::URA3-Tel(VII-L); ppr1::HIS3; leu2; lys2; trp1; ura3; his3; ade2; met15; sir2::KANMX)[56] strains, kindly provided by A. Bedalov, were grown to the exponential phase (0.5 OD/mL) in YPD medium, and 9000 cells were plated on SC medium containing 1 mg/mL 5-fluoorotic acid (5-FOA).[57] When appropriate, cells were grown in plates containing 0.18% DMSO and the specified (5.6, 16.7, and 25.0 μM) concentration of Sir2 inhibitor. The cell grown is evaluated (after 3−4 days of incubation at 30 °C), comparing the spot areas consisting of 3-fold dilution of growing cells on selective medium.

Supporting Information Available: Binding mode analysis, figure showing docked conformations, and results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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